Selected immune responses of adult cattle vaccinated with standard and reduced dosages of <u>Brucella abortus</u> strain 19 ISU 1981 by G428

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INTRODUCTION

Since the early 1900s, much of the research on bovine brucellosis has been directed toward the development of an effective immunizing product. The results of these studies with various experimental vaccines have determined strain 19 to possess the best combination of, 1) stable low pathogenicity, 2) relatively high immunogenicity, and 3) moderate antigenicity (54, 69, 86).

Consequently, in 1941, strain 19 vaccine became part of the USDA, Bureau of Animal Industries' (BAI) control program and has been the only official vaccine used in this country since that time. A dose containing 8×10^9 to 12 $\times 10^9$ living cells per ml (in a 5 ml dose) given subcutaneously was recommended (18), however, many vaccinated adult animals became reactors on diagnostic tests. A large proportion of animals vaccinated at four to eight months of age became negative within 13 months after vaccination (18). For these reasons, in the 1940s the BAI limited vaccination with strain 19 to calves between four and eight months of age and to adults only under certain conditions.

From the 1940s to the 1970s, a gradual shift in herd management toward large commercial dairies created a serious problem in establishing a successful brucellosis eradication program in these herds. A study conducted in calves, using reduced dosages of strain 19, indicated that the protection from 1/20th of a standard dose was comparable to that conferred by the standard dose and the problem of persisting vaccination

titers was reduced (70). Field studies were initiated in 1975 to evaluate strain 19 vaccination of adult cattle with various dosages and by different routes of administration. The use of the 1/20th dose of strain 19 in adult cattle appeared to give results comparable to those obtained in calves. As a result of these studies, the use of a reduced dose of strain 19 vaccine was approved¹, with certain restrictions, in adult female cattle in herds where a high prevalence of infection was reported.

While field studies indicated that reduced doses of vaccine administered to adult cows might be of value in controlling brucellosis in infected herds, accurate data concerning the optimum dose that provides adequate protection with little to no stimulation of diagnostically significant levels of antibodies was not provided. To gather such information it would be necessary to conduct vaccination trials under the best possible controlled conditions.

Therefore, this study was initiated to determine, under controlled conditions, the vaccinal titer patterns and the immunity produced by the standard dose and four different reduced dosages of strain 19. The study was designed to eliminate as many variables as possible and provide additional baseline data to aid in interpreting the antibody titers produced by reduced dosages. The cattle purchased for the study met strict nonexposure, age, pregnancy, and health requirements. Systematic

¹USDA, Animal and Plant Health Inspection Service, Veterinary Services, 1977.

serum, milk, vaginal swab and tissue collection schedules were developed and followed, including the cultural examination of tissues at the termination of the study.

LITERATURE REVIEW

History

Since 1897, the year Professor Bernard Bang (7) and his associate Stribolt, announced their discovery of the causal agent of "contagious abortion" in cattle, a concentrated effort has been made to find a method which would provide immunity to this costly disease. Bang suggested, in 1897, that artificial immunization was a possibility. Later, in 1906, Bang (8) reported on experiments using both living and killed suspensions of <u>Brucella abortus</u> as immunizing agents in cattle, sheep, and goats. His results with living vaccines were encouraging.

Since then, vaccination methods, dosages, and types of vaccines have received much attention by many investigators. McFadyean and Stockman (66), in 1909, injected single, massive doses of live <u>B</u>. <u>abortus</u> subcutaneously into virgin heifers and produced an active immunity.

A great controversy developed among researchers over the use of vaccination in cattle. Some felt that vaccinating infected, as well as noninfected cattle, with the virulent organism produced an effective immunity (82, 83). Others explained the beneficial results were accounted for by a saturation-tolerance (22). Huddleson reported that a nonvirulent, highly agglutinogenic strain of <u>Brucella</u> would produce a good immunity without the hazards that followed the use of extremely virulent vaccines (41).

A considerable volume of research on various vaccines has accumulated over the years. These efforts have been directed primarily toward the production of an effective, durable immunity. In addition to the goal of finding the most effective vaccination procedure, the problem of persisting agglutination titers in vaccinated animals became a major disadvantage in the use of vaccine.

Buck and Creech (17) studied the length of time that live <u>B</u>. <u>abortus</u> remained in the animal. A study was conducted in which two-thirds of the cattle in a large infected herd were vaccinated and the remaining one-third were used as controls. Their analysis indicated that heifers, which had a negative serological titer at the time of vaccination, had 5% fewer abortions during the first calving season and 7% fewer abortions during the second calving season than did the control cattle. A second more controlled and defined study was conducted on eight heifers and five cows. A 20 cc dose containing approximately one billion organisms per cc of live <u>B</u>. <u>abortus</u> was given. As a result, they observed that even though marked serological reactions were produced in susceptible heifers by the injections, the titers declined in four to five months.

Hart and Traum (38) reported on work using four strains of <u>B</u>. <u>abor-</u> <u>tus</u> (A, 80, 101, and a commercial strain) as vaccines. Suspensions containing 11 billion live organisms per ml were prepared and each animal was given 20 ml subcutaneously. The vaccine prevented abortions and

in addition, it was shown that organisms were shed prior to the development of a detectable agglutinin response. This pointed out the limitations of interpreting a negative agglutination test as an indication that the animals were free from infection. It was further observed that cattle had persistent titers of 1:100 or higher for several months to one year following injection at this dosage level.

Buck (16) vaccinated 18 heifer calves in order to determine the duration of immunity. Twelve were vaccinated subcutaneously with 20 cc of live <u>B</u>. <u>abortus</u> and the remaining six calves were used as controls. Three different lots of vaccine were prepared and used. One lot containing strains 8, 10, and 11 was administered to each of six animals; strain 88 was administered to three animals; and strain 19 was administered to three animals. Buck found that all three vaccine lots produced similar results. He felt that "medium virulence" strains such as <u>B</u>. <u>abortus</u> strain 19 should be used in place of the highly virulent strains. He concluded that the subcutaneous administration of the vaccine provided adequate immunity during the first pregnancy without producing sterility.

In 1933, Cotton and Buck (22) reported on the comparative virulence of three different strains of <u>B</u>. <u>abortus</u> (11, 19, and 484), when administered to guinea pigs and cattle. The results indicated that strains 11 and 19 did not infect the udders of nonpregnant cows and that all three strains were similar in their ability to produce immunity. Other investigators (29, 63, 85) searched for additional low virulence strains

that might be even more immunogenic. McEwen and Priestley found that a live vaccine prepared from a rough strain of <u>B</u>. <u>abortus</u> 45/20 provided adequate immunity, but that it tended to revert to its virulent smooth form.

Manthei et al. (57) and McDiarmid (60) compared the immune response in cattle vaccinated with varying doses of <u>B</u>. <u>abortus</u> strain 19 by the intradermal and subcutaneous routes. Manthei found that the maximum agglutinin titers were similar in animals inoculated with either 5 ml subcutaneously or 0.2 ml intradermally, and that the serological response from the 0.2 ml dose was considerably lower than the other two dosages. McDiarmid (60), however, failed to see any significant differences in the serological titers for these different dosages or routes. Both investigators found the degree of post-vaccinal agglutinin response was not related to the degree of immunity to brucellosis in cattle vaccinated with strain 19. The degree of immunity against brucellosis was similar in all the groups of vaccinated cattle.

In 1973, Worthington et al. (93) studied the persistence of antibody titers in vaccinated cattle. One hundred twenty-eight pregnant cows were vaccinated with 100 X 10^9 viable <u>B</u>. <u>abortus</u> strain 19 organisms and blood samples were collected and tested at monthly intervals for two years using the following procedures; the complement fixation (CF), mercaptoethanol (ME), rivanol (RIV), Coombs, and tube agglutination tests. The agglutination tests became positive during the first month

following vaccination and the titers persisted at levels above 64 I.U. in most of the cattle for over two years. The CF, RIV, and ME titers peaked one month post vaccination, then declined rapidly and became negative after five months. Seventy-five percent of the cattle remained negative on the CF test for the 18 month period of observation, whereas 25% had occasionally low titers. Application of the CF test six months following vaccination made it possible to determine whether the persistent agglutination titers were due to vaccination or natural infection in 90% of the cattle.

In 1976, Plommet and Fensterbank (77), and again in 1979, Fensterbank and Plommet (32), investigated a new route of vaccination, the conjunctival route, traditionally used as the site for challenge. In the first study, they used 74 heifers, 7-12 months old, and divided them into four groups; an unvaccinated control group; a second group vaccinated subcutaneously with 9 X 10^{10} <u>B</u>. <u>abortus</u> strain 19; a third group vaccinated subcutaneously with 9 X 10^{10} strain 19, then revaccinated by the conjunctival route six to eight months later with 5 X 10^9 strain 19 organisms; and a fourth group vaccinated twice by the conjunctival route with the same dose and time intervals as in the third group. Following challenge with <u>B</u>. <u>abortus</u> strain 544, the average degree of infection per group, as determined by culturing techniques, was significantly lower in the vaccine groups as compared to the Control Group. Groups 3 and 4 had a significantly lower degree of infection than the

group vaccinated subcutaneously. The second group developed agglutinin titers in which the geometric mean reached 1500 I.U. in 15 days post vaccination and receded to less than 30 I.U. seven months later. All cattle in Group 2 were negative 60 weeks post vaccination. The third group developed agglutinin titers similar to Group 2 until the booster vaccination was given by the conjunctival route. This caused a rise in titers for two months; however, the titers receded and became negative at about the same time period as the cattle in Group 2. The mean agglutination titers of Group 4 did not reach 30 I.U. after the first vaccination. Following the second vaccination, some titers were positive for a maximum of eight weeks with the group mean agglutinin titer of 30 I.U. lasting for one week. Flommet and Fensterbank felt the conjunctival route of vaccination could be used to vaccinate cattle at any age without serious interference in routine diagnostic tests.

After a lapse of approximately 30 years, adult vaccination has been reinstated in the southern United States as an immunizing procedure. Nicoletti et al. (74, 75) studied adult vaccination using various dosages and routes of administration of the strain 19 vaccine in large infected dairy herds. The serological tests used included the standard tube agglutination (STT), mercaptoethanol (ME), rivanol (RIV), card, and complement fixation (CF). The CF test became negative before the agglutination tests following vaccination. They concluded that a 0.2 ml dose of strain 19 given subcutaneously provided an adequate protection without

creating a persistent titer problem and that the CF test proved to be superior to other tests in correctly identifying culture positive cattle.

One of the hazards occasionally encountered with adult vaccination is vaccine induced abortion. Several reports of abortion following vaccination of cows in late gestation occur in the literature. Haring (35) induced uterine infection and abortion with strain 19 in one cow vaccinated during advanced gestation. Strain 19 was isolated from one heifer out of 40, which McDiarmid (60) vaccinated at approximately two months gestation. Manthei (52) reported strain 19 caused one abortion in 76 pregnant cattle inoculated with 5 ml of vaccine subcutaneously and one abortion in 27 pregnant cattle inoculated with a 0.2 ml of strain 19 intradermally. Deem and Gross (25) recovered organisms similar to strain 19 from two aborted fetuses collected from two herds containing a total of 116 cows vaccinated in late gestation following known exposure to brucellosis reactors.

Others (30, 68, 92) have reported abortions following vaccination of pregnant cows but could not prove that these abortions were due to strain 19 infection. The majority of abortions reported in pregnant cattle vaccinated with strain 19 have occurred in cattle vaccinated in late gestation.

Mode of Transmission

The transmission of <u>B</u>. <u>abortus</u> is primarily by ingestion of food or water contaminated directly or indirectly with infectious material

such as uterine discharge, aborted fetus or placenta. The organism may invade the surface of mucous membranes. Other means of spreading the organism include venereal and dam to offspring transmission (48). Brucellosis in man, caused by <u>B</u>. <u>abortus</u>, is primarily transmitted by handling infected cattle or ingestion of food products such as milk or meat (58, 67).

Diagnosis of Bovine Brucellosis

The diagnosis of brucellosis is primarily accomplished by serological tests and direct cultural examination of milk or tissue samples (2). Other diagnostic procedures include animal inoculation (2) and skin tests (44). The isolation and identification of <u>B</u>. <u>abortus</u> provides the best evidence that infection is present (2). Milk, placenta, vaginal mucous, fetal stomach contents, fetal lung, fetal spleen, meconium, and occasionally blood are cultured from live cows in order to isolate <u>Brucella</u> (2). Tissues collected from cattle at slaughter are also cultured for isolation of <u>Brucella</u>. These include, in order of importance as sources of <u>Brucella</u>; the supramammary lymph nodes, internal iliac lymph nodes, lumbar lymph nodes, parotid lymph nodes, spleen, uterus, and mammary tissues (2).

It is not always possible to isolate <u>Brucella</u> from infected cattle; therefore, the serological and milk antibody tests have been important in diagnosis. The tube agglutination and complement fixation tests were

first used in diagnosis of brucellosis (40, 84). In 1926, Huddleson et al. described the rapid plate agglutination test (43). The <u>Brucella</u> Ring test, used for detecting agglutinins in milk, was first reported in 1937 and used in field studies in the late 1940s (79). The acidified plate antigen (APA) test, reported by Roepke et al. (80) in 1956, uses acetic, lactic, or tartaric acid to reduce the pH of the serum-antigen mixture between the ranges of three to four. This low pH inhibits nonspecific agglutination reactions.

In the early 1960s, Anderson et al. (4) developed the rivanol precipitation plate agglutination test (Rivanol test). A 1% solution of rivanol and serum are mixed in equal quantities and centrifuged. The nonspecific (high molecular weight) agglutinins precipitate with the proteins; whereas, the specific (low molecular weight) agglutinins remain in the supernatant which is used for testing.

The card and mercaptoethanol (ME) tests were developed in 1964 (3, 76). The low pH of the buffered antigen used in the card test inhibits nonspecific agglutination reactions; whereas, the ME test utilizes 0.05 molar mercaptoethanol to inactivate the IgM class of antibodies.

MATERIALS AND METHODS

Brucella Organisms

Vaccine inoculum preparation

A lot of lyophilized vaccine was prepared, as previously described, from "Original Seed"¹ using liquid medium, sodium carboxymethyl-cellulose method for separating the cells and a lyophilizing stabilizer (2, 5). The original fill volume of single dose vials was 2 ml. It had previously been determined that after restoration of the vaccine to the original fill volume it could be stored at 4[°] C for several days with only a slight decrease in viability.²

Four days before vaccination of the animals, 20 vials of vaccine were restored to 2 ml volumes and the contents were then pooled and a viability count conducted. The results of the count indicated the pool contained 22 X 10^9 viable organisms per ml. On the day of vaccination, this concentrate was diluted in phosphate buffered saline pH 6.4 to make five lots of vaccine containing 78 X 10^9 , 5.0 X 10^9 , 9.2 X 10^8 , 1.4 X 10^8 , and 2.3 X 10^7 viable organisms/dose respectively as determined by viability counts post-dilution. After the vaccination was completed, a viability count was again made on an unused portion of each lot of vaccine and no appreciable difference was found between the two counts.

²R. D. Angus, unpublished data, Ames, IA.

^LUSDA, original stock culture of <u>B</u>. <u>abortus</u> strain 19 prepared by the Reagents Section, Scientific Services Laboratory, National Veterinary Services Laboratories, Ames, IA.

Challenge inoculum preparation

The challenge inoculum was prepared from a lyophilized culture of <u>B</u>. <u>abortus</u> strain 2308, the USDA Challenge Strain.¹ Prior to lyophilization, the culture had been passaged in guinea pigs and recovered. After the recovery from guinea pigs, the culture was passaged on artificial medium a minimum of times in order to prepare it for lyophilization.

The lyophilized culture was restored using sterile distilled water and then plated onto tryptose agar containing 5% bovine serum. The inoculated plates were incubated at 37° C for 96 hours and examined under a broad field dissecting microscope using a reflected light source. An area of typically smooth colonies was selected and inoculum was transferred to tryptose agar slants. The slants were incubated at 37° C for 24 hours after which the growth was washed from the surface of the agar using phosphate buffered saline, pH 6.4 (PBS). The density of the cell suspension was adjusted to give 66% light transmittance at a wave length of 600 nm on a B & L Spectronic 20 spectrophotometer,² then a 1:2000 dilution was made using PBS and duplicate viable counts were made using standard procedures (86). The viability count conducted on the day of challenge indicated the inoculum contained 8.27 X 10⁵ organisms per ml.

¹Prepared by the Reagents Section, Scientific Services Laboratory, National Veterinary Services Laboratories, Ames, IA.

²Bausch & Lomb, Inc., Rochester, NY.

Serological Procedures

Six serological tests were used to evaluate the antibody response to vaccination and challenge. The test procedures are described below. <u>Tube agglutination test</u>

Tube agglutination test antigen was a 4.5% (by volume) stock suspension of <u>B</u>. <u>abortus</u> strain 1119-3. When used in the laboratory, the stock antigen was diluted at 1:100 with saline containing 0.5% phenol. Serum quantities of 0.08, 0.04, 0.02, 0.01, and 0.005 ml were measured into five separate test tubes and 2.0 ml of diluted antigen added to each tube. This resulted in dilutions of 1:25, 1:50, 1:100, 1:200, and 1:400, respectively. The tubes were incubated at 37° C for 48 hours and read for clearing and agglutination against a dull black background, with light coming from behind the tubes. When the serum samples were positive at the 1:400 dilution, additional doubling dilutions, up to 1:1280, were made to determine the end point titer. Each dilution was read as positive, incomplete, or negative (89).

Plate agglutination test

The plate test has been standardized so that, by using the same quantities of serum as in the tube test, the results obtained are comparable to the results of the tube test. The antigen contained 11.0% (by volume) of <u>B</u>. <u>abortus</u> strain 1119-3 and was stained with a mixture of crystal violet and brilliant green. One drop (0.03 ml) of antigen was added to each quantity of serum (0.08, 0.04, 0.02, 0.01, and 0.005 ml) on a plate, mixed, rotated, and then incubated at room temperature for

eight minutes. The plate was rotated for four rotations immediately after mixing and after four and eight minutes of incubation. Immediately after the final rotation of the plate, the test was read against a black background with indirect lighting (89). Each dilution was read as positive, incomplete, or negative.

Rivanol precipitation test

In the rivanol precipitation-plate agglutination test, equal quantities of serum and 1.0% solution of rivanol¹ were mixed and centrifuged for five minutes. The supernatant was tested by placing five quantities (0.08, 0.04, 0.02, 0.01, and 0.005 ml) on a glass plate (such as the Minnesota test box) and one drop (0.03 ml) of rivanol antigen (prepared as outlined in the Diagnostic Reagents Manual 65-C (88)) was added to each quantity of supernatant and mixed. The results were read after the mixture on a plate was incubated at room temperature for 12 minutes. The plate was rotated four times at the beginning of incubation, after six minutes, and after 12 minutes incubation (just prior to reading). The results using five quantities of serum were considered comparable to dilutions of 1:25, 1:50, 1:100, 1:200, and 1:400, respectively, and the reaction of each dilution was read as negative, incomplete, or positive (90).

Mercaptoethanol test

The mercaptoethanol test (ME test) was performed similar to the standard tube test except that rather than adding 2 ml of a 1:100 dilution

¹Roussel Corporation, New York, NY.

of the concentrated tube antigen, 1 ml of 0.1 molar mercaptoethanol and 1 ml of a 1:50 dilution of the concentrated tube antigen were added (making a final ME concentration of 0.05 M). Phenol was not used as a preservative, as it caused cloudiness in the test suspension. The period of incubation and the reading of the results are the same as described for the tube test (90).

Complement fixation test

Serum samples were diluted 1:10 with veronal buffer (VB) and heated at 56° C for 30 minutes to inactivate resident complement. Tubes were placed in racks to provide for dilution of the serum up to 1:1280 in a doubling dilution scheme. The first tube for each sample was used as a serum control containing 0.5 ml inactivated serum and 1.0 ml complement. The second tube was the first test dilution (1:10) and contained 0.5 ml inactivated serum, 0.5 ml antigen (<u>Brucella</u> tube test antigen diluted 1:500) and 1.0 ml complement. Remaining tubes were the same as tube 2 except that the serum had been serially diluted in VB to provide for titration of the antibody activity. The racks of tubes were shaken and placed at 4° C overnight to allow for antigen-antibody reaction and fixation of the complement.

Following the reaction period, 1.0 ml of sensitized RBC's were added to each tube. The racks were shaken and then incubated in a waterbath for 30 minutes at 37° C and then read. No hemolysis indicated a "++++"

¹Conducted by the Serology and General Bacteriology Section, Diagnostic Bacteriology Laboratory, National Veterinary Services Laboratories, Ames, IA.

titer with 100% fixation. Twenty-five percent hemolysis was read as a "+++" titer with 75% fixation. Fifty percent hemolysis was read as a "++" titer with 50% fixation, and 75% hemolysis indicated a "+" titer with 25% fixation (40).

Card test

The card test was conducted on disposable, waxed cardboard sheets with ten teardrop indentations.¹ Equal quantities (0.03 ml) of serum and antigen were placed in an indentation and mixed. Each mixture was spread over the entire surface of the indentation. The card was then rocked for four minutes on a card test rocker² and the results were read immediately. The presence of an agglutination reaction indicated a positive test (90).

Experimental Animals

For this study 135 female beef cattle, $l_2^{\frac{1}{2}}$ to 2 years of age, were purchased from two Nebraska ranches. Prior to purchase, the cows were examined and found to be free of any clinical signs of disease. Blood samples were taken from each animal and the serum was tested for <u>Brucella</u> antibodies using the card, tube, plate, rivanol, mercaptoethanol, and complement fixation tests. The cattle were all serologically negative for brucellosis on all tests. In addition, they were certified by the owners as unvaccinated for or having no known exposure to brucellosis.

¹Hynson, Wescott & Dunning, Baltimore, MD. ²Tetracon Assn. Inc., Norman, OK. The cows were transported to Ames, Iowa and unloaded into an isolated cattle pen. Six days later, nine bulls were placed with the cattle for nine weeks (three estrus periods). The bulls were from an Iowa bull testing farm. They had no history of contact with <u>Brucella</u>, either from vaccination or exposure. Elood samples were drawn and subjected to the same tests as described above and were found to be negative. One week following removal of the bulls, each cow was examined for pregnancy. Ninety-five pregnant animals were randomly selected and transported to medium security animal housing facilities at the National Veterinary Services Laboratories (NVSL) for the vaccination-challenge phase of the study. The remaining 40 animals were transported to other animal housing facilities at NVSL and used for the vaccination-nonchallenge phase of the study. Any animals, which were diagnosed as being nonpregnant during the first examination, were reexamined 45 days later.

The 95 pregnant cows used in the vaccination-challenge phase of the study were housed separately in individual indoor stalls. Each animal had a separate headgate for restraint, separate feed containers, and separate water bowls. The cows were maintained in these stalls for the duration of the study. Great care was used to minimize contact between animals, caretakers, and equipment.

All personnel were required to wear rubber boots, coveralls, rubber gloves, and face shields. Boots and gloves were disinfected in a solution of Microbac¹ in water, after exiting from a stall and prior to the

¹Professional Products Division, Economics Laboratories, Inc., St. Paul, MN.

entry into other stalls or feed areas. The animals were bedded with wood shavings. The pens were scraped clean once every two days. The animals were fed a maintenance ration, supplemented with alfalfa hay or hay cubes.

The 40 cows used in the vaccination-nonchallenge phase were housed together in an open, dry lot with a loafing shed for shelter.

Vaccination Phase

Vaccination-challenge groups

Following serological testing and pregnancy examination (see Table 2), 95 pregnant animals were randomly assigned to six groups and vaccinated subcutaneously with B. abortus strain 19 vaccine as shown in Table 1.

| 0 | No. animals | Vaccine dosage | | |
|---------|----------------|---------------------------|------------------------|--|
| Group | | Volume | No, viable organisms | |
| VC-1 | 15 | 5 ml (standard dose) | 78.0 X 10 ⁹ | |
| VC-2 | 15 | 2 ml (6.4% of std. dose) | 5.0 x 10 ⁹ | |
| VC-3 | 15 | 2 ml (1.2% of std. dose) | 9.2 X 10 ⁸ | |
| VC-4 | 15 | 2 ml (0,18% of std. dose) | 1.4 X 10 ⁸ | |
| VC-5 | 15 | 2 ml (0.03% of std. dose) | 2.3 X 10 ⁷ | |
| Control | 20 | none | none | |

Table 1. Dosage schedule for vaccination-challenge groups

| Ùate | Time interval from vaccination | Blood sample collected | Comments |
|------------------|---------------------------------------|---------------------------|--|
| 9-15-77 | · · · · · · · · · · · · · · · · · · · | yes | 135 cows examined indi- vidually for clinical signs of disease |
| 9-16-77 | | | 135 cows delivered to site |
| 9 - 22-77 | week -12 | -4 | 9 bulls added to herd |
| 11-24-77 | week -3 | | bulls removed |
| 11-(28-29)-77 | week -2 | yes | pregnancy examination |
| 12-6-77 | week -1 | yes | |
| 12-13-77 | week 0 | yes | vaccinate 5 groups with different dosages |
| 12-20-77 | week l | yes | |
| 12-27-77 | week 2 | yes | · · |
| 1-3-78 | week 3 | yes | |
| 1-10-78 | week 4 | yes | reexamination of non- pregnant cows |
| 1-17-78 | week 5 | yes | Dr.eguante comp |
| 1-24-78 | week 6 | yes | • |
| 1-31-78 | week 7 | yes | · |
| 2-7-78 | week 8 | yes | |
| 2-21-78 | week 10 | yes | |
| 3-7-78 | week 12 | yes | |
| 3-21-78 | week 14 | yes | |
| 4-4-78 | week 16 | yes | challenge 95 animals |
| | | | |

Table 2. Weekly chronology of principal procedures performed with reference to vaccination

Table 2. (Continued)

| Date | Time interval from vaccination | Blood sample collected | Comments |
|----------------------|-----------------------------------|---------------------------|--|
| 4 -11- 78 | week 17 | yes | |
| 4-18-78 | week 18 | yes | |
| 4-25-78 | week 19 | yes | |
| 5-2-78 ^a | week 20 | yes | |
| 5-9-78 | week 21 | yes | |
| 5-16-78 | week 22 | yes | |
| 5-23-78 | week 23 | yes | |
| 5-30-78 ^a | week 24 | yes | |
| 6-6-78 | week 25 | yes | |
| 6-13-78 | week 26 | yes | |
| 6-20-78 | week 27 | yes | |
| 6-27-78 ^a | week 28 | yes | |
| 7-11-78 | week 30 | yes | |
| 7-25-78 ^a | week 32 | yes | |
| 8-8-78 | week 34 | yes | |
| 8-22-78 | week 36 | yes | last week that all cattle were sampled |
| 9-5-78 | week 38 | yes | |
| 9-19-78 ^a | week 40 | yes | |
| 8-22 to 10-15 | Cattle were s | old for slaugh | ter and tissues collected |

^aForty nonchallenged animals bled at one month intervals after challenge of the other 95 animals.

A blood sample was collected from each animal and serological tests (tube, card, mercaptoethanol, rivanol, plate, and complement fixation) were conducted at one week intervals for the first eight weeks after vaccination and at biweekly intervals for the next eight weeks (Weeks 9-16).

Vaccination-nonchallenge groups

The remaining 40 animals were randomly assigned to five groups of eight animals/group and vaccinated subcutaneously according to the schedule in Table 3. All but three animals were pregnant. For 16 weeks post vaccination, the blood sampling and testing followed the same schedule as for the vaccination-challenge groups; however, these animals were not challenged. Blood samples were collected and tested at four week intervals for Weeks 17-36 post-vaccination.

| | No. animals | Vaccine Dosage | | | |
|-------|----------------|---------------------------|------------------------|--|--|
| Group | | Volume | No. viable organisms | | |
| V-l | 8 | 5 ml (standard dose) | 78.0 X 10 ⁹ | | |
| V-2 | 8 | 2 ml (6.4% of std. dose) | 5.0 X 10 ⁹ | | |
| V-3 | 7 | 2 ml (1.2% of std. dose) | 9.2 X 10 ⁸ | | |
| V -4 | 8 | 2 ml (0.18% of std. dose) | 1.4 X 10 ⁸ | | |
| V-5 | 8 | 2 ml (0.03% of std. dose) | 2.3 X 10 ⁷ | | |

Table 3. Dosage schedule for vaccination-nonchallenge groups

When parturition occurred, the placental membranes, swabs of vaginal mucus and quarter milk samples from each animal were cultured for <u>Brucella</u>. Thereafter, quarter milk samples were cultured¹ at two week intervals from animals which strain 19 was isolated or with serologic evidence of persistent infection with strain 19. Tissues were collected from each animal at slaughter and cultured¹ for <u>B</u>. <u>abortus</u>.

Challenge Phase

Challenge procedure

Sixteen weeks after vaccination, 75 vaccinates and 20 nonvaccinates were exposed by inoculating 1 ml containing 8.27×10^5 viable organisms of <u>B</u>. <u>abortus</u> strain 2308 into the conjunctival sacs of each animal. Half the dose was placed into each eye and the eyelids were held shut and massaged gently for one minute to allow time for absorption.

Post-challenge examinations

The post-challenge antibody response of each animal was determined using the serological procedures previously mentioned. When parturition or abortion occurred, the aborted fetus, placental membrane, swabs of vaginal mucus, and quarter milk samples from each animal were cultured¹ for B. abortus.

Milk samples were collected and cultured¹ from the cows from which

¹Vaginal mucus, milk, and tissues taken from cows at slaughter were cultured by the Mycobacteria and Brucella Section, Diagnostic Bacteriology Laboratory, NVSL, Ames, IA. and fetal tissues and placental membranes were cultured by the Reagents Section, Scientific Services Laboratory, NVSL, Ames, IA.

<u>B</u>. <u>abortus</u> was not isolated, at two week intervals until <u>Brucella</u> organisms were isolated or until completion of the study. If isolations were made, the milk samples were collected and cultured until three negative weekly samples were collected.

Samples of the vaginal mucus were collected weekly from each animal following challenge and on the day of calving or abortion. Animals which remained negative for <u>Brucella</u> on culture for three continuous weeks, following calving or abortion, were not swabbed for the remainder of the study. Vaginal swabs were collected and cultured¹ at weekly intervals from each vaginal swab culture positive animal until negative results were obtained for three consecutive weeks.

Cattle that became serologically negative and those from which <u>B</u>. <u>abortus</u> was isolated were the first animals to be sent to slaughter following Week 36. Tissues (supramammary, retropharyngeal, iliac and lumbar lymph nodes and a section of the spleen, uterus and each quarter of the udder) were collected at slaughter from each animal and cultured² for <u>Brucella</u>.

Cultural Procedures

Bacteriologic culture mediums

The two mediums used to isolate the <u>Brucella</u> organisms by direct inoculation were designated No. 2 and No. 3 as described by Nelson et al. (73).

¹Amy Armbrust, Masters Thesis, ISU, 1979 (6).

²Tissues were cultured by the Mycobacteria and Brucella Section, Diagnostic Bacteriology Laboratory, NVSL, Ames, IA.

<u>Medium No. 2</u> (serum tryptose agar with antibiotics) Medium No. 2 contained 41 gm of Formula 0064¹ in one liter of water. After sterilization the medium was cooled to 50° C and immediately prior to dispensing the medium into petri dishes, 50 ml of sterile bovine serum with 1,800 units of polymixin B, 7,500 units of bacitracin, and 30 mg of cyclohexamide² were added to enrich the medium and aid in preventing the growth of contaminates.

<u>Medium No. 3</u> (serum tryptose agar with antibiotics and crystal violet) Medium No. 3 was prepared in the same manner as No. 2 except that 4 ml of 1:2600 solution of crystal violet was added to each liter of medium just prior to sterilization.

<u>Milk sample collection</u>

Samples of milk from each quarter of the udder were collected directly into sterile whirl-pak bags.³ Prior to collection the udder was washed with a H₂O and Betadine⁴ solution. To further reduce contamination, disinfected rubber gloves were worn while milking. The samples were placed in insulated containers and taken directly to the laboratory. Twenty milliliters of milk from each quarter sample were pipetted into a sterile tube and centrifuged at approximately 5,000 X g for 15 minutes. The cream and sediment from each sample was inoculated onto separate

¹Difco Laboratories, Detroit, MI.

²Acti-Dione, Calbiochem, Los Angeles, CA.

³Arthur H. Thomas Co., Philadelphia, PA.

⁴Purdue Frederick Co., Norwalk, CT.

plates of Medium No. 2 and No. 3. The plates were incubated in air at 37° C for a maximum of seven days and examined for the growth of <u>Bru</u>-cella-like colonies.

Tissue sample collection

The tissues obtained from each animal at slaughter were collected as aseptically as possible, placed in sterile whirl-pak bags, identified, and placed in insulated containers with dry ice. The frozen tissues were stored at -20° C and processed within three months following collection.

The methods described by Nelson et al. (73) were used to prepare and culture the tissue samples. A section of tissue was cleaned of all exterior fat, dipped in 95% ethyl alcohol and flamed. The tissue was incised with a sterile scalpel. Multiple cuts were made to increase the exposed surface area and then it was applied directly to the surface of the medium. Two plates, one containing Medium No. 2 and one containing Medium No. 3, were inoculated using this procedure. The plates were incubated at 37° C in an air environment and examined periodically for <u>Brucella</u>-like colonies for a maximum of seven days in an air environment. <u>Vaginal mucus collection</u>

Samples of vaginal mucus were aseptically collected on sterile disposable Kalayon guarded culture instruments,¹ placed in sterile labeled tubes and transported in insulated containers to the laboratory. The culture procedure and results are described and reported elsewhere by Armbrust (6).

W. A. Butler, Columbus, OH.

Identification and verification of Brucella isolates

The colonies which developed on selective mediums were isolated for study and those which had characteristics of <u>Brucella</u> were subjected to the established procedures (91) for accurate identification of the <u>Brucellae</u>. Antigenic and biochemical tests were conducted on each <u>Brucella</u> strain isolated following the procedures reported by Alton et al. (2). In order to distinguish between vaccination and challenge strains, the following differential characteristics were determined as described by Brown et al. (15).

Table 4. Tests for differentiation of <u>B</u>. <u>abortus</u> strain 19 and 2308

| Medium containing | Strain 19 | Strain 2308 |
|-----------------------|-----------|-------------|
| Thionin blue 1/50,000 | _aaaa | , + D |
| Penicillin 5 units/ml | - | + |
| Erythritol 1 mg/ml | - | + |

^aIndicates no growth in the presence of these factors. ^bIndicates growth in the presence of these factors.

Reading and Interpretation of Serologic Results

The card test results are based on the presence or absence of an agglutination reaction and are read as positive or negative. Therefore, the graphs depicting the card test results represent, as a percentage,

the total number of cows that were positive during each week.

The agglutination tests (tube, plate, ME, and rivanol) are conducted with serum dilutions of 1:25, 1:50, 1:100, 1:200, etc. After incubation, each serum dilution was read for clearing and agglutination. The highest dilution showing complete or incomplete agglutination was recorded as a positive test. Therefore, the graphs depicting these test results present the dilutions along the y-axis. Each dilution numbered is positive (i.e. +25) while halfway between the positive dilutions is an incomplete positive (i.e. I50).

The CF test was conducted with serum dilutions of 1:10, 1:20, 1:40, 1:80, etc. After incubation, each serum dilution was read and the degree of hemolysis was recorded. A "+" indicates 25% of the Red Blood Cells (RBC's) are hemolyzed. A "++" indicates 50% are hemolyzed, a "+++" indicates 75% are hemolyzed, and a "++++" indicates 100% of the RBC's are hemolyzed. Therefore, the graphs depicting the CF test results have the dilutions listed along the y=axis.

RESULTS

The results are divided into four sections: 1) the antibody responses of the vaccination-nonchallenge groups, 2) the antibody responses of the vaccination-challenge groups and the nonvaccination-challenge control group, 3) a comparison of the results of the tube and mercaptoethanol tests is made to depict the difference between the IgG and IgM response for each group of cattle, and 4) pertinent clinical observations and individual serologic and cultural results

Vaccination-Nonchallenge Phase

The antibody levels in blood samples collected weekly or biweekly from each cow were determined using six serological tests and the results are presented in graphic form.

Card test results

Figure 1 displays the antibody response of the five vaccinationnonchallenge groups (eight animals/group) as judged by the card test. The card test is read as a positive or negative; therefore, the graph represents the percentage of cows that were positive on the test, by week, for 36 weeks following vaccination.

Group V-1 received the standard dose of <u>B</u>. <u>abortus</u> strain 19 vaccine (78 X 10^9 organisms). These cattle became positive on the card test one week following vaccination and remained positive until after Week 18. One animal became negative during Week 20. On Week 22, all of the

Results of biweekly and monthly card tests for the five vaccination-nonchallenge Figure 1.

groups following <u>B</u>. <u>abortus</u> strain 19 vaccination Group V-1 (+) received 78 X 10⁹ organisms (8 animals per group) Group V-2 (*) received 5 X 10⁹ organisms (8 animals per group) Group V-3 (#) received 9.2 X 10⁸ organisms (7 animals per group) Group V-4 (D) received 1.4 X 10⁸ organisms (8 animals per group) Group V-5 (X) received 2.3 X 10⁷ organisms (8 animals per group)

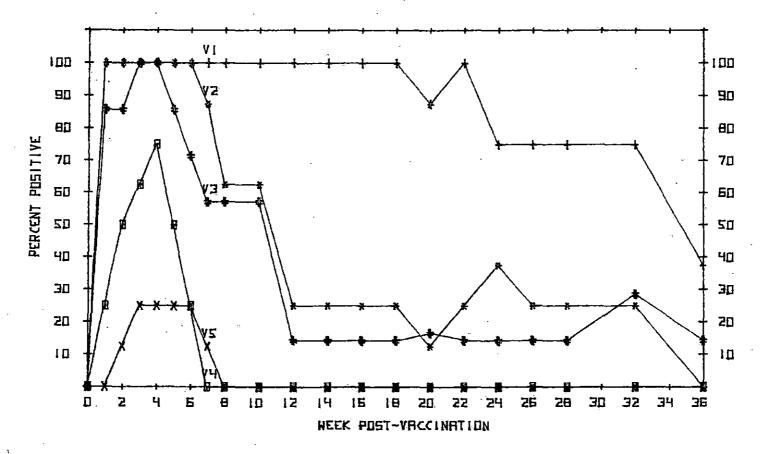


FIGURE I

cattle were positive but decreased to 75% positive during Weeks 24-32. Thereafter, the percentage of positive cattle declined to 37% by Week 36.

Cattle in Group V-2 received a vaccine dose of 5 X 10⁹ organisms. All animals were positive on the card test after one week and remained positive until Week 6. After the sixth week, the percent positive declined rapidly until Week 12; where only 25% of the cattle were positive (2 animals). The card test reactions tended to remain at about 25% positive from the 12th to the 32nd week, however, there was some fluctuation. For example, at the 20th week only 23% were positive (1 animal) and at Week 24, 37% reacted (3 animals). All animals were negative by the 36th week.

Group V-3 cattle $(9.2 \times 10^8 \text{ B. abortus} \text{ strain 19 organisms})$ were over 85% positive during Weeks 1 and 2, and all were positive during Weeks 3 and 4. Thereafter, the percentage of positive reactions declined sharply until Week 12 when 12% of the cattle (1 animal) remained positive until Week 36. One additional animal became positive during Week 32.

The percentage of positive reactions in the Group V-4 cattle (1.4 X 10^8 <u>B</u>. <u>abortus</u> strain 19 organisms) peaked at 75% by Week 4, then declined until all cattle were negative on the card test by the seventh week. Group V-5 received 2.3 X 10^7 <u>B</u>. <u>abortus</u> strain 19 organisms, which was the lowest dosage used. Only 25% (2 animals) were

positive by Week 3 and they reverted to negative by Week 8.

The percentage of cattle responding on the card test and the persistence of antibodies as detected by the card test were directly correlated with the dosage of vaccine given. The highest dosage group remained over 35% positive by Week 36, whereas Groups V-2 and V-3 were almost negative by Week 36. The lowest two dosages were negative by Week 8.

Tube test results

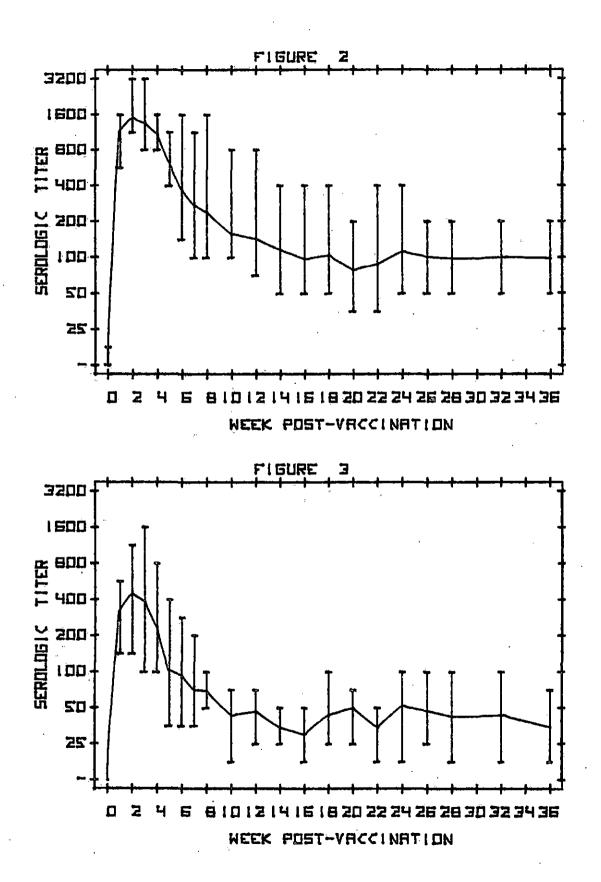
The serologic results for the groups of vaccination-nonchallenge cows on the tube test are presented in Figures 2-6. The graphs represent the geometric mean titer of each group. The titer range for each sampling date is also depicted. All animals in the vaccination-nonchallenge groups responded to the vaccine as evidenced by positive reactions in the tube test. The antibody titers in all cows were highest during Week 2. The overall trend of the titers was similar for each group, regardless of the dosage of vaccine given. The major difference between the five vaccine groups was the degree of antibody response, which correlated with the dose of vaccine.

Figure 2 displays the tube test results for Group V-1. The range of titers for each week is included and was approximately two to four dilutions. The highest individual titer was a positive at 1:3200 (+3200) during Week 2 while the lowest titer, that week, was an incomplete positive at 1:800 (1800). The maximum geometric mean titer was a positive

Results of biweekly tube tests for Group V-l (vaccination-nonchallenge) following <u>B. abortus</u> strain 19 vaccination with 5 ml containing 78 X 10⁹ organisms (8 animals per group) Figure 2.

Results of biweekly tube tests for Group V-2 (vaccinationnonchallenge) following <u>B</u>. <u>abortus</u> strain 19 vaccination with 2 ml containing 5×10^9 organisms (8 animals per group)

Figure 3.



at a 1:1600 (+1600) during Week 2. The mean titer gradually receded to a positive at a 1:100 (+100) between Weeks 24 and 36.

Group V-2 (Figure 3) had mean titers that were lower than Group V-1 with the maximum titer being a +400. This mean titer then receded following Week 2 until Week 16 when it was an 150. It fluctuated slightly at that level through Week 36.

The range in titers (approximately four dilutions) was greatest for Group V-2 during the first seven weeks. After Week 7, the range was approximately two dilutions. The highest individual titer was a +1600 during Week 3.

The mean titers for Group V-3 (Figure 4) were similar to those in Group V-2; however, the range of individual titers was considerably different. The range for Group V-3 varied between three to four dilutions during the entire study, except for Week 16 when the range was approximately one dilution. The highest individual titer was a +1600 during Weeks 1 and 2. By Week 10 the titers began to decline to an I50 and remained at that level through Week 36.

Figure 5 shows the mean titers for Group V-4. The maximum mean titer was a +100. During Week 8 the mean titer receded to a +25, thereafter, it fell below the +25 dilution. The range was five dilutions during the first week but decreased to one or two dilutions (-25 to +50) during the last 16 weeks.

Group V-5 (Figure 6) had the lowest mean titer response, with the

Figure 4. Results of biweekly tube tests for Group V-3 (vaccinationnonchallenge) following B. <u>abortus</u> strain 19 vaccination with 2 ml containing 9.2 X 10⁸ organisms (7 animals per group)

Figure 5. Results of biweekly tube tests for Group V-4 (vaccinationnonchallenge) following B. <u>abortus</u> strain 19 vaccination with 2 ml containing 1.4 X 10⁸ organisms (8 animals per group)

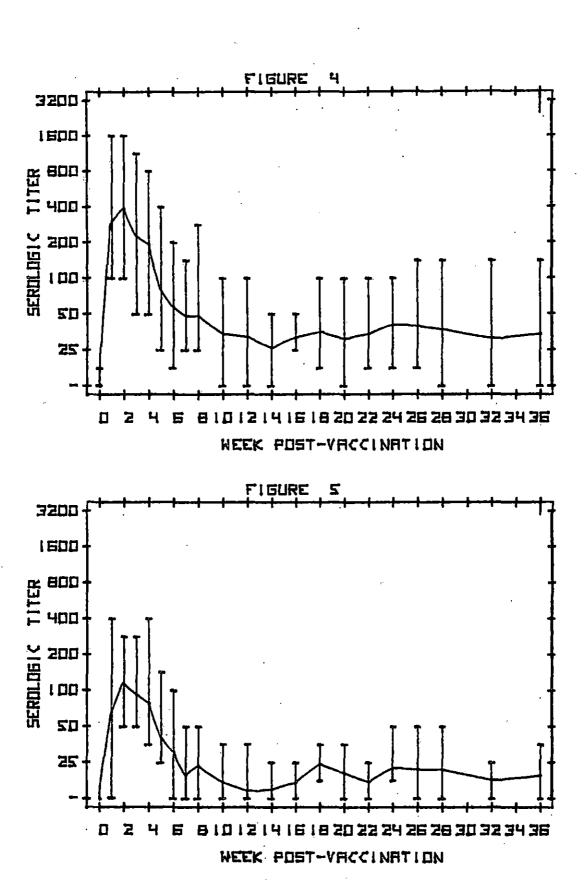
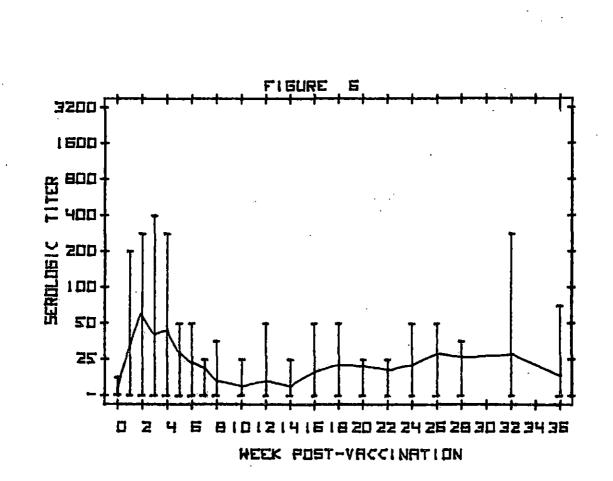


Figure 6. Results of biweekly tube tests for Group V-5 (vaccinationnonchallenge) following B. <u>abortus</u> strain 19 vaccination with 2 ml containing 2.3 X 107 organisms (8 animals per group)

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maximum being a +50 during the second week. The mean titer receded to almost negative at a 1:25 dilution during Weeks 8-14, increased to a +25 during Weeks 26-32, then decreased to an I25 on Week 36.

The greatest range in titers among individual animals was five dilutions during Week 3 (-25 to 4400). However, there was a variation of only one to two dilutions from Week 5 through Week 28. One animal's titer increased from an 150 during Week 28 to a 4400 during Week 32, then decreased to an Il00 during Weeks 36 and 40.

Plate test results

The highest dilution used in the plate test was a 1:400, which conforms with established procedures. The serologic results for the groups of vaccinated-nonchallenged cows on the plate test were similar in each group when compared with the corresponding tube titers, except that the geometric mean plate titer was lower than the tube titer because the plate titers were not conducted at the highest reacting dilutions. Therefore, the plate test results are not presented in the graphs. Individual animal plate test results are included in the Appendix.

Mercaptoethanol test results

Figures 7-11 depict the geometric mean serologic results for the five vaccination-nonchallenge groups of cows on the mercaptoethanol (ME) test. The maximum mean antibody titers occurred during Week 3 for the four higher dosage groups and at Week 4 for the lowest dosage group. The general titer patterns were similar for each group. The major

difference between the five vaccine groups was the degree and persistence of the antibody response.

Figure 7 shows the ME test results for Group V-1. During Week 1 there was no response, which differed from the tube test results. A peak titer of a +400 was reached during Week 3; it gradually declined to a -25 by Week 36. The range of individual titers fluctuated from over five dilutions during Week 2 (+50 to I3200) to half a dilution during Week 4 (+200 to I400). The range increased again to four dilutions (I25 to +400) by Week 16 then gradually declined to two dilutions (-25 to +50) at Week 32.

Group V-2 (Figure 8) had mean ME titers that were significantly lower (99% probability)¹ than Group V-1. Although all of the cows in this group responded to the vaccine, there was no response during the first week. A peak mean titer of an Il00 was reached during Week 3. The titer declined rapidly and was negative at a 1:25 by Week 12. One animal reacted at an I25 during Week 16. The highest individual titer was a +400. The range in individual titers was five dilutions (+25 to +400) during Week 3, but decreased rapidly as did the group mean titer.

The mean titer response for Group V-3 (Figure 9) was similar to Group V-2; however, the peak titer was only an 150 during Week 3 and returned to a -25 by Week 12. One cow in this group failed to respond.

Figure 10 shows the antibody response produced by Group V-4 on the

¹Pearson chi-square was used to determine significance level.

Figure 7. Results of biweekly mercaptoethanol tests for Group V-1 (vaccination-nonchallenge) following <u>B</u>. <u>abortus</u> strain 19 vaccination with 5 ml containing 78 X 10⁹ organisms (8 animals per group)

Figure 8. Results of biweekly mercaptoethanol tests for Group V-2 (vaccination-nonchallenge) following <u>B</u>. <u>abortúk</u> strain b9 vaccination with 2 ml containing 5 X 10⁹ organisms (8 animals per group)

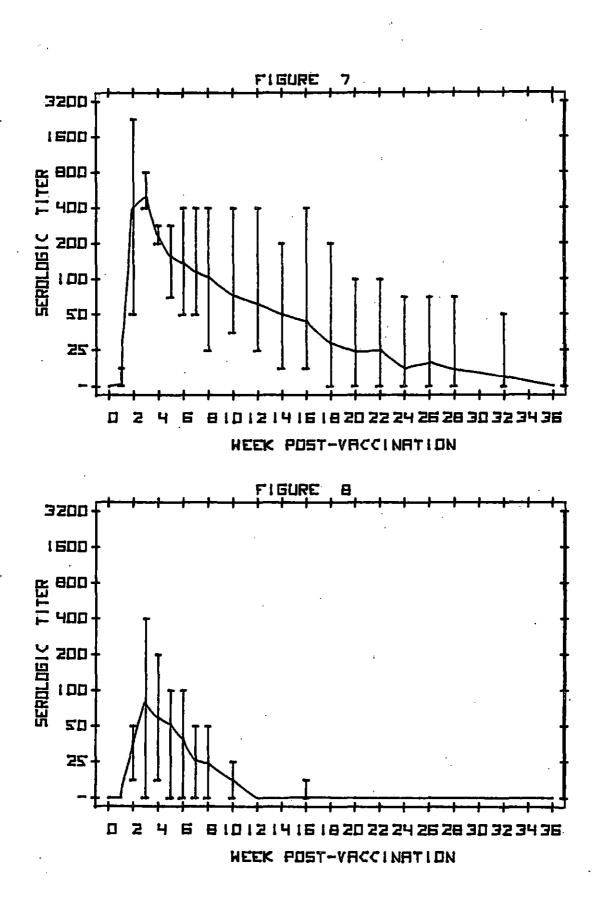
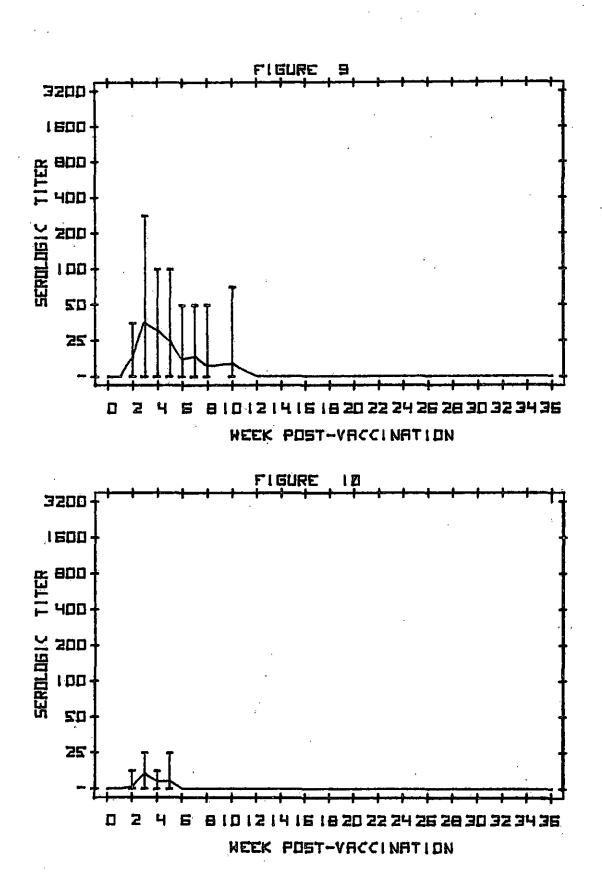


Figure 9. Results of biweekly mercaptoethanol tests for Group V-3 (vaccination-nonchallenge) following <u>B</u>. <u>abortus</u> strain 19 vaccination with 2 ml containing 9.2 X 10⁸ organisms (7 animals per group)

Figure 10. Results of biweekly mercaptoethanol tests for Group V-4 (vaccination-nonchallenge) following <u>B</u>. <u>abortus</u> strain 19 vaccination with 2 ml containing 1.4 X 10⁸ organisms (8 animals per group)



ME test. The highest titer was a +25, which occurred in three cows during Weeks 3 and 5. Group V-5 had only one cow respond at a +25 during Week 4 and one at a I25 during Week 5 (Figure 11).

Rivanol test results

Antibody responses evaluated on the rivanol (RIV) test are presented in Figures 12-16. The response pattern of each group is similar to the ME test results. However, endpoint titers were not determined at dilutions greater than a 1:400. This affected the geometric mean results in Group V-1 for eight weeks and Group V-2 for two weeks.

Group V-1 (Figure 12) showed a response the first week following vaccination but did not reach the peak mean titer (1400) until Week 4. The mean titer decreased more slowly than did the ME titer but was negative at a 1:25 dilution by Week 36. A range in individual titers of one to two dilutions increased after Week 10 (I100 to +400) to over four dilutions from Weeks 12 to 20 (-25 to I400). The range gradually declined to one-half dilution (-25 to I25) by Week 36.

Figure 13 (Group V-2) shows there was no response at Week 1. However by Week 2 a +100 peak mean titer was reached and persisted until Week 4, then gradually declined. The mean titer became negative by Week 20; whereas, the mean ME test titer was negative by Week 12. The range of titers (a +400 individual titer was reached during Weeks 3 and 4) was over three dilutions during Weeks 3-8 and decreased to half a dilution by Week 16 (-25 to I25).

Figure 11. Results of biweekly mercaptoethanol tests for Group V-5 (vaccination-nonchallenge) following <u>B. abortus</u> strain 19 vaccination with 2 ml containing 2.3 X 107 organisms (8 animals per group)

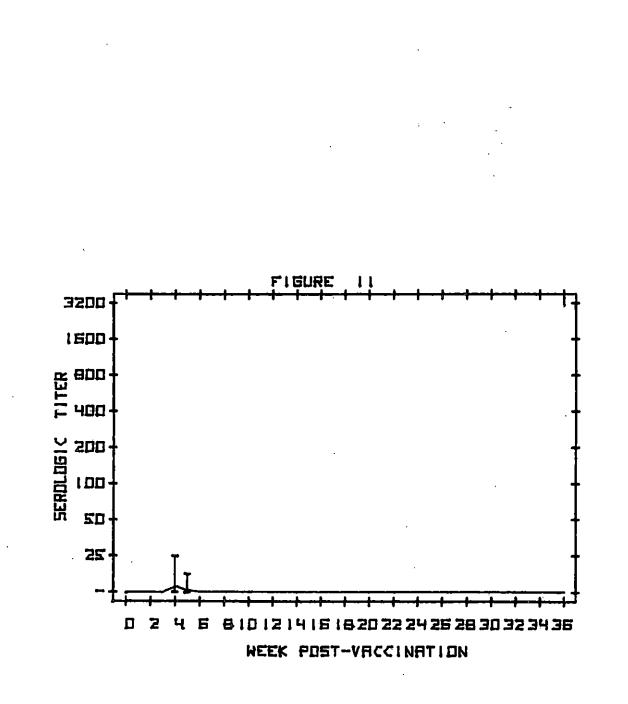
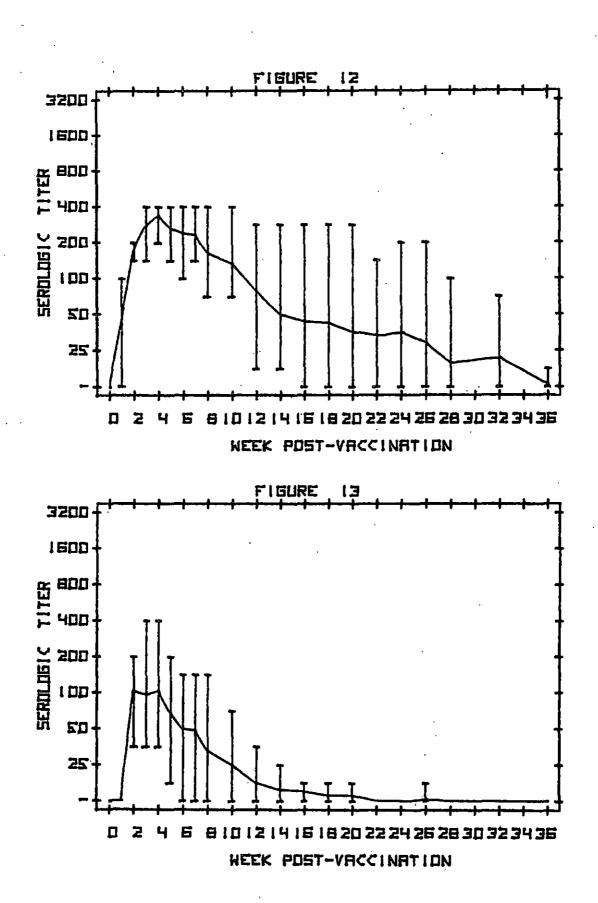


Figure 12. Results of biweekly rivanol tests for Group V-1 (vaccinationnonchallenge) following <u>B</u>. <u>abortus</u> strain 19 vaccination with 5 ml containing 78 X 10⁹ organisms (8 animals per group)

Figure 13. Results of biweekly rivanol tests for Group V-2 (vaccinationnonchallenge) following <u>B</u>. <u>abortus</u> strain 19 vaccination with 2 ml containing 5×10^9 organisms (8 animals per group)



Group V-3 (Figure 14) had a response during the first week which peaked at a +50 during Weeks 2 to 4. The mean titer gradually declined until Week 14 when it was almost negative at a 1:25 dilution. The range of titers was from a -25 to an I400. Two cows had titers which fluctuated from a -25 to an I50 on Weeks 16 and 18.

Figure 15 shows the mean antibody response for Group V-4. Only four of the eight cows in the group responded to the vaccine. The highest individual titer was an I50 during Weeks 3 and 4. The maximum mean titer was an I50 and all animals were negative by Week 8.

Two of the eight cows in Group V-5 (Figure 16) responded to the vaccine with titers of an I25 during Weeks 2 to 5.

Complement fixation test results

The complement fixation (CF) test results are depicted in Figures 17-21. The CF test uses doubling dilutions from a 1:10 to a 1:1280, and the results are recorded as the degree of fixation in the highest reacting dilution (25% fixation (1+), 50% fixation (2+), 75% fixation (3+), and 100% fixation (4+)). The general trend for each group response is similar as with the other tests used, with each higher dosage giving a greater response. The peak titers were reached during Week 3.

Figure 17 shows the CF antibody response for Group V-1. The maximum mean titer was a 25% fixation at the 1:160 dilution (1+160). The mean titer gradually decreased until it became negative at a 1:10 dilution (-10) on Week 32. A range of approximately four dilutions occurred until Week 18, it then decreased to three dilutions (-10 to 4+40).

Figure 14. Results of biweekly rivanol tests for Group V-3 (vaccinationnonchallenge) following B. <u>abortus</u> strain 19 vaccination with 2 ml containing 9.2 X 10⁸ organisms (7 animals per group)

Figure 15. Results of biweekly rivanol tests for Group V-4 (vaccinationnonchallenge) following B. <u>abortus</u> strain 19 vaccination with 2.ml containing 1.4 X 10⁸ organisms (8 animals per group)

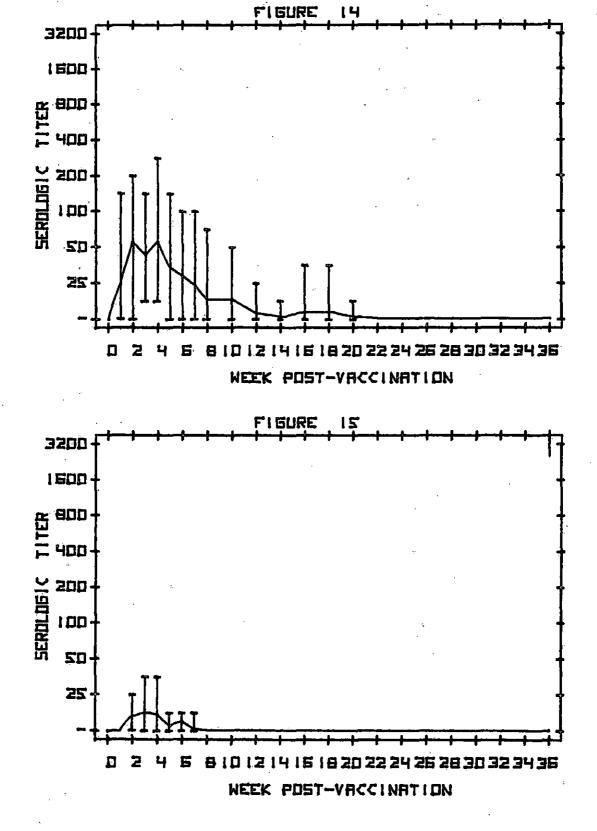


Figure 16. Results of biweekly rivanol tests for Group V-5 (vaccinationnonchallenge) following B. <u>abortus</u> strain 19 vaccination with 2 ml containing 2.3 X 107 organisms (8 animals per group)

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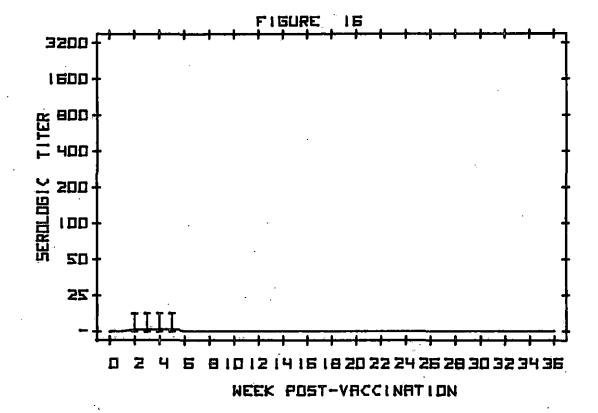
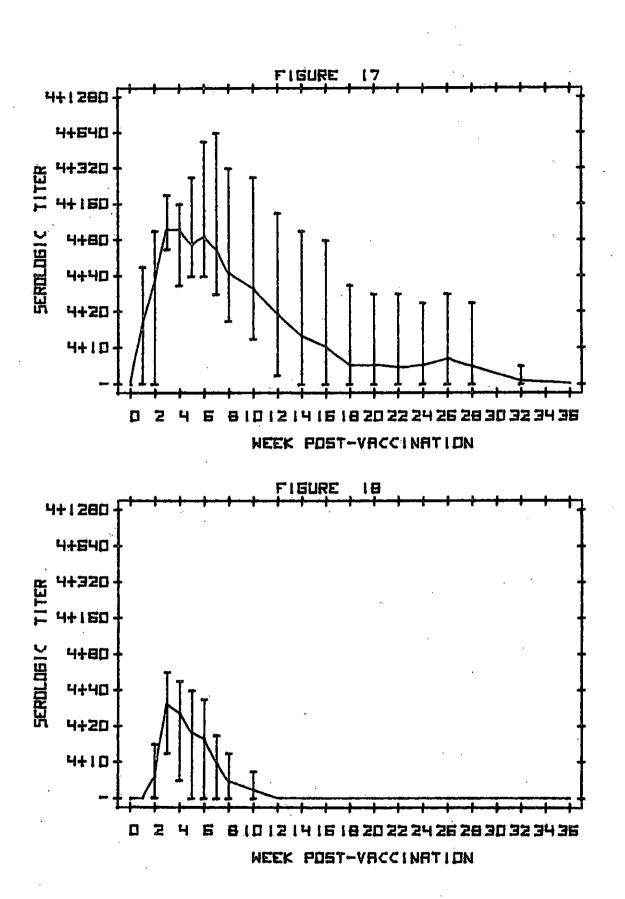


Figure 17. Results of biweekly complement fixation tests for Group V-1 (vaccination-nonchallenge) following <u>B</u>. <u>abortus</u> strain 19 vaccination with 5 ml containing 78 X 10⁹ organisms (8 animals per group)

Figure 18. Results of biweekly complement fixation tests for Group V-2 (vaccination-nonchallenge) following <u>B</u>. <u>abortus</u> strain 19 vaccination with 2 ml containing 5 X 10⁹ organisms (8 animals per group)



The Group V-2 (Figure 18) CF antibody response was much lower than Group V-1. The peak mean titer was 75% fixation at a 1:40 (3:440). The highest individual titer was a 2:80. All cattle were negative at a 1:10 dilution by the 12th week.

Group V-3 (Figure 19) had a lower mean titer (4+10) than Group V-2; however, the highest individual titer (2+80) was the same. All animals were negative at a 1:10 dilution by Week 12. Two cows failed to produce antibodies detectable by the CF test.

Figure 20 shows the antibody response for Group V-4. Only half of the cows (4) showed a response, with the highest response being a 2+20during the third week. Group V-5 (Figure 21) had only two out of eight animals respond on the CF test for two to four weeks. The highest individual titer was a 3+20.

Table 5 and 6 summarize, by group, the antibody responses to <u>B</u>. <u>abortus</u> strain 19 vaccine for the tube, ME, rivanol, CF, and card tests.

Table 5. Summary, by group, of the peak antibody response and duration of response to the <u>B</u>. <u>abortus</u> strain 19 vaccine for the card test

| Group | Test | Highest percent positive | Duration of positive response |
|--------------|-------------------|--------------------------|-------------------------------|
| V-1 | Card ^a | 100% positive | 37% positive at Week 36 |
| V-2 | Card | 100% positive | Negative by Week 36 |
| V− 3 | Card | 100% positive | 14% positive at Week 36 |
| V-4 | Card | 75% positive | Negative by Week 7 |
| · V-5 | Card | 25% positive | Negative by Week 8 |

^aThe card test is read as either positive or negative.

Figure 19. Results of biweekly complement fixation tests for Group V-3 (vaccination-nonchallenge) following <u>B</u>. <u>abortus</u> strain 19 vaccination with 2 ml containing 9.2 X 10^8 organisms (7 animals per group)

Results of biweekly complement fixation tests for Group V-4 Figure 20. (vaccination-nonchallenge) following <u>B</u>. <u>abortus</u> strain 19 vaccination with 2 ml containing 1.4×10^8 organisms (8 animals per group)

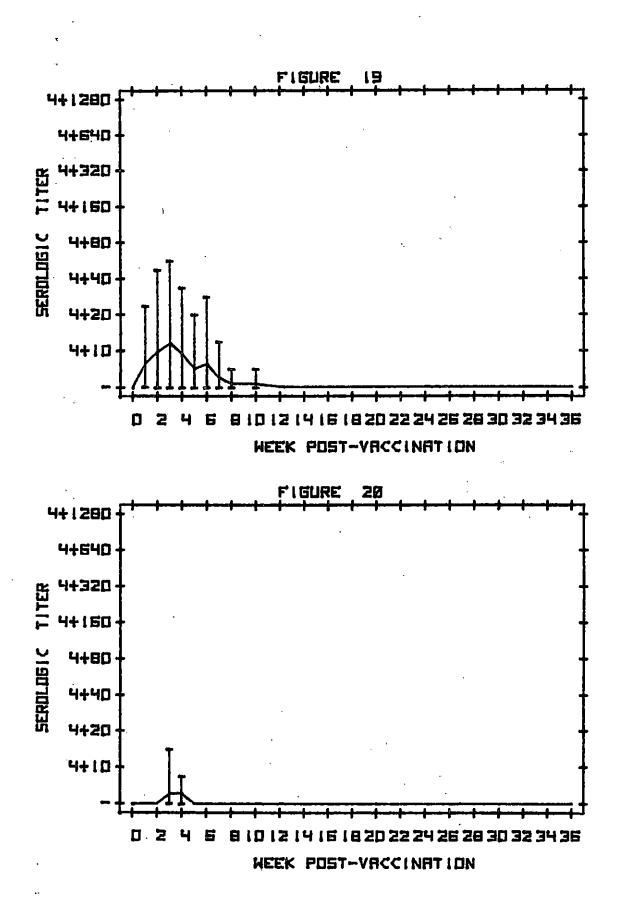
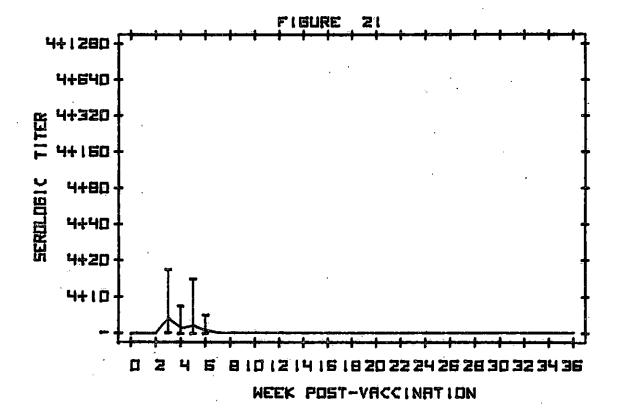


Figure 21. Results of biweekly complement fixation tests for Group V-5 (vaccination-nonchallenge) following <u>B</u>. <u>abortus</u> strain 19 vaccination with 2 ml containing 2.3 X 10⁷ organisms (8 animals per group)



| Group | Test | Highest mean titer | Duration of group mean tite |
|-------------|-----------------|--------------------|-----------------------------|
| V-1 | Tubea | +1600 | +100 at Week 36 |
| V-2 | tt | +400 | I50 at Week 36 |
| V-3 | 11 | +400 | I50 at Week 36 |
| v-4 | U U | +100 | I25 at Week 36 |
| V −5 | 11 | +50 | I25 at Week 36 |
| V-l | ME ^b | 1800 | Negative by Week 36 |
| V-2 | н | I100 | " " 12 |
| V-3 | н | I50 · | " " 12 |
| V-4 | | I25 | IT 11 IP 6 |
| V-5 | ft | ×125 | " " 6 |
| V-l | RIVC | I400 | Negative by Week 36 |
| V-2 | 11 | +1.00 | "" 22 |
| v-3 | 11 | +50 | " " 22. |
| v-4 | H | I25 | u u u 8 |
| V-5 | н | <125 | """8 """6 |
| V-l | CF ^d | 1+160 | Negative by Week 34 |
| V-2 | ti | 2+160 | " " 12 |
| V-3 | n | 1+20 | ", " " 12 |
| v-4 | tı | 1+10 | " " " 5 ". " " 7 |
| V-5 | 11 | 2+10 | |

Table 6. Summary, by group, of the peak antibody response and duration of response to <u>B</u>. <u>abortus</u> strain 19 vaccine for the tube, ME, rivanol, and CF tests

^a+50 = negative; Il00 to I200 = suspect; +200 or > = reactor.

^bNot an official test.

 $c_{\geq+50} = reactor; <+50 = negative.$

 $d \ge 1+40$ = reactor; 2+10 to 4+20 = suspect; <2+10 = negative.

Vaccination-Challenge Phase

The serological responses for the five vaccination-challenge groups (15 animals/group) were basically the same as those in the vaccinationnonchallenge groups through Week 16. During Week 16 the five vaccination-challenge groups and a nonvaccinated control group (20 animals) were instilled into the conjunctival sac with 8.27 X 10^5 <u>B</u>. <u>abortus</u> strain 2308 organisms.

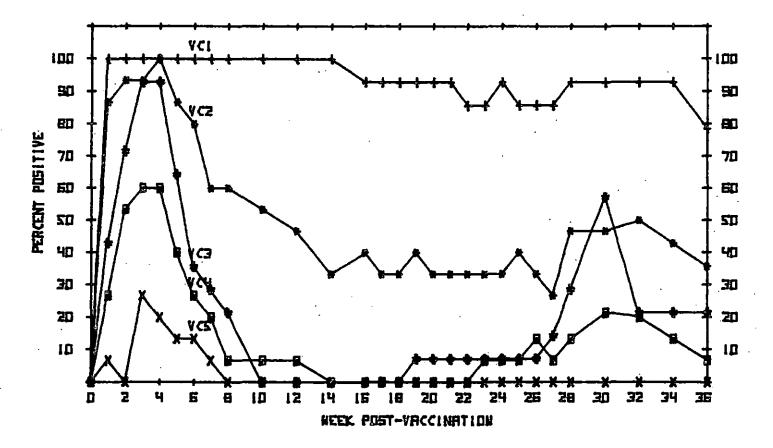
Card test results

Figure 22A displays the antibody response for the five vaccine dosage groups and the nonvaccinated controls as detected by the card test. All of the animals in Group VC-1 were positive on the card test one week after vaccination and remained positive through Week 14. During Weeks 16-34 over 90% of the cattle remained positive (one cow had an intermittent response for six weeks). At Week 36 the percentage of positive cattle declined to less than 80%.

More than 90% of the cows in Group VC-2 were positive on the card test after two weeks, and all were positive by the fourth week. The percent of reactions declined rapidly to about 30% between Weeks 4 and 14 and stabilized at that level until Week 26. Between Weeks 26-36 there were some fluctuations due to three animals titers. Thirty-five percent of the cattle were still positive during Week 36.

Over 90% of the cows in Group VC-3 were positive during Weeks 2-4. Thereafter, the percentage declined sharply and all were negative by

Figure 22A. Results of biweekly and monthly card tests for the five vaccination-challenge groups following B. abortus strain 19 vaccination (Week 0) and challenged with 8.27 X 10⁵ B. <u>abortus</u> strain 2308 organisms (Week 16) Group VC-1 (+) received 78 X 10⁹ organisms (14 animals per group) Group VC-2 (*) received 5 X 10⁹ organisms (15 animals per group) Group VC-3 (#) received 9.2 X 10⁸ organisms (14 animals per group) Group VC-4 () received 1.4 X 10⁸ organisms (15 animals per group) Group VC-5 (X) received 2.3 X 10⁷ organisms (15 animals per group)



FIEURE 22A

Week 10. After this group was challenged, one animal (7%) became positive during Weeks 19-26. The number of animals with positive responses then increased to 60% during Week 30, but declined again to 7% by Week 36. <u>Brucella abortus</u> strain 2308 was isolated from two of the cows in this group.

The percent of card test positive reactions in the cows from Group VC-4 reached 60% by Weeks 3 and 4, then declined until all cattle were negative by Week 14 (seven weeks after being challenged). One animal (7%) had an agglutination response at Week 23. Approximately 20% (3 animals) were positive during Week 30, then the percentage declined to 7% by Week 36. <u>Brucella abortus</u> strain 2308 was isolated from one cow in Group VC-4. Twenty-five percent of the cattle in Group VC-5 (Figure 22A) were positive by Week 3, but reverted to negative by Week 8 and remained negative through Week 36.

Figure 22B displays the card test responses of the vaccinationchallenge control group. Isolations of <u>B</u>. <u>abortus</u> strain 2308 were made from three cows in this group. All animals remained negative on the card test until three weeks following challenge (Week 19). Fifteen percent of the animals were positive on Week 19 and 30% were positive during Weeks 22-26. Only 10% of the animals were positive between Weeks 32-36.

<u>Tube</u> <u>test</u> <u>results</u>

The results of the tube test for the groups of vaccinated and nonvaccinated cows are presented in Figures 23-27. As depicted in the

Figure 22B. Results of biweekly and monthly card tests for the nonvaccination-challenge Control Group (20 animals per group) preceding and following challenge during Week 16 with 8.27 X 10⁵ B. abortus strain 2308 organisms

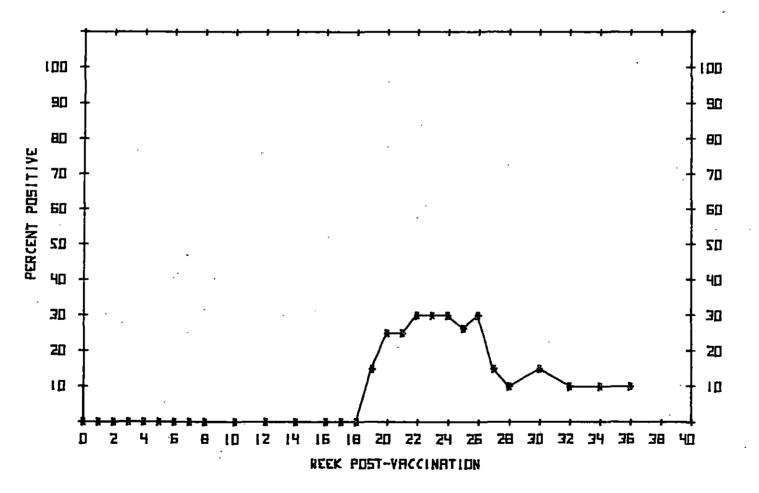


FIGURE 22B

vaccination-nonchallenge groups, the maximum antibody titers occurred in all cows during Week 2. The major difference among the five vaccine groups during Weeks 0-16 was the degree and persistence of the antibody response for each group.

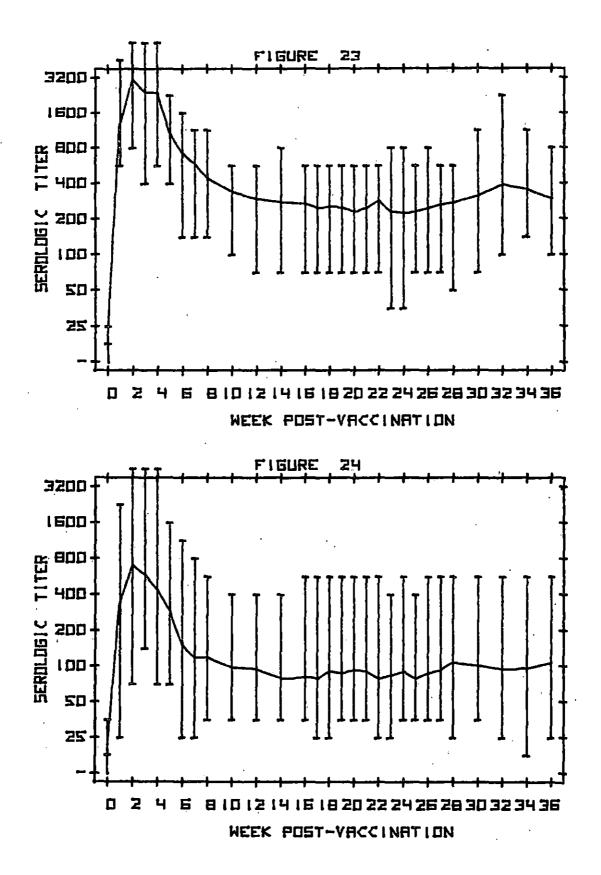
Figure 23 contains the tube test results for Group VC-1. The range of titers for each week was approximately three dilutions. The highest individual titer was a +6400 during Week 2, while the lowest titer that week was an I800. The maximum geometric mean titer was a +1600 during Week 2, but the titer gradually receded to a +200 by Week 12. Following Week 24, the mean titer increased minimally through Week 32. One individual titer increased after challenge from an I800 (Week 26) to a +1600 (Week 32), but then receded to an I800 by Week 36.

The mean tube test titers for Group VC-2 (Figure 24) were lower than Group VC-1 with the maximum being a +400 on Week 2. The mean titer then receded until Week 14 when it was positive at a +50 and remained at that level through Week 26. Between Weeks 28-36 the mean titer gradually increased to an Il00. The variation of responses of individual animals was observed to be greater in Group VC-2 than in Group VC-1.

The mean tube titers were lower for Group VC-3 (Figure 25) than for the previous two groups. The highest mean titer was a +200 (Week 2) which receded to a +25 by Week 14. Following Week 17 the mean titer began to gradually increase until Week 30 when the titer peaked at an Il00. The mean titer decreased slightly by Week 36. Following Week 16

Figure 23. Results of biweekly tube tests for Group VC-1 (vaccinationchallenge) following vaccination with 78 X 10⁹ <u>B. abortus</u> strain 19 organisms and challenged with <u>B. abortus</u> strain 2308 during Week 16 (14 animals per group)

Figure 24. Results of biweekly tube tests for Group VC-2 (vaccination-challenge) following vaccination with 5 X 10⁹ <u>B</u>. <u>abortus</u> strain 19 organisms and challenged with B. abortus strain 2308 during Week 16 (15 animals per group)



until Week 36 the range of titers increased to over eight dilutions (-25 to I6400). <u>Brucella abortus</u> strain 2308 was isolated from two of the cows at the time of abortion or calving.

Figure 26 shows the mean tube test results for Group VC-4. The maximum mean titer prior to challenge was a +100. By Week 14 the mean titer had receded to an I25. During the first ten weeks, 12 out of the 15 cows responded to the vaccine. The individual titers ranged from an I25 to a +400 during Week 2. At the time the cows were challenged the highest titer was a +50. Following the challenge during Week 16, the mean titer increased slightly to an I50 by Week 34. One of three cows which responded to the challenge had a titer which increased to a +1600 by Week 36. She aborted and <u>B</u>. <u>abortus</u> strain 2308 was isolated from the fetus.

Group VG-5 (Figure 27) showed the lowest mean titer response, with the maximum being an incomplete positive at a 1:50 during Week 4. The mean titer receded to almost negative at a 1:25 during Weeks 14 and 17, and increased minimally to an I25 during Weeks 18-36. As in Group VC-4, some animals had a minimal response (I25) to the vaccine; however, six cows had titers of a +50 to an I400. After challenge, the titer of the three cows increased to a +50 and a +100. Five cows did not respond to the challenge and seven cows maintained fluctuating titers between a -25 and an I50.

The tube test results for the nonvaccinated control group (C) are

Figure 25. Results of biweekly tube tests for Group VC-3 (vaccinationchallenge) following vaccination with 9.2 X 10⁸ <u>B</u>. <u>abortus</u> strain 19 organisms and challenged with <u>B</u>. <u>abortus</u> strain 2308 during Week 16 (14 animals per group)

Figure 26. Results of biweekly tube tests for Group VC-4 (vaccinationchallenge) following vaccination with 1.4 X 10⁸ <u>B</u>. <u>abortus</u> strain 19 organisms and challenged with <u>B</u>. <u>abortus</u> strain 2308 during Week 16 (15 animals per group)

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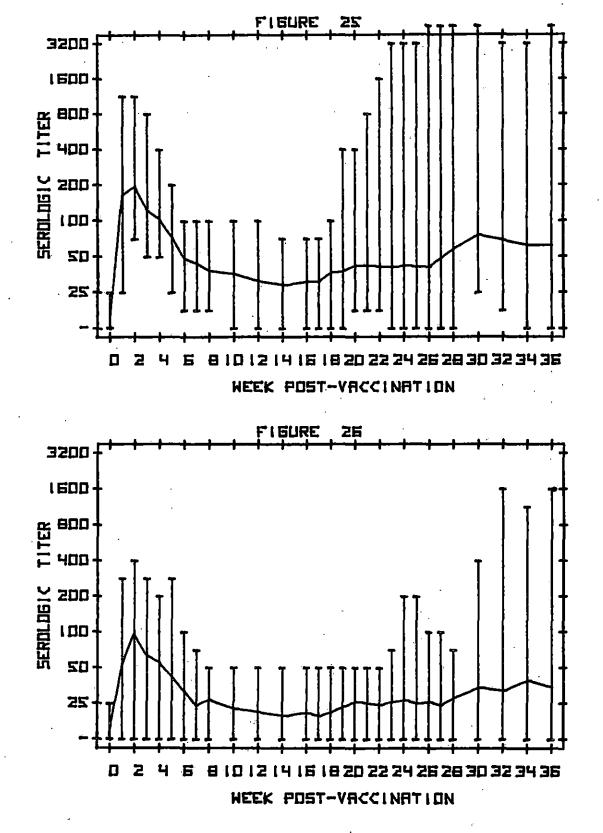
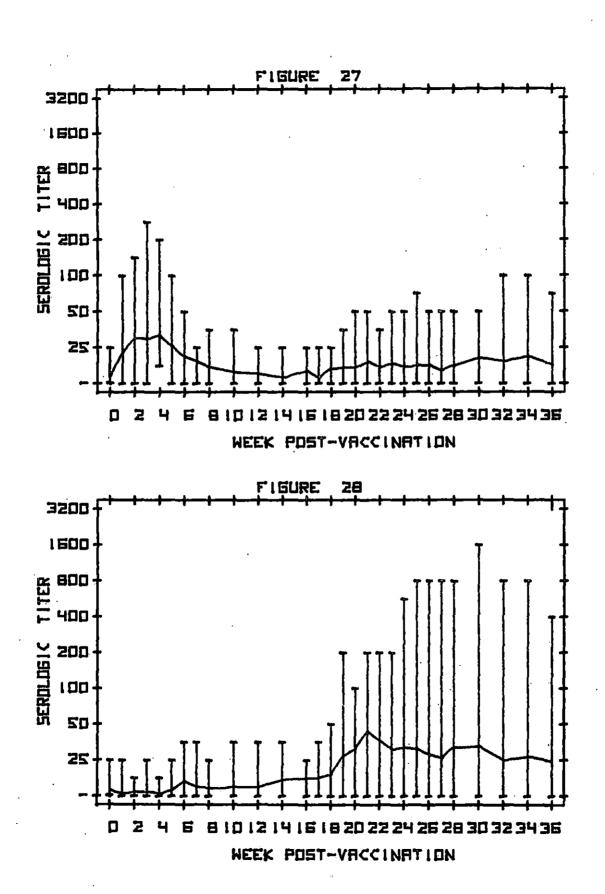


Figure 27. Results of biweekly tube tests for Group VC-5 (vaccination-challenge) following vaccination with 2.3 X 10⁷ <u>B</u>. <u>abortus</u> strain 19 organisms and challenged with <u>B</u>. <u>abortus</u> strain 2308 during Week 16 (15 animals per group)

Figure 28. Results of biweekly tube tests for the Control Group (nonvaccination-challenge) following challenge with <u>B</u>. <u>abortus</u> strain 2308 during Week 16 (20 animals per group)



shown in Figure 28. Nine of the cattle had fluctuating titers prior to challenge that ranged from a -25 to a +25. The highest individual titer was an 150. After being challenged with <u>B</u>. <u>abortus</u> strain 2308, 19 of the 20 animals had a detectable increase in antibody response (+25 to +1600). Those that had been negative at the 1:25 dilution before challenge (10 cows) developed titers between a +25 and a +100 after challenge. <u>Brucella abortus</u> strain 2308 was isolated from the lymph nodes of one of these cows. Two cows that had intermittent I25 responses before challenge developed titers of an I50 to an I100, three to five weeks after challenge. Five cows had a maximum pre-challenge titer of a +25; one maintained a titer of an I25; two developed responses of a +50; one developed a titer of a +200 and one a titer of a +1600 following challenge (<u>B</u>. <u>abortus</u> strain 2308 was isolated from the cow with the +1600 titer).

Three cows developed nonspecific titers of an I50 during the two weeks before challenge. After challenge, one cow maintained a titer of a +25; one developed a response of a +100 for three weeks; the third cow developed a peak titer of a +800 two weeks following abortion and isolation of <u>B. abortus</u> strain 2308.

Plate test results

As indicated before, the highest plate test dilution used was a 1:400, which is one dilution higher than that used in routine test procedures. The plate titers were similar for each vaccine group when

compared with the corresponding tube test titers, except that the geometric mean plate titers were lower because dilutions above a 1:400 were not used. Therefore, the plate test results are not presented, but are included in the Appendix.

<u>Mercaptoethanol test results</u>

Figures 29-34 depict the geometric mean antibody responses on the ME test for the five vaccination-challenge groups and the controls. All of the cows failed to respond on the ME test during the first week. Following vaccination, Groups VC-1 and VC-2 developed higher overall antibody responses with longer duration of titers on the ME test than the three lower dosage groups.

Group VC-1 (Figure 29) developed a maximum mean titer of a +400 during Week 2. The mean titer declined during Weeks 4-23. Twelve to 16 weeks following the challenge the mean titer increased from a -25 to a +25. The range in individual titers during the first week was from an I25 to an I6400 (8 dilutions). Seven of 15 cows had a minimal serological response to the challenge, with titers increasing from a -25 to a maximum of either a +25 or a +50.

Cows in Group VC-2 (Figure 30) had a maximum mean titer of a +50 during Week 4. Only three of the 15 cows had detectable antibody responses following challenge (I25 to I50), indicating the challenge had little effect, as measured by the ME test.

Group VC-3 (Figure 31) had a minimal antibody response following vaccination. The highest individual titer was a +50. The mean titer

Figure 29. Results of biweekly mercaptoethanol tests for Group VC-1 (vaccination-challenge) following vaccination with 78 X 10⁹ <u>B. abortus</u> strain 19 organisms and challenged with <u>B. abortus</u> strain 2308 during Week 16 (14 animals per group)

Figure 30. Results of biweekly mercaptoethanol tests for Group VC-2 (vaccination-challenge) following vaccination with 5 X 10' <u>B. abortus</u> strain 19 organisms and challenged with <u>B. abortus</u> strain 2308 during Week 16 (15 animals per group)

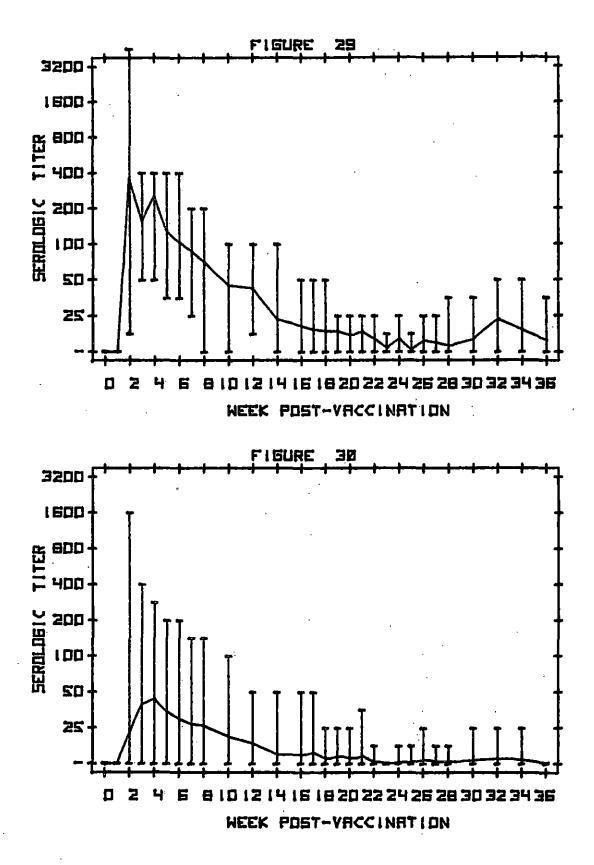
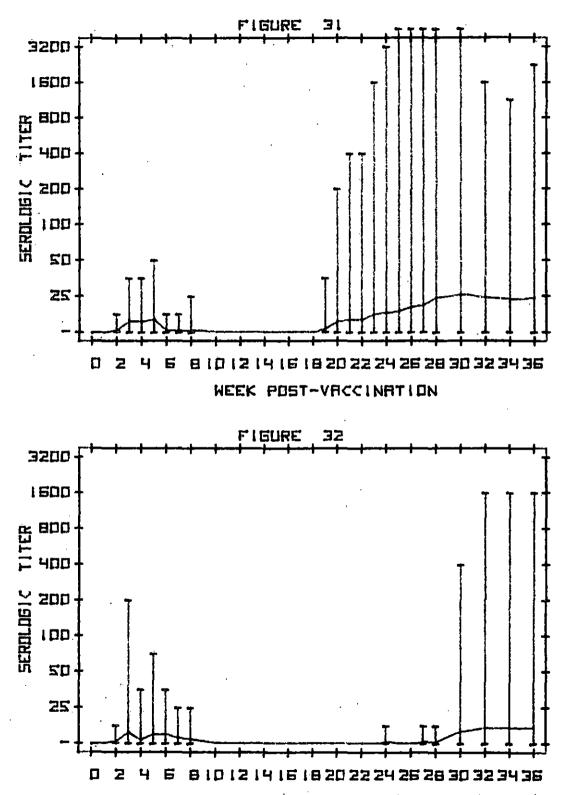


Figure 31. Results of biweekly mercaptoethanol tests for Group VC-3 (vaccination-challenge) following vaccination with 9.2 X 10⁸ <u>B. abortus</u> strain 19 organisms and challenged with <u>B. abortus</u> strain 2308 during Week 16 (14 animals per group)

Figure 32. Results of biweekly mercaptoethanol tests for Group VC-4 (vaccination-challenge) following vaccination with 1.4 X 10⁸ <u>B. abortus</u> strain 19 organisms and challenged with <u>B. abortus</u> strain 2308 during Week 16 (15 animals per group)



WEEK POST-VACCINATION

was a +50. The mean titer following vaccination was below the I25 level and lasted only four weeks (Weeks 2-6). Following challenge only two cows (Nos. 67 and 69) responded on the ME test. Their titers increased from a -25 to as high as a +12,800 by Week 26. Isolations of <u>B</u>. <u>abortus</u> strain 2308 were made from these two cows. The individual serological results for these two animals are presented in Figures 56 and 58.

Figure 32 (Group VC-4) had a response to vaccination similar to Group VC-3. Ten out of 15 cows in Group VC-4 had no response on the ME test during the study. Two animals responded to vaccination, with one cow developing a titer of a +200 during Week 3; both animals were negative at a 1:25 dilution by Week 10. One cow developed a post-challenge titer of a +1600 by Week 36. All the other cows remained negative. <u>Brucella abortus</u> strain 2308 was isolated from two cows in this group.

The only response in Group VC-5 on the ME test were two cows which developed a I25 titer for one week each.

The Control Group (Figure 34) remained serologically negative on the ME test throughout the study, except for two cows. The individual titers of those cows reached an I800 and a +1600. Individual results for these two animals are shown in Figures 53 and 54.

<u>Rivanol test results</u>

Figures 35-40 display the results of the rivanol test for each vaccination-challenge group and the controls. The results of the rivanol test for each group were similar to those reported for the ME test, and therefore, will not be presented in detail.

Complement fixation test results

The complement fixation (CF) test results are depicted in Figures 41-46. In Group VC-1 (Figure 41) the maximum mean titer of a 3+80 was reached during Week 4, then declined to negative at a 1:10 dilution by Week 20. The range in titers was five dilutions (-10 to 4+160) during the first week, then decreased as the mean titer declined. Seven cows responded to the challenge at a 1+10 or a 2+10 during Weeks 26 and 27.

Group VC-2 (Figure 42) had four cows that failed to respond on the CF test. The remaining 11 cows developed a maximum mean titer of a 3+20 during Week 4. The mean titer decreased rapidly following Week 6 and was essentially negative by Week 14. Cow No. 106 developed the highest individual titer of a 3+160 during Week 3. Only two cows responded beyond Week 8. After Week 12 only one cow (No. 106) responded (at a 2+10 and a 1+10) until Week 24.

Ten cows in Group VC-3 (Figure 43) did not respond to the vaccination or challenge on the CF test. Only two cows (Nos. 55 and 56) responded to the vaccination with the highest titer being a 4+20 during Week 5. Following challenge, the only cows which responded were those from which B. abortus strain 2308 was isolated (Nos. 67 and 69).

Figure 44 (Group VC-4) includes the results of 15 cows; 10 of which did not respond to the CF test. Four cows responded to the vaccination for five weeks (Weeks 3-7) with a maximum titer of a 2+40 during Week 3. After the challenge only two cows responded on the CF test. Cow No. 23

Figure 33. Results of biweekly mercaptoethanol tests for Group VC-5 (vaccination-challenge) following vaccination with 2.3 X 10⁷ <u>B. abortus</u> strain 19 organisms and challenged with <u>B. abortus</u> strain 2308 during Week 16 (15 animals per group)

Figure 34. Results of biweekly mercaptoethanol tests for the Control Group (nonvaccination-challenge) following challenge with <u>B. abortus</u> strain 2308 during Week 16 (20 animals per group)

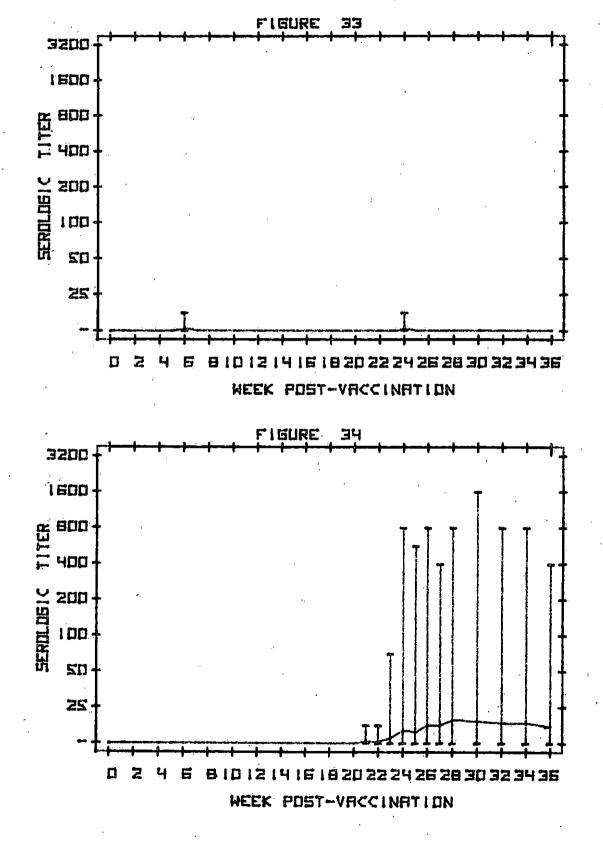
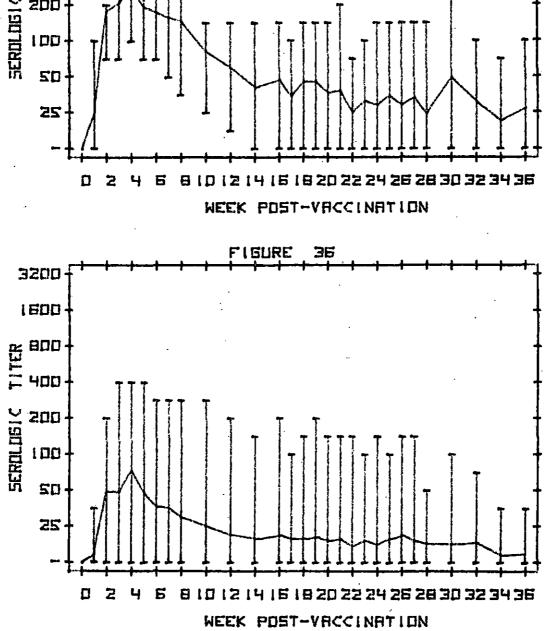


Figure 35. Results of biweekly rivanol tests for Group VC-1 (vaccination-challenge) following vaccination with 78 X 10⁹ <u>B. abortus</u> strain 19 organisms and challenged with <u>B.</u> <u>abortus</u> strain 2308 during Week 16 (14 animals per group)

Figure 36. Results of biweekly rivanol tests for Group VC-2 (vaccination-challenge) following vaccination with 5 X 10⁹ <u>B. abortus</u> strain 19 organisms and challenged with <u>B</u>. abortus strain 2308 during Week 16 (15 animals per group)



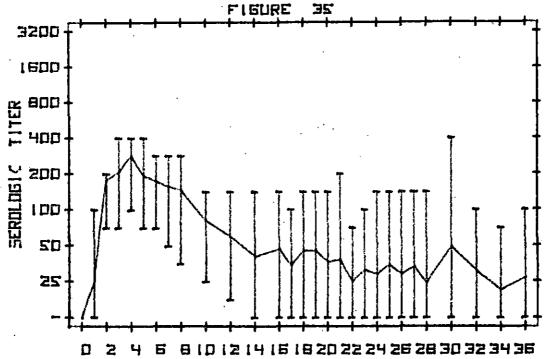


Figure 37. Results of biweekly rivanol tests for Group VC-3 (vaccination-challenge) following vaccination with 9.2 X 10⁸ <u>B. abortus</u> strain 19 organisms and challenged with <u>B.</u> <u>abortus</u> strain 2308 during Week 16 (14 animals per group)

Figure 38. Results of biweekly rivanol tests for Group VC-4 (vaccination-challenge) following vaccination with 1.4 X 10⁸ <u>B. abortus</u> strain 19 organisms and challenged with <u>B</u>. abortus strain 2308 during Week 16 (15 animals per group)

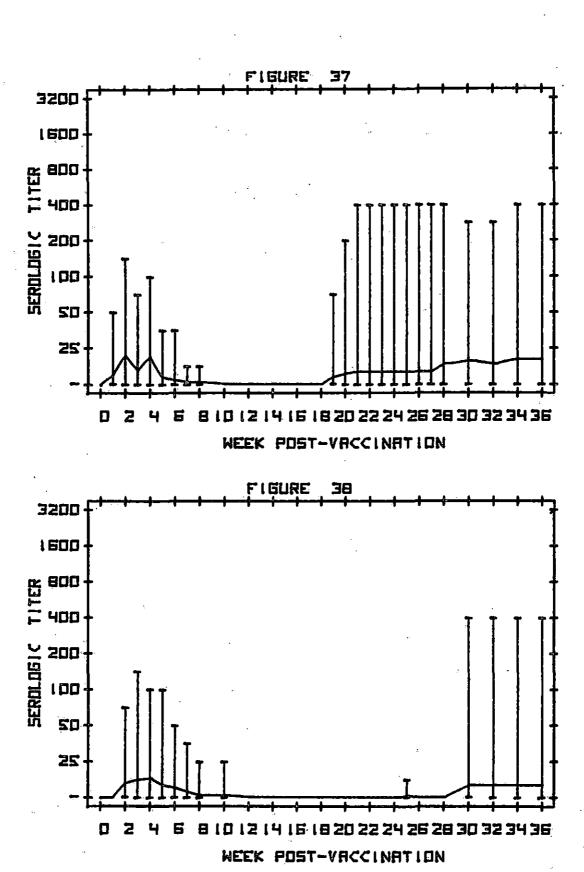


Figure 39. Results of biweekly rivanol tests for Group VC-5 (vacci-nation-challenge) following vaccination with 2.3 X 10⁷ <u>B. abortus</u> strain 19 organisms and challenged with <u>B.</u> <u>abortus</u> strain 2308 during Week 16 (15 animals per group)

Figure 40. Results of biweekly rivanol tests for the Control Group (nonvaccination-challenge) following challenge with B. abortus strain 2308 during Week 16 (20 animals per group)

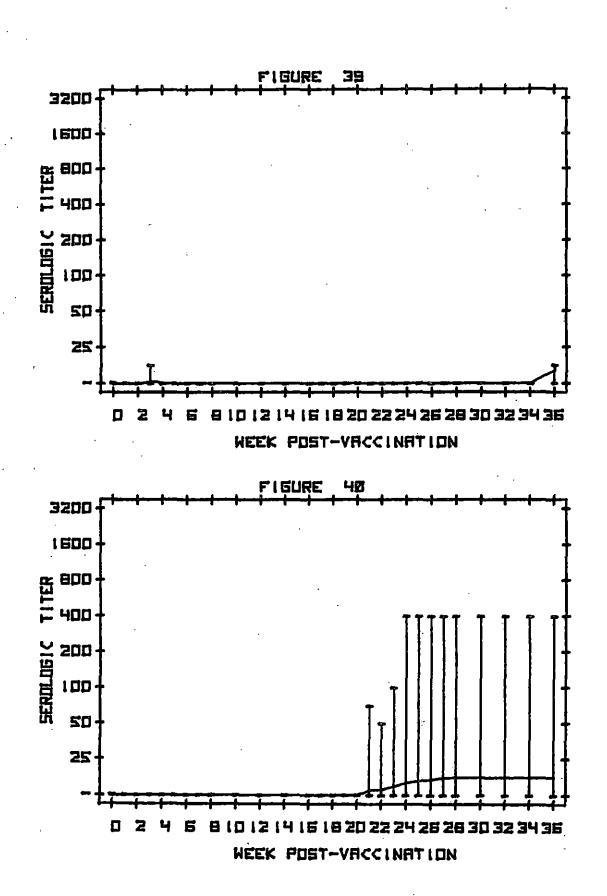


Figure 41. Results of biweekly complement fixation tests for Group VC-1 (vaccination-challenge) following vaccination with 78 X 10⁹ <u>B. abortus</u> strain 19 organisms and challenged with <u>B. abortus</u> strain 2308 during Week 16 (14 animals per group)

Figure 42. Results of biweekly complement fixation tests for Group VG-2 (vaccination-challenge) following vaccination with 5 X 10' <u>B. abortus</u> strain 19 organisms and challenged with <u>B. abortus</u> strain 2308 during Week 16.(15 animals per group)

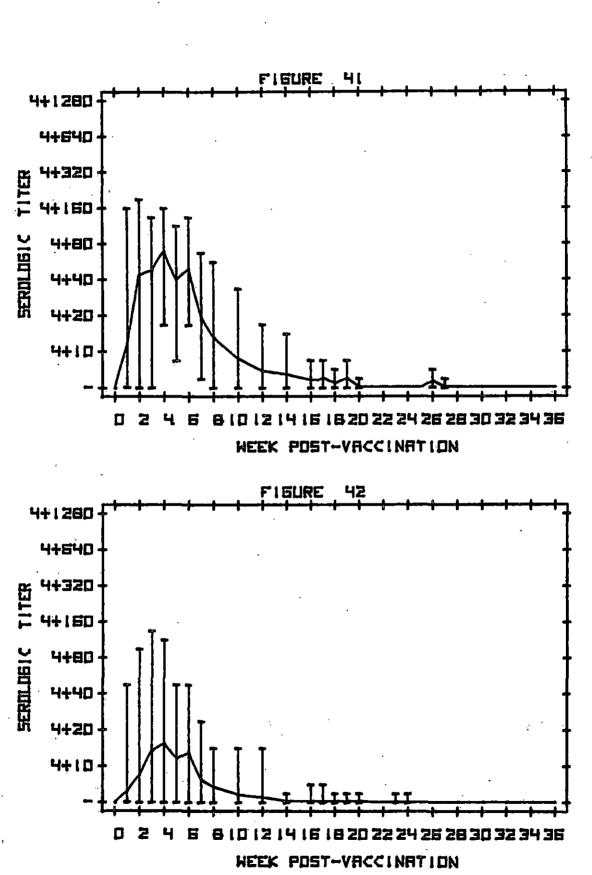
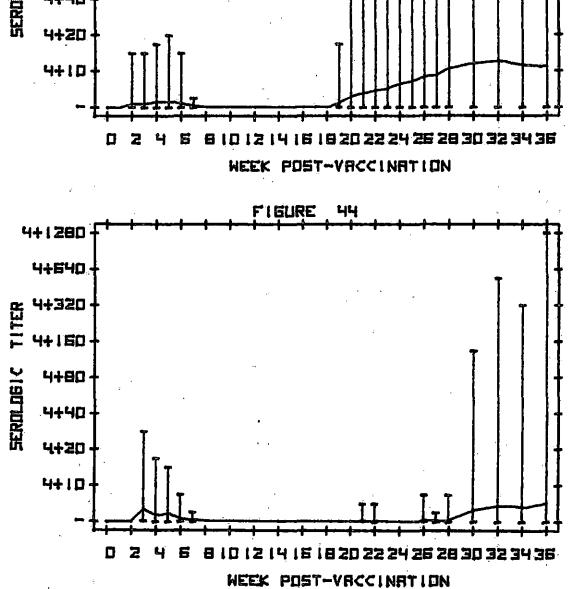
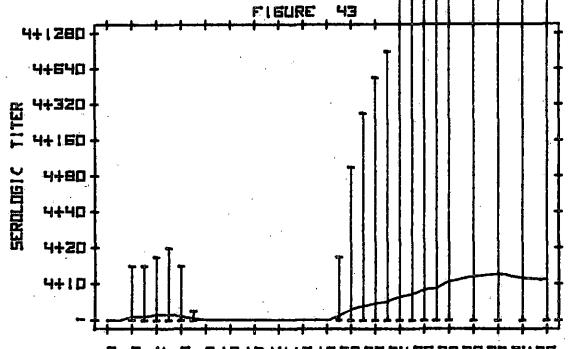


Figure 43. Results of biweekly complement fixation tests for Group VC-3 (vaccination-challenge) following vaccination with 9.2 X 10⁸ <u>B. abortus</u> strain 19 organisms and challenged with <u>B. abortus</u> strain 2308 during Week 16 (14 animals per group)

Figure 44.

Results of biweekly complement fixation tests for Group VC-4 (vaccination-challenge) following vaccination with 1.4 X 10^8 <u>B. abortus</u> strain 19 organisms and challenged with <u>B. abortus</u> strain 2308 during Week 16 (15 animals per group)





had a 2+10 titer during Weeks 21 and 22, and Cow No. 20 developed a titer at Week 26 which continued to rise until Week 36. Group VC-5 (Figure 45) did not respond on the CF test during the 36 week study.

Only two out of the 20 cows (Nos. 75 and 82) in the Control Group (Figure 46) developed a detectable CF titer. The first response was at Week 22, six weeks following challenge, and a maximum titer of a 2+1280 was reached by Week 30 (14 weeks after challenge). <u>Brucella abortus</u> strain 2308 was isolated from both of these animals.

Comparison of Tube and Mercaptoethanol Tests

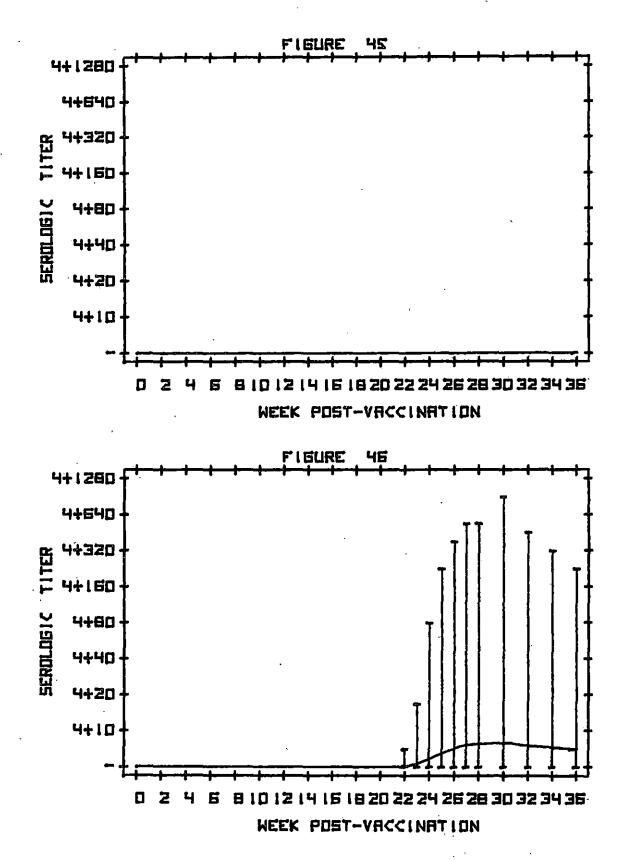
Figures 47-51 are comparisons of the results of the ME and tube tests for each of the five groups of vaccinated cows. In Figure 47 (Group V-1) the results of the ME and tube tests have the same general pattern; however, the tube test titers are significantly higher than the ME titers and appeared one week before the ME test. The results of the other four groups had similar titer patterns between the two tests; however, as the vaccine dosages decreased, the corresponding titers also decreased.

Persistently Infected Cows

Isolations of <u>B</u>. <u>abortus</u> were made from only ll cows in this study (Table 7). Strain 19 was isolated from four cows and strain 2308 from seven cows. The cultural results for each of these eleven animals are presented in Figures 52-62, along with the individual antibody responses and clinical observations. The tube and ME test results are presented

Results of biweekly complement fixation tests for Group VC-5 (vaccination-challenge) following vaccination with 2.3 X 107 Figure 45. <u>B. abortus</u> strain 19 organisms and challenged with <u>B. abortus</u> strain 2308 during Week 16 (15 animals per group)

Figure 46. Results of biweekly complement fixation tests for the Control Group (nonvaccination-challenge) following challenge with B. abortus strain 2308 during Week 16 (20 animals per group)



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Figure 47.

Comparison of the results of the mercaptoethanol (ME) and tube tests for Group V-1 following vaccination with 78 $\times 10^9$ <u>B</u>. <u>abortus</u> strain 19 organisms (8 animals per group)

Figure 48. Comparison of the results of the mercaptoethanol (ME) and tube tests for Group V-2 following vaccination with 5 X:10⁹ <u>B. abortus</u> strain 19 organisms (8 animals per group)

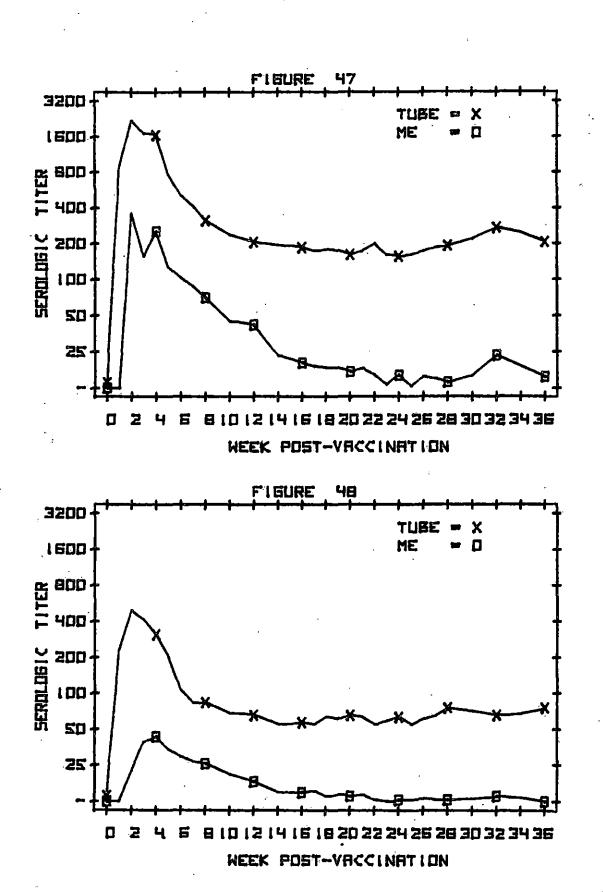


Figure 49. Comparison of the results of the mercaptoethanol (ME) and tube tests for Group V-3 following vaccination with 9.2 X 10⁸ <u>B. abortus</u> strain 19 organisms (7 animals per group)

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Figure 50. Comparison of the results of the mercaptoethanol (ME) and tube tests for Group V-4 following vaccination with 1.4 X 10⁸ <u>B. abortus</u> strain 19 organisms (8 animals per group)

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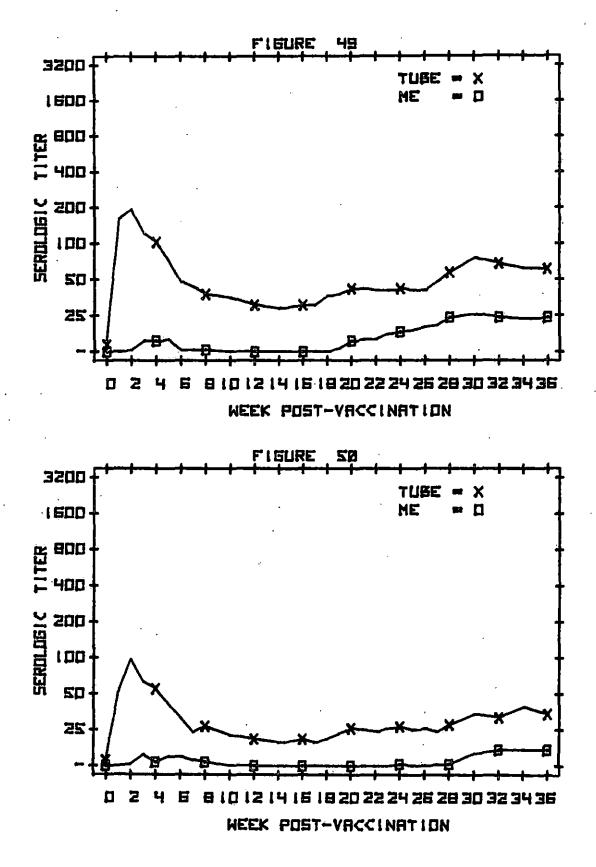
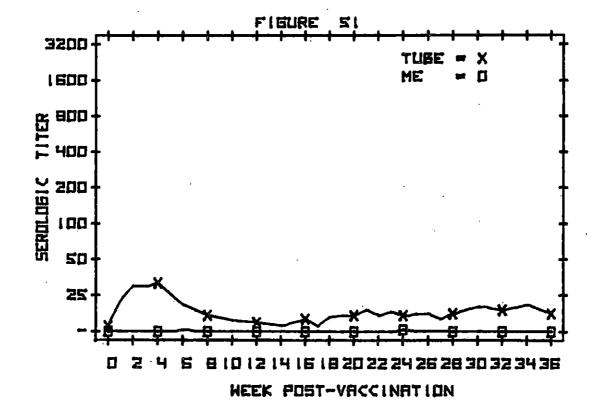


Figure 51. Comparison of the results of the mercaptoethanol (ME) and tube tests for Group V-5 following vaccination with 2.3 X 10⁷ <u>B. abortus</u> strain 19 organisms (8 animals per group)



as line graphs for each infected cow. The card, rivanol, and CF test results are presented using the negative, suspect, or positive classification as specified in the Brucellosis Eradication, Federal Uniform Methods and Rules.¹

| Group | Animal No. | Isolated strain | Source of isolation | | | |
|--------------|---------------|--------------------|----------------------|--------------------|------------|------------------|
| | | | Tissues ^a | Fetus ^b | Milk | Vaginal mucus |
| . с у | 73 | 2308 | +c,d | _C,e | - | |
| CL. | 75 | 2308 | | + | + | + |
| C | 82 | 2308 | | + | , + | + |
| VC-1 | 30 | 19 | | + ^c | - | + |
| V-2 | 105 | 19 | + | - | - | _ |
| V-3 | 62 | 19 | _ | + | - | ÷ |
| VC-3 | 67 | 2308 | | _c | - | , + |
| VC-3 | 116 | 19 | | ຼີ | + | - |
| VC-3 | 69 | 2308 | | + | + | + |
| VC -4 | 16 | 2308 | | _c | - + | - |
| VC -4 | 20 | 2308 | | + | + | + |

Table 7. List of the cows, by group, from which <u>B</u>. <u>abortus</u> was isolated and the source of the isolations

^aTissues collected at slaughter.

^bFetus includes fetal membranes and meconium from live calves.

c Live calf.

 d_{+} = an isolation of <u>B</u>. <u>abortus</u> was made.

- = an isolation of B. abortus was not made.

¹USDA, Animal and Plant Health Inspection Service, 1979 (92).

<u>Brucella abortus</u> strain 2308 was isolated from three cows in the Control Group (Nos. 73, 75, and 82). Cow No. 73 (Figure 52) did not develop a detectable titer on the card, ME, rivanol, or CF tests throughout the duration of the study. Tube and plate test titers peaked at a +25 during Weeks 20-22 and that was the only significant antibody response developed toward the challenge. She calved normally, and attempts to isolate <u>Brucella</u> from the milk, placenta, meconium, and vaginal mucus were negative. However, <u>B. abortus</u> strain 2308 was isolated from the retropharyngeal lymph nodes collected at slaughter.

Cow No. 75 (Figure 53) developed a ± 25 to an I50 titer on the tube test prior to challenge. Following challenge, the titer increased to a ± 800 by Week 24. Seven weeks later (Week 23) she aborted. Isolations of <u>B</u>. <u>abortus</u> strain 2308 were made from the fetal tissues, milk, and vaginal mucus at the time of abortion. Three weeks following abortion, <u>B</u>. <u>abortus</u> strain 2308 was isolated from the vaginal mucus swabs only. The ME titer was detected one week before abortion or six weeks following the challenge. It then reached a ± 800 but began to gradually decline following Week 26. The card test became positive three weeks following challenge and remained positive throughout the remainder of the study. The rivanol test reached the reactor classification (± 50) two weeks before the abortion (five weeks post-challenge) and remained in a reactor status. The CF titer was in the suspect category one week before the abortion. It then increased after the abortion to a reactor status for the remainder of the study.

Figure 52. Serologic and cultural results for cow No. 73 (Control Group) preceding and following challenge during Week 16 with 8.27 X 10⁵ <u>B</u>. <u>abortus</u> strain 2308 organisms The tube and ME test results are presented as line graphs

The card test is read as positive (+), a weak positive (W), or negative (-)

A +50 or higher dilution on the rivanol test is classified as a reactor (+); I50 or less is negative (-)

The CF test dilution of 1:40 with a 25% fixation or higher is classified as a reactor (+); 50% fixation in a dilution of 1:10 but less than 25% fixation in a 1:40 dilution is a suspect (S); less than 50% fixation in a 1:10 dilution is negative (-)

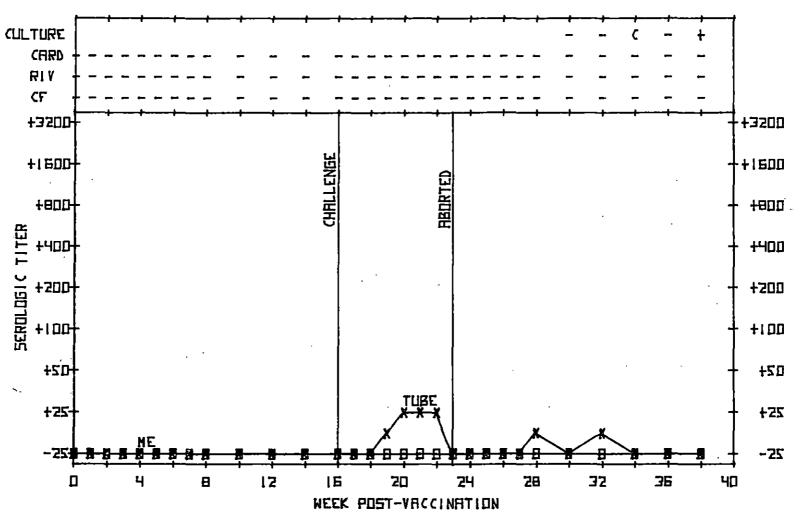


FIGURE SZ

Figure 53. Serologic and cultural results for cow No. 75 (Control Group) preceding and following challenge during Week 16 with 8.27 X 10⁵ <u>B</u>. <u>abortus</u> strain 2308 organisms The tube and ME test results are presented as line graphs The card test is read as positive (+), a weak positive (W), or negative (-) A +50 or higher dilution on the rivanol test is classified as a reactor (+);

I50 or less is negative (-)

The CF test dilution of 1:40 with a 25% fixation or higher is classified as a reactor (+); 50% fixation in a dilution of 1:10 but less than 25% fixation in a 1:40 dilution is a suspect (S); less than 50% fixation in a 1:10 dilution is negative (-)

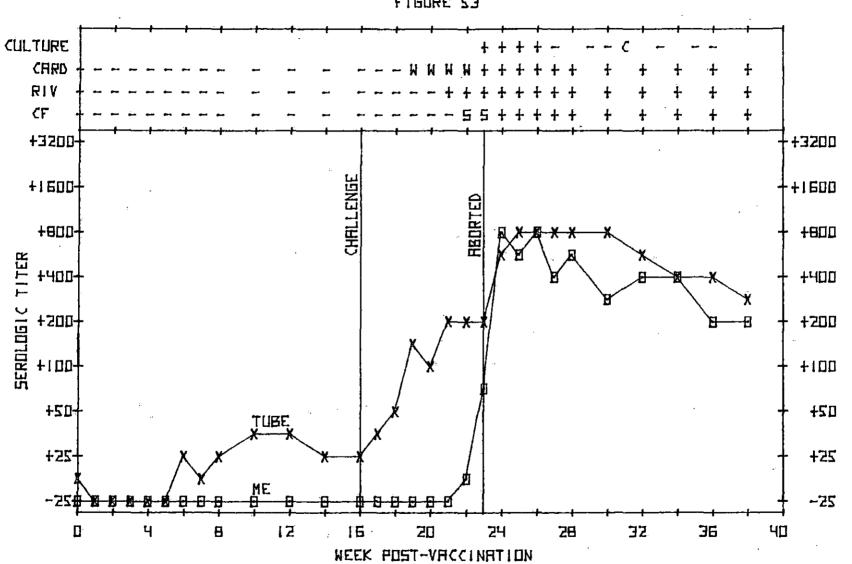


FIGURE 53

Cow No. 82 (Figure 54) developed a low transient tube titer of a +25 between Weeks 5 and 14. Her titer was negative again at the time of challenge (Week 16). Following challenge, the tube test titer increased to a +25 for three weeks then continued to increase until Week 30 when it was positive at a 1:1600 dilution. By Week 36 it had declined to She aborted 11 weeks after challenge (Week 27). A reaction to +400. the ME test occurred during Week 24 and was a +400 at the time of the Thereafter, it was identical to the tube titer. abortion. The card test became weakly positive six weeks following challenge (Week 22) then became a strong positive one week before the abortion. It remained positive throughout the remainder of the study. The rivanol and CF tests detected titers in the reactor category ten weeks after challenge (one week before the abortion) and remained positive for at least 12 weeks. At the time cow No. 82 aborted, B. abortus strain 2308 was isolated from the fetal tissues, milk, and vaginal mucus. Strain 2308 continued to be isolated from the vaginal mucus for three weeks following the abortion.

Only one cow in Group VC-1 was positive on cultures for <u>Brucella</u>. <u>Brucella abortus</u> strain 19 was isolated from cow No. 30 (Figure 55) during Weeks 29-33. She calved normally 13 weeks after challenge (Week 29). The first isolation was made at the time of calving from the placenta and vaginal mucus. <u>Brucella</u> was not isolated from the milk. The vaginal mucus yielded <u>B</u>. <u>abortus</u> strain 19 for four weeks after the

Figure 54. Serologic and cultural results for cow No. 82 (Control Group) preceding and following challenge during Week 16 with 8.27 X 10⁵ <u>B</u>. <u>abortus</u> strain 2308 organisms The tube and ME test results are presented as line graphs The card test is read as positive (+), a weak positive (W), or negative (-)

A +50 or higher dilution on the rivanol test is classified as a reactor (+); I50 or less is negative (-)

The CF test dilution of 1:40 with a 25% fixation or higher is classified as a reactor (+); 50% fixation in a dilution of 1:10 but less than 25% fixation in a 1:40 dilution is a suspect (S); less than 50% fixation in a 1:10 dilution is negative (-)

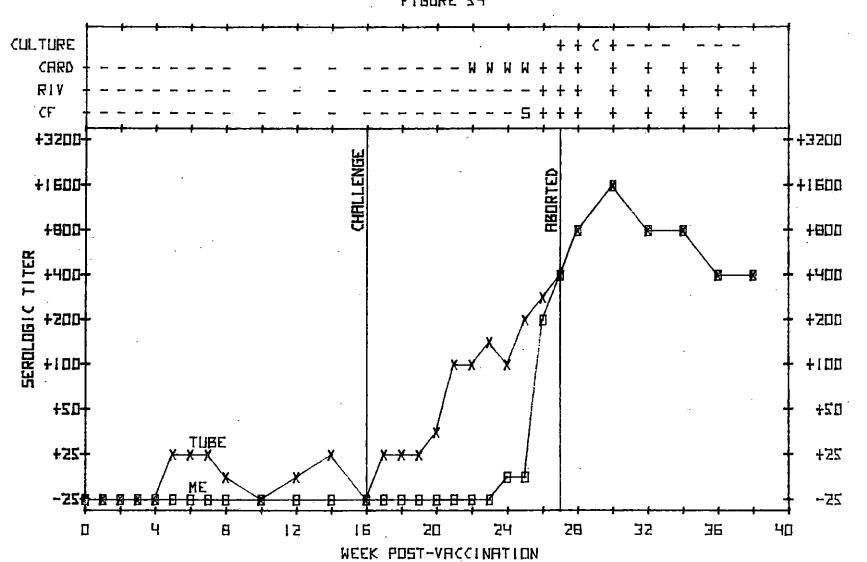


FIGURE 54

Figure 55. Serologic and cultural results for cow No. 30 (Group VC-1) following vaccination with 78 X 10⁹ B. <u>abortus</u> strain 19 organisms (Week 0) and challenge during Week 16 with 8.27 X 10⁵ <u>B</u>. <u>abortus</u> strain 2308 organisms

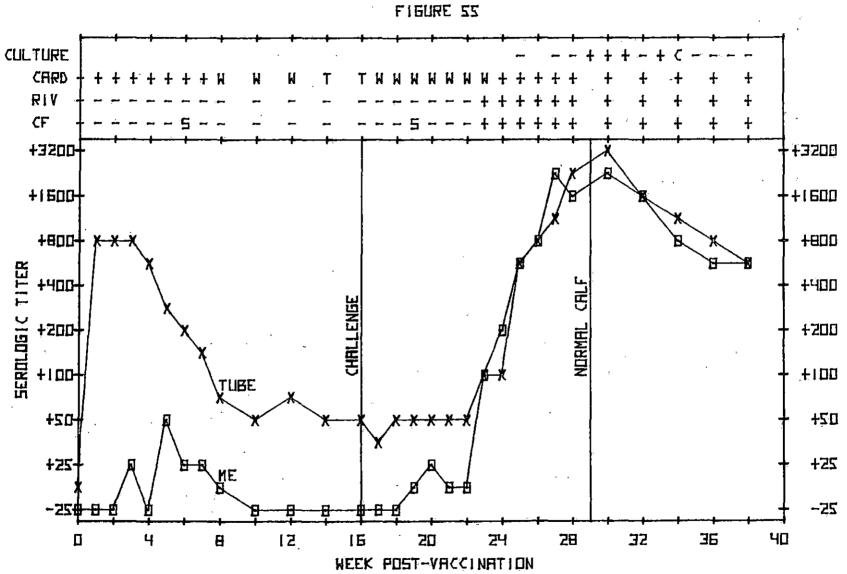
The tube and ME test results are presented as line graphs

The card test is read as positive (+), a weak positive (W), or negative (-)

A +50 or higher dilution on the rivanol test is classified as a reactor (+);

I50 or less is negative (-)

The CF test dilution of 1:40 with a 25% fixation or higher is classified as a reactor (+); 50% fixation in a dilution of 1:10 but less than 25% fixation in a 1:40 dilution is a suspect (S); less than 50% fixation in a 1:10 dilution is negative (-)



abortion. Following vaccination cow No. 30 developed a +800 tube titer, a +50 ME titer, and was positive on the card test. At the time of challenge, her titer had receded to a +50 on the tube test and was negative on the ME and card tests. The ME test titer was a +25 three weeks after challenge, increased to an I3200 during Weeks 27 and 30, and declined to an I800 by Week 38. The tube test titer increased to a +3200 by Week 30 (one week after calving) then declined. The rivanol and CF tests were positive in the reactor category following the 23rd week.

In Groups VC-3 and V-3, four cows were positive on culture for <u>Brucella</u>. Cows No. 67 and 69 shed <u>B</u>. <u>abortus</u> strain 2308; whereas, cows No. 62 and 116 shed <u>B</u>. <u>abortus</u> strain 19. Cows No. 69 and 62 aborted, while cows No. 67 and 116 had normal calves.

Cow No. 67 (Figure 56, Group VC-3) reacted on the tube test at an Il00 during Week 2, then her titer receded to a +25 until Week 25. Thereafter it increased to an I800 by Week 32 and then leveled off. The card, rivanol, CF, and ME tests did not show reactions until Week 27 (nine weeks after challenge). The ME titer increased to an Il600 from Week 27 to Week 32 and then began to decline. After Week 26 the card test remained positive. The CF test titer was in the suspect classification during Week 27 and then increased to the reactor status during Weeks 28-38 as did the rivanol test. Isolations of <u>Brucella</u> were made, from the vaginal mucus, at the time of normal calving (Week 29) and for 11 weeks thereafter.

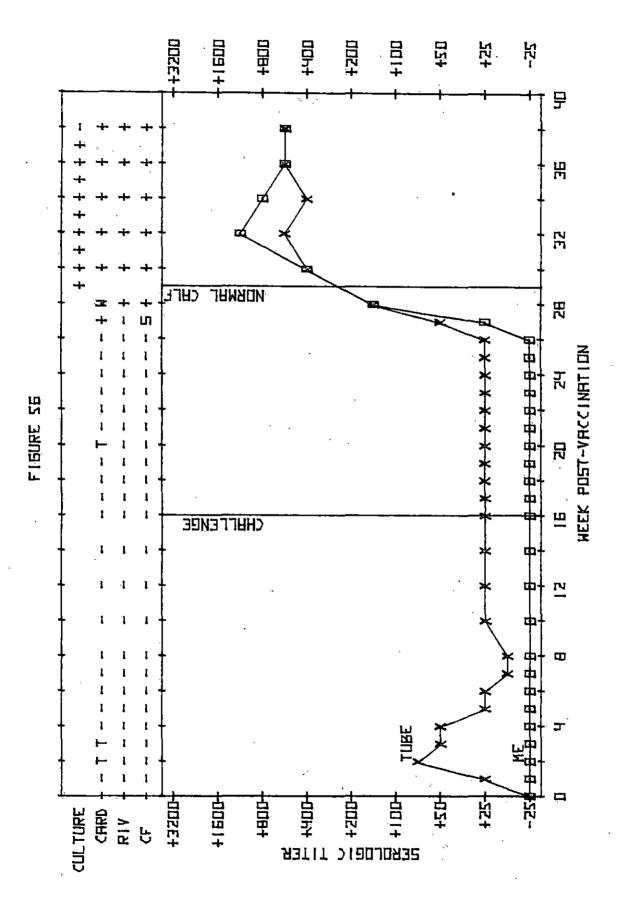
Figure 56. Serologic and cultural results for cow No. 67 (Group VC-3) following vaccination with 9.2 X 108 B. abortus strain 19 organisms (Week 0) and challenge during Week 16 with 8.27 X 10⁵ B. abortus strain 2308 organisms

The tube and ME test results are presented as line graphs

The card test is read as positive (+), a weak positive (W), or negative (-) A +50 or higher dilution on the rivanol test is classified as a reactor (+);

I50 or less is negative (-)

The CF test dilution of 1:40 with a 25% fixation or higher is classified as a reactor (+); 50% fixation in a dilution of 1:10 but less than 25% fixation in a 1:40 dilution is a suspect (S); less than 50% fixation in a 1:10 dilution is negative (~)



Cow No. 116 (Group VC-3, Figure 57) calved normally during Week 30, at which time <u>B</u>. <u>abortus</u> strain 19 was isolated only from the milk. The tube titer increased rapidly to a #400 after vaccination, then decreased to a #50 until Week 12 when it began to rise again and was a #200 during Weeks 17-23. Thereafter, the tube titer fluctuated between a #100 and a #200. The ME titer became positive four weeks after vaccination at a 1100. During Weeks 17-24 it was a #200, after that it fluctuated between an Il00 and an I200. The card and rivanol tests remained in the reactor status after Week 1. The CF test titer was in the suspect category during Weeks 2, 3, and 5 and was classified as a reactor thereafter.

Cow No. 69 (Figure 58, Group VC-3) aborted eight weeks after challenge and <u>B</u>. <u>abortus</u> strain 2308 was isolated from the fetus, milk, and vaginal mucus. Isolations of strain 2308 were made from the vaginal mucus for three weeks following abortion. Eleven weeks following abortion strain 2308 was isolated from the milk. The card test was positive during Weeks 2-5 and again on Weeks 19-38. The tube test titer peaked at a #400 and declined to a #50 at the time of challenge. It increased to a #3200 during Week 23 and remained at that level throughout the study. There were no responses on the ME, rivanol, or CF tests until Week 19. After Week 19 the rivanol and CF tests remained in the reactor status and the ME test increased to a #3200 by Week 24 (time of abortion). By Week 36 it was still an I3200.

Figure 57. Serologic and cultural results for cow No. 116 (Group VC-3) following vaccination with 9.2 X 10⁸ <u>B. abortus</u> strain 19 organisms (Week 0) and challenge during Week 16 with 8.27 X 10⁵ <u>B. abortus</u> strain 2308 organisms

The tube and ME test results are presented as line graphs

The card test is read as positive (+), a weak positive (W), or negative (-) A +50 or higher dilution on the rivanol test is classified as a reactor (+);

I50 or less is negative (-)

The CF test dilution of 1:40 with a 25% fixation or higher is classified as a reactor (+); 50% fixation in a dilution of 1:10 but less than 25% fixation in a 1:40 dilution is a suspect (S); less than 50% fixation in a 1:10 dilution is negative (-)

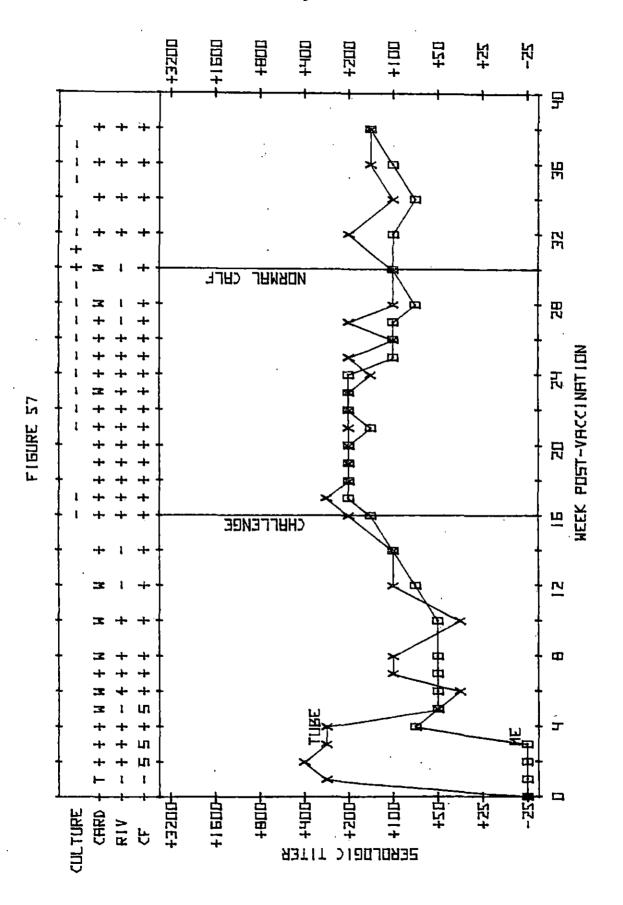


Figure 58. Serologic and cultural results for cow No. 69 (Group VC-3) following vaccination with 9.2 X 10⁶ <u>B. abortus</u> strain 19 organisms (Week 0) and challenge during Week 16 with 8.27 X 10⁵ <u>B. abortus</u> strain 2308 organisms

The tube and ME test results are presented as line graphs

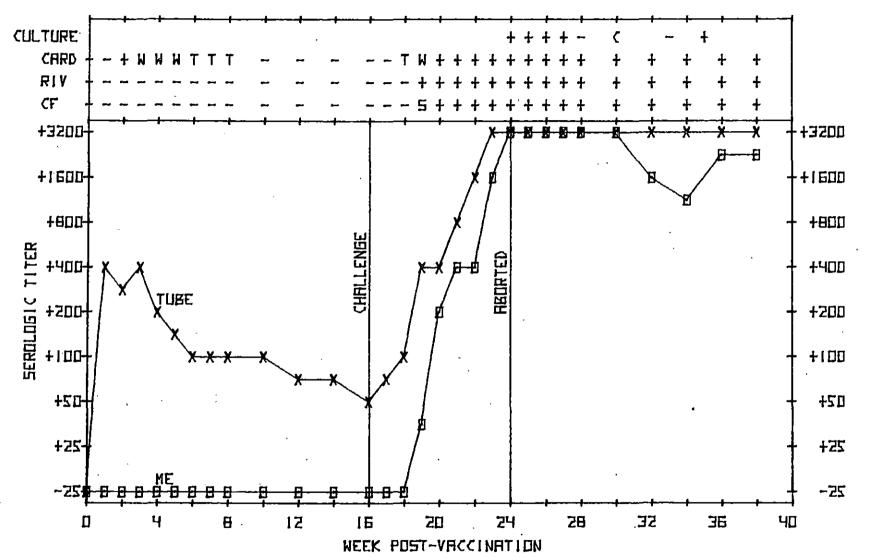
The card test is read as positive (+), a weak positive (W), or negative (-)

A +50 or higher dilution on the rivanol test is classified as a reactor (+); 150 or less is negative (-)

The CF test dilution of 1:40 with a 25% fixation or higher is classified as a reactor (+); 50% fixation in a dilution of 1:10 but less than 25% fixation in a 1:40 dilution is a suspect (S); less than 50% fixation in a 1:10 dilution is negative (-)

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Cow No. 62 was originally in Group VC-3; however, the post-vaccination serological response decreased until Week 12 and then began to increase (Figure 59). This response was indicative of strain 19 infection; therefore, she was replaced with another cow from Group V-3. Cow No. 62 aborted on Week 21 and strain 19 was isolated from the fetus and vaginal mucus. Strain 19 was isolated from the vaginal mucus for two weeks following abortion. The tube and ME test titers were a +800 from Weeks 14-28, then they began to decline. The CF titer increased to the suspect range during Weeks 4-6, then returned to negative until Week 12. Thereafter, the titer remained in the reactor category. The rivanol test titer was in the reactor status during Weeks 2-5 and after Week 11. Cow No. 62 was sacrificed during Week 30.

Cow No. 16 (Group VC-4, Figure 60) was negative on the CF, ME, and rivanol tests throughout the study. She was positive on the card test during Weeks 2-4. A peak tube titer of an I200 during Weeks 2 and 3 declined to a +25 at the time of challenge. After challenge, the tube test titer fluctuated between a +25 and a +50. Cow No. 16 calved normally during Week 29. <u>Brucella abortus</u> strain 2308 was only isolated from the milk, during that one week. Additional isolation attempts were negative. Based on the serologic response, this animal would not have been classified as either a reactor or suspect.

Cow No. 20 (Group VC-4, Figure 61) did not develop a serologic response to vaccination. One contributing factor may have been stress.

Figure 59. Serologic and cultural results for cow No. 62 (Group V-3) preceding and following challenge during Week 16 with 8.27 X 10⁵ <u>B</u>. <u>abortus</u> strain 2308 organisms The tube and ME test results are presented as line graphs The card test is read as positive (+), a weak positive (W), or negative (-)

A +50 or higher dilution on the rivanol test is classified as a reactor (+); I50 or less is negative (-)

The CF test dilution of 1:40 with a 25% fixation or higher is classified as a reactor (+); 50% fixation in a dilution of 1:10 but less than 25% fixation in a 1:40 dilution is a suspect (S); less than 50% fixation in a 1:10 dilution is negative (-)

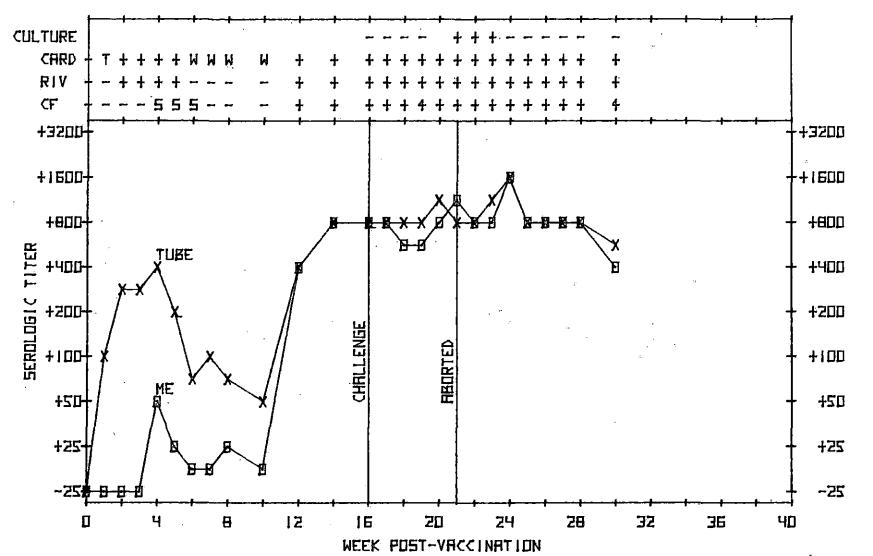


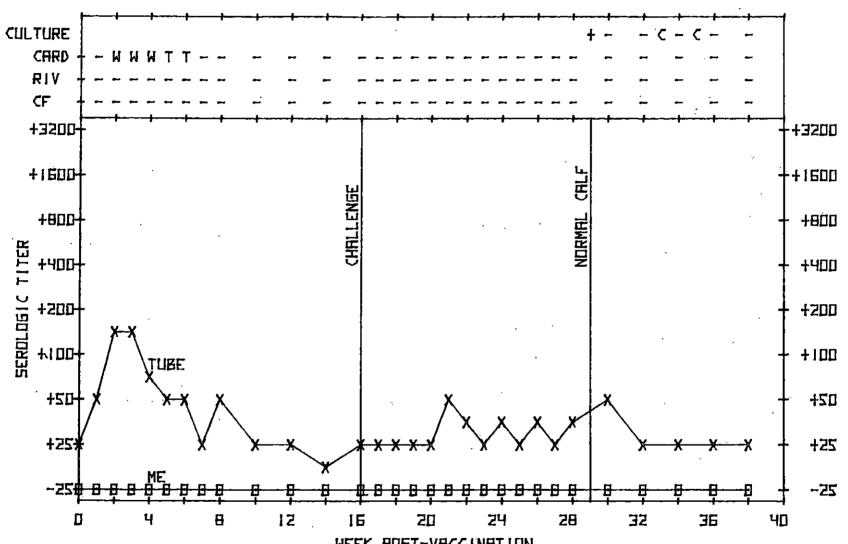
FIGURE 59

Figure 60. Serologic and cultural results for cow No. 16 (Group VC-4) following vaccination with 1.4 X 10° <u>B</u>. <u>abortus</u> strain 19 organisms (Week 0) and challenge during Week 16 with 8.27 X 10⁵ <u>B</u>. <u>abortus</u> strain 2308 organisms

The tube and ME test results are presented as line graphs

The card test is read as positive (+), a weak positive (W), or negative (-) A +50 or higher dilution on the rivanol test is classified as a reactor (+); I50 or less is negative (-)

The CF test dilution of 1:40 with a 25% fixation or higher is classified as a reactor (+); 50% fixation in a dilution of 1:10 but less than 25% fixation in a 1:40 dilution is a suspect (S); less than 50% fixation in a 1:10 dilution is negative (-)



WEEK POST-VACCINATION

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FIGURE 60

She was wild, mean, and difficult to handle (as many cows were) especially in the isolation stalls. Two weeks before being vaccinated she tried to escape from the headgate and developed a pressure necrosis on both sides of her neck. During the next several weeks she was anorexic and became very weak. She was treated with penicillin and given special care until she regained strength. Following challenge she developed a tube test titer of a +25 during Week 20. After that, the titer increased to an 13200 by Week 38. The ME test titer increased from an 125 before abortion to a +400 the week following abortion. The CF test was in the suspect category for two weeks before the abortion. After abortion the card, CF, and rivanol test titers remained in the reactor category. Cow No. 20 aborted 13 weeks after challenge (Week 29) and <u>B</u>. <u>abortus</u> strain 2308 was cultured from the fetus, milk, and vaginal mucus. The card test became positive on Week 23.

Cow No. 105 (Group V-4, Figure 62) responded to vaccination on all tests. She developed a tube titer of an I800 two weeks following vaccination. The titer gradually declined to an I25 by Week 16, but increased again and fluctuated between a +25 to an I100 the remainder of the study. The card test was positive between Weeks 1 and 7. The rivanol test showed agglutination titers beginning Week 2 (+50), which peaked during Week 4 (I200) and returned to negative by Week 17. Cow No. 105 responded on the ME test during Weeks 2 to 10 with a peak titer of a+100 during Week 5. There was a CF response between Weeks 3 to 10

Figure 61. Serologic and cultural results for cow No. 20 (Group VC-4) following vaccination with 1.4 X 10⁸ <u>B.</u> <u>abortus</u> strain 19 organisms (Week 0) and challenge during Week 16 with 8.27 X 10⁵ <u>B</u>. <u>abortus</u> strain 2308 organisms

The tube and ME test results are presented as line graphs

The card test is read as positive (+), a weak positive (W), or negative (-)

A +50 or higher dilution on the rivanol test is classified as a reactor (+); 150 or less is negative (-)

The CF test dilution of 1:40 with a 25% fixation or higher is classified as a reactor (+); 50% fixation in a dilution of 1:10 but less than 25% fixation in a 1:40 dilution is a suspect (S); less than 50% fixation in a 1:10 dilution is negative (-)

FIGURE BI

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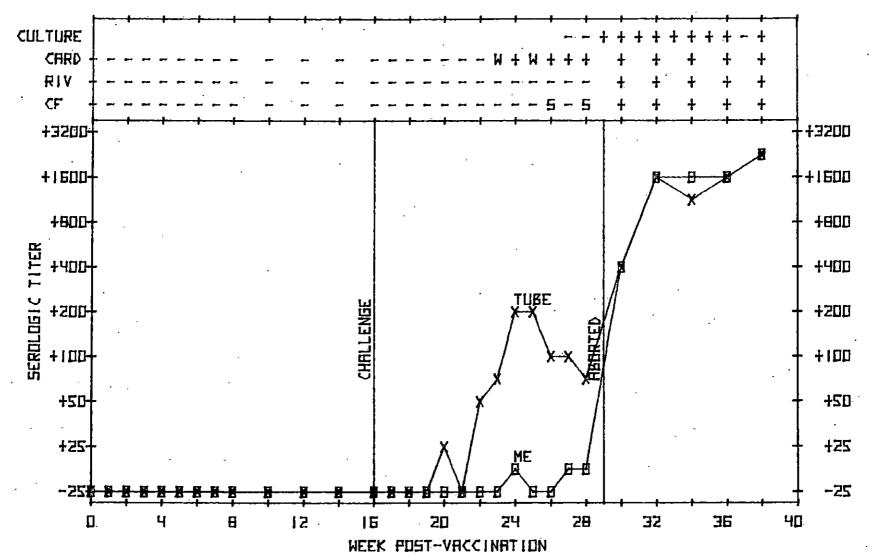


Figure 62. Serologic and cultural results for cow No. 105 (Group V-2) following vaccination with 5 X 10⁹ B. abortus strain 19 organisms

The tube and ME test results are presented as line graphs

The card test is read as positive (+), a weak positive (W), or negative (-)

A +50 or higher dilution on the rivanol test is classified as a reactor (+); I50 or less is negative (-)

The CF test dilution of 1:40 with a 25% fixation or higher is classified as a reactor (+); 50% fixation in a dilution of 1:10 but less than 25% fixation in a 1:40 dilution is a suspect (S); less than 50% fixation in a 1:10 dilution is negative (-)

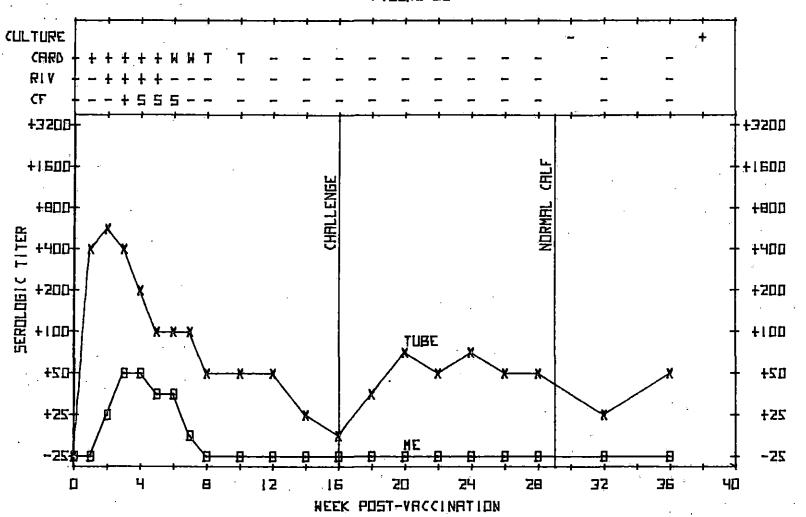


FIGURE 62

with the peak titer of a 4+40. Attempts to isolate <u>Brucella</u> from milk, vaginal mucus, placenta, and meconium were negative. However, strain 19 was isolated from lymph nodes collected at slaughter.

Replacement Cattle

The cattle were vaccinated on Week 0 (12-13-77) and developed titers following vaccination that began to recede after Week 2. All cows were reexamined for pregnancy one week following vaccination. Cow No. 68 had an abnormal uterus and ovaries upon palpation. Because of this abnormality, she was replaced, by random selection, with cow No. 116.

Ten weeks post-vaccination the titer of cow No. 62 (Group VC-3) began to increase rather than continue to recede. This response was indicative of strain 19 infection. She was replaced, by random selection, with cow No. 119 of Group V-3.

During Week 14, cow No. 18 (Group VC-4) developed a prominent actinomycotic lesion on her jaw. This introduced a possible variable in her response to the vaccination and challenge. Therefore, she was replaced, by random selection, with cow No. 124 (originally in Group V-4). Cow No. 133 (Group V-5) also developed actinomycosis and was sold for slaughter during Week 16.

On 3-27-78 (Week 15), cow No. 51 (Group VC-2) aborted. Attempts to isolate <u>Brucella</u> were negative and her post-vaccination titer had

1,38

receded to almost negative. Since it was desirable to challenge pregnant cattle, she was exchanged, by random selection, with cow No. 106 from Group V-2 before the challenge (Week 16).

On 4-4-78 (Week 16), the five vaccine dosage groups and the nonvaccinated group were challenged with strain 2308. Two weeks later cow No. 42 (Group VC-2) aborted. Attempts to isolate <u>Brucella</u> were negative. The serological titers remained stable; therefore, the serologic and cultural data indicated that she aborted from another cause.

A summary of the clinical signs observed in the cattle challenged with <u>B. abortus strain 2308</u> is presented in Table 8.

| Group | Animal No. | No. of weeks post challenge ^a | Clinical signs observed |
|-------|---------------|---|---|
| C | 75 | 7 | Aborted, gestation-213 days |
| C | 76 . | 14 | Calved near term, calf was weak and died at 7 days of age |
| C · | 77 | 1 <u>4</u> | Calved near term, calf weak |
| C | 81 | 14 | Calved near term, calf weak |
| Ċ | 82 | 11 | Aborted, gestation-252 days. |
| C | 86 | 16 | Calved near term, calf weak and died at 4 days of age |
| C | 89 | 15 | Calved near term, calf born dead |
| C | 90 | 14 | Calved near term, calf weak |
| VC-1 | 27 | 12 | Dystocia, live calf manually delivered |
| VC-1 | 32 | 13 | Calved near term, calf born dead |
| VC-l | 38 | 14 | Dystocia, live calf manually delivered |
| VC-2 | 41 | .14 | Calved near term, calf died at 2 days of age |
| VC-2 | 42 | 2 | Aborted, gestation-203 days |
| VC-2 | 46 | 12 | Dystocia, dead calf manually delivered |
| VC-2 | 50 | 12 | Dystocia, live calf manually delivered |
| VC-2 | 51 | -1 | Aborted before challenge date, gestation-182 days |
| VC-2 | 52 | 14 | Calved near term, calf born dead |
| VC-2 | 53 | 15 | Dystocia, dead calf manually delivered |

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Table 8. Summary of clinical signs observed in the cattle challenged with <u>B</u>. <u>abortus</u> strain 2308

| VC-3 | 61 | 12 | Dystocia, cow paralyzed, calf killed later | |
|--------|--------------|-----------------|--|--|
| VC-3 · | 62 | 5 | Aborted, gestation-231 days | |
| VC-3 | 69 | 8 | Aborted, gestation-223 days | |
| VC-4 | 17 | 15 ⁻ | Calved near term, calf died at 1 day of age | |
| VC-4 | 20 | 13 | Aborted, gestation-264 days | |
| VC-4 | 72 | 12 | Calved early, calf weak | |
| VC -4 | 9 3 , | 15 | Calved near term, calf died at 3 days of age | |
| VC-5 | 5 | ·- | Nonpregnant | |
| VC-5 | 10 | 14 | Calved near term, calf weak | |
| VC -5 | 11 | 15 | Calved near term, calf died at 2 days of age | |
| VC-5 | 13 | 14 | Dystocia, live calf manually delivered | |
| | | | | |

^aOccurrence of clinical signs.

DISCUSSION

The antibody response of adult cows to vaccination with <u>Brucella</u> <u>abortus</u> strain 19 was dose related with respect to both the degree and the duration. The group of cows that received the highest (standard) dosage developed the highest geometric mean tube test titer (\pm 1600); whereas, the reduced dosage groups developed correspondingly lower mean titers (\pm 50 in Group V-5). This same relationship was true with respect to the duration of mean titers for each group. The group receiving the highest dosage produced a detectable mean titer that persisted 18 to 20 weeks longer than the groups that received reduced dosages of vaccine.

Studies by others, using various dosages of <u>B</u>. <u>abortus</u> strain 19 in yearling heifers (26), calves (70), and pregnant cows (75), have reported similar results. In each of these studies the antibody response was found to be related to the dosage of vaccine. However, Manthei et al. (57) did not find as great a difference between mean titers in two groups of yearling heifers injected with different vaccine dosages. However, the dosages used in that study were relatively close together compared to those used by other investigators, but corresponded approximately to the two highest dosages used in this study. Nevertheless, Manthei concluded that the antibody response is lower and declines to a negative status more rapidly in cattle when the dose of strain 19 is reduced. The results of the present study, along with the results of others, agree that reduced dosages of strain 19 produce correspondingly

lower antibody responses which persist for shorter periods of time.

In addition to the monitoring of the antibody responses stimulated by various dosages of strain 19, the cattle were challenged with virulent organisms to determine whether or not reduced dosages would produce adequate immunity. A challenge dosage of 8.27×10^5 strain 2308 was used. This dose was slightly greater than that recommended in 1947 (54) by the USDA as the challenge standard.

Direct evidence of the existence of <u>Brucella</u> infection includes the production of clinical signs (abortion and weak or dead calves) and the isolation of <u>Brucella</u> from the secretions, excretions, or tissues. When these two criteria are applied to the results of challenge, the vaccinated animals were significantly $(P < 0.005)^1$ more resistant to the challenge than the control cattle. However, no significant difference was found among the vaccination groups when they were compared with each other.

If the absence of clinical signs is considered separately as a criterion of immunity, there is also a significant difference (P< 0.025) between the vaccinates and the controls, but no significant difference among the vaccinates (P< 0.5). However, if the cultural results are used as the only basis of evaluating resistance to challenge, the resistance of the vaccinates and controls are not significantly different at the 0.01 level, even though there was a higher percentage of recoveries from the control cattle versus the vaccinates.

¹Pearson chi-square was used to determine the significance level.

The overall effect of the challenge was somewhat less than expected. Only nine out of 20 control cattle had serologic evidence of exposure (positive on the card test). <u>Brucella abortus</u> strain 2308 was isolated from only three of the nine cattle. These expectations were based on results of other investigators (11, 12, 26, 49, 53) who reported higher rates of abortion and infectivity. While many of the conditions in this investigation were the same as those reported by others, there were several differences which could have influenced the results. Differences such as number of viable organisms in the challenge dose, virulence of the organism, age of the cattle, pregnancy status, time interval between vaccination and challenge, housing, breed variation, stress, and genetic background, were not uniform among the various investigations and could account for some of the differences in results.

A traditional method for evaluating an animal's "immunity" has been the measurement of the antibody response. In this study, the tube agglutination response of the control cattle to challenge was significantly higher (P < 0.05) than the response of the cattle in the vaccinationchallenge (VC) groups. The tube test titers of 12 out of the 20 control cows (60%) increased from negative to a 1:50 or greater, following challenge, whereas only 16% of the vaccinated-challenged cattle had similar responses. Devoe et al. (26) reported similar serological responses following challenge in cattle vaccinated with reduced dosages of strain 19; however, the titer increase of the control cattle was from negative to a 1:200 or greater. Manthei et al. (57) and Berman and Irwin (12).

however, reported increases in the mean agglutinin titer from negative to a 1:100 or greater following challenge of vaccinated cattle.

There was no significant difference, following challenge, in the tube test titers of the cattle in each of the VC groups when compared with those of the corresponding groups that were not challenged. These results indicate that a significant anamnestic response was not produced by the exposure of vaccinated cattle to strain 2308.

In studies (9, 10, 27) using the anamnestic response to differentiate vaccinal and infection titers, it was proposed that infected cattle may be saturated with antigen (B. abortus) which stimulates the antibodyproducing cells to capacity and creates a "static phase." Therefore, an injection of strain 19 during this static phase would not elicit the formation of additional antibodies. If this can be expanded to include recently vaccinated animals exposed to a challenge, then perhaps the time interval between vaccination and challenge in this study was short enough to provide a "static phase." One difficulty with this theory is that several cows, especially those in the lower dosage groups did not develop an antibody response when challenged. It has recently been established (45, 47) that cell-mediated mechanisms are significant in providing immunity to Brucella. The possibility must be considered that cellmediated mechanisms may have eliminated the challenge dose of Brucella before antibody-forming cells were stimulated to activity. Another factor, which might favor the rapid elimination of the challenge organisms, would be a reduction in virulence of the challenge strain.

A persistent elevated antibody response is a good indication of exposure and/or infection. The most pronounced and persistent increase in antibody response in this study, following challenge, occurred in those cows in which the infection became established and Brucella was isolated. Strain 19 was isolated from four cows and strain 2308 from another seven cows. Eight of the 11 cows, from which Brucella was isolated, produced characteristic antibody responses on all six serological tests which are recognized as diagnostic evidence of an established persistent infection. Five of these eight cows aborted and three had normal calves. However, three of the ll cows, with an established Brucella infection (two cows with strain 2308 and one with strain 19) had low antibody responses which would not have been interpreted as reactors by usual serological methods. These cows also had normal calves. One cow shed strain 2308 in her milk for one week after calving. The other two cows did not shed Brucella, but had localized infections and Brucella was isolated from individual tissues. Whether these cows would have overcome the infection or succumbed and aborted during a later pregnancy is unknown.

Other vaccination-challenge studies have reported similar findings. Berman and Irwin (12) stated that "an interesting and important aspect of the work with the lower exposure level (6 X 10^5 strain 2308 organisms) is the number of infected animals which failed to develop agglutinins of diagnostic significance." Deyoe et al. (26) used a higher challenge dosage of strain 2308 and reported the same observations. Both

investigators theorized that this lack of response was related to the severity and extent of infection in which some localization in the tissues had occurred with little or no contact with antibody forming cells. This fact is consistent with field observations where it has been difficult to eliminate the disease from herds through the use of serological tests as the only means of diagnosis.

Apparently, when cattle are naturally exposed or challenged, the dosage and/or virulence may be critical in the production of antibody and cell-mediated responses. Therefore, cattle in infected herds that are exposed to low numbers of organisms may develop localized infections that persist for unknown periods of time before the organisms are eliminated or cause abortion and/or shedding. This points out the problem that regulatory personnel and cattle owners face if they depend entirely on the serological tests to identify infected cattle. It stresses the importance of collecting milk, vaginal mucus, and tissue samples, if possible, when attempting to detect all of the cattle which are shedding the organism.

It is appropriate to look at the protection produced in other studies using reduced dosages of <u>B</u>. <u>abortus</u> strain 19. Nicoletti et al. (75), using infected dairy herds, reported "there were no apparent differences in protection afforded by the standard vaccine dose (5.9×10^{10}) and the reduced dose (2×10^9) of strain 19 administered subcutaneously." These finding are supported by trials in England (70) in which there

were no differences in protection between calves inoculated with 0.25 cc (2.4×10^9) and 5 cc (4.4×10^{10}) . The calves were challenged ten months after vaccination with strain 544. However, in the same study, a dosage of 3.6 $\times 10^7$ (1/1000 of the normal dose) reportedly failed to provide "protection." The method of evaluating protection was by the isolation of <u>Brucella</u> from tissues and by the serological response which was "slow to develop, reached a low peak, and had virtually disappeared two months after vaccination." Since the calves were not pregnant, any effect the challenge may have had upon reproduction was not evaluated.

Manthei et al. (57) stated in 1952 that the minimum dose of viable strain 19 organisms that will produce a serviceable resistance to brucellosis in cattle was unknown at that time. Yet, based on their study, they found that the subcutaneous inoculation of 2.4 X 10^9 viable strain 19 provided an immunity equal to that produced by the subcutaneous inoculation of 6 X 10^{10} viable organisms. Devoe et al. (26) stated in 1979 that a vaccine dosage as low as 1 X 10^7 colony forming units (or 1/5000, of the current minimum recommended dose) given subcutaneously was fully as protective as a standard dose. These studies provide evidence that a dosage greater than 3.6 X 10^7 should provide adequate immunity.

One problem occasionally encountered in cattle vaccinated with strain 19 is the establishment of persistent infections which stimulate diagnostically significant serological titers. Strain 19 was isolated from four of the 115 cattle (3.5% infection rate) vaccinated in this

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study. Three of the four cows were classified as reactors on the basis of their serological titers. One of the three aborted and strain 19 was isolated from the fetus. Other studies (25, 35, 52, 60, 68, 93) have also reported abortions and persistent infections in cows vaccinated during pregnancy. Apparently strain 19 does not cause as many persistent infections when given to nonpregnant cattle (53, 68).

Strain 19 was not isolated from cows in the two groups receiving the lowest dosages $(2.3 \times 10^7 \text{ and } 1.4 \times 10^8 \text{ organisms})$. Perhaps the number of organisms at these dosage levels was insufficient to produce persistent infections. However, the next highest dosage $(9.2 \times 10^8 \text{ organisms})$ produced strain 19 infection in two cows; one cow aborted and one had a normal calf. Whether or not this dosage level will routinely produce more persistent strain 19 infections or clinical problems than other dosages is difficult to evaluate without further testing.

Each of the groups that received the two highest vaccine dosages, contained one strain 19 infected animal. One of these had a persistent antibody titer and the other had no serological evidence of persistent strain 19 infection eight weeks after vaccination. Both cows calved normally. An explanation for the failure of the one cow to produce an antibody response was undetermined.

Six serological tests were included in this study to monitor the antibody responses due to vaccination with varied dosages and to observe their usefulness in distinguishing infection due to virulent strain 2308 versus vaccination with strain 19. The CF, rivanol, and ME tests

gave similar results in correctly identifying cattle (72%) from which <u>Brucella</u> was isolated. However, in those cattle that had transient infections, the CF test titer returned to a negative status earlier than the other five tests. This reduced time interval between vaccination and the return to a negative status is the main advantage of using reduced dosages of strain 19. When the supplemental tests were used with cattle given a standard dosage of strain 19, most vaccinated cows from which <u>Brucella</u> was not isolated could be differentiated from those in which <u>Brucella</u> was isolated, 16 weeks following vaccination. However, by using reduced dosages, this time interval was reduced to ten weeks. Therefore, under the conditions of this study, it was possible to identify and remove infected cattle ten weeks after vaccination. If similar results were obtained under field conditions, this practice would aid in reducing the exposure potential.

The tube and plate tests were the most sensitive test procedures for the detection of antibody responses to both vaccination and challenge earlier and longer than the other four tests. The card test was the next most sensitive test for detecting antibodies produced from either vaccination or infection. However, the test was not as selective in differentiating between vaccination and infection as were the CF, rivanol, or ME tests, but was more selective than the plate and tube tests in revealing infected animals.

SUMMARY

The purpose of this study was to evaluate the effect of varied dosages of <u>B</u>. <u>abortus</u> strain 19 in adult pregnant cattle. One hundred thirty-five beef cows were purchased and bred for this study. Forty cows were used in the vaccination-control phase and 95 were used in the challenge phase. The 95 cows were randomly divided into six groups. One group of 20 cows was used as nonvaccinated-challenged controls. The remaining 75 cows were divided into five groups of 15 animals and each group was given a different dose of vaccine.

Four months following vaccination the 95 cows were challenged with <u>B. abortus</u> strain 2308, administered into the conjunctival sacs. Blood samples were collected and evaluated using six tests. Vaginal swabs were taken at weekly intervals following challenge. Milk samples were collected following abortion or calving. All cows were slaughtered at the end of the study and tissues were cultured for <u>Brucella</u>.

The antibody titers produced against the strain 19 organisms varied in proportion to the dose given. The vaccinated animals were found to be significantly more resistent to challenge than the control cattle (P < 0.005), as determined by the combination of clinical signs and cultural recovery. However, isolations of <u>B</u>. <u>abortus</u> strain 2308 were made from only three of 20 control cattle (15%). Sixty percent of the control cattle developed a two dilution tube test titer increase following challenge. Generally, Brucella was not usually isolated from vaccinatedchallenged animals which did not have persistent elevated antibody responses.

<u>Brucella</u> was isolated from milk, vaginal swabs, fetal tissues, and/or tissues collected at slaughter from 11 cows. Three of these cows had low antibody titers and were not suspected as carriers by serological procedures. <u>Brucella abortus</u> strain 19 was isolated from four cows within the three highest dosage groups, and was responsible for an abortion in one. Persistently high antibody titers were observed in three of the four cows from which strain 19 was isolated.

CONCLUSIONS

- 1. The maximal antibody response to <u>B</u>. <u>abortus</u> strain 19 vaccine was proportional to each dosage given.
- 2. The duration of the antibody response corresponded directly to the dosage of vaccine given.
- 3. A high persistent titer is an indication of an active <u>Brucella</u> infection.
- 4. The CF, rivanol, and ME tests were better predictors of an active established <u>Brucella</u> infection than the card, tube, and plate tests.
- 5. The CF titer returned to a negative status more rapidly than the other tests in vaccinated cattle from which <u>Brucella</u> was not iso-lated.
 - 6. The tube and plate tests were the most sensitive and detected the antibody responses to both vaccination and challenge earlier and for a longer period than the other four tests.

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APPENDIX

| | | | | oup | | | |
|--------|--|-------------|-----------|--------------|-----|---|--|
| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL | VSL TUBE | VSL CF |
| 73 | 0 1 2 3 4 5 6 7 8 1 2 4 5 6 7 8 1 2 1 4 5 6 7 8 1 2 2 1 2 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 16 7 8 9 0 12 4 5 6 7 8 0 12 4 16 7 8 9 0 12 4 5 6 7 8 9 0 12 4 16 7 8 9 0 12 4 16 7 8 9 0 12 4 16 7 8 9 0 12 4 16 7 8 9 0 12 4 16 7 8 9 0 12 2 12 8 9 0 12 2 12 8 9 0 12 2 12 8 9 0 12 2 12 2 12 2 12 2 12 2 12 2 12 2 | | | | | | $\begin{array}{c} -10 \\$ |
| 74 | 0 1 2 3 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | - I + - I | | - 2225555555555555555555555555555555555 | -10 - 10 - 10 - 10 - 10 - 10 - 10 - 10 |

| DRUGELL | USIS SERUE | UGI FUK | CONCLOT GT | oup | | | |
|---------|---|---------------|------------|--|--|---|---|
| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
| 75 | 0 12 3 4 5 6 7 8 0 2 14 16 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 2 3 4 5 6 7 8 0 2 2 3 4 5 6 7 8 0 2 2 3 4 5 6 7 8 0 2 2 3 4 5 6 7 8 0 2 2 3 4 5 6 7 8 0 2 2 3 4 5 6 7 8 0 12 4 6 7 8 0 12 2 12 1 2 3 4 5 6 7 8 0 12 2 12 2 12 2 12 2 12 2 12 2 12 2 | ++++++++M&&&A | | - 25 - 25 - 25 - 25 - 25 - 25 - 25 - 25 | -2255555555555555555555555555555555555 | I 25 - 225 - 255 - 200 - | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |
| 76 | 0 1 2 3 4 5 6 7 8 0 12 4 6 7 8 0 12 4 6 7 8 0 12 4 6 7 8 0 12 4 5 6 7 8 0 12 8 9 0 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | - 1 - 2555555555555555555555555555555555 | | | $ \begin{array}{r} -10 \\ -10 $ |

| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL RIV | VSL Tube | VSL CF |
|--------|---|-------------|-----------|--------------|------------|-------------------------|--|
| 77 | 0 1 2 3 4 5 6 7 8 10 12 14 16 7 8 10 12 14 16 7 8 9 20 1 22 24 5 6 7 8 0 24 5 6 7 8 0 24 5 6 7 8 9 20 1 22 3 4 5 6 7 8 9 20 1 22 3 4 5 6 7 8 9 20 1 22 3 4 5 6 7 8 9 20 1 22 3 4 5 6 7 8 9 20 1 22 3 4 5 6 7 8 9 20 1 22 3 4 5 6 7 8 9 20 1 22 1 2 2 4 5 6 7 8 9 20 1 2 2 1 2 2 3 4 5 6 7 8 9 20 1 2 2 1 2 2 3 4 5 6 7 8 9 20 1 2 2 1 2 2 3 4 5 6 7 8 9 20 1 2 2 1 2 2 3 4 5 6 7 8 9 2 2 1 2 2 3 4 5 6 7 8 9 2 2 1 2 2 3 4 5 6 7 8 9 2 2 1 2 2 3 4 5 6 7 8 9 2 2 1 2 2 3 4 5 6 7 8 9 2 2 1 2 2 3 4 5 6 7 8 9 2 2 1 2 2 3 4 5 6 7 8 9 2 2 1 2 2 3 4 5 8 9 2 2 1 2 2 3 4 5 8 9 2 2 1 2 2 3 2 2 3 2 2 3 2 2 3 3 2 3 3 2 3 3 2 3 3 2 3 3 2 3 3 2 3 3 2 3 3 2 3 3 2 3 | | | | | | $ \begin{array}{r} -10 \\ -10 $ |
| 78 | 0 1 2 3 4 5 6 7 8 1 2 1 4 1 6 7 8 1 2 2 1 2 3 4 5 6 7 8 1 2 2 1 2 3 4 5 6 7 8 0 1 2 1 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 2 1 2 3 4 5 6 7 8 0 2 2 2 2 2 4 5 6 7 8 0 2 2 2 2 2 2 4 5 6 7 8 0 2 2 2 2 2 4 5 6 7 8 0 2 2 2 2 2 2 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | | | I I + I I + + + + + + + | $\begin{array}{c} -10 \\$ |

166

| BRUCELL | USIS SERU | LUGY FUR | Control | Group | | | , |
|---------|---|-------------|-----------|--|---------------------------------------|--|--|
| ANIMAL | WEEK | VSL Card | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
| 79 | 0 1 2 3 4 5 6 7 8 0 12 14 5 6 7 8 0 12 14 6 7 8 9 0 1 2 2 3 4 5 6 7 8 0 2 2 2 2 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | - | - - - - - - - - - - | | $ \begin{array}{r} -10 \\ -10 $ |
| 80 | 0 1 2 3 4 5 6 7 8 0 2 4 6 7 8 0 2 4 6 7 8 0 2 4 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | - + - + | +50 1550 1550 1550 1550 1550 1550 1550 1 | | ++I+I+I5555555555555555555555555555555 | $\begin{array}{c} -10 \\$ |

.

| ANTAL | | | VSL | VSL | VSL | VSL | VSL |
|----------|---|-------------|--|---|--|--|---|
| ANIMAL . | WEEK | VSL CARD | ME | PLATE | RIV | TUBE | CF |
| 81 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 18 9 221 223 4 5 6 7 8 0 12 14 16 17 18 9 221 223 4 5 6 7 8 0 221 223 4 5 6 7 8 0 221 23 4 5 6 7 8 0 221 23 4 5 6 7 8 0 221 223 4 5 6 7 8 0 221 223 4 5 6 7 8 0 221 223 4 5 6 7 8 0 221 223 4 5 6 7 8 0 221 223 4 5 6 7 8 0 221 223 4 5 6 7 8 0 221 223 4 5 6 7 8 0 221 223 4 5 6 7 8 0 221 223 4 5 6 7 8 8 0 221 223 225 6 7 8 8 0 221 223 225 8 2 22 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | - 25 - 222555555555555555555555555555555 | | | $ \begin{array}{r} -10 \\ -10 $ |
| 82 | 0 1 2 3 4 5 6 7 8 10 12 14 16 7 8 10 12 14 16 7 8 19 21 22 3 4 5 6 7 8 10 12 14 16 7 8 19 21 22 3 4 5 6 7 8 10 12 14 16 7 8 10 12 14 16 7 8 10 12 14 16 7 8 19 0 12 14 16 7 8 19 0 12 14 16 7 8 19 0 12 14 16 7 8 19 0 12 14 16 7 8 19 0 12 14 16 7 8 19 0 12 14 16 7 8 19 0 12 14 16 7 8 19 0 12 14 16 7 8 19 0 12 14 16 7 8 19 0 12 14 16 7 8 19 0 21 22 3 4 5 6 7 8 19 0 21 22 3 4 5 6 7 8 19 0 21 22 3 4 5 6 7 8 19 0 21 22 3 4 5 6 7 8 19 0 21 22 3 4 5 6 7 8 19 0 21 22 3 4 5 6 7 8 19 0 21 22 3 4 5 6 7 8 19 2 2 1 2 2 7 8 9 2 2 1 2 2 3 4 5 8 3 2 4 5 8 3 2 4 5 8 3 2 4 5 8 3 2 4 5 8 3 2 4 5 8 3 2 4 5 8 3 2 4 5 8 3 2 4 5 8 3 2 4 5 8 3 2 4 5 8 3 2 4 5 8 3 2 4 5 8 3 2 4 5 8 3 2 3 2 3 2 3 2 3 3 2 3 3 2 3 3 2 3 | | -255 -225 -225 -225 -225 -225 -225 -225 | -25 -25 -25 -25 -25 -225 -225 -225 -225 | -255 -255 -25 | $\begin{array}{c} -25\\ -225\\ $ | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |

| ANIMAL | WEEK | VSL CARD | VSL ME | V SL PLATE | VSL | VSL TUBE | VSL CF |
|--------|--|-------------|-----------|---------------|----------|--|--|
| 83 | 0 1 2 3 4 5 6 7 8 0 12 14 16 7 8 9 21 22 34 5 6 7 8 0 12 14 16 7 8 9 21 22 34 5 6 7 8 0 24 5 6 7 8 0 24 5 6 7 8 0 24 5 6 7 8 0 24 5 6 7 8 0 22 4 5 6 7 8 0 22 4 5 6 7 8 0 22 4 5 6 7 8 0 22 4 5 6 7 8 0 22 4 5 6 7 8 0 22 4 5 6 7 8 0 22 4 5 6 7 8 0 22 4 5 6 7 8 0 22 4 5 6 7 8 0 22 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | 1+ | - | -2555555555555555555555555555555555555 | $ \begin{array}{r} -10 \\ -10 $ |
| | 0 1 2 3 4 5 6 7 8 10 12 14 16 7 8 10 12 14 16 7 8 90 21 23 4 5 6 7 8 0 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 2 12 10 10 10 10 10 10 10 10 10 10 10 10 10 | | | | <u> </u> | I 2555555555555050000 - I 22555555555050000 + I 225555555555555555555555555555555555 | $\begin{array}{c} -10 \\$ |

| ANIMAL | WEEK | VSL | VSL | VSL | VSL | VSL | VSL |
|--------|---|---------------------------------------|-----|--|--|--|--|
| 85 | 0 1 2 3 4 5 6 7 8 10 12 4 6 7 8 10 12 4 6 7 8 10 12 4 5 6 7 8 10 12 4 5 6 7 8 10 21 22 3 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 22 22 22 22 22 22 22 22 22 22 22 22 | CARD | M | PLATE - 25 - 255 - 2255 - 2555 - 2555 - 2555 - 2555 - 2555 - 25555 - 25555 - 25555 - 25555 - 25555 - 25555 - 2555 | R IV - 222222222222222222222222222222222222 | TUBE -2555555555555555555555555555555555555 | CF = 10 - 10 - 10 - 10 - 10 - 10 - 10 - 10 |
| 86 | 0 1 2 3 4 5 6 7 8 0 2 4 6 7 8 0 2 4 6 7 8 0 2 4 6 7 8 0 2 4 6 7 8 0 2 4 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 6 7 8 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 0 2 4 5 8 9 2 4 5 8 9 2 4 5 8 9 2 4 5 8 9 2 4 5 8 9 2 4 5 8 9 2 4 5 8 9 2 4 5 8 9 2 4 5 8 9 2 4 5 8 9 2 4 5 8 9 2 8 9 2 4 5 8 9 2 4 5 8 9 2 8 9 2 8 9 2 8 9 2 8 9 2 8 9 2 8 9 2 8 9 2 8 9 2 8 9 2 8 9 2 8 9 2 8 9 8 9 | ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ | | | | | $\begin{array}{c} -10 \\$ |

| ANIMAL | WEEK | VSL | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|--|-----|-----------|--|------------|---|--|
| 87 | 0 1 2 3 4 5 6 7 8 10 12 4 6 7 8 10 12 4 6 7 8 10 21 22 3 4 5 6 7 8 10 21 22 3 4 5 6 7 8 10 21 22 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | -2555555555555555555555555555555555555 | | -25 -225 -225 -225 -225 -225 -225 -225 | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |
| 88 | 0 123456780 10246789012345678024680 1121222222222233333 33533340 | | | | | | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |

| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL Řív | VSL TUBE | VSL CF |
|--------|--|-------------|-----------|---|------------|--|--|
| 89 | 0 1 2 3 4 5 6 7 8 10 12 14 16 7 8 10 12 14 16 17 8 90 21 223 4 5 6 7 80 24 5 6 7 80 24 5 6 7 80 21 23 4 5 6 7 80 21 23 4 5 6 7 80 21 2 3 4 5 6 7 80 221 23 4 5 6 7 80 221 23 4 5 6 7 80 221 23 4 5 6 7 80 221 23 4 5 6 7 80 221 23 4 5 6 7 80 221 23 4 5 6 7 80 221 223 4 5 6 7 80 221 223 4 5 80 221 223 4 5 6 7 80 221 223 4 5 80 221 223 225 223 225 223 225 223 225 225 225 | | | - 25 - 225555555555555555555555555555555 | | -2555555555555555555555555555555555555 | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |
| 90 | 0 1 2 3 4 5 6 7 8 12 14 16 7 8 9 0 12 14 16 7 8 9 0 12 2 2 3 2 4 5 6 7 8 0 2 2 2 3 2 4 5 6 7 8 0 2 2 2 2 3 4 5 6 7 8 0 2 2 2 2 3 4 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 16 7 8 9 0 12 14 16 7 8 9 0 12 14 16 7 8 9 0 2 2 2 2 2 2 4 5 6 7 8 9 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | | | | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |

| BRUCELL | 0515 SERUL | LUGI PUR | COULTOT OF | roup | | | s * |
|----------|--|--------------|---|---|------------|-------------|--|
| ANIMAL , | WEEK | V SL CARD | VSL ME | VSL Plate | VSL RIV | VSL TUBE | VSL CF |
| 91 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 8 9 20 12 23 4 5 6 7 8 10 21 22 3 4 5 6 7 8 0 12 14 6 7 8 9 20 12 23 4 5 6 7 8 0 22 3 4 5 6 7 8 0 22 3 4 5 6 7 8 0 22 3 4 5 6 7 8 0 22 3 4 5 6 7 8 0 22 3 4 5 6 7 8 0 22 3 4 5 6 7 8 0 21 2 2 5 6 7 8 0 21 2 2 5 6 7 8 0 21 2 2 5 6 7 8 0 21 2 2 5 6 7 8 0 2 1 2 2 5 6 7 8 0 2 1 2 2 5 6 7 8 0 2 1 2 2 5 6 7 8 9 20 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | - 2255555555555555555555555555555555555 | -25 12255 -2255 -2255 12255 -2255 12255 12255 12255 12255 12255 12000 11000 1500 15 | | | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |
| 92 | 0 1 2 3 4 5 6 7 8 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 6 7 8 9 0 2 4 5 8 9 0 2 4 5 8 9 0 2 4 5 8 9 0 2 4 5 8 9 2 2 4 5 8 9 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | | | | -10 - 10 - 10 - 10 - 10 - 10 - 10 - 10 |

BRUCELLOSIS SEROLOGY FOR GROUP VC-1

| | · | | | | | | |
|--------|--|---|--|---|---|---|--|
| ANIMAL | WEEK | | V SL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
| 25 | 0 1 2 3 4 5 6 7 8 0 12 14 6 7 8 0 12 14 6 7 8 0 12 14 6 7 8 0 12 14 6 7 8 0 12 14 6 7 8 0 12 14 6 7 8 0 21 23 4 5 6 7 8 0 24 5 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ | -25 -25 +300 +4000 +2000 +550 +550 +550 +550 -2255 -2555 -25 | -25 +400 +400 +400 +400 +400 +200 +200 +200 | -25 +100 14000 12000 1500 1500 1500 1500 1500 1500 | -25 +800 +3200 +3200 +3200 +800 +800 +800 +400 +400 +400 +400 +4 | -10 4+160 1+320 3+160 2+160 2+80 1+40 1+20 1+20 1+10 1-10 |
| 26 | 0 1 2 3 4 5 6 7 8 10 12 14 16 7 8 9 20 12 12 23 4 5 6 7 8 9 20 12 21 22 3 24 5 6 7 8 9 20 12 21 22 3 4 5 6 7 8 9 20 12 21 2 3 4 5 6 7 8 9 20 12 21 2 3 4 5 6 7 8 9 20 12 21 2 3 4 5 6 7 8 9 20 12 21 2 3 4 5 6 7 8 9 20 12 21 2 3 4 5 6 7 8 9 20 12 21 2 21 2 20 12 21 2 20 12 21 2 20 2 20 2 2 2 2 | ~ * * * * * * * * * * * * * * * * * * * | -25 -25 +200 +400 +400 +2000 +1000 +505 12555555555555555555555555555555555555 | -25 +400 +400 +400 +400 1400 1400 1400 1400 | -25 -25 +200 1400 1400 1400 1200 1200 1200 1200 1 | I 25 + 800 + 3200 + 3200 + 1600 + 800 + 800 + 800 + 400 + | $\begin{array}{c} -10\\ 1+40\\ 2+80\\ 1+160\\ 1+160\\ 1+160\\ 2+80\\ 2+40\\ 2+40\\ 2+40\\ 2+40\\ 2+10\\ 1+10\\ 1+10\\ -110\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ $ |

BRUCELLOSIS SEROLOGY FOR GROUP VC-1

| DRUCELL | USIS SERUE | UGT PUR | | 4 | • | | |
|---------|--|---|--|---|--|---|--|
| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
| 27 | 0 1 2 3 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 6 7 8 0 12 4 5 8 9 0 1 2 2 8 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | - ~ ~ ~ + + + + + + + + + + + + + + + + | -25 -1100 +5500 +5500 55555555555555555555555 | $\begin{array}{c} -25\\ 1400\\ 1400\\ +400\\ +200\\ +200\\ +100\\ +50\\ +50\\ 1100\\ +50\\ 1100\\ +50\\ 150\\ 150\\ 150\\ 150\\ 150\\ 150\\ 150\\ 1$ | -25 1100 +100 1100 150 1555 12255 12555 125555 122555 122555 122555 1225555 1255 | $\begin{array}{c} -25 \\ +800 \\ 11600 \\ +1600 \\ +400 \\ +200 \\ +200 \\ +100 \\ +100 \\ +100 \\ +100 \\ +100 \\ +100 \\ +100 \\ +50 \\ 1100 \\ +100 \\ +50 \\ 1100 \\ +10$ | $ \begin{array}{r} -10\\ 1+40\\ 1+80\\ 1+40\\ 3+40\\ 4+20\\ 1+10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -$ |
| 28 | 0 1 2 3 4 5 5 7 8 0 12 14 16 7 8 9 0 12 14 16 7 8 9 0 12 2 2 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 12 14 5 6 7 8 9 0 12 2 3 4 5 6 7 8 9 0 2 2 2 2 3 4 5 6 7 8 0 2 2 3 4 5 6 7 8 9 0 12 14 5 6 7 8 9 0 12 14 5 6 7 8 9 0 12 14 15 6 7 8 9 0 12 14 15 16 7 8 9 0 12 2 12 2 14 5 6 7 8 9 0 12 2 12 2 14 5 6 7 8 9 0 12 14 15 16 7 8 9 0 12 2 12 2 12 2 12 2 12 2 12 2 12 2 | ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ | -25 +1600 +4000 +1000 +1000 +1505 12255 5555 5555 5555 225555 225555 225555 225555 225555 225555 2255555 2255555 22555555 | - 25 + 400 + 400 + 400 + 400 + 400 + 400 + 200 1 | -25 +100 +200 1400 +200 1200 1200 1200 1200 1200 1200 12 | $\begin{array}{c} -25\\ +3200\\ +3200\\ +3200\\ +3200\\ +3200\\ +3200\\ +3200\\ +3200\\ +3200\\ +3200\\ +3200\\ +3200\\ +400\\ +400\\ +400\\ +400\\ +400\\ +400\\ +400\\ +300\\ +400\\ +300\\ +400\\ +300\\ +400\\ +300\\ +400\\ +300\\ +400\\ +300\\ +400\\ +300\\ +40\\ +4$ | $\begin{array}{c} -10\\ 1+40\\ 2+80\\ 2+160\\ 3+80\\ 2+40\\ 2+20\\ 3+10\\ 1+10\\ -10\\ -10\\ 1+10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -$ |

| Producto officiation (M-T | | | | | | | | | | | |
|---------------------------|--|--|--|---|---|---|--|--|--|--|--|
| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF | | | | |
| 29 | 0 1 2 3 4 5 6 7 8 10 12 14 5 6 7 8 10 12 14 5 6 7 8 10 212 34 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 12 3 4 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 16 7 8 9 0 12 12 12 12 12 12 12 12 12 12 12 12 12 | %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% | -25 -+2000 +2000 +2000 +2000 +1255 +22255 | -25' I400 +400 +400 +400 I400 +200 I200 I200 I200 I200 I200 I200 I2 | -25 125 +2000 +4000 140000 140000 1400000000 | $\begin{array}{c} -25 \\ 11600 \\ 13200 \\ +800 \\ +200 \\ +200 \\ +200 \\ +100 \\ +100 \\ +100 \\ 1100 \\ +50 \\ +50 \\ +50 \\ +50 \\ +50 \\ +50 \\ +50 \\ +50 \\ +100 \\ 150 \\ +50 \\ +100 \\ 100 \\ 1100 \\ 1100 \end{array}$ | -10 -10 1+80 2+160 1+160 1+160 3+80 2+40 2+10 2+10 -10 | | | | |
| 30 | 0 1 2 3 4 5 6 7 8 12 14 16 7 8 9 0 12 14 16 7 8 9 0 12 24 5 6 7 8 9 0 12 24 5 6 7 8 9 0 21 23 4 5 6 7 8 9 0 21 23 4 5 6 7 8 9 0 21 23 4 5 6 7 8 9 0 21 23 4 5 6 7 8 9 0 21 23 4 5 6 7 8 9 0 21 2 21 2 21 2 2 2 2 2 2 2 2 2 2 2 2 | _ + + + + + * * * * * * * * * * * * * * | -25 -25 +25 +25 +25 +25 +25 +25 +25 +25 +25 + | -25 +200 +200 +200 +200 +100 +50 +50 +50 +50 +50 +50 +50 +50 +50 + | -25 150 150 150 1525 1525 1525 1525 1525 | I 25 +800 +800 I 400 +200 I 200 I 100 +50 +50 +50 +50 +50 +50 +50 +50 +50 + | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ 1+10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -$ | | | | |

BRUCELLOSIS SEROLOGY FOR GROUP VO-1

| ANIMAL | WEEK | VSL CARD | V SL ME | VSL PLATE | VSL RIV | V SL TUBE | VSL CF |
|--------|---|--|--|--|---|---|---|
| 31 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 18 19 20 1 22 3 4 5 6 7 8 10 12 14 16 17 8 19 20 1 22 24 5 6 7 8 30 24 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 12 14 16 17 8 10 21 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 4 5 6 7 8 10 12 14 16 17 8 10 2 1 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 | 所满满满满满满酒酒的风雨风暖满满 十十十十十十十十十十十十一 | | - 25 +200 +200 +200 +200 1400 1200 1200 +100 +100 +100 +100 +100 +1 | -25 -25 1100 12000 +400 1400 1400 1200 1100 1100 1100 1100 1 | $\begin{array}{c} -25 \\ +400 \\ +800 \\ 16400 \\ +800 \\ +400 \\ +400 \\ +400 \\ +200 \\ +200 \\ +200 \\ +200 \\ +200 \\ +200 \\ +200 \\ +200 \\ +200 \\ +200 \\ +200 \\ +200 \\ +200 \\ +200 \\ +1$ | $\begin{array}{c} -10\\ -10\\ 2+20\\ 1+80\\ 2+80\\ 4+40\\ AC\\ AC\\ AC\\ AC\\ AC\\ -10\\ -10\\ 3+10\\ -10\\ 3+10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -$ |
| 32 | 01234567802468 10124678901234567802468 33340 | ++++MM%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% | -25 -2600 +2600 12000 12000 +1000 +55055555555555555555555555555 | -25 I200 +400 +400 I400 I400 I400 I200 I200 I200 I200 I | -25 +2000 +4000 +4000 +1 | -25 +400 +3200 13600 +800 +800 +800 +400 +400 +400 +400 +4 | $\begin{array}{c} -10\\ -10\\ 2+80\\ 1+160\\ 3+40\\ 4+40\\ 1+20A\\ 1+10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -$ |

| ANIMAL | WEEK | V SL CARD | V SL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|--|----------------------------------|---|---|--|---|---|
| 33 | 0 1 2 3 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 2 3 4 5 6 7 8 0 1 2 2 3 4 5 6 7 8 0 1 2 2 3 4 5 6 7 8 0 1 2 2 1 2 3 4 5 6 7 8 0 1 2 2 1 2 3 4 5 6 7 8 0 1 2 2 1 2 2 3 4 5 6 7 8 0 1 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | 王一府内风佑—— 工烧上工成池内烧烧烧烧烧烧烧烧烧 | -255000 1415025555555555555555555555555555555555 | -25 +200 I400 +400 +200 +100 1100 1100 +100 I100 +50 I100 +50 I100 +50 I100 +50 I100 +50 I100 +50 I100 +50 I100 +50 I100 +50 I100 +50 I100 +50 I100 +50 I100 +50 I100 +50 I100 +100 I100 +100 I100 +100 I100 I10 | -25 -22000 +1000 1000 10000 100000 10000 10000 10000 10000 1000000 | $\begin{array}{c} -25 \\ +800 \\ I 1600 \\ 1800 \\ +800 \\ 1400 \\ +200 \\ 1400 \\ +200 \\ 1400 \\ +200 \\ 1400 \\ +100 \\ +$ | $\begin{array}{c} -10 \\ -10 \\ -10 \\ 2+40 \\ 3+10 \\ 2+10 \\ -1$ |
| 34 | 0 1 2 3 4 5 6 7 8 0 2 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 2 3 4 5 6 7 8 0 2 12 2 3 4 5 6 7 8 0 2 12 2 3 4 5 6 7 8 0 2 12 2 3 4 5 6 7 8 0 2 12 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | M.M.M.M.M.M.M.++M.M.+++++++++ | -25 -14000 +100 +1000 +1 | -25 I4000 +4000 +4000 I40000 I4000 I4000 I4000 I4000 I4000 I4000 I4000 I4000 I4000 I4000 I | -25 I100 I200 I400 I400 I400 I400 I400 I400 I100 I1 | $I 25 + 1600 \\I 3200 + 1600 \\+ 1600 \\+ 800 \\+ 400 \\+ 400 \\I 400 $ | $\begin{array}{c} -10\\ 1+40\\ 2+80\\ 3+160\\ 3+160\\ 2+80\\ 2+160\\ 2+160\\ 2+10\\ 2+10\\ 1+20\\ 2+10\\ 1+10\\ -110\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ $ |

| ANIMAL | WEEK | V SL CARD | V SL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|--|---|---|--|---|--|---|
| 35 | 0 1 2 3 4 5 6 7 8 0 2 4 5 6 7 8 0 2 1 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 4 5 6 7 8 0 2 14 6 7 8 9 0 1 2 3 4 5 6 7 8 0 2 14 5 6 7 8 0 2 14 5 6 7 8 0 2 14 6 7 8 9 0 12 3 4 5 6 7 8 0 2 14 6 7 8 9 0 12 2 3 4 5 6 7 8 0 2 14 6 7 8 9 0 12 2 3 4 5 6 7 8 0 2 14 6 7 8 9 0 12 2 3 4 5 6 7 8 0 2 12 8 9 0 12 2 2 2 2 2 2 4 5 6 7 8 0 2 12 8 9 0 12 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | - AAAAAAAAAAAAAAAAAAA++++++++++ | -25 -2500 +10000 +1000 +1000 +1000 +1000 +1000 +1000 +1000 +1000 +1000 +1000 + | -25 I400 +400 I400 +400 I400 +200 I | -25 +2200 120000 120000 120000 120000 1200000000 | $\begin{array}{c} -25 \\ +1600 \\ 13200 \\ +1600 \\ +1600 \\ +800 \\ 1400 \\ +400 \\ +400 \\ +400 \\ +100 \\ +5$ | $\begin{array}{c} -10\\ 1+40\\ 1+40\\ 2+80\\ 3+40\\ 2+20\\ 2+10\\ 1+10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -$ |
| 36 | 01234567802466789012345678024680 10246789012345678024680 333345678024680 | - * * * * * * * * * * * * * * * * * * * | -25 -26400 14400 +22000 +1550 1+550 1+++++++ 11++-+ | -25 1200 +400 +400 +400 +400 1400 +200 1200 1200 1200 1200 1200 1200 12 | -25 -25 +2000 14000 14000 14000 14000 11000 1000 1000000 | $I 25 \\ I 800 \\ + 6400 \\ + 3200 \\ + 6400 \\ + 1600 \\ I 1600 \\ + 400 \\ + 400 \\ + 400 \\ + 400 \\ + 400 \\ + 400 \\ + 400 \\ I 400 \\ + 400 \\ I 400 \\ I 400 \\ + 400 \\ - 400 \\ $ | $\begin{array}{c} -10\\ -10\\ 1+320\\ 1+160\\ 2+160\\ 3+40\\ 3+80\\ 2+40\\ 3+20\\ 3+10\\ 2+10\\ 1+10\\ -110\\ -110\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |

| ANIMAL | WEEK | V SL C ARD | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|---|--|--|--|--|---|--|
| .37 | 0 1 2 3 4 5 6 7 8 0 2 4 6 7 8 9 0 1 2 3 4 5 6 7 8 0 2 4 6 7 8 9 0 1 2 3 4 5 6 7 8 0 2 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 2 2 4 5 6 7 8 9 0 1 2 2 2 4 5 6 7 8 9 0 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | 成牛牛牛猪牛牛猪 + + + + + + + + + + + + + + + + | - 25 1 100 + 2000 + 2000 + 100 + 100 + 550 + 555 555 555 555 555 555 555 555 555 5 | -25 1200 14000 14000 14000 14000 14000 120000 12000 120000 1200000000 | $\begin{array}{c} -25\\ 125\\ +200\\ 1400\\ 1400\\ 1400\\ 1200\\ 1200\\ 1200\\ 1200\\ 1100\\ +50\\ +50\\ 150\\ 150\\ 150\\ 150\\ +50\\ 150\\ +50\\ 150\\ 150\\ 1100\\ +100\\ $ | -25 +800 +3200 13200 +1600 11600 +800 1800 1800 +400 +400 +400 +400 +400 +400 +400 1400 +400 1400 +400 1400 +400 1400 +400 1400 +400 + | -10 -10 1+80 2+80 2+160 3+160 3+20 2+20 2+10 3+10 2+10 2+10 2+10 2+10 -10 |
| | 32 34 36 38 40 | W W W | +25 125 -25 -25 -25 | +200 1200 +200 +100 +100 | I 50 +25 +25 I 50 I 25 | 1400 +200 +200 1400 +200 | - 10 - 10 - 10 - 10 - 10 - 10 |
| 38 | 0 12 3 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 2 2 2 2 2 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | - + + + + + + + + + + + + + + + + + + + | -25 -2500 1200 1200 +100 +100 +505 2255 2255 2255 2255 2255 2255 22 | -25 I200 +4000 +4000 I4000 I4000 I4000 I2000 +2000 +2000 +2000 +2000 +2000 +2000 +2000 +2000 +4000 14000 14000 14000 +4000 140000 140000 140000 140000 140000 140000 140000 140000 140000 140000 140000 140000 140000 1400000000 | -25 -25 +200 1400 +200 1200 1200 +100 125 150 1200 +255 100 1200 +255 100 1200 +250 1200 1200 +250 1200 1200 +250 +2000 +200 +100 +25 +100 +25 +100 +25 +100 +25 +100 +25 +100 +25 +100 +25 +100 +25 +100 +25 +100 +25 +100 +25 +25 +25 +100 +25 +25 +100 +25 +25 +100 +25 +25 +100 +25 | $\begin{array}{c} -25 \\ +800 \\ I 6400 \\ +1500 \\ +3200 \\ +1600 \\ +800 \\ +400 \\ +80 \\ +800 \\$ | $\begin{array}{c} -10 \\ -10 \\ 2+80 \\ 1+40 \\ 2+80A \\ 4+40 \\ 2+80A \\ 1+10 \\ -10$ |

| ANIMAL | WEEK | VSĽ CARD | V SL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|---|-------------|---|---|--|---|--|
| 39 | 0 1 2 3 4 5 6 7 8 0 2 4 6 7 8 9 0 1 2 3 4 5 6 7 8 0 2 4 6 7 8 9 0 1 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 3 4 5 6 7 8 0 2 4 5 6 7 8 9 0 1 2 2 2 4 5 6 7 8 0 2 4 5 6 7 8 9 0 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | -25 +5000 +100 +15225555555555555555555555555555555555 | -25 +200 1400 +200 +200 +100 1100 1100 1100 +100 +1 | -25 +2000 +1000 +1000 +1000 +1000 +12000 +1000 +12000 +1000 +12000 +12000 +1000 +12000 +10000 +10000 +10000 +10000 +10000 +10000000 +10000 +100000000 | $\begin{array}{c} 125\\ 1800\\ 1400\\ +400\\ +100\\ +100\\ +100\\ +100\\ +100\\ +100\\ +50\\ +50\\ +50\\ +50\\ +50\\ 150\\ +50\\ +50\\ 150\\ +$ | $\begin{array}{c} -10\\ -140\\ 1+20\\ 3+10\\ 4+10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -$ |

| ANIMAL | WEEK | VSL CARD | V SL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|---|---------------------|---|---|---|---|---|
| 96 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | ₩₩₩+++++++ | - 25 - 25 + 800 + 400 I 400 + 100 + 100 + 50 + 50 + 50 + 50 + 25 I 25 + 25 | -25 +400 +400 +400 +200 +200 1200 1200 1200 +100 1100 11 | -25 150 +200 +400 1400 1400 1400 1200 +100 +50 +25 125 | -25 +800 +1600 +3200 +800 +400 +200 +200 +100 +100 +100 +100 +50 | -10 $1+20$ $2+80$ $4+80$ $3+40$ $4+40$ $4+40$ $2+40$ $1+40^{\circ}$ $2+20$ $3+20$ $2+10$ -10 |
| | 18 19 | W | -25 | 1100 | 125 | +100 | -10 |
| | 20 21 | W. | - 25 | 1100 | -25 | +50 NT | -10 |
| | 22 23 | W | -25 | +10,0 | -25 | +50 | -10 |
| | 24 25 | W | 125 | I 100 | I 25 | +100 | -10 |
| | 26 27 | W | -25 | +100 | -25 | +100 | 1+10 |
| | 28 30 | W | -25 | +100 | -25 | +100 | -10 |
| | 30 32 34 | W | - 25 | 0051 | 125 | +100 | -10 |
| | 36 | - | -25 | +100 | -25 | I100 | -10 |
| | 38 40 | _ | -25 | +50 | -25 | +50 | -10 |
| 97 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | M MMM + + + + + + + | -25 -25 +1600 +400 +200 I100 +50 +50 +50 +25 I50 +25 I25 I25 | +100 +50 +50 | | -25 11600 11600 +800 +400 1200 +100 +100 +100 +100 +100 +100 +100 | -10 1+80 1+80 3+80 1+160 4+40 2+40 3+20 1+20 1+20 2+20A -10 -10 |
| | 18 19 | W | -25 | +50 | -25 | I 100 | -10 |
| | 20 21 | _ | -25 | +50 | -25 | +50 | - 1 Q |
| | 22 23 | W | -25 | I 100 | -25 | 1100 | -10 |
| | 24 25 | - | -25 | +50 | -25 | +100 | -10 |
| | 26 27 | | -25 | I 100 | -25 | +100 | -10 |
| | 28 30 | - | -25 | 150 | -25 | 1100 | -10 |
| | 32 34 | - | -25 | I 100 | -25 | +100 | -10 |
| | 36 38 40 | т | -25 | 1100 | -25 | +100 | -10 |

| ANIMAL | WEEK | VSL | VSL | VSL | VSL | VSL | VSL |
|-------------|---|--|---|--|---|---|--|
| | | CARD | ME | PLATE | RIV | TUBE | CF |
| 98 . | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | — + + + + + + + W | -25 I25 I3200 +800 +200 +200 1200 +200 I200 I200 I200 +200 I100 I100 +50 +25 | -25 +200 +400 +400 +400 1400 +200 +200 1200 +100 +100 1100 | -25 +100 +200 +400 1400 1400 1400 1200 +100 1100 150 | -25 +1600 +1600 +1600 +1600 +400 1400 +200 +200 +200 +100 1100 +50 +50 | $ \begin{array}{c} -10\\ 1+80\\ 3+160\\ 3+160\\ 4+80\\ 4+80\\ 4+80\\ 4+80\\ 4+20\\ 2+20\\ 3+10\\ 1+10\\ \end{array} $ |
| | 18 19 | * | -25 | I100 | 1100 | +50 | -10 |
| | 20 21 | W | I 25 | +50 | 150 | 150 [°] | -10 |
| • • | 22 23 | W | 125 | 1100 | +25 | +50 | -10 |
| | 24 25 | T | -25 | 1100 | 150 | +50 | -10 |
| | 26 27 | | -25 | 1100 | -25 | +50 | -10 |
| · | 28 30 | - | - 25 | +50 | -25 | +50 | -10 |
| ۰. | 32 34 | - | -25 | 1100 | I 25 | I 100 | -10 |
| | 36 38 40 | - | -25 | +50 | -25 | +50 | -10 |
| 99 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | - + + + + + + + + + + + + + + + + + + + | -25 -25 11600 +800 1400 +200 +200 +200 +200 +100 +100 +100 +1 | - 25 + 200 + 400 + 400 + 400 + 400 + 400 I 400 I 400 + 200 I 200 I 200 I 200 | -25 +100 +200 +400 +400 +400 +400 +400 1400 1400 1200 1200 | -25 +1600 +1600 +800 +800 +800 +800 +400 +400 +200 +200 1200 +100 | $ \begin{array}{r} -10 \\ 1+40 \\ 1+320 \\ 4+160 \\ 4+80 \\ 1+160 \\ 3+80 \\ 1+80 \\ 4+40 \\ 1+40 \\ 3+20 \\ 2+20 \\ \end{array} $ |
| | 18 19 | + | +50 | +100 | 1200 | +100 | 2+10 |
| | 20 21 | W | +25 | +100 | 1100 | +100 | 2+10 |
| | 22 23 | W | 15 0 / | +100 | I100 | +100 | 1+10 |
| | 24 25 | + | 125 | +100 | I100 | +100 | 2+10 |
| | 26 27 | W | +25 | +100 | I100 | +100 | 3+10 |
| | 28 30 | W | 125 | 1100 | 150 | +100 | 1+10 |
| | 32 34 | W | -25 | +100 | 1100 | 1200 | — 1 O |
| ĩ | | W | -25 | 1100 | -25 | +100 | -10 |
| | 40 | + | - 25 | 100 | +25 | +100 | -10 |

| • | - | | | |
|-------------|----------|-----|-------|-----|
| BRUCELLOSIS | SEROLOGY | FOR | GROUP | V-l |

| ANIMAL | WEEK | VSL CARD | V SL ME | VSL PLATE | VSL RIV | VSL. TUBE | VSL ČF |
|--------|---|---|--|---|---|--|---|
| 100 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | _ + + + + + + + + + + + + + + + + + + + | -25 -25 +50 +200 +200 +100 +100 +100 1100 +50 +50 +25 | -25 +200 +400 1400 +400 +200 1400 +200 1200 +100 +100 +100 | -25 -25 1200 1400 +200 +200 +200 1200 1100 +50 +25 150 | -25 +800 11600 +800 +1600 11600 +400 +200 +200 +200 +200 +200 +200 | $ \begin{array}{r} -10 \\ -10 \\ 1+40 \\ 4+80 \\ 2+160 \\ 3+80 \\ 4+80 \\ 1+80 \\ 3+40 \\ 3+40 \\ 3+10 \\ 2+10 \\ 1+10 \\ \end{array} $ |
| | 18 19 | + | +25 | 1200 | +25 | +400 | 1+10 |
| | 20 21 | W | 125 | +100 | +25 | +200 | -10 |
| | 22 23 | 2 | +25 | I 200 | 150 | 1400 | -10 |
| | 24 25 | + | -25 | +100 | +50 | +200 | 1+10 |
| | 26 27 | W | 125 | +100 | +25 | +200 | 2+10 |
| | 28 30 | W | -25 | +100 | 125 | +200 | 1+10 |
| | 32 34 | W | -25 | +100 | +25 | +200 | -10 |
| | 36 38 | W | -25 | 1500 | -25 | +200 | -10 |
| | 40 | т | -25 | +100 | -25 | +100 | -10 |
| 101 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | _ * * * * * * * * * * * * | -25 -25 +50 +200 +100 +100 +100 +100 100 100 100 +100 +100 | -25 +200 +200 +400 I400 I400 +200 +200 +100 +100 +100 I200 | -25 1100 1200 1400 1200 1400 1200 +100 1200 +100 1100 1100 | -25 +1600 I1600 +800 +800 +400 +200 +200 +200 +100 +100 +100 | -10 -10 -10 3+80 2+80 2+80 2+80 2+80 4+40 1+80 3+40 3+40 1+40 |
| | 18 19 = | ₩ | 150 | I 100 | +50 | 1100 | 3+10 |
| | `20 21 | W | +50 | +50 | 1100 | :+50 | 1+20 |
| | 22 23 | W | 150 | +50 | 1100 | +50 | 3+10 |
| | 24 25 | W | 125 | 1100 | 150 | +50 | 1+20 |
| | 26 27 | W | +25 | 1100 | 150 | I100 · | 2+20 |
| | 28 30 | W | 125 | +50 | -25 | 1100 | 4+10 |
| | 32 34 | W | -25 | +50 | -25 | +50 | -10 |
| | 36 38 | T | -25 | 1100 | -25 | I100 | -10 - 10 |
| | 40 | - | -25 | 150 | ÷25 | +50 | -10 |

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| ANIMAL | WEEK | VSL CARD | VSL Me | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|---|--|--|--|--|---|--|
| 102 | 0 1 2 3 4 5 6 7 8 10 12 14 16 | _ + + + + + + + + | -25 -25 I 100 +400 I 400 +400 +400 +400 +400 +400 +400 +400 | -25 +200 +400 +400 +400 +400 +400 +400 +400 | -25 -25 +200 +400 +400 +400 +400 +400 +400 1400 I400 I400 I400 | 125 1800 +3200 +1600 11600 +1600 +1600 +1600 +800 +800 +800 +400 +400 | $-10 \\ -10 \\ 1+160 \\ 3+160 \\ 3+320 \\ 3+640 \\ 4+640 \\ 4+320 \\ 3+320 \\ 3+320 \\ 3+160 \\ 1+160 \\ 4+80 \\ $ |
| | 17 18 | + | +200 | +200 | 1400 | +200 | 3+40 |
| - | 19 | + | +100 | 1400 | 1400 | +200 | 2+40 |
| | 21 22 | +* | +100 | +200 | 1200 | +400 | 2+40 |
| | 23 | + | 1100 | +200 | +200 | +400 | Í+40 |
| ; | 25 26 | + | I 100 | +100 | +200 | +200 | 2+40 |
| · · · | 27 28 30 | + | 1100 | 1200 | +100 | +200 | 1+40 |
| • | 32 34 | ÷ | +50 | +100 | +50 | +200 | 2+10 |
| | 36 38 | W | -25 | 1100 | 125 | 1200 | -10 |
| _ | 40 | + | +25 | Í 100 | I 25 | 1200 | -10 |
| 103 | 103 1 2 3 4 5 6 7 8 10 12 14 16 17 | 8888 + + + + + + + + - + - + + - + + - + | -25 -25 +800 +400 1400 +200 +100 150 150 +25 +25 | -25 I400 I400 +400 I400 +200 I200 +100 I100 +50 I100 | -25 150 1200 1400 +400 1200 1200 +100 150 125 125 | -25 +1600 11600 +800 +200 +200 +200 +200 +200 +100 1100 11 | $ \begin{array}{r} -10 \\ 1+40 \\ 2+40 \\ 2+160 \\ 2+160 \\ 4+80 \\ 4+80 \\ 2+80 \\ 3+40 \\ 2+20 \\ 1+10 \\ -10 \\ -10 \end{array} $ |
| | 18 19 | W. | +25 | +50 | 25 | 1100 | -10 |
| | 20 21 | W | 125 | 1100 | -25 | °1100 | -10 |
| | 22 23 | W | 125 | +50 | -25 | 150 | -10 |
| | 24 25 | W | -25 | +50 | -25 | +100 | -10 |
| | 26 27 | ₩ . | -25 | +50 | I 25 | 1100 | -10 |
| | 28 30 | W | -25 | +50 | -25 | 1100 | -10 |
| | 32 34 | W | -25 | +50 | -25 | +50 | -10 |
| | 36 38 40 | - | -25 | +50 | -25 | +100 | -10 |

| DRUCELL | .U313 SERUL | UGI FUR | GROUP VU- | ~ | | | |
|----------|--|--|--|---|--|--|--|
| ANIMAL . | WEEK | VSL CARD | VSL ME | VSL Plate | VSL RIV | VSL TUBE | VSL CF |
| 40 | 0 1 23 34 5 6 7 8 10 12 46 7 8 10 12 46 7 8 10 12 46 7 8 10 21 23 4 56 7 8 0 12 46 7 8 0 22 22 34 56 7 8 0 22 22 34 56 7 8 0 22 2 22 34 56 7 8 0 22 2 22 34 56 7 8 0 22 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | —————————————————————————————————————— | | +25 I4000 I2000 I2000 +1000 I1000 +1000 I1000 +5500 I1000 +5500 I1000 +5500 I1000 +5500 I1000 +5500 I1000 +5500 I1000 +5500 I1000 +5000 I1 | -25000005555555555555555555555555555555 | +25 1400 1800 +400 +400 +400 +100 +100 +100 +100 +100 1100 1100 1100 1100 1100 1100 1100 1100 1100 1200 1200 1200 1200 1200 1200 1200 1200 150 | $ \begin{array}{r} -10 \\ -10 $ |
| 41 | 0 1 2 3 4 5 6 7 8 10 12 14 16 7 8 9 0 12 14 16 7 8 9 0 12 12 14 16 7 8 9 0 12 22 3 4 5 6 7 8 9 0 12 23 4 5 6 7 8 9 0 12 24 5 6 7 8 9 0 12 24 5 6 7 8 9 0 12 24 5 6 7 8 9 0 12 24 5 6 7 8 9 0 12 2 14 5 6 7 8 9 0 12 2 14 5 6 7 8 9 0 12 2 14 5 6 7 8 9 0 12 2 14 5 6 7 8 9 0 12 2 14 5 6 7 8 9 0 12 2 14 5 6 7 8 9 0 12 2 14 5 6 7 8 9 0 12 2 12 2 12 2 12 2 12 2 12 2 12 2 | | - 25555000055555555555555555555555555555 | -25 12000 12000 +1000 11000 +1000 1500 +2505555 222225555555555555555555555 | -2550 2250 5250 5250 5255555555555555555 | -25 14000 +4000 +1000 +1000 +15255555555555555555555555555555555555 | $\begin{array}{c} -10 \\ -10 \\ -10 \\ 24 \\ 20 \\ 34 \\ 20 \\ -10$ |

| DAUCCEC | | | 011001 10 | ~ | | | · · |
|-----------|---|--|--|--|--|--|---|
| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
| 42 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 18 9 21 22 24 5 6 7 8 10 12 14 16 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 38 4 5 6 7 8 10 21 22 23 4 5 6 7 8 10 21 22 23 24 5 6 7 8 38 4 38 4 5 6 7 8 38 4 5 6 7 8 5 8 5 8 10 8 10 8 10 8 10 8 10 10 10 10 10 10 10 10 10 10 | - T W+WWWTT | | -25 +2000 11000 1100 +2505 12255 12255 12255 1550 152500 1500 1 | -25 -25000 15000 1505555555555555555555555555 | -25 +14000 +14000 +1500 +550505 +550505 +550505 +5505 +5505 +5505 +5505 +5505 +5505 +5505 +5505 +1500 +1000 +1500 +1000 | $\begin{array}{c} -10 \\ -10 \\ -10 \\ 2+20 \\ 1+10 \\ 1+10 \\ -1$ |
| 43 | 0 1 2 3 4 5 6 7 8 10 12 14 16 7 8 9 0 12 14 16 7 8 9 0 12 24 26 7 8 0 24 5 6 7 8 9 0 21 2 34 5 6 7 8 9 0 21 2 34 5 6 7 8 9 0 21 2 34 5 6 7 8 9 0 21 2 34 5 6 7 8 9 0 21 2 34 5 6 7 8 9 0 21 2 34 5 6 7 8 9 0 21 2 34 5 6 7 8 9 0 21 2 34 5 6 7 8 9 0 21 2 34 5 6 7 8 9 0 21 2 34 5 6 7 8 9 0 21 2 34 5 6 7 8 9 0 21 2 34 5 6 7 8 9 0 21 2 34 5 6 7 8 9 0 21 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | ─₩★★★★★★★★★★★★★★★★★★★★★★★★★★★★★ | -25 -25 125 +500 +500 +500 +505 1225 255 555 555 555 555 555 555 555 5 | -25 +100 I 400 I 400 I 200 I 200 I 200 I 200 I 200 I 200 I 100 + 100 I 100 + 100 I 100 + 100 I 100 + 100 I 100 I 200 I 200 I 200 I 200 I 200 I 200 I 200 I 100 I 100 | -25 -25 12000 12000 11000 12000 11000 12255 12255 12255 12255 12255 125555 125555 125555 125555 125555 125555 12555555 1255555555 | $\begin{array}{c} -25 \\ I400 \\ I1600 \\ +800 \\ +2$ | $\begin{array}{c} -10 \\ -10 \\ 1+40 \\ 2+40 \\ 2+40 \\ 2+40 \\ 2+20 \\ -10 \\ $ |

| ORUCCLL | USIS SERUE | | GROUP VG- | 2 | | | |
|-----------|---|--|-----------|---|---|---|---|
| ANIMAL | WEEK | VSL CARD | VSL ME | VSL | VSL RIV | VSL TUBE | VSL CF |
| 44 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 18 9 20 21 22 23 4 5 6 7 8 10 12 14 16 17 18 9 20 21 22 24 5 6 7 8 32 34 5 6 7 8 10 12 14 16 17 18 9 20 1 22 3 4 5 6 7 8 10 12 14 16 17 18 9 20 21 22 3 4 5 6 7 8 10 12 14 16 17 18 9 20 21 22 3 4 5 6 7 8 10 12 14 16 17 18 9 20 21 22 3 4 5 6 7 8 10 20 21 22 3 4 5 6 7 8 10 12 14 16 17 18 9 20 21 22 24 5 6 7 8 10 22 1 22 24 25 6 7 8 10 20 12 21 22 23 24 5 6 7 8 10 20 20 20 20 20 20 20 20 20 20 20 20 20 | -++++WWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWW | | $\begin{array}{c} -25\\ I200\\ +200\\ +200\\ I400\\ +100\\ +100\\ +100\\ +100\\ +100\\ +100\\ +100\\ +50\\ +50\\ +50\\ +50\\ 1100\\ +100\\ 1100\\ +50\\ +50\\ +50\\ 1100\\ 1100\\ \end{array}$ | -25 -2500 11000 +1 | $\begin{array}{c} -25 \\ I 400 \\ +800 \\ +400 \\ +400 \\ +100 \\ 1100 \\ +100 \\ +100 \\ +100 \\ +100 \\ +100 \\ +100 \\ +100 \\ I 100 \\ I 100 \\ I 100 \\ 1100 \\ +50 \\ 1100 \\ +100 \\ $ | $\begin{array}{c} -10 \\ -10 \\ 1+20 \\ 3+20 \\ 3+20 \\ 3+20 \\ 2+10 \\ -10 \\ $ |
| 45 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 18 90 12 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 32 4 5 6 7 8 32 4 5 6 7 8 32 4 5 6 7 8 32 4 5 6 7 8 32 4 5 6 7 8 32 4 5 6 7 8 32 4 5 6 7 8 3 2 3 4 5 6 7 8 3 3 3 4 5 6 7 8 3 3 3 4 5 6 7 8 3 3 4 5 6 7 8 3 8 3 3 4 5 6 7 8 3 8 3 3 4 5 6 7 8 3 8 3 8 3 8 4 5 6 7 8 3 8 3 8 3 8 3 8 4 5 6 7 8 3 8 3 8 4 5 6 7 8 8 3 8 4 5 6 7 8 8 3 8 8 8 8 8 8 8 8 8 8 8 8 8 | WT1WT-T | | -25 1100 150 +50 1550 1555 1555 12555 12555 125555 125555 125555 125555555555 | | -250 150000 1++152555555555555555555555555555555555 | $ \begin{array}{r} -10 \\ -10 $ |

| ANIMAL | WEEK | VSL | V SL ME | ~ VSL PLATE | VSL RIV | VSL TUBE | VSL - CF, |
|--------|--|---------------------------|--|--|---|---|---|
| 46 | 0 1 2 3 4 5 6 7 8 10 12 4 6 7 8 10 12 14 16 7 8 10 21 223 4 5 6 7 8 0 21 223 4 5 6 7 8 0 21 223 4 5 6 7 8 0 21 23 4 5 6 7 8 0 21 23 4 5 6 7 8 0 21 2 21 2 22 2 22 2 2 2 2 2 2 2 2 2 2 | -++++++WWTT | -25550055555555555555555555555555555555 | -25 I400 | -250 15500 15500 155255555555555555555555 | -25 +400 1800 +200 +100 +100 +525 1550 1550 1550 1550 1550 1550 1550 1 | $\begin{array}{r} -10 \\ -10 \\ -10 \\ 2+20 \\ 2+10 \\ -10$ |
| 47 | 0 1 2 3 4 5 6 7 8 10 12 14 16 7 8 9 0 12 14 16 7 8 9 0 12 2 12 3 4 5 6 7 8 0 2 2 2 3 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 12 2 14 6 7 8 9 0 12 2 12 2 12 2 12 2 12 2 12 2 12 2 | _ + + + + + ₩₩₩₩ ™ | - 255500 - + + + + + 5500555555555555555555555555 | -25 12000 12000 12000 12000 11000 11000 1550 155 | -25 -25 150000 +50000 +50000 +1005555555555555 | -25 +2000 +4000 +2000 +1000 +2000 +1000 +5505 +555050505 +250505055505 +250505550555 +250505550555 +25055555555 +2505555555555 | -10 - 10 - 10 - 10 - 10 - 10 - 10 - 10 |

| ANIMAL | ₩EEK | V SL C ARD | V SL ME | VSL PLATE | VSL RIV | VSL Tubé | VSL CF |
|-------------|---|---|--|---|---|--|--|
| 48 | 0 12345678024667802468024680 10246789012345678024680 22222222223334680 33024000000000000000000000000000000000 | -++++WWWTT | | I 25 + 100 I 200 + 100 + 50 + 50 + 50 I 50 I 50 I 50 I 50 I 50 I 50 I 50 I | | $\begin{array}{c} 125\\ +100\\ +2000\\ +100\\ +50\\ 150\\ 150\\ +50\\ 150\\ +50\\ +50\\ +50\\ +50\\ +50\\ +50\\ +50\\ +$ | $\begin{array}{c} -10 \\$ |
| 49 · | 0 1 2 3 4 5 6 7 8 0 12 14 6 7 8 9 0 12 14 6 7 8 9 0 12 2 2 2 3 4 5 6 7 8 9 0 12 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | 系统系统系统系统系统系统系统系统系统系统 + + + + + + + + + + | -25 +1600 I4000 +100 +500 +500 +500 +500 +500 +500 + | -25 +400 1400 +400 +400 +400 +400 +200 +200 1200 +200 1200 +100 1200 +100 1200 +100 1200 12 | -25 +200 +400 +400 +400 +200 +200 +200 +100 +100 1100 +100 1100 +100 1100 +100 1100 +100 1100 +100 1100 +100 1100 +100 +100 1100 +100 | -25 +800 +3200 +3200 +3200 +400 +400 +400 +400 +200 +200 +200 + | $\begin{array}{c} -10\\ 1+20\\ 1+20\\ 2+80A\\ 2+80A\\ 1+80A\\ 3+40\\ 2+20A\\ 1+10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -$ |

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| ANIMAL | WEÉK | VSL CARD | V SL ME | VSL PLATE | VSL RIV | VSL Tube | V SL. CF |
|--------|---|---|--|---|---|---|--|
| 50 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 18 90 21 23 45 6 7 8 10 21 23 45 6 7 8 10 21 23 45 6 7 8 10 21 23 45 6 7 8 10 21 23 45 6 7 8 10 21 23 45 6 7 8 10 21 23 45 6 7 8 10 21 23 45 6 7 8 10 21 22 34 5 6 7 8 10 21 22 34 5 6 7 8 10 21 22 34 5 6 7 8 10 21 22 34 5 6 7 8 10 21 22 34 5 6 7 8 10 21 22 34 5 6 7 8 10 21 22 32 4 5 6 7 8 10 21 22 34 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 24 5 6 7 8 10 24 5 6 7 8 10 24 5 6 7 8 10 24 25 6 7 8 10 24 5 6 7 8 10 24 5 6 7 8 10 24 5 6 7 8 10 24 5 6 7 8 10 24 5 6 7 8 10 24 5 6 7 8 10 24 5 6 7 8 10 24 5 6 7 8 10 24 5 6 7 8 10 24 5 8 10 10 10 10 10 10 10 10 10 10 | + * * * * * * * * * * * * * * * * * * * | -25 -42500000 +10000 +10000 +++++++11 +1+1+1+1+1 | -25 1400 1400 +400 1400 1400 1400 +200 +200 +200 1200 | -25 -225 +2000 +20 | -25 1400 +1600 +1600 11600 1800 +400 +400 +400 +400 +200 +200 +200 +2 | $\begin{array}{c} -10 \\ -10 \\ 1+40 \\ 2+80 \\ 3+40 \\ 3+20 \\ 1+20A \\ 1+20A \\ 1+10 \\ -1$ |
| 106 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 18 9 221 223 4 5 6 7 8 10 12 14 16 17 18 9 221 223 4 5 6 7 8 32 4 5 6 7 8 10 12 14 5 6 7 8 10 12 14 16 7 8 9 221 223 4 5 6 7 8 9 221 223 4 5 6 7 8 9 221 223 4 5 6 7 8 9 221 223 4 5 6 7 8 9 221 223 4 5 6 7 8 9 221 223 4 5 6 7 8 9 221 223 4 5 6 7 8 9 221 223 4 5 6 7 8 9 221 223 4 5 6 7 8 9 221 223 4 5 6 7 8 9 221 223 225 8 22 8 225 8 22 22 22 22 22 22 22 22 22 22 22 22 2 | - + + + + + + + + + + + + + + + + + + + | -25 -250000000 1+22000000 1+5500555555555555555555555555555 | -25 +400 +400 +400 +400 1400 +200 +200 +200 +200 +200 +200 +200 + | -25 1500 +4000 14000 14000 14000 1200 1200 | $\begin{array}{c} -25 \\ +1600 \\ +3200 \\ +3200 \\ +3200 \\ +800 \\ +800 \\ +800 \\ +400 \\ +200 \\$ | -10 1+80 1+160 3+160 2+160 1+40 1+40 2+20A 2+20A 2+20A 1+10 2+10 1+10 1+10 1+10 -110 -10 |

| ANIMAL | WEEK | VSL CARD | VSL ME | VSL . PLATE | VSL RIV | VSL Tube | VSL CF |
|--------|--|----------------|---|---|---|--|---|
| 52 | 01234567802466789012345678024680 10246789012345678024680 33333340 | - + + + + WWWW | + + + + + | I 25 +4000 +1000 +1000 I 100 I 1500 I 5500 I 5505 I 555555555 I 5500 I 5555555555 | -25000 1+150055555555555555555555555555555 | I25 I400 +200 +200 +100 I100 +50 I100 +50 I550 I550 I550 I550 I550 I550 I55 | $\begin{array}{c} -10 \\ -10 \\ -10 \\ 1+20 \\ 3+10 \\ -10$ |
| 53 | 0 1 2 3 4 5 6 7 8 0 2 4 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 4 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 2 1 2 2 1 2 2 2 2 2 2 2 2 2 2 2 | | - 255 - 2255 2255 2255 2255 2255 2255 22 | -++1000 115500505055555555555555555555555 | -++I+++ | -25 12100 141000555505555555555555555555555555 | $ \begin{array}{r} -10 \\ -10 $ |

| 54 025 -25 -25 -25 - | -10 |
|--|---|
| $\begin{array}{cccccccccccccccccccccccccccccccccccc$ | $ \begin{array}{r} -10 \\ -10 $ |

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| ANIMAL | WEEK | V SL CARD | V SL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|---|--------------|--|--|---|---|---|
| 104 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | | -25 -25 +200 +200 +100 +100 +50 150 +25 -25 125 | -25 +200 1400 1400 1200 +100 1100 +100 1100 1100 +50 1100 | -25 -25 1200 1200 1200 1200 +100 1200 1100 150 125 125 | -25 +400 I1600 +800 1400 +200 I200 +100 I100 +50 +50 +50 | $ \begin{array}{r} -10 \\ -10 \\ -10 \\ 1+80 \\ 3+40 \\ 1+40 \\ 2+40 \\ 3+20 \\ 4+10 \\ 3+10 \\ -10 \\ -10 \\ -10 \\ -10 \\ \end{array} $ |
| | 18 19 | W | -25 | +50 | 125 | +100 | -10 |
| | 20 21 | W | -25 | +50 | 125 | I100 | -10 |
| | 22 | W · | -25 | +50 | ~25 | +50 | -10 |
| | 24 25 | W | -25 | 1100 | -25 | I 100 | -10 |
| | 26 27 | т | -25 | 1100 | -25 | +100 | -10 |
| | 28 | T | -25 | I 100 | -25 | +100 | -10 |
| | 30 32 | ŷŃ | -25 | 1100 | -25 | +100 | - 10 |
| | 34 36 | - | -25 | + 50 | -25 | 1100 | -10 |
| | .38 40 | | -25 | +50 | -25 | +100 | -10 |
| 105 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | -+*++WWTT | - 25 - 25 + 25 + 550 1555 - 225 1525 - 225 - 225 - 25 | -25 +200 +200 +100 I 200 +100 I 100 +50 I 50 I 50 I 50 I 50 I 50 | -25 -25 1200 1100 +50 +50 +25 -25 -25 -25 -25 -25 -25 | -25 +400 1800 +200 +100 +100 +100 +50 +50 +50 +25 125 | $ \begin{array}{r} -10 \\ -10 \\ -10 \\ 3+20 \\ 4+20 \\ 1+20 \\ 1+10 \\ -10 \\ $ |
| | 18 19 | - | -25 | 150 | -25 | 150 | -10 |
| | 20 21 | | -25 | +25 | -25 | 1100 | - 10 |
| | 22 23 | - | -25 | +50 | -25 | +50 · | -10 |
| | 24 25 | - | -25 | I 50 | ~ 25 | I100 | -10 |
| | 26 27 | - | -25 | 150 | -25 | +50 | -10 |
| | 28 30 | · | -25 | I 50 | -25 | +50 · | -10 |
| | 32 | - | -25 | +50 | -25 | +25 | -10 |
| • . | 34 36 38 40 | - | -25 | 150 | -25 | +50 | -10 |

| ANIMAL | WEEK | | VSL ME | VSL | VSL RIV | VSĽ TUBE | V SL CF |
|--|--|-----------------|---|--|---|---|---|
| 51 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | 1 2 3 4 5 6 7 8 10 12 14 16 17 18 | _ + + + + ₩ ₩ T | -25 -25 +25 +25 +100 +100 +50 125 +25 +25 -25 -25 -25 -25 -25 | -25 +200 1200 +100 +100 1100 1100 1100 150 150 150 155 125 +25 125 | -25 -25 +50 1200 1100 1100 150 +25 125 125 125 125 -25 | -25 1200 1200 +200 +100 +100 150 1100 +50 1100 150 +25 125 +25 | $ \begin{array}{r} -10 \\ -10 \\ -10 \\ 3+40 \\ 1+80 \\ 4+40 \\ 3+40 \\ 3+20 \\ 1+20 \\ 3+10 \\ -10$ |
| | 19 20 21 | | -25 | -25 | -25 | +25 | -10 |
| | 22 | - | -25 | 125 | -25 | +25 | |
| | 23 24 25 | - | -25 | +25 | -25 | 125 | -10 |
| ۲ ۲ | 26 | - | | +25 | -25 | +25 | -10 |
| | 27 28 30 | - | -25 | +25 | -25 | +25 | -10 |
| ĉ. | 30 32 34 | - | -25 | I 50 | -25 | 125 | -10 |
| | 34 36 38 40 | - | -25 | + 25 | -25 | [25 | -10 -10 |
| 107 0 1 2 3 4 5 6 7 8 10 12 12 | 1 2 3 4 5 6 7 8 10 12 | - + + + + W W W | - 25 - 25 1 50 + 50 1 25 + 25 + 25 + 25 - 25 - 25 - 25 | -25 +200 +200 +100 +100 +50 +50 +50 +25 +25 +25 +25 +25 | - 25 - 25 I 100 I 50 I 50 I 25 I 25 I 25 - 25 - 25 - 25 - 25 | -25 1400 +400 1400 +50 +50 1100 +50 150 +50 150 +50 +25 +25 | $ \begin{array}{r} -10 \\ -10 \\ 2+20 \\ 1+40 \\ 3+20 \\ 2+20 \\ 4+10 \\ -10 \\$ |
| · . | 18 19 | _ | -25 | +25 | -25 | +25 | -10 |
| | 20 21 | - | -25 | +25 | -25 | +25 | -10 |
| | 22 | - | -25 | + 25 | -25 | I 25 | -10 |
| | 24 25 | - | -25 | +25 | -25 | 150 | -10 |
| | 26 27 | - . | -25 | 125 | -25 | +25 | -10 |
| | 28 | - | -25 | 1.25 | -25 | 125 | - t o |
| | 32 34 | - · | -25 | +25 | -25 | 150 | - t o |
| | 34 36 38 40 | | -25 | +25 | -25 | +25 | -1.0 |

| ANIMAL | WEEK | VSL CARD | V SL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|---|---|--|--|---|---|--|--|
| 108 | 0 1 2 3 4 5 6 7 8 10 12 14 14 14 14 14 17 | 1 11111111111111111111111111111111111 | -25 +25 +400 1200 +100 +100 +50 +50 125 -25 -25 -25 | -25 +200 1400 +400 +200 +200 1200 +100 1100 1100 +50 +50 | -25 -25 +200 +400 1400 +200 1200 1200 1200 1200 150 +25 125 | -25 1800 11600 +1600 +800 +400 1400 +200 +100 1100 +50 +50 +50 | $ \begin{array}{r} -10 \\ -10 \\ -10 \\ 2+80 \\ 1+80 \\ 1+40 \\ 3+40 \\ 3+20 \\ 2+10 \\ -10 $ |
| | 18 19 | W | -25 | +50 | 125 | +50 | -10 |
| | 20 21 | 1 | -25 | 1100 | 125 | I 100 | -10 |
| | 22 | W | -25 | I 100 | -25 | +50 | -10 |
| | 23 24 | W | -25 | I 100 | -25 | +100 | -10 |
| | 25 26 | W | - 25 | 150 | 125 | +100 | -10 |
| | 27 28 | W | -25 | +50 | -25 | 1100 | -10 |
| , | 30 32 | W | -25 | 1100 | -25 | +100 | -10 |
| | 34 36 38 | <u> </u> | -25 | 150 | -25 | +50 | -10 |
| | 40 | <u>-</u> | -25 | +50 | -25 | 150 | -10 |
| 109 0 1 2 3 4 5 6 7 8 10 12 14 16 | - W+++WWW+ | - 25 - 25 + 50 + 100 125 + 25 - 25 - 25 - 25 - 25 - 25 - 25 - 25 | -25 I100 I200 +100 1100 +50 +50 +50 I50 +25 I50 I50 | -25 -25 12000 11000 +50 +50 +50 +50 +25 -25 -25 -25 -25 | -25 1200 +200 1200 +50 150 150 +50 150 +50 150 +25 +25 +25 | $ \begin{array}{r} -10\\ -10\\ 1+20\\ 2+10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -$ | |
| | 17 18 19 | т | -25 | +25 | ~25 | +50 | -10 |
| | 20 21 | - | -25 | +25 | -25 | +50 | -10 |
| ÷ | 22 | - | -25 | 150 | -25 | 150 | -10 |
| | 24 25 | - | -25 | 150 | -25 | +50 | -10 |
| | 26 27 | - | -25 | 150 | -25 | 150 | -10 |
| | 28 30 | - | -25 | +25 | -25 | 150 | -10 |
| | 32 34 | - | -25 | 150 | -25 | ×I 50 | -10 |
| • ? | 36 38 40 | | -25 | I 25 | -25 | +25 | -1 Ó |
| | | | | | | | |

| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL . RIV | VSL TUBE | VSL CF |
|--------|---|--|--|--|---|---|---|
| 110 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | —————————————————————————————————————— | 25 25 +225 +225 +225 -225 -225 -225 | -25 +100 +100 1100 +50 150 150 +25 +25 +25 +25 +25 +25 | -25 -25 150 155 -25 -25 -25 -25 -25 -25 -25 -25 -25 - | -25 1400 +200 +100 150 +50 150 +50 125 +25 +25 125 | $ \begin{array}{r} -10 \\ -10 \\ 2+10 \\ 3+20 \\ 2+40 \\ 3+10 \\ -10 \\ $ |
| | 18 19 | - | -25 | +25 | -25 | 150 | -10 |
| | 20 21 | - | - 25 | -150 | -25 | +50 | -10 |
| | 22 23 | - | -25 | +25 | -25 | I 25 | -10 |
| | 24 25 | - | -25 | I 50 | -25 | +50 | -10 |
| • • | 26 27 | - | - 25 | I 50 | -25 | I 50 | -10 |
| ÷ | 28 30 | _ | -25 | +25 | -25 | 150 | -10 |
| | 32 34 | - | -25 | I 50 | -25 | +50 | -10 |
| ÷ | 36 38 40 | - | -25 | I 25 | -25 | +25 | -10 |
| 111 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | L - L M M + + + + + - | -25 -25 150 +100 +100 +50 +50 +50 +25 -25 -25 -25 | -25 +200 +400 1400 1200 +100 1100 1100 150 +50 | -25 -25 +200 1400 +400 1200 +100 +100 +50 +50 125 125 125 | -25 1800 +800 1200 1200 +100 +100 +50 1100 +50 +50 +50 | -10 -10 1+20 2+80 3+40 2+40 2+20 2+10 1+10 -10 -10 |
| | 18 19 | - | -25 | +25 | -25 | 1100 | -10 |
| | 20 21 | Т | -25 | · +25 | -25 | 1100 | -10 |
| | 22 | T | -25 | I 50 | -25 | +50 | 10 |
| | 24 25 | W K | -25 | I 50 | -25 | I100 | -10 |
| · | 26 27 | W | -25 | 150 | -25 | +50 | -10 |
| , | 28 30 | M | -25 | 150 | -25 | +50 | -10 |
| | 32 · 34 | | -25 | · 150 | -25 | ;+ 50 | · - 1 0 |
| | 36 38 40 | - | -25 | I 50 | -25 | 150 | -10 |

| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL | TUBE | VSL CF |
|--------|--|-------------------|-----------|--|-----|---|---|
| 55 | 01234567802466789012345678024680 112146789012345678024680 3333533340 | - W + + W W V T T | | - 25 - 55 - 55 | | I 25 I 200 I 200 I 500 I 100 I 100 I 100 I 2255555555555555555555555555555555555 | $ \begin{array}{c} -10 \\ -10 $ |
| 56 | 0 1 2 3 4 5 6 7 8 0 12 4 6 7 8 0 12 4 6 7 8 0 12 4 6 7 8 0 12 4 6 7 8 0 12 4 6 7 8 0 12 4 5 6 7 8 0 12 2 12 2 3 4 5 6 7 8 0 12 2 12 2 12 2 2 2 2 2 2 2 2 2 2 2 2 | | | -25 +100 1100 +50 +50 +50 +225 1255 555 555 555 555 555 555 555 1225 1225 555 55 | | -25 +500 +2000 1525 +2500 +2250 +2550 +2550 +2550 +2550 +5500 +5500 +5500 +5500 +5500 +5500 +5500 +5500 +5500 +5000 +5000 +5000 +2500 +2000 +2000 +2000 +2000 +2500 +2000 +2500 +2000 +250 | $ \begin{array}{r} -10 \\ -10 $ |

| ANIMAL | WEEK | VSL CARD | V SL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|---|--------------------|------------|---|------------|--|--|
| 57 | 0 1 2 3 4 5 6 7 8 10 12 4 6 7 8 10 12 4 6 7 8 10 21 22 3 4 5 6 7 8 10 21 22 3 4 5 6 7 8 10 21 22 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | ₩+₩₩ | | - 25 + 100 + 500 + 500 + 500 + 500 + 255 + 225 + | | -+++++++ ++++++++ -+++++++++++++++++++ | -10 - 10 - 10 - 10 - 10 - 10 - 10 - 10 |
| 58 | 0 1 2 3 4 5 6 7 8 10 12 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 0 21 23 4 5 6 7 8 0 21 23 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 9 0 12 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | _+++++WWWTTTTWWWWW | | - 25 +400 1400 +1200 +500 +500 +500 +500 +500 +500 +500 + | -+++II | I 25 I 1600 I 1600 +400 +200 +100 I 100 +50 +50 +100 I 100 +50 +50 +100 I 100 +50 +50 +100 I 100 +50 +100 I 100 +200 +100 I 100 +50 +100 I 100 +100 I 100 I | $\begin{array}{c} -10 \\$ |

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| ANIMAL | WEEK | VSL | V'SL | VSL PLATE | VSL . RIV | VSL TUBE | VSL CF |
|--------|--|--|---|--|--------------|---|--|
| 59 | 0 1 2 3 4 5 6 7 8 10 12 14 16 7 8 10 12 14 16 7 8 9 0 12 23 4 5 6 7 8 10 12 14 16 7 8 9 0 21 22 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | T W W W W W W W W W W W W W W W W W W W | | -250 150 +550 +550 550 5555 550 55255 550 55255 55255 55255 552555 55255555 52555555 | | -2500 +2000 +550 +550 +550 15255 +550 1550 1550 1550 1550 1550 1550 15 | $\begin{array}{c} -10 \\$ |
| 60 | 0 1 2 3 4 5 6 7 8 0 12 4 5 8 9 0 12 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | -++++%%% | - 25 - 2255 - 2555 - 25555 - 25555 - 2555 - 2555 - 2555 - 2555 - 2555 - 2555 - 2555 - | +25 +50 1500 +500 +500 +500 +500 +500 +500 | ~ + + | -25000000 +12000 +12000 +1100 +1550005500505050 +15522500505050 +1552500 +1552500 +155250 +++++ +1++ +55500 +++++ +55500 +++++ +55500 ++++++ +55500 +++++++ +55500 ++++++++ | $\begin{array}{c} -10 \\$ |

| ANIMÁL | WEEK | VSL | VSL | VSL | VSL | VSL | VSL |
|----------|--|---------|-----|--|-----|--|---|
| . ANTRAL | | CARD | ME | PLATE | RIV | TUBE | CF |
| 61 | 0 1 2 3 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 2 3 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 12 8 9 0 12 2 3 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | -2500 1+100 +5500 +522255555555555555555555555555 | | I2500 I44000 I44000 I25 | $ \begin{array}{r} -10 \\ -10 $ |
| 119 | 0 1 2 3 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 2 3 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 3 4 5 6 7 8 0 2 2 3 4 5 6 7 8 0 2 2 3 4 5 6 7 8 0 2 2 3 4 5 6 7 8 0 2 2 3 4 5 6 7 8 0 2 2 2 2 2 4 5 6 7 8 0 2 2 2 2 2 4 5 6 7 8 0 2 2 2 2 2 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | -+++W T | | -25 ++1000 ++15505555555555555555555555555 | ++ | *1442100505555555555555555555555555555555 | -10 - 10 - 10 - 10 - 10 - 10 - 10 - 10 |

| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL RIV | VSL TUBE. | VSL CF |
|--------|---|-------------|-----------|--|------------|---|--|
| 63 | 0 1 2 3 4 5 6 7 8 10 14 6 7 8 10 14 6 7 8 10 2 12 3 4 5 6 7 8 10 2 12 3 4 5 6 7 8 10 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 14 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 2 3 4 5 6 7 8 0 2 1 2 2 3 4 5 6 7 8 0 2 1 2 2 3 4 5 6 7 8 0 2 1 2 2 3 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | -25 +200 1100 1100 1100 1100 1100 150 +50 150 +50 150 +50 150 +50 150 +50 150 +50 150 +50 150 +50 150 +50 150 +50 150 +50 150 +50 1100 +50 150 150 +50 150 150 +50 150 150 150 +50 150 150 150 150 150 150 150 150 150 1 | | $\begin{array}{c} 125 \\ +100 \\ +200 \\ +200 \\ +200 \\ +1000 \\ +1000 \\ +1000 \\ +50 \\ 1100 \\ +50 \\ 1100 \\ +50 \\ 1100 \\ +50 \\ 1100 \\ +100$ | $\begin{array}{c} -10 \\$ |
| 64 | 0 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | - 11111 | -25 +1000 11000 1500 +5500 155000 15000 15000 15000 1500000000 | | -25 +100 +100 +100 +100 +50 1100 +50 1100 +50 +50 +50 +50 +50 +50 +50 +50 +50 + | $\begin{array}{c} -10 \\$ |

| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|--|-------------------------|-----------|---|--|--|--|
| 65 | 0 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 14 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 14 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 12 5 6 7 8 0 2 12 12 12 12 12 12 12 12 12 12 12 12 1 | -++++WWWT | | -242000 122000 122000 1422000 14420000 1442000 1440000 1440000 1440000 14400000 14400000000 | -25 152000 1+100 155555555555555555555555555555 | -25 +4000 +2000 +22000 +5500 +52555 +225555 +2500 +52555 +250555 11000 11000 1100 1100 1100 1100 1 | $\begin{array}{c} -10\\ -10\\ 2+20\\ 3+20\\ 4+20\\ 1+10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -$ |
| 66 | 0 1 2 3 4 5 6 7 8 0 2 4 6 7 8 0 2 4 6 7 8 0 2 4 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 4 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | - . ₩ ₩ . | | -2500 141000 14151000 14151000 14151000 14151000 14151000 152222222222222222222222222222222 | | -250000 ++1552222222222222222222222222222222222 | $\begin{array}{c} -10 \\$ |

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| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL RIV | VSL Tube | VSL CF |
|--------|---|--|--|---|--|--|--|
| 67 | 0 1 2 3 4 5 6 7 8 12 14 16 7 8 12 14 16 12 14 16 7 8 19 221 23 4 5 6 7 8 12 14 16 7 8 9 221 23 4 5 6 7 8 10 12 3 4 5 6 7 8 10 12 14 16 7 8 10 12 14 16 7 8 9 221 23 4 5 6 7 8 10 12 14 16 7 8 10 12 14 16 7 8 10 12 14 16 7 8 10 12 14 16 7 8 10 12 14 16 7 8 10 12 14 16 7 8 10 22 12 23 4 5 6 7 8 10 22 14 16 7 8 10 22 12 23 4 5 6 7 8 10 22 14 16 7 8 10 22 12 23 4 5 6 7 8 10 2 21 2 23 4 5 6 7 8 10 2 21 2 23 4 5 6 7 8 10 2 21 2 23 4 5 6 7 8 10 2 2 12 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | -25 +1000 +5055055555555555555555555555555 | | -22500 +11500555555555555555555555555555555 | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |
| 116 | 0 1 2 3 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 12 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | + + + + + WM + + + + + + + + + + + + W M + H M + + + | -25 -25 -25 -25 -25 +50 +50 +50 +200 +2000 +1000 +2000 +2000 +2000 +2000 +2000 +2000 +2000 +2000 +2000 +2000 +2000 +2000 +2000 +1000 +2000 +2000 +1000 +1000 +2000 +2000 +1000 +1000 +2000 +2000 +10000 +1000 +10000 +10000 +10000 +100000 | -25 +100 1200 +100 +50 1100 +50 1100 +50 1100 +100 1100 1 | -25 -25 1100 1500 1500 11000 12000 1550 12000 | $\begin{array}{c} -25\\ 1400\\ 1400\\ 1400\\ 150\\ 150\\ +100\\ 150\\ +100\\ 150\\ +100\\ 1400\\ +200\\ +2000\\$ | $\begin{array}{c} -10\\ -10\\ 1+20\\ 1+40\\ 2+40\\ 1+440\\ 3+440\\ 3+1460\\ 1+160\\ 3+160\\ 1+160\\ 3+160\\ 3+160\\ 3+160\\ 3+160\\ 3+160\\ 3+160\\ 3+160\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0$ |

| ANIMAL | WEEK | VSL CARD | V SL ME | VSL PLATE | V SL R I V | VSL TUBE | VSL CF |
|--------|---|---|--|---|---|--|--|
| 69 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 18 10 21 223 45 6 7 8 10 12 14 16 17 18 10 21 223 45 6 7 8 10 21 223 45 6 7 8 10 21 223 45 6 7 8 10 21 223 45 6 7 8 10 21 223 45 6 7 8 10 21 223 45 6 7 8 10 21 223 45 6 7 8 10 21 223 45 6 7 8 10 21 223 45 6 7 8 10 21 223 45 5 6 7 8 10 21 223 45 5 6 7 8 10 21 223 45 5 6 7 8 10 21 223 45 5 6 7 8 10 21 223 245 5 6 7 8 32 245 5 8 10 21 225 245 5 8 10 21 225 245 5 267 8 32 34 5 8 10 25 25 25 25 25 25 25 25 25 25 | ┘ - +₩₩₩⊤⊤т Т₩+ + + + + + + + + + + + + + + + + + + | $\begin{array}{c} -25 \\$ | $\begin{array}{c} -25 \\ +100 \\ 1200 \\ +100 \\ +100 \\ +100 \\ 100 \\ 100 \\ 150 \\ 150 \\ 150 \\ 150 \\ 150 \\ 150 \\ 150 \\ 150 \\ +400 \\$ | -25 -22555555555 -225555555 -22555555 -2255555 -225555 -22555 -22555 -2255 -2400 -2440000000000 | -25 +400 1400 +200 1200 +100 +100 +100 1100 +100 1100 +100 + | $\begin{array}{c} -10 \\$ |

| ANIMAL | WEEK | VSL CARD | VSL ME | VŠL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|---|--------------------------------|---|--|---|---|---|
| 112 | 0 1 2 3 4 5 6 7 8 10 12 14 16 | T T W W T T | -25 -25 -25 -25 -25 -25 -25 -25 -25 -25 | - 25 + 50 + 50 + 50 + 550 + 250 + 250 1 525 I 525 I 525 I 225 I 225 | -25 -25 -225 -225 -225 -225 -225 -225 - | -25 +100 +50 +50 +255 +255 +255 +225 +225 +225 | $ \begin{array}{r} -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ \end{array} $ |
| | 17 18 | - | -25 | -25 | -25 | +25 | -10 |
| | 19 20 21 | _ | -25 | 125 | -25 | +25 | -10 |
| •. | 22 | | -25 | +25 | -25 | 125 | -10 |
| | 23 | | - 25 | 125 | -25 | +25 | -10 |
| , | 25 | | -25 | 125 | -25 | 125 | ÷10 |
| . · | 27 | | -25 | +25 | -25 | +25 | -10 |
| | 30 32 34 | - | -25 | 1 50 | ~25 | I 25 | +10 |
| | 34 36 38 | - | -25 | +25 | -25 | 150 | -10 |
| | 40 | - | -25 | 125 | -25 | 150 . | -10 |
| 113 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | - + + + + W W | - 25 - 25 - 255 - 225 - 225 + 225 - 225 - 225 - 225 - 225 - 225 - 225 - 225 | -25 1100 +100 1200 150 +25 +25 125 125 125 125 125 | - 25 - 255 I 255 + 225 - 255 - | -25 +200 +200 +200 +225 +25 +25 +25 -25 -25 +25 | $ \begin{array}{r} -10 \\ -10 \\ 1+10 \\ 3+10 \\ -1$ |
| ۶. | 18 19 | - | -25 | -25 | -25 | +25 | -10 ¹ |
| | 20 21 | - | -25 | -25 | -25 | +25 | -10 |
| | 22 | - | -25 | I 25 | -25 | +25 | -10 |
| | 24 25 | - | ~25 | +25 | -25 | 150 | -10 |
| | 26 27 | <u> </u> | -25 | 125, | -25 | 125 | -10 |
| * | 28 | , - | -25 | +25 | -25 | +25 | -10 |
| | 32 34 36 38 40 | - | -25 -25 | +25 125 | -25 -25 | +25 -25 | -10 -10 |
| | | | | | | | |

| ANIMAL | WEEK | VSL CARD | VSL ME | VSL Plate | VSL RIV | VSL TUBE | VSL CF |
|--------------|---|-------------------|---|--|--|---|--|
| 1 t 4 | 1 2 3 4 5 6 7 8 10 12 | ×+++++ | - 25 - 25 + 200 + 100 I 50 + 50 + 50 I 100 - 25 - 25 - 25 | -25 1400 +400 +400 1400 +200 1200 +100 +100 1100 1100 | -25 +100 +200 1200 1400 1200 +100 +100 +100 +100 +100 +25 125 -25 | -25 +1600 +1600 +800 +400 +200 1200 1400 +100 +100 +50 +50 | -10 1+40 1+80A 2+80A 3+40 4+20 2+40 1+20A 2+10 2+10 -10 -10 |
| | 17 18 19 | Ψ | -25 | +100 | [50 | +100 | -10 |
| | 20 21 | W | -25 | I 100 | I 25 | +100 | -10 |
| | 22 23 | W | -25 | 1100 | -25 | +100 | - 10 |
| • | 23 24 25 | W | -25 | +100 | -25 | +100 | -10 |
| | 26 27 | W | -25 | +100 | -25 | 1200 | -10 |
| | 28 30 | W | -25 | +100 | -25 | 1200 | -10 |
| | 32 34 | W . | - 25 | +100 | -25 | 1200 | -10 |
| • • | 36 38 40 | Ψ, | -25 | +100 | -25 | 1200 | -10 |
| 115 | 115 0 1 2 3 4 5 6 7 8 10 12 14 16 | 1 - 1 M M + + + + | - 25 - 25 I 25 I 400 + 50 I 50 - 25 - 25 - 25 - 25 - 25 - 25 - 25 - 25 | -25 +100 I400 +200 I200 +100 +50 +50 +50 +50 I50 +25 I50 | -25 -25 I100 +100 I200 I100 +50 I50 +25 +25 -25 -25 -25 | -25 +400 +400 1400 +100 +50 150 +25 +25 +25 +25 +25 +25 | $ \begin{array}{r} -10 \\ -10 \\ 1+20 \\ 2+40 \\ 2+20A \\ 3+20 \\ 3+10 \\ 1+10 \\ -10$ |
| | 18 19 | - | -25 | +25 | ~25 | +50 | -10 |
| | 20 21 22 23 | | -25 | 125 | -25 | +25 | -10 |
| | | т | -25 | +25 | -25 | +50 | -10 |
| 24 25 | - | -25 | +25 | -25 | +50 | -10. | |
| | 26 27 | - | ~25 | +25 | -25 | 150 | -10 |
| | 28 30 | - | -25 | ÷25 | -25 | I50 | -10 |
| | 32 34 | - | -25 | +25 | -25 | -25 | -10 |
| | 36 38 40 | | -25 | +25 [.] | -25 | 150 | -10 |

| ANIMAL | WEEK | VSL CARD | V SL ME | VSL PLATE | VSL RIV | VSL Tube | VSL CF |
|--------|---|--|---|--|---|---|---|
| 68 | 0 1 2 3 4 5 6 7 8 0 12 14 16 17 | | - 25 - 25 - 25 - 25 - 25 + 100 I 25 + 25 - 25 - 25 - 25 - 25 - 25 | -25 1400 1200 1100 +50 +50 +50 150 +25 150 +25 +25 +25 | -25 -25 155 1255 -225 -225 -225 -225 -22 | 125 +100 +200 +50 +50 +50 150 150 150 +25 125 +25 125 | $ \begin{array}{r} -10 \\ -10 $ |
| | 18 19 20 | | -25 -25 -25 | 125 | -25 -25 | 125 +25 -25 | -10 -10 -10 |
| | 21 22 | - | -25 | 125 | -25 | 125 | |
| | 23 24 | - <u>-</u> | -25 | +25 | -25 | I 25 | -10 |
| | 25 26 | | -25 | -25 | -25 | 125 | -10 |
| | 27 28 30 | _ | -25 | I 25 | -25 | -25 | -10 |
| | 30 32 34 | _ | -25 | 125 | -25 | -25 · | -10 |
| | 36 38 40 | | -25 | +25 | ~25 | -25 | -10 |
| 117 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | —————————————————————————————————————— | -25 -25 125 +25 +25 125 125 125 -25 -25 -25 -25 -25 -25 | -25 +100 1200 +100 +100 +50 +50 150 150 150 +25 +25 | -25 -25 1100 +500 150 +50 +25 125 125 -25 -25 | -25 1400 1800 +400 +200 +100 +50 +50 +50 150 +50 | $ \begin{array}{r} -10 \\ -10 $ |
| | 18 19 | | -25 | +25 | -25 | +50 | -1.0 |
| | 20 21 | _ | -25 | 125 | -25 | +25 | -10 |
| | 22 | <u></u> | -25 | +25 | -25 | +50 | -10 |
| | 24 25 | - | -25 | 150 | -25 | +50 | -10 |
| | 26 27 | · · · · · · · · · · · · · · · · · · · | -25 | 150 | ~25, | I 100 | -10 |
| | 28 30 | | -25 | 150 | -25 | 150 | -10 |
| . 1 | 32 34 | <u></u> | -25 | I 50 | -25 | +50 | -1.0 |
| | 36 38 40 | - | -25 | 150 | -25 | I 50 | -10 |

| ANIMAL | WEEK | V SL CARD | V SL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL. CF |
|---|--|---|---|--|---|---|--|
| 118 0 1 2 3 4 5 6 7 8 10 12 14 14 | 1 2 3 4 5 6 7 8 10 12 14 | | -25 -25 150 +50 -25 -25 -25 -25 -25 -25 -25 -25 -25 | -25 1400 4100 +100 +100 1100 +50 +50 150 +25 +25 +25 | -25 1200 +200 150 150 150 150 +255 -225 -255 -255 -255 | -25 +400 +800 1800 +100 +100 +100 +50 +50 150 150 150 | $ \begin{array}{r} -10 \\ 1+40 \\ 1+40 \\ 1+40 \\ 1+20A \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ \end{array} $ |
| | 18 19 | - | -25 | 150 | -25 | +25 | -10 |
| | 20 21 | - | -25 | +25 | -25 | +50 | -10 |
| | 22 23 | - | -25 | +25 | -25 | +25 | -10 |
| | 24 25 | т | -25 | 150 | -25 | +50 | -10 |
| | 26 27 | - | -25 | 150 | -25 | 1100 | -10 |
| | 28 30 | - | -25 | +50 | -25 | 1100 | -10 |
| | 32 34 · | W | -25 | 1100 | -25 | +50 | -10 |
| | 36 38 | - | -25 | I 100 | -25 | +50 | -10 |
| | 40 | - | -25 | 150 | -25 | +50 | -10 |
| 62 | 0 1 2 3 4 5 6 7 8 10 12 14 6 7 8 90 12 22 23 4 5 6 7 8 10 12 14 6 7 8 90 12 22 24 5 6 7 8 90 21 223 4 5 6 7 8 90 21 223 4 5 6 7 8 90 21 223 4 5 6 7 8 90 21 223 4 5 6 7 8 90 21 223 4 5 6 7 8 90 21 223 4 5 6 7 8 90 21 223 4 5 6 7 8 90 21 223 4 5 6 7 8 90 21 223 4 5 6 7 8 90 21 223 225 6 7 8 90 21 2 223 225 6 7 8 90 21 2 223 225 8 9 223 225 225 225 225 225 225 225 225 225 | - T + + + + W W W W + + + + + + + + + + + | -25 -25 -25 +25 +25 +25 +25 +25 +25 +25 +25 +25 + | -25 +100 1200 1200 1100 1100 1100 1100 1400 +400 1400 +400 + | -25 +50 1100 +100 +50 150 1550 1550 1550 15 | -25 +100 1400 +400 +200 1100 +100 +100 +50 +800 +800 +800 +800 +800 +800 +80 | -10 -10 -10 -10 2+20A 2+10 -10 -10 1+320 2+640 1+1280 4+540 3+1280 4+140 4+1280 4+10 4 |

| ANIMAL | WEEK | VSL CARD | V SL ME | VSL PLATE | VSL RIV | VSL TUBE | V SL CF |
|--------|--|-------------|------------|--|------------|--|--|
| 16 | 0 1 2 3 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 2 2 2 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | WWWFTT | | -210000 1+110000 1+110000 1+110000 1+110000 1+110000 1+11111 1+11111 1+11111 1+11111 1+11111 1+111111 | | ++++++++++++++++++++++++++++++++++++++ | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |
| 17 | 0 1 2 3 4 5 6 7 8 10 12 14 6 7 8 10 12 14 6 17 18 9 0 12 23 4 5 6 7 8 0 21 22 3 4 5 6 7 8 0 21 23 4 5 6 7 8 0 21 23 4 5 6 7 8 0 21 23 4 5 6 7 8 0 21 2 3 4 5 6 7 8 0 21 2 3 4 5 6 7 8 0 21 2 3 4 5 6 7 8 0 21 2 3 4 5 6 7 8 0 21 2 3 4 5 6 7 8 0 21 2 3 4 5 6 7 8 0 21 2 2 3 4 5 6 7 8 0 2 2 1 2 2 3 4 5 6 7 8 0 2 2 1 2 2 3 4 5 6 7 8 0 2 2 1 2 2 3 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | | | | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |

| 0 | 0010 02000 | | | | | | • |
|--------|--|--------------|------------|--------------|------------|---|---|
| ANIMAL | 冬田田 | V SL CARD | V SL ME | VSL PLATE | VSL RIV | VSL Tube | VSL. CF |
| 124 | 0 1 2 3 4 5 6 7 8 102 14 5 6 7 8 102 14 16 7 18 9 0 1 2 2 2 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 16 7 8 0 2 2 2 2 4 5 6 7 8 0 12 14 16 7 8 0 12 2 3 4 5 6 7 8 0 12 14 16 7 8 0 12 2 14 16 7 8 0 2 2 2 2 4 5 6 7 8 0 2 2 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | - 111+ | | 550555555555555555555555555555555555555 | $ \begin{array}{r} -10 \\ -10 $ |
| 19 | 0 1 2 3 4 5 6 7 8 0 2 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 1 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 1 2 1 2 1 2 1 2 3 4 5 6 7 8 0 2 1 2 1 2 1 2 1 2 2 2 2 2 2 2 2 2 2 2 | | | | | +++++I | $ \begin{array}{r} -10 \\ -10 $ |

| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|--|--|-----------|---|------------|---|---|
| 20 | 0 1 2 3 4 5 6 7 8 0 12 14 5 6 7 8 0 12 14 16 7 8 9 0 12 23 4 5 6 7 8 0 12 14 16 7 8 9 0 12 23 4 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 16 7 8 9 0 12 2 3 4 5 6 7 8 0 12 14 16 7 8 9 0 12 2 3 4 5 6 7 8 9 0 12 14 16 7 8 9 0 12 2 3 4 5 6 7 8 9 0 12 14 16 7 8 9 0 12 2 3 4 5 6 7 8 9 0 12 2 14 16 7 8 9 0 12 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | ++++++******************************** | | -25 12255555 12255555 12255555 12222255555555 | | -2555555555555555550000000 -2225555555555 | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |
| 21 | 0 1 2 3 4 5 6 7 8 12 14 16 7 8 9 0 12 14 16 7 8 9 0 12 22 3 4 5 6 7 8 0 12 14 16 7 8 9 0 1 2 2 2 3 4 5 6 7 8 0 12 14 5 6 7 8 0 12 14 5 6 7 8 0 12 14 16 7 8 9 0 12 2 2 2 3 4 5 6 7 8 0 12 14 16 7 8 9 0 2 2 2 3 4 5 6 7 8 9 0 2 2 2 3 4 5 6 7 8 9 0 2 2 2 3 4 5 6 7 8 9 0 2 2 2 3 4 5 6 7 8 9 0 2 2 2 3 4 5 6 7 8 9 0 2 2 2 3 4 5 6 7 8 9 0 2 2 2 3 4 5 6 7 8 9 0 2 2 2 3 4 5 6 7 8 9 0 2 2 2 3 4 5 6 7 8 9 0 2 2 2 2 3 4 5 6 7 8 9 0 2 2 2 2 3 4 5 6 7 8 9 0 2 2 2 2 3 4 5 6 7 8 9 0 2 2 2 2 2 2 2 3 4 5 6 7 8 9 0 2 2 2 2 3 4 5 6 7 8 9 0 2 2 2 2 3 4 5 6 7 8 9 0 2 2 2 2 3 4 5 6 7 8 9 2 2 2 2 2 3 2 2 2 3 2 2 3 2 2 3 2 2 3 2 2 3 3 2 3 | | | - I I I I I I - + - I I I + I I - + - I I I - + I I - + + - I I I + I I - + + - I I + | | II+++I+II++I+II+++++++++++ 2552222222222222222 | $ \begin{array}{c} -10 \\ -10 $ |

| ANIMAL | WEEK | V SL CARD | VSL Me | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|---|--------------|-----------|--|------------|--|--|
| 22 | 0 1 2 3 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 6 7 8 0 12 4 5 6 7 8 0 12 2 3 4 5 6 7 8 0 12 2 4 5 6 7 8 0 12 2 4 5 6 7 8 0 12 2 4 5 6 7 8 0 12 2 3 4 5 6 7 8 0 12 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | - 25 + 1100 11555222222255555555555555555555555555 | | -25 11100 +5000 +15000 +15000 +5525555555555555555555555555555555 | $ \begin{array}{r} -10 \\ -10 \\ -10 \\ 10 \\ -10 \\$ |
| 23 | 0 1 2 3 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | -+++I 1100005555555555555555505555000505555555 | | I25 +5000 I1000 +1000 I1000 +5500 I1000 +5500 I100 I100 I1000 I1000 I1000 I1000 I1000 I1000 I100 I1000 I100 I1000 | $\begin{array}{c} -10 \\ -10 \\ -10 \\ 2+10 \\ 1+10 \\ 1+20 \\ 3+10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ 2+10 \\ -10 \\ $ |

| ANIMAL | WEEK | VSL CARD | VSL ME | V SL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|---|--|-----------|---|------------|--|--|
| 24 | 0 1 2 3 4 5 6 7 8 12 14 16 17 8 9 0 12 14 16 17 8 9 0 12 2 2 2 3 2 4 5 6 7 8 10 2 12 2 2 2 2 3 2 4 5 6 7 8 10 2 14 5 6 7 8 10 2 14 5 6 7 8 10 2 14 5 6 7 8 10 2 14 5 6 7 8 10 2 14 5 6 7 8 10 2 14 5 6 7 8 10 2 14 5 6 7 8 10 2 14 5 6 7 8 10 2 14 5 6 7 8 10 2 12 2 14 5 6 7 8 10 2 12 2 12 2 12 2 12 2 12 2 12 2 12 | | | -25500055555555555555555555555555555555 | | -1+1+1+++++++++++++++++++++++++++++++++ | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |
| 70 | 0 1 2 3 4 5 6 7 8 0 12 4 6 7 8 0 12 4 6 7 8 0 12 4 6 7 8 0 12 4 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 12 3 4 5 6 7 8 0 2 12 12 10 12 12 2 12 2 12 2 12 2 1 | T + W W T T - - - - - - - - - - - - - - - - | | -1++++++++++++++++++++++++++++++++++++ | | -22000 +22000 +100 +100 +100 +100 +250 +100 +100 +100 +100 +100 +100 +100 +1 | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |

| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|--|-------------|-----------|---|------------|---|---|
| 71 | 0 1 2 3 4 5 6 7 8 0 2 4 6 7 8 0 2 4 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 4 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | -+W+WWWT | | -25 14000 +1000 1500 1500 1500 1500 1500 1500 | | -25000 1440000 1150000 15500000 15500000 15500000 15500000 15500000000 | $ \begin{array}{r} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |
| 72 | 0 1 2 3 4 5 6 7 8 0 2 4 6 7 8 0 2 4 6 7 8 0 2 4 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | -+₩+₩ | | -25 I400 I1000 I500 I550 I55555555555555555555 | | -++1+++++1+11++++1+1++++++++++++++++++ | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |

| ANIMAL | WEEK | VSL CARD | VSL Me | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|---|--|--|--|---|---|--|
| 93 | 0 12 3 4 5 6 7 8 12 14 16 7 8 9 0 12 12 3 4 5 6 7 8 12 12 14 16 7 8 9 0 12 3 4 5 6 7 8 0 12 12 3 4 5 6 7 8 0 12 12 12 14 5 6 7 8 0 12 12 14 5 6 7 8 0 12 12 12 12 12 12 12 12 12 12 12 12 12 | - W + + + W W W | -25 -22500 1+2000 15255555555555555555555555555555555 | -++1200000 ++12000000 ++12000000 ++12000000 ++12000000 ++120000000 ++120000000 ++120000000 ++1200000000 ++12000000000 ++120000000000 | -25 12000 +10000 +1000 +1000 +1000 +1000 +1000 +1000 +1000 +1000 + | -25 +2000 +22000 +22000 +100 +550 +550 +550 +550 +550 +550 + | $\begin{array}{c} -10\\ -10\\ -10\\ 2+40\\ 3+20\\ 2+20\\ 1+10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -$ |
| 94 | 0 1 2 3 4 5 6 7 8 0 12 16 7 8 0 12 16 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 12 3 4 5 6 7 8 0 2 2 2 2 2 2 3 4 5 6 7 8 0 2 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 2 1 2 3 4 5 6 7 8 0 2 1 2 3 4 5 6 7 8 0 2 1 2 2 1 2 2 3 4 5 6 7 8 0 2 1 2 2 1 2 2 1 2 2 2 2 2 2 2 2 2 2 2 | ► + + ₩ ₩ ₩ ₩ ► | | I 25 + 1000 I 1000 + 5500005555255000 I 1550000555525000 I 1550000 I 15500000 I 1550000 I 15500000 I 155000000 I 155000000 I 155000000 I 155000000 I 155000000 I 1550000000 I 15500000000000000000000000000000000000 | | -25 +4000 12000 +1000 +1000 +1000 +500 +500 +500 +50 | $\begin{array}{c} -10 \\$ |

| ANIMAL | WEEK | VSL CARD | VSL ME | VSL Plate | VSL RIV | VSL TUBE | V SL CF |
|--------|--|-------------|-----------|---|------------|--|---|
| 95 | 0 1 2 3 4 5 6 7 8 12 14 16 7 8 9 0 12 14 16 7 8 9 0 12 22 22 4 5 6 7 8 0 22 12 22 24 5 6 7 8 0 22 4 5 6 7 8 9 0 22 24 5 6 7 8 9 0 22 24 5 6 7 8 9 0 21 22 3 4 5 6 7 8 9 0 21 22 3 4 5 6 7 8 9 0 21 22 3 4 5 6 7 8 9 0 21 2 2 3 4 5 6 7 8 9 0 21 2 2 3 4 5 6 7 8 9 0 21 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | -+++WWF | | +100 I100 +50 I25 +25 I25 I25 -25 -25 | | -25 +2000 +2000 +1200 +525 +2255 +2555 +2255 +2555 +2255 +25555 +25555 +25555 +25555 +255555 +255555 +255555 +255555555 | $ \begin{array}{r} -10 \\ -10 $ |

| ANIMAL | WEEK | VSL CARD | VSL ME | VSL Plate | VSL RIV | VSL Tuse | VSL CF |
|--------|---|--------------------------------------|---|---|---|---|---|
| 120 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | - + + W W T - - | -25 -225 -225 -225 -225 -225 -225 -225 | -25 +50 +100 +100 +50 +50 150 +50 150 +25 +25 +25 | -25 -25 +250 1505 1255 1255 1255 -225 -225 -255 -255 -25 | -25 +100 +200 +200 +50 +50 +50 +50 +50 -25 I25 -25 | $ \begin{array}{r} -10 \\ -10 \\ -10 \\ 1+10 \\ -10$ |
| | 18 19 | - | -25 | +25 | -25 | I50 | -10 |
| | 20 21 | · - . | -25 | +25 | ~25 | Į50 | -10 |
| | 22 | - ; | - 25 | +25 | -25 | I 25 | -10 |
| | 24 25 | <u>`</u> | -25 | +25 | -25 | +50 | -10 |
| | 26 27 | | -25 | +25 | -25 | +50 | -10 |
| | 28 30 | - | -25 · | +25 | -25 | +50 | -10 |
| | 32 | - | -25 | [50 | -25 | -25 | -10 |
| | 34 36 38 40 | • : • • | -25 | 150 | -25 | 150 | -10 |
| 121 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | T T W W T - - | - 2255 - 225555 - 222555 - 222555 - 2225555555555 | - 25 I 25 + 50 I 100 I 50 I 25 + 25 I 25 I 25 I 25 I 25 I 25 | -2555555555555555555555555555555555555 | -25 -25 1200 1100 +25 +25 -25 -25 -25 -25 -25 -25 | $ \begin{array}{r} -10 \\ -10 $ |
| | 18 | · | -25 | 125 | -25 | I25 | -10 |
| | 19 20 21 | : | - 25 | 125 | -25 | -25 | -1 Ó |
| | 22 | · | - 25 | 125 | -25 | 125 | -10 |
| · · · | 23 24 | _ | -25 | -25 | -25 | I 25 | -10 |
| | 25 26 | | -25 | I 25 | -25 | -25 | -10 |
| | 27 28 | - | -25 | -25 | -25 | -25 | -10 |
| | . 30 . 32 | <u> </u> | -25 | I 25 | -25 | 125 | -10 |
| | 34 36 | - | -25 | -25 | -25 | -25 | -10 |
| | _38 40 | ÷. | -25 | 125 | -25 | 125 | -10 |

| ANIMAL | WEEK | VSL CARD | V SL ME | VSL PLATE | VSL | VSL Tube | VSL CF |
|--------|---|-----------------|---|--|---|--|---|
| 122 | 2 0 1 2 3 4 5 6 7 8 10 12 14 16 | | - 25 - 225 + 255 - 225 - 225 - 225 - 225 - 225 - 225 - 225 - 255 - 255 - 255 - 255 - 255 - 255 - 255 | -25 I100 +50 +50 +25 I50 +25 I25 I25 I25 I25 I25 I25 | -2555555555555555555555555555555555555 | -25 +100 1200 +50 +25 +25 125 +25 125 -25 +25 125 +25 +25 | $ \begin{array}{r} -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ \end{array} $ |
| | 17 | - | -25 | I 25 | -25 | +25 | -10 |
| | 19 20 | - | -25 | +25 | -25 | +25 | -10 |
| | 21 22 | ~ | -25 | +25 | -25 | I 25 | -10 |
| | 23 24 | - | -25 | 125 | -25 | 125 | -10 |
| | 25 26 | - | -25 | +25 | -25 | -25 | -10 |
| | 27 28 | | -25 | 125 | -25 | 125 | -10 |
| • | 30 32 | - | - 25 | +25 | -25 | I 25 | -10 |
| | 34 36 38 40 | - | -25 | -25 | -25 | -25 | -10 |
| 123 | | · ₩ ₩ | - 25 - 255 - 225 - 255 - | -25 +25 150 150 125 +25 +25 125 +25 125 -25 -25 | - 2255 - 2255555555555555555555555555555 | -25 -25 +50 +50 +25 +25 +25 -225 125 125 +25 | $ \begin{array}{r} -10 \\ -10 $ |
| | 18 19 | - | -25 | -25 · | -25 | +25 | -10 |
| | 20 21 | - | - 25 | I 25 | -25 | 125 | -10 |
| | 22 23 | - | -25 | 125 | -25 | 125 | -10 |
| | 24 25 | - | -25 | 125 | -25 | 125 | -10 |
| | 26 27 | - | -25, | +25 | -25 | +25 | -10 |
| | 28 30 | <u></u> | - 25 | 125 | -25 | +25 | -10 |
| | 32 34 | · <u></u> . | -25 | +25 | -25 | +25 | -10 |
| | 36 38 40 | <u> </u> | -25 | 125 | -25 | +25 | -10 |

| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|---|--------------|--|--|---|---|---|
| 18 | 0 1 2 3 4 5 6 7 8 102 14 16 17 8 9 0 12 2 4 5 6 7 8 102 14 16 17 8 9 0 2 12 3 4 5 6 7 8 0 2 2 2 3 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 9 0 2 2 2 2 4 5 6 7 8 9 0 2 2 2 2 4 5 6 7 8 9 0 2 2 2 2 4 5 6 7 8 9 0 2 2 2 2 2 4 5 6 7 8 9 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | -25 -225 -225 -2255 -2255 -2255 -2255 -2255 -2255 -2255 -2255 -2255 | - 25 + 50 + 100 I 100 + 50 I 50 + 25 I 25 I 25 I 25 I 25 | -255 -225555555555555555555555555555555 | -25 150 +100 +50 150 +25 125 +25 +25 -25 -25 | $ \begin{array}{r} -10 \\ -10 $ |
| 125 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | | -25 -225 -225 +225 -225 -225 -225 -225 - | -25 1200 +200 1400 1200 1100 +50 +50 +25 150 +25 125 125 | -25 -25 +25 150 125 125 -25 -25 -25 -25 -25 | -25 +400 I400 +400 1200 +100 I50 +50 I50 I50 +25 +25 | $ \begin{array}{r} -10 \\ -10 \\ -10 \\ 2+20 \\ 3+10 \\ -1$ |
| | 18 | - | -25 | 125 | -25 | +25 | -10 |
| | 20 21 | - | -25 | +25 | -25 | +25 | -10 |
| | 22 | | -25 | 125 | -25 | -25 | -10 |
| | 24 25 | . | -25 | +25 | -25 | 125 | -10 |
| | 26 27 | - | -25 | +25 | -25 | +25 | -10 |
| | 28 30 | - | -25 -25 | -25 | -25 | 150 | -10 |
| | 32 34 | - | -25 | +25 | -25 | 125 | -10 |
| | 36 38 40 | | -25 | +25 | - 25 | +25 | -10 |

| Difected | | 02001 . 01 | | • | | | • |
|----------|---|-------------|---|---|--|--|---|
| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
| 126 | 0 1 2 3 4 5 6 7 8 0 12 14 16 17 | + + + % % - | - 25 - 25 - 225 I 25 I 25 - 225 - 255 - 25 | -25 +100 +100 +50 +50 150 +25 150 125 125 125 +25 | -25 -25 125 -225 -225 -225 -225 -225 -22 | -25 +200 +100 +50 +50 150 -25 -25 -25 -25 -25 -25 | $ \begin{array}{r} -10 \\ -10 \\ 3+10 \\ 2+10 \\ -1$ |
| | 18 | - | - 25 | I 25 | -25 | +25 | -10 |
| | 19 20 | | -25 | 125 | -25 | 125 | -10 |
| • | 21 22 | _ · | -25 | +25 | -25 | +25 | -10 |
| | 23 24 | · | -25 | +25 | -25 | +25 | -10 |
| | 25 26 | - | -25 | .1 25 | -25 | +25 | -10 |
| | 27 28 | - | - 25 | 150 | -25 | I 25 | -10 |
| · , | 30 32 | - | -25 | +25 | -25 | +25 | -10 |
| | 34 36 38 40 | | -25 | I 50 | -23 | +25 | -10 |
| ι27 | 0 1 2 3 4 5 6 7 8 0 2 4 6 1 2 4 6 1 2 4 5 1 2 7 8 0 2 4 5 7 8 0 2 4 5 6 7 8 0 1 2 7 4 5 6 7 8 0 1 2 7 4 5 6 7 8 0 1 2 7 4 5 6 7 8 9 1 2 7 8 9 1 9 1 9 1 9 1 9 1 9 1 9 1 9 1 9 1 9 | | - 25 - 25 - 25 - 25 - 225 - 2255 - 225 - 255 - 2555 - 2555 | -25 1500 1550 15225 +2255 1225 1225 1225 1225 1225 12 | -255 -2255 -22255 -22255 -22255 -22225 -22225 -22225 -22225 -22225 -22255 -22555 -22255 -22555 -255555 -255555 -255555 -255555 -255555 -2555555 -2555555 -255555555 | -25 +50 +50 +50 +25 -25 125 125 -25 -25 -25 | $ \begin{array}{r} -10 \\ -10 $ |
| | 18 19 | - | -25 . | -25 | -25 | 125 | -10 |
| | 20 21 | | -25 | -25 | -25 | 125 | -10 |
| | 22 2 3 | - | -25 | -25 | ~25 | ~-25 | -10 |
| • | 2 4 25 | ***** | ~25 | 125 | -25 | +25 | -10 |
| | 26 27 | - | ~25 | 125 | -25 | 125 | -10 |
| | 28 30 | - | -25 | -25 | -25 | -25 | -10 |
| | 32 34 | | -25 | -25 | -25 | -25 | -10 |
| | 36 38 40 | - | -25 | I 25 | -25 | -25 | -10 |

· .

| ANIMAL | WEEK | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VŞL CF |
|--------|---|-----------|-------------------------------|---------------------------------------|-------------|--|
| 1 | 0 1 2 3 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 4 5 6 7 8 0 12 2 3 4 5 6 7 8 0 12 2 3 4 5 6 7 8 0 12 2 3 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | - I + + - I I - I - I - I - I | , , , , , , , , , , , , , , , , , , , | | -10 - 10 - 10 - 10 - 10 - 10 - 10 - 10 |
| 2 | 0 1 2 3 4 5 6 7 8 0 2 4 6 7 8 0 2 4 6 7 8 0 2 4 6 7 8 0 2 4 6 7 8 0 2 4 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 1 2 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 8 0 2 4 5 6 7 8 0 2 4 5 8 0 2 4 5 6 7 8 0 2 4 5 8 0 2 4 5 6 7 8 0 2 4 5 8 0 2 4 5 8 0 2 4 5 8 0 2 4 5 8 0 2 4 5 8 0 2 4 5 6 7 8 0 2 4 5 8 0 2 2 4 5 8 0 2 2 4 5 8 0 2 2 4 5 8 0 2 2 4 5 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | | | -10 - 10 - 10 - 10 - 10 - 10 - 10 - 10 |

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| ANIMAL | WEEK | VSL CARD | VSL ME | V SL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|---|-------------|-----------|---|------------|--|--|
| 3 | 012345678024678901223456780246880 1024678901223456780246880 33333333333333333333333333333333333 | WF | | I 25 I 1000 + 1000 I 1500 I 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 | | +25 +100 12000 +1555 12225 12225 12255 12550 12550 12550 12500 12500 12500 12500 12500 12500 12500 12500 12500 12500 12500 12500 12500 12500 12500 12500 12500 12500 12555 12555 12500 12500 12555 12555 12500 12500 125555 125555 125555 125555 125555 125555 1255555 1255555555 | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |
| 4 | 0123456780246789012345678024680 112146789012345678024680 3333334 | ─₩₩₩ | | - +5 10000 +5 10000 + + + + + + + + + + + + + + + + + + | | -25 +100 +1000 +505 +2255 5555555555555555555555555 | $\begin{array}{c} -10 \\$ |

| DROCLE | | | ••••• | <i>,</i> | | | |
|--------|--|---------------|-----------|--|------------|---|---|
| ANIMAL | WEEK | VSL - CARD | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
| 5 | 0 1 2 3 4 5 6 7 8 1 2 1 4 5 6 7 8 1 2 1 4 6 7 8 9 0 1 2 1 2 3 4 5 6 7 8 1 2 1 4 5 6 7 8 1 2 1 4 5 6 7 8 1 2 1 4 5 6 7 8 10 2 14 5 6 7 8 10 2 14 5 6 7 8 10 2 14 5 6 7 8 9 0 12 14 5 6 7 8 9 0 12 14 5 6 7 8 9 0 12 14 6 7 8 9 0 12 2 14 5 6 7 8 9 0 12 14 6 7 8 9 0 12 2 14 5 6 7 8 9 0 12 2 14 6 7 8 9 0 12 2 14 6 7 8 9 0 12 2 14 6 7 8 9 0 1 2 2 1 2 2 4 5 6 7 8 9 0 1 2 2 1 2 2 1 2 2 2 4 5 6 7 8 9 0 1 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | -+11552555555555555555555555555555555555 | | -++15525555555555550000 ++15522225500555555550000 ++1555222222555555550000 00000000000000000 | $ \begin{array}{r} -10 \\ -10 $ |
| 6 | 0 1 2 3 4 5 6 7 8 10 14 16 7 8 9 20 12 24 5 8 9 22 2 22 2 22 2 22 2 22 2 22 2 22 | | | - 255 - 155000 + 55000 + 550555555555555555555555 | | -25550005555555555555555555555555555555 | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |

| BRUCELL | US15 SERUL | UGI FUR | GROUP VG- | .7 | | | <i>.</i> |
|---------|--|-------------|-----------|--|------------|---------------|---|
| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
| 7 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 18 90 21 223 4 5 6 7 8 30 2 12 24 5 6 7 8 30 2 12 34 5 6 7 8 10 21 2 34 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 2 12 14 16 7 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 14 16 7 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 5 6 7 8 10 2 12 2 3 4 5 5 6 7 8 3 3 2 4 5 8 5 7 8 10 2 12 2 3 4 5 5 8 3 2 2 2 2 2 3 2 2 3 3 3 3 3 3 3 3 3 3 | | | | | - I - I I I I | $ \begin{array}{r} -10 \\ -10 $ |
| 8 | 0 1 2 3 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 8 0 2 4 5 6 7 8 0 2 2 4 5 6 7 8 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | - 25000050555555555555555555555555555555 | | -++L++L | $ \begin{array}{r} -10 \\ -10 $ |

| ANIMAL | WEEK | VSL CARD | V SL ME | VSL PLATE | VSL RIV | VSL TUBE | V.SL. CF |
|--------|--|----------|------------|---|------------|--|---|
| 9 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 18 19 21 223 4 5 6 7 8 10 12 14 6 17 18 19 21 223 4 5 6 7 8 32 34 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 20 12 21 20 12 21 20 12 21 20 12 21 20 12 21 20 12 21 20 12 21 20 12 21 20 12 21 20 20 20 20 20 20 20 20 20 20 20 20 20 | | | -250 1+50 +5505555555555555555555555555555 | | 125 1500 +550 +555 +2255 +2555 +22555 +22555 +22555 +22555 +22555 +22555 +225555 +225555 +225555 +2255555 +2255555555 | $ \begin{array}{r} -10 \\ -10 $ |
| 10 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 18 19 21 223 4 5 6 7 8 10 21 223 4 5 6 7 8 10 21 223 4 5 6 7 8 10 21 223 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 21 2 3 4 5 6 7 8 10 2 12 14 16 17 18 9 0 21 2 23 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 12 2 3 4 5 6 7 8 10 2 1 2 2 3 4 5 6 7 8 10 2 1 2 2 3 4 5 6 7 8 1 2 2 3 4 5 8 1 2 2 1 2 2 3 4 5 8 1 3 2 2 3 2 2 3 4 5 8 3 2 2 3 3 2 3 3 2 3 3 3 3 3 3 3 3 3 3 | | | - 250 1 + 550 1 + 550 1 + 550 1 + 550 1 + 225 2 2 2 2 2 55 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 | | +++2555555555555555555555555555555555 | $ \begin{array}{r} -10 \\ -10 $ |

| | | | | ; | | | |
|--------|--|------|-----------|---|------------|--|--|
| ANIMAL | WEEK | CARD | VSL ME | | VSL RIV | VSL TUBE | VSL CF |
| 11 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 18 19 20 21 22 24 5 6 7 8 10 12 14 16 17 18 19 20 21 22 24 5 6 7 8 10 12 14 16 17 8 19 20 21 22 24 5 6 7 8 10 12 14 16 17 8 19 20 21 22 24 5 6 7 8 10 12 14 16 17 8 19 20 21 22 24 5 6 7 8 10 12 14 16 17 8 10 12 14 16 17 8 19 20 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 8 10 21 22 24 5 6 7 28 5 28 5 7 28 5 8 6 8 7 8 5 8 7 8 7 8 8 8 8 8 8 8 8 8 8 8 8 8 | | | - I + I I - I - I - I - I - I - I - I - | | LI+I++++I-II-II+I+I+++I-III-+III 2222222222 | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |
| 12 | 0 1 2 3 4 5 6 7 8 0 1 2 4 5 6 7 8 0 1 2 4 5 6 7 8 0 2 1 4 5 6 7 8 0 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 | | | - I + + I I I I | | | $\begin{array}{c} -10 \\$ |

| | BRUCELLUSIS SERVEUUI TUR BRUUP W-J | | | | | | | | |
|--------|---|-------------|-----------|---|------------|---|---|--|--|
| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL RIV | VSL Tube | VSL CF | | |
| 13 | 0 1 2 3 4 5 6 7 8 10 12 14 16 17 18 9 0 12 23 4 5 6 7 8 10 12 14 16 17 18 9 0 12 23 4 5 6 7 8 0 22 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 23 4 5 6 7 8 10 21 22 3 4 5 6 7 8 10 21 22 3 4 5 6 7 8 10 21 22 3 4 5 6 7 8 10 21 22 3 4 5 6 7 8 10 21 22 22 22 22 22 22 22 22 22 22 22 22 | | | -+++II+II | | - I + I + I + I + I + I + I + I - I - I | $ \begin{array}{r} -10 \\ -10 $ | | |
| 14 | 0 1 2 3 4 5 6 7 8 0 12 14 6 7 8 9 0 12 14 5 6 7 8 0 12 14 6 7 8 9 0 12 3 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 0 2 4 5 6 7 8 9 0 12 3 4 5 6 7 8 9 0 12 3 4 5 6 7 8 9 0 12 3 4 5 6 7 8 9 0 12 3 4 5 6 7 8 9 0 12 3 4 5 6 7 8 9 0 12 14 6 7 8 9 0 12 2 3 4 5 6 7 8 9 0 12 2 3 4 5 6 7 8 9 0 12 2 3 4 5 6 7 8 9 0 12 14 16 7 8 9 0 12 2 3 4 5 6 7 8 9 0 12 12 12 12 12 12 12 12 12 12 12 12 12 | | | - 2555555555555555555555555555555555555 | | -++ | $ \begin{array}{r} -10 \\ -10 $ | | |

| ANIMAL | WEEK | VSL CARD | V SL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|--------|---|-------------|------------|--|------------|--|--|
| 1.5 | 0 1 2 3 4 5 6 7 8 10 12 4 6 7 8 10 12 4 6 17 8 10 21 23 4 5 6 7 8 10 21 2 34 5 6 7 8 10 21 2 34 5 6 7 8 10 2 2 3 4 5 6 7 8 10 2 2 3 4 5 6 7 8 10 2 2 3 4 5 6 7 8 10 2 2 3 4 5 6 7 8 10 2 2 3 4 5 6 7 8 10 2 2 3 4 5 6 7 8 10 2 2 3 4 5 6 7 8 10 2 2 1 2 3 4 5 6 7 8 10 2 2 1 2 3 4 5 6 7 8 10 2 12 8 10 2 12 12 12 12 12 12 12 12 12 12 12 12 1 | | | - 255 + 255 + 255 - 2255 - 2555 - 25555 - 2555 - 2555 - 2555 - 2555 - 2555 - 2555 - 25 | | -25 -255 -255 -255 -255 -255 -255 -2555 -2255 -25555 -2555 -2555 -2555 -2555 -2555 -2555 -2555 -2555 -2555 -2555 - | $\begin{array}{c} -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\ -10\\$ |
| | 40 | | | | | | • • |

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| BRUGELL | U212 2CH | OLUGT FUR | GROUP | -5 | | | |
|------------|------------------------------------|----------------|--|--|--|---|--|
| ANIMAL | WEEK | VSL CARD | V SL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
| 128 | 0 1 2 3 4 5 | - - - | -25 -25 -25 -25 -25 -25 | -25 +25 +50 +50 150 +25 | -25 -25 -25 -25 -25 -25 | -25 +50 1200 +50 +50 150 | -10 -10 -10 -10 -10 -10 |
| · · | 5 6 7 8 10 12 14 | | -25 -25 -25 -25 -25 -25 | +25 +25 125 -25 125 -25 | -25 -25 -25 -25 -25 -25 | +25 +25 -25 -25 -25 -25 | -10 -10 -10 -10 -10 -10 |
| | 16 17 | - | -25 | -25 | -25 | -25 | -10 |
| | 18 19 | | ~25 | -25 | -25 | -25 | -10 |
| | 20 21 | · _ | -25 | -25 | -25 | 125 I | -10 |
| 1 , | 22 | - | -25 | -25 | -25 | -25 | -10 |
| | 24 25 | - | -25 | -25 | -25 | 25 | -10 |
| | 26 27 | - | -25 | -25 | -25 | -25 | -10 |
| | 28 30 | _ , | - 25 | - 25 | -25 | -25 | -10 |
| | 32 34 | - | -25 | +25 | -25 | -25 | -10 |
| | 36 38 | - | -25 | 125 | -25 | -25 | -10 |
| | 40 | - | -25 | 125 | -25 | -25 | -10 |
| 129 | 0 1 2 | - T | -25 -25 -25 | -25 125 +50 | -25 -25 -25 | -25 +25 +100 | -10 -10 -10 |
| | 2 3 4 5 6 | | -25 -25 -25 -25 | +50 +25 +25 +25 | -25 -25 -25 -25 | +50 +50 150 +25 | -10 -10 -10 -10 |
| | 7 8 10 12 | | -25 -25 -25 -25 | +25 +25 +25 +25 | -25 -25 -25 -25 | -25 -25 125 | -10 -10 -10 |
| | 14 16 17 | - | -25 -25 | +25 +25 | -25 -25 | -25 -25 +25 | -10 -10 -10 |
| | 18 19 | | -25 | 125 | -25 | +25 | -10 |
| | 20 21 | - | -25 | +25 | -25 | +25 | -10 |
| | 22 23 | | -25 | +25 | -25 | +25 | -10 |
| | 24 25 | - | ~25 | +25 | -25 | +25 | -10 |
| | 26 27 | ~ | -25 | +25 | -25 | +50 | -,1,0 |
| | 28 30 | - | - 25 | 150 | -25 | 150 | -10 |
| | 32 34 | · | ~25 | +25 | ÷25 | . +25 | -10 |
| | 36 38 40 | - | -25 | +25 | -25 | I 25 | -10 |

| ANIMAL | WEEK | VSL CARD | VSL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|---|---|---------------------------------------|--|---|---|---|---|
| 8 10 12 14 | 1 2 3 4 5 6 7 8 10 12 | — — — — — — — — — — — — — — — — — — — | - 25 - 2255 - 22255 - 22255 | -25 1500 +50 1550 +255 +255 +255 +255 +255 +255 +255 + | -25 -225 -225 -225 -225 -225 -225 -225 | -25 1100 1400 +50 +100 +25 +25 125 125 125 +25 125 +25 +25 +25 | $ \begin{array}{r} -10 \\ -10 \\ 2+20 \\ 2+10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ -10 \\ \end{array} $ |
| | 18 19 | | 25 | 125 | -25 | +25 | -10 |
| . , | 20 21 | _ | -25 | +25 | -25 | +25 | -10 |
| | 22 | - | -25 | 125 | -25 | +25 | -10 |
| | 23 24 25 | - | -25 | +25 | -25 | +25 | -10 |
| · . | 25 26 | - | -25 | +25 | -25 | +50 · | -10 |
| | 27 28 | - | -25 | +25 | -25 | 150 | -10 |
| | 30 32 | - | -25 | +25 | -25 | +25 | -10 |
| | 34 36 38 | - | -25 | 125 | -25 | 125 | -10 |
| 40 131 0 1 2 3 4 5 6 7 8 10 12 14 16 17 | 0 1 2 3 4 5 6 7 8 10 12 14 | | - 25 - 225 - 2225 - 2255 - 22555 - 2255 - 2255 - 2255 - 2255 - 2255 - 2255 - 2255 - 2255 - 2 | - 25 I 25 I 50 + 25 I 255 - 255 I 225 I 255 I 25 | - 25 - 225 - 255 - | I 25 I 25 I 50 I 505 + 255 I 2 | $ \begin{array}{r} -10 \\ -10 $ |
| | 18 19 | | -25 | +25 | -25 | +25 | 10 |
| | 20 21 | - | -25 | +25 | -25 | +25 | -10 |
| | 22 23 | - | -25 | +25 | -25 | +25 | -10 |
| | 24 25 | - | -25 | +25 | -25 | +25 | -10 |
| | 26 27 | | -25 | +25 | -25 | 150 | -10 |
| | 28 30 | - | -25 | +25 | -25 | 150 | -10 |
| | 32 34 | - | ~25 | +25 | -25 | +25 | -10 |
| | 36 38 | - . | ~25 | 125 | -25 | -25 | -10 |
| | 40 | - | -25 | -25 | -25 | -25 | -10 |

| ANIMAL | WEEK | VSL CARD | V SL ME | VSL PLATE | VSL RIV | VŠĽ TUBE | VSL CF |
|--------|--|-------------|---|--|--|---|---|
| | 0 1 2 3 4 5 6 7 8 10 12 14 16 14 | | -25 -25 -25 -25 -25 -25 -25 -25 -25 -25 | -25 I100 +100 I100 I100 +50 +50 +25 +25 I50 +25 +25 +25 | -25 -225 -2255 -2255 -2255 -2255 -2255 -2255 -2255 -2255 -2255 -2255 -2255 | I 25 +200 +200 I 400 I 50 +50 +25 I 50 +25 I 50 +25 +50 +25 +50 | $ \begin{array}{r} -10 \\ -10 \\ -10 \\ 3+20 \\ 3+10 \\ 2+20A \\ 2+10 \\ -10 \\$ |
| | 18 | - | -25 | +25 | -25 | +50 | -10 |
| | 20 21 | - | -25 | +25 | -25 | +25 | -10 |
| · ^ · | 22 | | -25 | +25 | -25 | +25 | -10 |
| | 24 25 | - | -25 | +25 | -25 | +25 | -10 |
| . : | 26 27 | - | -25 | +25 | -25 | +25 | -10 |
| | 28 30 | - | -25 | +25 | -25 | 150 | -10 |
| | 30 32 34 | - | -25 | I 50 | -25 | 125 | -1.0 |
| | 36 38 40 | - | -25 | +25 | -25 | I 25 | - 1 |
| 133 | 0 1 2 3 4 5 6 7 8 10 12 4 16 17 | | - 25 - 225 - 255 - | -25 12255 122255 -22255 -22255 -22255 -22555 -22555 -22555 -225555 -2255555555 | | - 25 - 25 I 255 - 255 - 255 I 255 I 255 - 255 - 255 - 255 - 255 - 255 - 255 | $ \begin{array}{r} -10 \\ -10 $ |
| | 17 18 19 20 21 223 24 25 26 27 28 30 324 36 38 | | н В В В В В В В В В В В В В В В В В В В | | | · ·· · | |

| ANIMAL | WEEK | VSL | V SL ME | VSL PLATE | VSL RIV | VSL TUBE | VSL CF |
|---|---|-------------|---|--|---|--|---|
| 134 | 0 1 2 3 4 5 6 7 8 10 12 14 16 | | - 25 - 255 - 255 - 255 - 225 - 225 | - 25 1 25 1 25 1 25 1 225 1 225 1 225 1 225 1 225 1 225 1 225 1 225 1 225 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 | - 25 - 225 - 255 - | -25 -225 -225 +225 +225 +225 +225 +225 + | $ \begin{array}{r} -10 \\ -10 $ |
| | - 17 18 | - | -25 | +25 | -25 | +25 | -10 |
| | 19 20 | - | -25 | +25 | -25 | +25 | -10 |
| | 21 22 | - | -25 | +25 | -25 | +25 | -10 |
| | 23 24 | · , | -25 | 150 | -25 | +50 | -10 |
| | 25 26 | - | -25 | 1 50 | -25 | [50 | -10 |
| | 27 28 | - | -25 | I 25 | -25 | 150 | -10 |
| | 30 32 | + | -25 | +100 | -25 | I400 | -10 |
| | 34 36 | - | -25 | +50 | -25 | I100 | -10 |
| | 38 40 | + | -25 | +50 | -25 | 1100 | -10 |
| 135 0 1 2 3 4 5 6 7 8 10 12 14 | 1 2 3 4 5 6 7 8 10 12 | | - 25 - 255 - 225 - 255 - | - 25 - 25 I 25 + 50 I 50 + 25 + 25 I 25 I 25 I 25 I 25 I 25 - 25 - 25 | -25 -225 -225 -225 -225 -225 -225 -225 | -25 -225 +2255 +22255 +2255 -2555 -25555 -25555 -25555 -25555 -25555 -25555 -25555 -255555 -25555 -25555 -25555 -255555 -255555 -255555 -255555 -255555 -255555 -25555555 -255555555 | $ \begin{array}{r} -10 \\ -10 $ |
| | 18 | | -25 | -25 | -25 | -25 | -10 |
| | 20 21 | - | -25 | -25 | -25 | -25 | -10 |
| | 22 | - | ~25 | -25 | -25 | -25 | -10 |
| | 24 25 | - | -25 | +25 | -25 | -25 | -10 |
| | 26 27 | - | -25 | I 25 | -25 | -25 | -10 |
| | 28 30 | - | -25 | -25 | -25 | -25 | - 1.0 |
| | 32 34 | - | -25 | +25 | -25 | -25 | -10 |
| | 36 38 40 | - | -25 | ť 25 | -25 | -25 | -10 |