A new selective medium for isolating *Brucella* sp. from bovine milk

by

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INTRODUCTION

Bovine brucellosis is a disease caused by the microorganism, Brucella abortus (B. abortus) and results in a significant economic loss to livestock producers due to abortions, infertility and reduced milk production. Brucellosis is also a public health problem because it can be transmitted to man by consumption of infected milk or milk products or exposure to infected materials (15, 60). At the present, a high priority program in the Animal and Plant Health Inspection Service of the U. S. Department of Agriculture is the eradication of bovine brucellosis. Accurate diagnosis of infected animals is essential in an eradication program. This is accomplished by both serological and bacteriological methods.

Several serological tests are widely used for the identification of infected animals; however, they have limitations. One of the main problems with these tests is that they are unable to differentiate between vaccinated animals and those infected with a field strain of Brucella. Also, there are reports of serologically negative animals which were found to be shedding Brucella.

The isolation and biotyping of Brucella from infected animals is used in epidemiological studies, adult vaccination programs, research projects and identifying carrier animals in problem herds. Luchsinger et al. call attention to the importance of determining the biotype of the infecting Brucella organism in the eradication program which is used in Minnesota (43). This is helpful in the epidemiological study of a newly infected herd. A variety of animal materials may be examined

for the presence of Brucella, some of which are milk, tissue, vaginal discharge and aborted fetal material.

Diagnosis of brucellosis by bacteriological techniques is not without its limitations. A lower than expected isolation rate of Brucella may be due to several causes: 1) low number of organisms present in chronic infections, 2) intermittent shedding of Brucella, and 3) overgrowth of the isolation medium by non-brucella organisms.

Milk is a good source for isolating Brucella because the microorganism is often shed in the milk and it is easy to collect. One of the problems with milk samples is that they often contain more non-brucella organisms than do other types of samples such as tissue, therefore requiring a selective medium for the isolation of Brucella. The shedding of Brucella in milk, ease in obtaining samples and the contamination problem are the reasons that milk was used in this project.

The objectives of this research were: 1) to develop an improved isolation medium for Brucella from milk samples, 2) to develop a medium which can control the growth of non-brucella organisms by the addition of various antimicrobial agents, and 3) to compare the efficiency of such a medium to three media presently being used for the isolation of Brucella from milk samples.

This thesis is divided into two related parts:

1) Development and evaluation of a new selective medium for the isolation of Brucella from milk samples.
2) Comparison of the new selective medium to three established media.

LITERATURE REVIEW

A historical review

The Brucella organism was first described by Sir David Bruce in 1887 and referred to as Micrococcus melitensis (11). He isolated the organism on a peptone-beef infusion agar medium from the spleens of soldiers who had died of Malta fever. Bruce described various characteristics of the organism and was able to reproduce a similar disease in monkeys (12).

Brucella abortus (Bacterium abortus) was first described by Bang in 1897 as the agent which caused epizootic abortion in cattle (5). He isolated the organism from uterine exudate and fetal tissue on a serum-gelatine agar medium. By injecting this organism into pregnant cows, he was able to induce abortions. In 1906, Bang reported various routes of infection, which were the vagina, blood vascular system and the alimentary tract (6). He recommended the segregation of the infected animals and the disinfection of the site as the main procedures for preventing the spread of brucellosis. These procedures are still followed today.

Evans, in 1918, determined that the organisms described by Bruce (Micrococcus melitensis) and Bang (Bacterium abortus) were very closely related and should be in the same genus (21). She recommended changing the name of Micrococcus melitensis to Bacterium melitensis. The genus name was later changed to Brucella in honor of Sir David Bruce.

Isolation of Brucella from bovine milk

Brucella was first isolated from milk by Schroeder and Cotton in 1911 (57). They discovered lesions in the spleens of guinea pigs which had been injected with milk from cows suspected of having tuberculosis. They isolated a gram negative coccobacillus which was identified as the organism which causes contagious bovine abortion. They also noted that infected cows could shed Brucella in their milk for several years. Fabyan confirmed the findings of Schroeder and Cotton by isolating Brucella from the milk of two cows (22). One cow had aborted at 8 months gestation and the other had normally calved 11 months previously.

In 1913, Cotton reported the isolation of Brucella from 18 out of 19 cows (18). Fifteen of these shedders had aborted. Some of these cows had shed Brucella in their milk continuously for at least $4\frac{1}{2}$ years while others shed intermittently. Thompson in 1934 studied 10 "carrier" animals which showed no clinical symptoms of brucellosis and were high milk producers (62). By culturing at 30 day intervals for an entire lactation period, he found 8 out of 10 cows shed Brucella constantly.

Gilman correlated the agglutination titer of milk with the presence of Brucella organisms (29). He isolated Brucella from 53.7% of the quarters which had a titer of 1:80 or higher. The study also revealed that infected cows did not necessarily shed Brucella from all four quarters. Thompson studied the shedding patterns of Brucella of individual quarters of infected cows (63). He found that B. abortus may localize in one quarter of the udder, usually the right hind, and be

continuously shedding in the milk. He recommended culturing individual quarter milk samples to prevent the dilution factor which occurs with composite samples.

Caldwell et al. conducted a study on an infected herd consisting of 86 vaccinated cows and 77 non-vaccinates (14). They noted a correlation between blood serum titers and the presence of Brucella in milk. They isolated Brucella from the milk of 23.6% of the cows which showed agglutinins in the blood serum. In 1960, Morgan and McDiarmid studied the excretion of Brucella in the milk from 45 experimentally infected cows of which 29 were vaccinated and 16 were not (47). On the basis of weekly cultural examinations during the first lactation period following challenge, intermittent shedding was observed in all except five vaccinated cows which remained negative throughout the testing period. Another 10 cows from an earlier experiment that were in their second lactation period, were examined and nine were still shedding Brucella.

In 1954, Huddleson and White isolated B. abortus, biotype 2 from milk (36). They reported that it was more virulent than B. abortus, biotype 1 since it produced macroscopic inflammatory changes in the udder which are not observed in biotype 1 infections.

Isolation methods

From 1911 through about 1932, the usual method of isolating Brucella from milk was by guinea pig inoculation. Smillie, in 1918, improved the guinea pig method of isolating Brucella by determining the optimum incubation time (59). The guinea pigs were necropsied at various time intervals following inoculation in order to determine the number of

Brucella organisms present and the visibility of lesions. Fitch and Lubbehusen used guinea pig inoculations to compare the isolation efficiency when either whole milk or milk sediment was used as inoculum (26). Although 43.2% of the isolations were made from the milk sediment, neither type of inoculum yielded Brucella isolations from all the known shedders.

In 1920, Huddleson developed a selective medium which had an isolation rate comparable to that of the guinea pig method as cited by Robertson et al. (53). In 1932, Henry et al. compared the isolation efficiency of a direct culture method to guinea pig inoculation (32). They found the guinea pig method was better although it was more expensive and required a greater time interval than the direct culture method. These workers suggested that the direct culture method could supplement the guinea pig procedure.

Development of basal media

Bruce (11) first isolated B. melitensis on a peptone-beef infusion agar medium and Bang (5) isolated B. abortus on a semi-solid gelatin agar containing 33% serum. Schroeder and Cotton used an agar containing 6% glycerin and 5% ox bile to isolate B. abortus from guinea pig spleens (57). These were the first media used to grow Brucella.

According to Robertson et al., a medium developed by Stafseth in 1920, which was a spleen and liver infusion agar enriched by the addition of 1% glucose or starch, was the basis of a selective medium developed later (53). During World War I, the variability in quality of commercial peptones and meat infusion broths prompted studies into

the use of peptic and tryptic digest media. This research developed the liver digest infusion agar widely used until the development of serum-dextrose agar. In 1937, Gould and Huddleson prepared a peptone from a pancreatic digest of casein which gave satisfactory results for the growth of Brucella as cited by Robertson et al. (53).

In 1941, Ardrey studied the effect of peptones on the growth of Brucella (2). He compared four peptones at various concentrations to determine their growth promoting properties. He found the addition of more than 0.5% peptone to beef liver infusion agar retarded the growth of Brucella. He also compared a commercial agar (tryptose agar) and found it to be highly satisfactory as a basal medium.

Gerhardt and Wilson developed a simple chemically defined medium for the growth of B. abortus, strain 19 (28). Their medium consisted of mineral salts, four accessory growth factors, lactate, glycerol and DL asparagine. They evaluated the medium for its ability to support all Brucella spp. by testing it with 28 different isolates. All except two CO_2 dependent B. abortus grew on the medium. Sanders et al. developed a chemically defined medium for B. melitensis which contained six amino acids, glucose, four inorganic salts and two vitamins (56). This medium supported the abundant growth of B. melitensis but moderate to poor growth for B. suis and B. abortus.

The chemically defined media were useful in determining the nutritional requirements of Brucella. In 1958, Gerhardt reviewed the literature and concluded that the Brucella spp. have basically simple nutritional needs but are susceptible to certain inhibitory and toxic

substances such as fatty acids and elementary sulfur (27).

Since chemically defined media were not suitable for isolating Brucella species from animals, improvements of undefined media were investigated. Huddleson studied the various factors which affect the growth of Brucella. He found the pH level (7.4 - 7.5) and the CO_2 concentration influenced the growth of Brucella (33). The growth of B. abortus, biotype 2 was enhanced by the addition of killed bacterial cells (2×10^9 per ml), aged blood serum (0.2%), crystalline serum albumin (0.02%) or Tween 40 (0.1%) (33, 34, 35).

Pacheco and DeMello, in 1950, compared various commercial broths and digested media which were made from bovine heart, placenta, liver and spleen (50). They found that the dextrose tryptose broth and veal infusion broth were as good if not better than the digest media in supporting the growth of Brucella. The addition of 5% horse serum to either medium improved their efficiency as an isolation medium.

The joint FAO/WHO expert committee on brucellosis, in their 1964 report, recommended the use of five media (serum dextrose agar, serum potato infusion agar, trypticase soy agar plus serum, tryptose agar plus serum and sheep blood agar) for the basal media for the growth and isolation of Brucella (38). In 1975, Alton et al. recommended the use of serum-dextrose agar, serum-tryptose agar and serum-trypticase-soy agar as the best non-selective basal media (1). They also listed nutrient agar, glycerol-dextrose (2:1) agar and potato agar as good basal media.

Selective media

One of the advantages of guinea pig inoculation as a method of detecting Brucella sp. in specimens collected from infected hosts is the ability of the animal to destroy any contaminating organisms while allowing the Brucella to grow and produce lesions. By contrast, the culture media was often overgrown by the contaminants thus preventing the isolation of any Brucellae which might be present. A selective medium which would control the growth of non-brucella organisms would clearly have an advantage over the costly and time-consuming guinea pig method. In 1920, Huddleson developed a liver infusion agar containing gentian violet (1:10,000) which was comparable to the isolation rates from milk samples using guinea pig inoculation as cited by Robertson et al. (53). Gould and Huddleson later modified the medium to contain a 1:200,000 dilution of gentian violet as cited by Robertson et al. (53). It wasn't until the general availability of antibiotics increased that numerous selective media were developed.

Elberg et al. in 1946, developed a medium for the isolation of B. suis (19). This medium consisted of a tryptose agar base to which was added 0.025 mg/ml of tyrothricin and 0.0125 mg/ml sodium azide.

In 1951, Felsenfeld et al. developed a selective medium for the isolation of Brucella from chicken faeces by using circulin, polymyxin B sulfate, bacitracin and sulphadiazine in a tryptose agar base as previously cited (42, 44, 53). Kuzdas and Morse (1953) developed a selective medium which became the basis for several media formulated later (42). They added polymyxin B sulfate (6 units/ml), cycloheximide (0.1 mg/ml),

bacitracin (25 units/ml), circulin (15 units/ml) and crystal violet (1.4 mg/l) to albimi brucella agar.

In 1955, Mair developed a selective medium for the isolation of Brucella from milk (44). This medium contained polymyxin B sulfate, penicillin, cycloheximide, horse blood and gentian violet. He compared the selective medium to guinea pig inoculation and found the isolation rates for both were similar. Morris (1956), by comparing the ability of several basal media to support the growth of Brucella, developed a new selective medium (48). To either tryptose agar or Hartley digest agar he added 5-nitrofurfurylmethyl ether, bacitracin, polymyxin B and cycloheximide.

Most of the selective media developed would not support the fastidious B. abortus, biotype 2 strains. Huddleson had reported that this strain required extra additives to the medium (33, 34, 35). In 1958, Jones and Morgan addressed the problem of isolating B. abortus, biotype 2 by first evaluating basal media and then basal media plus antimicrobial agents (39). They reported that serum-dextrose agar containing bacitracin, polymyxin B and cycloheximide was the most effective medium for the isolation of B. abortus, biotype 2 from contaminated sources.

Morgan (1960) compared several previously described selective media (46). From his comparative study, he arrived at several conclusions: 1) although Tween 40 could replace serum in basal medium for the growth of B. abortus, biotype 2, it could not do so in the presence of antimicrobial agents, 2) the addition of ethyl violet to serum-dextrose agar inhibited the growth of B. abortus, biotype 2 and adversely

affected some strains of B. melitensis, and 3) the serum-dextrose agar plus antibiotics was the most effective in isolating Brucella. By 1964, the recommended selective media for the isolation of Brucella were serum-dextrose agar, serum-potato infusion agar, serum-trypticase-soy agar, serum-tryptose agar or blood agar containing bacitracin, polymyxin B sulfate, cycloheximide and with ethyl violet optional (38). Amphotericin B could replace cycloheximide or be used in addition to it.

Cabelli and Levin in 1964 developed a medium for the isolation of Pasteurella and Brucella (13). Their medium was a peptic digest-starch agar with brilliant green and cycloheximide.

Painter et al. compared various media in 1966 (51). They concluded that a minimum of two different media should be used for the isolation of Brucella. They recommended tryptose agar plus bovine serum containing four antimicrobial agents (polymyxin B, bacitracin, cycloheximide and ethyl violet) and either tryptose serum agar, trypticase-soy serum agar or albimi-serum agar. Nelson et al. (1966) in studying the epizootiologic factors of brucellosis described their modification of a selective medium recommended by Painter et al. (49). They used two media: 1) tryptose agar containing 5% serum, 10 gm/l dextrose, 1800 units/l polymyxin B, 7500 units/l bacitracin and 30 mg/l cycloheximide, 2) the same as the first medium plus 1:650,000 dilution of crystal violet. These two media are presently being used at the National Veterinary Services Laboratories with a minor modification.

In 1966, Keppie et al. studied the effect on Brucella growth by the addition of erythritol to two media (41). They found that the

addition of 1 uM/ml of erythritol to Morris' medium enhanced the growth of B. abortus and B. melitensis by the appearance of colonies 1 day earlier than the medium without erythritol but did not affect B. suis growth. Also, the growth of B. melitensis was accelerated on albimi agar with erythritol but not the other two species.

In 1967, Ryan developed a selective medium for the isolation of Brucella from milk samples (55). His medium contained penicillin (or bacitracin), polymyxin B sulfate, Spontin (replaced by vancomycin), nalidixic acid, cetrimide, cycloheximide and nystatin in a blood agar medium.

Farrell developed a highly selective medium for the isolation of Brucella from contaminated sources (23). After determining the minimum inhibitory concentration of each antimicrobial agent for Brucella he added bacitracin, polymyxin B, nalidixic acid, vancomycin, cycloheximide and nystatin to a basal medium of serum-dextrose agar.

Brown et al. developed a selective medium for the isolation of B. ovis from ram semen in 1971 (10). They modified Thayer-Martin medium by eliminating IsoVitaleX and adding furadantin and 2% ion agar #2. This medium is being used at the present time for the isolation of B. ovis from ram semen.

Farrell and Robertson, in 1972, compared the isolation efficiency of Ryan's medium, Farrell's medium, Mair's medium and serum-dextrose agar with antimicrobial agents (25). Their study showed that Farrell's and Ryan's media had the highest isolation rates, although Ryan's medium inhibited the growth of B. abortus, biotype 2. Mair's

medium and the serum dextrose agar with antimicrobial agents were not as effective in controlling contamination and therefore their isolation rates were lower. Mair's medium was also found to inhibit B. abortus, biotype 2. They recommended the use of Ryan's and Farrell's media for the isolation of Brucella. These two media are presently being used in the United Kingdom (54).

In 1975, Brodie and Sinton developed a selective broth medium for the enrichment of Brucella in milk samples (9). They modified Farrell's medium by decreasing the concentration of some of the antimicrobial agents and by adding cycloserine. They reported a 10 - 16% increase in the isolation rate of Brucella by the use of their fluid enrichment technique.

Hunter and Kearns, in 1977, compared serum-dextrose agar, Barrow and Peel's medium and Farrell's medium for the isolation of Brucella from bovine milk and vaginal mucus (37). Farrell's medium proved superior to the other two media with Barrow and Peel's medium second and the serum-dextrose agar third. It was observed that Brucella colonies were visible and easily identified after only 3 days of incubation on the Barrow and Peel medium. It was concluded that this medium had a more enriched basal medium.

Berkhoff and Nicoletti, in 1978, compared the isolation efficiency of a modified Brodie-Sinton broth and a solid selective medium (8). Their report indicated the enrichment broth method was inferior to the direct culture onto a solid medium. They recommended more research into broth enrichment of milk samples. Also in 1978, Shin et al.

reported the use of a new basal medium (Schaedler agar medium) with three antimicrobial agents (bacitracin, polymyxin B sulfate and cycloheximide) and 5% fetal calf serum (58). They compared it to brucella (albimi) agar with the same antimicrobials and 5% whole cow blood. In their study, Schaedler's medium was superior.

In 1979, Armbrust (3) and Armbrust et al. (4) developed a selective broth for the isolation of B. abortus from vaginal secretions of cattle. The liquid medium was composed of tryptose broth, hemin, bacitracin, cycloheximide, nalidixic acid, polymyxin B sulfate, vancomycin and sodium polyanetholesulfonate. The study showed the enrichment broth increased the isolation rate of B. abortus when compared to two solid media. Although more contaminants were encountered in the enrichment broth cultures, the contamination did not interfere with the isolation of B. abortus.

Antimicrobial agents

Numerous antimicrobial agents have been studied and used for the selective isolation of Brucella spp. or as chemotherapeutic drugs to treat brucellosis. Gentian violet and ethyl violet are two dyes which were used in the early formulations of selective media (53). Several workers have reported that B. abortus, biotype 2 is sensitive to ethyl violet (39, 46).

In 1939, Hamann and Huddleson studied the effect of sulfapyridine on B. abortus in vitro and in vivo (31). They found it was bacteriostatic to Brucella in vitro but was not effective against Brucella

when administered orally to infected guinea pigs.

Farrell and Robertson, in 1967, studied the sensitivity of B. abortus to polymyxin B sulfate, bacitracin, and amphotericin B (24). Brucella abortus, biotype 2 was observed to be more sensitive to the antimicrobial agents than were the other biotypes of B. abortus.

In 1970, Hall and Manion studied the sensitivity of 27 strains of six species of Brucella to 29 antimicrobial agents (30). Brucella spp. were found to be relatively insensitive to the penicillin-cephalosporin group except ampicillin, the polypeptide group, chloramphenicol, lincomycin, cycloserine and sulfadiazine. They were sensitive to the tetracycline class of antibioltics, erythromycin, gentamicin, streptomycin kanamycin and rifampin.

Kaur and Gupta (1972) studied the sensitivity of B. melitensis to eight antimicrobial agents (40). They found B. melitensis to be the most sensitive to tetracycline, followed by erythromycin, kanamycin, chloramphenicol, penicillin, colistin, polymyxin B sulfate and lincomycin.

In 1973, Robertson et al. determined the sensitivity of B. abortus to six antimicrobial agents (52). The minimum inhibitory concentration (MIC) range of each antimicrobial agent for B. abortus is as follows: gentamicin (1 to 2 ug/ml), kanamycin (2 to 4 ug/ml), streptomycin (0.8 to 12.8 ug/ml), tetracycline (0.4 to 0.8 ug/ml), ampicillin (1 to 16 ug/ml) and carbenicillin (2 to 64 ug/ml).

Farrell (1974) determined the MIC of various antimicrobial agents for 95 Brucellae strains (23). His results are as follows: polymyxin B sulfate (10 to 160 units/ml), colistin (up to > 500 units/ml), nalidixic

acid (10 to 30 ug/ml), bacitracin (50 to 500 units/ml) and amphotericin B (10 to 20 ug/ml). All of the Brucella strains tested were resistant to 1,000 ug/ml of vancomycin, 500 units/ml of nystatin and 500 ug/ml cyclohexamide.

In 1974, Corbel tested the sensitivity of 185 Brucellae strains to spectinomycin (16). All B. canis, B. neotomae, B. ovis, B. abortus, biotypes 1, 5 and 9 and B. suis, biotypes 1 and 5 were sensitive to spectinomycin. Brucella melitensis and the remaining biotypes of B. abortus and B. suis showed variable sensitivity. In 1976, Corbel reported the sensitivity of 107 strains of Brucellae to rifampicin (rifampin; 3-4-methylpiperazinyliminomethyl rifamycin SV) (17). The MIC range for this antimicrobial agent is 0.15 to 2.5 ug/ml.

Terakado et al. (1978) studied the sensitivity of 90 strains of B. canis to 38 antimicrobial agents (61). Brucella canis was found to be resistant to colistin, polymyxin B sulfate, bacitracin and cycloserine. Of the strains tested, 24.4% were found to be resistant to erythromycin, oleandomycin, kitasamycin, spiramycin, tytosin, phenethicillin, cloxacillin, oxacillin, novobiocin and lincomycin. Brucella canis was sensitive to the other 24 antimicrobial agents tested.

PART I DEVELOPMENT AND EVALUATION OF A NEW SELECTIVE MEDIUM FOR THE ISOLATION OF *BRUCELLA* FROM MILK SAMPLES

MATERIALS AND METHODS

Brucella cultures

Forty-nine cultures of Brucella spp. consisting of 20 B. abortus, biotype 1, 7 B. abortus, biotype 2, 9 B. abortus, biotype 4, 10 B. abortus, strain 19, 2 B. canis and 1 B. suis, biotype 1 were used in the development of a selective medium for the isolation of Brucella from milk samples. The Brucella cultures included 5 reference strains and 44 recent isolates kept by the Diagnostic Bacteriology Laboratory.[1] Table 1 lists the Brucella cultures used in this investigation and the background data of each. All cultures were kept as stocks on potato infusion agar slants and stored at -20°C except for the reference strains which were stored at 4°C. In order to obtain a working culture of actively growing organisms, each stock was inoculated onto a plate of tryptose agar with 5% bovine serum. Unless otherwise stated, Brucella cultures in this project were incubated for 48 hours at 37°C in 10% CO_2.

Cultures of other microorganisms

The ability of the experimental media to inhibit the growth of microorganisms commonly found in mastitis or as contaminants of milk samples was determined by the use of representative species of these microorganisms. The microorganisms were obtained from the culture collection kept by the Veterinary Microbiology and Preventive Medicine

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[1] National Veterinary Services Laboratories, Animal and Plant Health Inspection Service, U. S. Department of Agriculture, Ames, IA.

Table 1 Species, biotypes, and source of *Brucella* stock cultures

NVSL Identification	*Brucella* species and biotype	Source	State or Country and year
0-1421	*B. abortus*, biotype 1	Bovine	Mississippi 1980
0-1422	*B. abortus*, biotype 1	Ovine	Kansas 1980
0-1424	*B. abortus*, biotype 1	Bovine	Arizona 1980
0-1457	*B. abortus*, biotype 1	Bovine	Puerto Rico 1980
0-1480	*B. abortus*, biotype 1	Bovine	Oregon 1980
0-1481	*B. abortus*, biotype 1	Bovine	Kentucky 1980
o-1487	*B. abortus*, biotype 1	Bovine	Massachusetts 1980
0-1490	*B. abortus*, biotype 1	Bovine	Massachusetts 1980
0-1492	*B. abortus*, biotype 1	Bovine	Missouri 1980
0-1493	*B. abortus*, biotype 1	Bovine	Missouri 1980
0-1512	*B. abortus*, biotype 1	Bovine	Nebraska 1980
0-1513	*B. abortus*, biotype 1	Bovine	Wisconsin 1980
0-1516	*B. abortus*, biotype 1	Bovine	Arkansas 1980
0-1529	*B. abortus*, biotype 1	Bovine	Minnesota 1980
0-1600	*B. abortus*, biotype 1	Bovine	Oklahoma 1980
0-1651	*B. abortus*, biotype 1	Bovine	Kentucky 1980
0-1652	*B. abortus*, biotype 1	Canine	Kentucky 1980
0-1653	*B. abortus*, biotype 1	Bovine	Puerto Rico 1980
0-1655	*B. abortus*, biotype 1	Bovine	Puerto Rico 1980
2308	*B. abortus*, biotype 1	USDA Challenge Strain	
0-1082	*B. abortus*, biotype 2	Bovine	Missouri 1980
0-1171	*B. abortus*, biotype 2	Bovine	Alabama 1980
0-1288	*B. abortus*, biotype 2	Bovine	Nevada 1980
0-1494	*B. abortus*, biotype 2	Bovine	Missouri 1980
0-1528	*B. abortus*, biotype 2	Bovine	Texas 1980
0-1618	*B. abortus*, biotype 2	Bovine	Idaho 1980

Table 1. (continued)

NVSL Identification	Brucella species and biotype	Source	State or Country and year	
Biotype II	B. abortus, biotype 2	Brucellosis Lab Control		
0-1657	B. abortus, biotype 4	Bovine	Nebraska	1980
0-1858	B. abortus, biotype 4	Canine	Canada	1980
0-1647	B. abortus, biotype 4	Bovine	Kentucky	1980
0-1863	B. abortus, biotype 4	Canine	Canada	1980
0-1862	B. abortus, biotype 4	Canine	Canada	1980
0-1859	B. abortus, biotype 4	Canine	Canada	1980
0-1866	B. abortus, biotype 4	Bovine	Canada	1980
0-1857	B. abortus, biotype 4	Canine	Canada	1980
0-1861	B. abortus, biotype 4	Canine	Canada	1980
0-1396	B. abortus, strain 19	Bovine	Texas	1980
0-1430	B. abortus, strain 19	Bovine	Montana	1980
0-1485	B. abortus, strain 19	Bovine	Colorado	1980
0-1530	B. abortus, strain 19	Bovine	Minnesota	1980
0-1592	B. abortus, strain 19	Bovine	Iowa	1980
0-1608	B. abortus, strain 19	Bovine	Oklahoma	1980
0-1614	B. abortus, strain 19	Bovine	Oklahoma	1980
0-1733	B. abortus, strain 19	Bovine	Indiana	1980
0-1804	B. abortus, strain 19	Bovine	Florida	1980
Strain 19	B. abortus, strain 19	NVSL Vaccine Strain		
0-1533	B. canis	Canine	Tennessee	1980
Canis	B. canis	Strain RM6/66	New York	1966
Suis	B. suis, biotype 1	Strain 1330		

Department.[1] The cultures were Enterobacter sp., Staphylococcus epidermidis, Klebsiella pneumoniae, Escherichia coli, Bacillus subtillis, Pseudomonas aeruginosa, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Staphylococcus aureus, Listeria monocytogenes, Torulopsis glabrata, and Proteus mirabilis. To provide a working culture, each microorganism was streaked onto a separate plate of tryptose agar containing 5% bovine serum and incubated overnight at 37°C.

Media

Tryptose agar[2] enriched with 5% bovine serum (filter sterilized)[3] (TS) was the basal medium used for the incorporation of the antimicrobial agents and for the continuing propagation of all microorganisms. Tryptose broth[2] containing 5% bovine serum (TSB) was used in the production of standardized suspensions of microorganisms. One percent peptone broth[2], containing 0.5% NaCl, was used as a diluent and a suspending medium. Mueller-Hinton medium was prepared and poured into plates to a depth of 4 mm and was used in an antimicrobial disk diffusion test.

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[1] Iowa State University, Ames, IA.

[2] Difco Laboratories Inc., Detroit, MI.

[3] National Animal Disease Laboratory, Ames, IA.

Screening for antimicrobial agent sensitivity

The antimicrobial disk diffusion test was used to determine the sensitivity of 49 Brucella spp. to 27 different antimicrobial agents (7). The inoculum was prepared by transferring each Brucella culture to a different slant of TS in a 25 x 200 mm culture tube. Following incubation 10 ml of sterile saline (0.85%) was added to each tube in order to suspend the cells. Each bacterial suspension was diluted by combining 0.5 ml of the suspended cells with 20 ml sterile saline. The required number of TS plates per Brucella culture were inoculated with a sterile cotton swab which was saturated with the suspension of Brucella organisms. The cotton swab was streaked across each TS plate in three directions with the swab being dipped into the cell suspension between each direction of streaking. This gave a lawn of Brucella growth over the entire surface of the agar plate. A dispenser[1] was used to place eight antimicrobial agent disks[1] on each inoculated TS plate. The disks were pressed firmly onto the agar surface with a flame-sterilized forcep. Following incubation, any zone of growth-inhibition surrounding a disk was considered to show some degree of sensitivity and the absence of an inhibition zone was an indicator of resistance.

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[1]Difco Laboratories Inc., Detroit, MI.

Minimal inhibitory concentrations

The minimal inhibitory concentrations (MIC) of lincomycin (lincomycin hydrochloride)[1], nystatin[2] and dicloxacillin (dicloxacillin sodium monohydrate)[3] were determined in order to calculate the concentration of each antimicrobial agent for incorporation into the experimental media. The procedure used was described by Ericsson and Sherris and is as follows (20).

Preparation of antimicrobial agar plates A stock solution of each antimicrobial agent was made by mixing the powdered antimicrobial with sterile distilled water. Depending on the antimicrobial used, the concentration of the stock solution was either 2000 units or 2000 ug per ml.

lincomycin

Commercial lot (1 million units/vial)

Assay 840 units/mg

238.1 mg + 100 ml H_2O = 2000 units/ml

nystatin

Commercial lot (500,000 units/vial)

500,000 units + 10 ml H_2O = 50,000 units/ml

1 ml of 50,000 units/ml + 9 ml H_2O = 5,000 units/ml

5 ml of 5,000 units/ml + 5 ml H_2O = 2,500 units/ml

8 ml of 2,500 units/ml + 2 ml H_2O = 2,000 units/ml

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[1]Sigma Chemical Company, St. Louis, MO.

[2]E. R. Squibb and Sons, Inc., Princeton, N.J.

[3]Bristol Laboratories, Syracuse, N.Y.

dicloxacillin

Commercial lot (500,000 ug/capsule)

500,000 ug + 250 ml H_2O = 2,000 ug/ml

Tryptose agar was prepared and 90 ml aliquots were dispensed into 250 ml flasks. The agar was kept liquid by placing the flasks in a 48° to 50°C shaker water bath.

Serial two-fold dilutions, ranging from 1:2.5 to 1:1280 of each antimicrobial stock solution were prepared according to the scheme described by Ericsson and Sherris, pg. 68 (20). Ten milliliters of each antimicrobial dilution were added to separate flasks of 90 ml of agar in the water bath. Five milliliters of bovine serum were added to each flask. The liquid agar-antimicrobial mixture was poured immediately into petri dishes to an approximate depth of 5 mm and allowed to cool. The agar plates were stored at 4°C and used within 24 hours.

Brucella inoculum for MIC determination Starting with the initial transfer from the stock culture of each Brucella strain, a second transfer to a TS plate was made. Following incubation, a loopful of growth was transferred to 10 ml of TSB and incubated for 18 to 24 hours. The bacterial density of each culture was adjusted to approximately 71% T on a spectrophotometer[1] by the addition of uninoculated TSB. Each culture was then diluted 1:20 in sterile saline. Within 30 minutes a 0.001 ml drop from each standardized suspension was pipetted

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[1]Wavelength 420 nm on Coleman Spectrophotometer, Model 6/20. Coleman Instruments, Maywood, IL.

onto the appropriately marked area on the series of antimicrobial agar plates to give a circle of inoculum with a diameter of 5 to 8 mm. This procedure was repeated for each antimicrobial agent plus a TS control plate for each series. The plates were incubated for 48 hours and observed for inhibition of growth.

Experimental and standard media

Five experimental media, designated A through E were developed in order to determine if there was any antagonism or enhancement among the antimicrobial agents which would affect the growth of Brucella. In Table 2 are listed the ingredients and differences between each experimental medium and the two standard media, TS with antimicrobial agents (TSA) and TSA with ethyl violet (TSA EV). Tryptose serum agar was the basal medium to which the various combinations of antimicrobial agents were added. Table 3 gives the dilution scheme used for obtaining the desired concentrations of each antimicrobial agent.

Viability counts of Brucella on the experimental media

The United States Department of Agriculture method of determining viability counts was used to test the ability of the different experimental media to support the growth of Brucella (1). Brucella abortus, strain 2308 and B. abortus, strain 19 were used for this purpose. A loopful of 48 hour growth from a plate of each strain was transferred into separate tubes of TSB (10 ml) and incubated for 18 hours. Dilutions of 10^{-2}, 10^{-4}, 10^{-6}, and 10^{-7} in peptone broth were made from the culture of each Brucella strain. Two TS plates and two plates of

Table 2. The composition of the experimental and standard media

Component	Medium						
	A	B	C	D	E	TSA	TSAEV
Bacitracin[a] (units/ml)	25	-	-	25	25	7.5	7.5
Cycloheximide[b] (ug/ml)	-	100	-	100	100	30	30
Lincomycin (units/ml)	6	6	6	6	6	-	-
Nystatin (units/ml)	100	100	100	100	100	-	-
Polymyxin B[c] (units/ml)	-	5	5	-	5	1.8	1.8
Ethyl Violet[d] (ug/l)	-	-	-	-	-	-	1.4
Bovine serum	5%	5%	5%	5%	5%	5%	5%

[a]Diagnostic Reagents, National Veterinary Services Laboratories, Animal and Plant Health Inspection Service, US Dept. of Agriculture, Ames, IA.

[b]Pfizer Laboratories, Clifton, N.J.

[c]Upjohn, Kalamazoo, MI.

[d]Certified by the National Biological Stains Dept.

Table 3. A method for obtaining the desired antimicrobial drug or dye concentration for each medium

Antimicrobial	Preparation of stock solution		Preparation of medium		
	Concentrate[a]	Distilled Water	Stock	TS[b]	Final Concentration
Bacitracin[c]	150,000 units	100 ml	5 ml	1000 ml	7.5 units/ml
			16.6 ml	1000 ml	25 units/ml
Cycloheximide	4 gms	400 ml	3 ml	1000 ml	30 ug/ml
			10 ml	1000 ml	100 ug/ml
Lincomycin	1×10^6 units	100 ml	0.6 ml	1000 ml	6 units/ml
Nystatin	500,000 units	10 ml	2 ml	1000 ml	100 units/ml
Polymyxin B	500,000 units	100 ml	0.36 ml	1000 ml	1.8 units/ml
			1 ml	1000 ml	5 units/ml
Ethyl Violet	1 gm	1000 ml	1.4 ml	1000 ml	1.40 ug/l

[a]Preparation as received from supplier.

[b]tryptose agar enriched with 5% bovine serum.

[c]5×10^6 units/vial, weight varies.

each experimental medium were inoculated with 0.1 ml of 10^{-6} dilution, likewise four TS plates and four experimental media plates were inoculated with 10^{-7} dilution for each culture. The inoculum was spread over the entire surface of the agar with a flame-sterilized glass spreader. Following incubation the colonies were counted and the viability count calculated.

Inhibition of non-brucella organisms by the experimental media

The ability of the five experimental media to inhibit the growth of 13 non-brucella microorganisms was determined.

a. Two different concentrations (approximately 10^3 and 10^6 viable microorganisms/ml) of inoculum were used. In order to determine the appropriate dilutions of an 18 hour broth culture to obtain these concentrations the procedure of Miles and Misra was followed (45). An 18 hour broth culture of each microorganism was made from a TS plate. After incubation, eight 10-fold dilutions of each culture were made in peptone broth. TS plates, six for each culture, were dried for 2 hours at 37°C and then the bottom of each plate was marked into eight squares and appropriately labeled. Into its designated square 0.02 ml of each dilution was dropped onto the surface of the agar. The inoculum was allowed to absorb for 20 minutes before incubation. The dilution selected for counting was the one on each plate which showed no confluence or excessive overcrowding of colonies. The viable count per ml was calculated by multiplying the average number of colonies on the six plates by 50 times the dilution factor.

b. From the results obtained above dilutions containing

approximately 1 x 10^3 and 1 x 10^6 microorganisms per ml were calculated for each culture. A second 18 hour broth culture of each microorganism was grown and the appropriate dilutions were made in milk.[1] A 0.1 ml. aliquot of each dilution was inoculated onto each of the experimental media and a TS control plate. The inoculum was spread over the surface of the agar with a flame-sterilized glass spreader and incubated for 24 hours except for Listeria monocytogenes which was incubated for 48 hours and Staphylococcus epidermidis for 96 hours. The number of colonies were counted and recorded.

Antimicrobial agent sensitivity of other microorganisms

The standard disk diffusion test as described by Bauer et al. was used to determine the sensitivity of 13 microorganisms to 3 of the antimicrobial agents used in the experimental media (7). The inoculum was prepared by transferring four or five colonies from a 24 hour plate culture of each microorganism to separate tubes of tryptose broth (5 ml). The broth cultures were incubated for 2 to 8 hours at 37°C and the turbidity was then standardized to match a McFarland tube 0.5[2] with sterile saline. Each culture was inoculated onto a separate plate of Mueller-Hinton medium and the antimicrobial disks placed on the agar as previously described. The plates were incubated overnight at 37°C and any zone of growth inhibition was measured.

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[1]Heated in a 62°C water bath for 60 minutes.

[2]Mix 0.5 ml of 1.175% barium chloride dehydrate ($BaCl_2$ 2 H_2O) solution and 99.5 ml of 0.36N (1%) sulfuric acid.

Milk samples

A total of 277 milk samples from 100 cows was used for evaluating the experimental media. Some of the samples were from an adult-vaccination study conducted by the National Veterinary Services Laboratories (NVSL) and the remaining samples submitted from the field by cooperative state-federal program personnel for diagnostic purposes. The samples were centrifuged and cultured according to the procedures outlined by Alton et al. and are as follows (1). If the sample was larger than 20 ml, it was mixed and 20 ml was removed for culturing purposes. The samples were centrifuged for 15 minutes at 7,700 X g. Approximately equal portions of the cream layer were inoculated onto one plate of each experimental medium and each standard medium by using a sterile cotton swab. The skim milk was decanted and the sediment was inoculated by the same procedure given for the cream layer. After 7 days of incubation, the plates were observed under 7.5x magnification with light coming at a 45° angle from underneath the plate. Colonies resembling those of the genus Brucella were transferred to a TS plate and identified to species and biotype by the following tests: dye tolerance, growth on penicillin and erythritol, Tb phage, urease, H_2S production, A and M antigen, CO_2 dependence and catalase (1).

RESULTS

Screening for antimicrobial agents to which Brucellae are resistant

The resistance or sensitivity of the 49 Brucellae cultures to 27 antimicrobial agents is shown in Table 4. All of the cultures were resistant to dicloxacillin, lincomycin, and nystatin with the exception of one B. abortus, biotype 2 culture which was sensitive to dicloxacillin. Most of the cultures were resistant to methicillin except nine cultures of B. abortus, strain 19 and all of the cultures of B. abortus, biotype 2. Variation in resistance within some biotypes was noted in the case of seven of the antimicrobial agents. Brucella abortus, biotype 1 cultures were resistant to gantrisin but all of the other Brucella cultures were sensitive. Brucella canis and B. suis, biotype 1 were resistant to furadantin whereas the cultures of B. abortus were all sensitive. All of the cultures were sensitive to 14 antimicrobial agents except one B. canis culture which was resistant to polymyxin B. Table 5 summarizes the results of these tests.

Minimal inhibitory concentrations (MIC)

The MIC for dicloxacillin, lincomycin and nystatin was determined. Nystatin did not inhibit the growth of any of the Brucella cultures at the dilutions tested. The results are shown in the Appendix, Table 14.

The results of the MIC test for dicloxacillin are summarized in Figure 1. Thirty cultures out of the 49 tested were resistant to the 128 ug/ml concentration of dicloxacillin; however, the remaining 19

Table 4. Results of the antimicrobial agent disk diffusion test on 49 Brucella cultures

Antimicrobial Agent[a]		Brucella abortus								B. canis		B. suis	
		biotype 1 (20)[b]		biotype 2 (7)		biotype 4 (9)		Strain 19 (10)		(2)		biotype 1 (1)	
		R[c]	S	R	S	R	S	R	S	R	S	R	S
Amikacin	30mcg	0	20	0	7	0	9	0	10	0	2	0	1
Carbenicillin	30mcg	0	20	0	7	0	9	0	10	0	2	0	1
Cephaloridine	30mcg	0	20	0	7	0	9	0	10	0	2	0	1
Cephalothin	30mcg	0	20	0	7	0	9	0	10	0	2	0	1
Clindamycin	2mcg	16	4	4	3	9	0	9	1	2	0	1	0
Cloxacillin	1mcg	5	15	2	5	0	9	5	5	1	1	0	1
Coly-Mycin	5mcg	5	15	0	7	2	7	7	3	1	1	0	1
Dicloxacillin	1mcg	20	0	6	1	9	0	10	0	2	0	1	0
Doxycyline	30mcg	0	20	0	7	0	9	0	10	0	2	0	1
Erythromycin	2mcg	18	2	0	7	5	4	9	1	0	2	0	1
Furadantin	50mcg	0	20	0	7	0	9	0	10	2	0	1	0
Gantrisin	150mcg	20	0	0	7	0	9	0	10	0	2	0	1
Gentamicin	10mcg	0	20	0	7	0	9	0	10	0	2	0	1
Lincomycin	2mcg	20	0	7	0	9	0	10	0	2	0	1	0
Mandelamine	3mcg	0	20	0	7	0	9	0	10	0	2	0	1
Methicillin	5mcg	20	0	0	7	9	0	1	9	2	0	1	0
Nafcillin	1mcg	20	0	0	7	8	1	4	5	0	2	0	1

Novobiocin	5mcg	0	20	0	7	0	9	0	10	0	2	0	1
Nystatin	100 units	20	0	7	0	9	0	10	0	2	0	1	0
Oxacillin	1mcg[d]	10	1	1	3	4	0	2	1	1	1	1	0
Oxolinic Acid	3mcg	16	4	5	2	8	1	9	1	0	2	0	1
Polymyxin B	300 units	0	20	0	7	0	9	0	10	1	1	0	1
Rifampin	5mcg	0	20	0	7	0	9	0	10	0	2	0	1
SXT	25mcg	0	20	0	7	0	9	0	10	0	2	0	1
Sulfadiazine	300mcg	0	20	0	7	0	9	0	10	0	2	0	1
Sulfathiazole	300mcg	0	20	0	7	0	9	0	10	0	2	0	1
Triple Sulfa	300mcg	0	20	0	7	0	9	0	10	0	2	0	1

[a]Difco Laboratories, Product names.

[b]Number of cultures tested.

[c]R= resistant, S= sensitive.

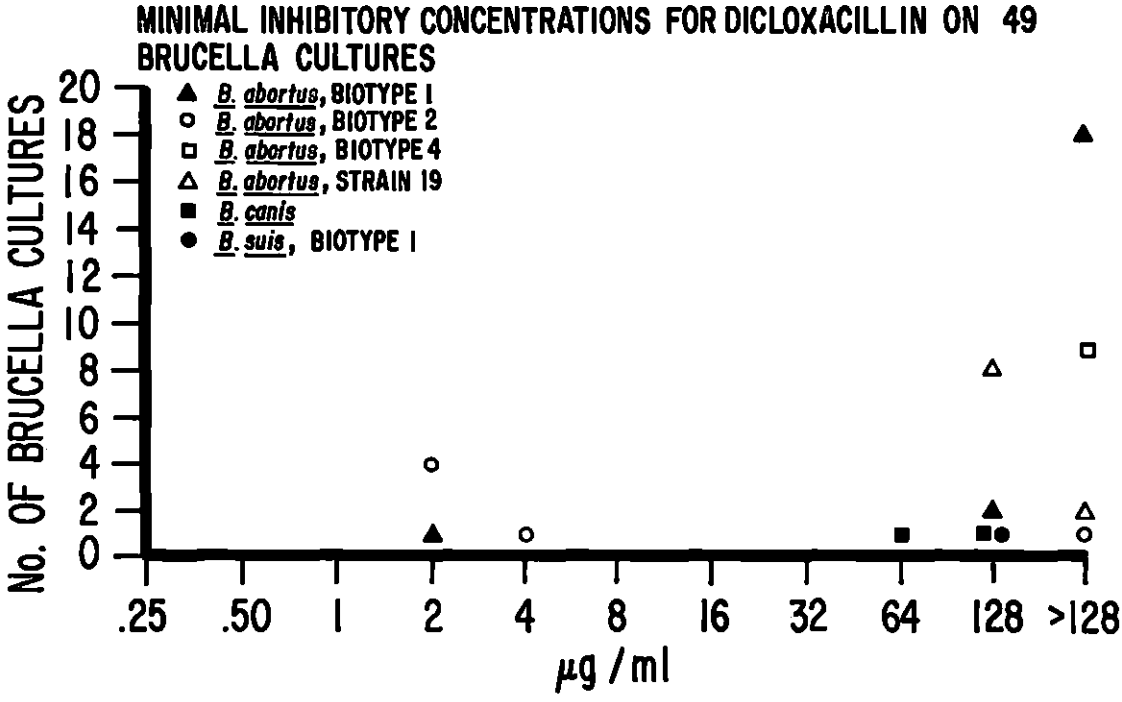
[d]Oxacillin disks in the lot used were contaminated.

Table 5. Summary of the antimicrobial agent sensitivity tests on 49 *Brucella* cultures

All Sensitive To	Variable Sensitivity	All Resistant To
Amikacin	Clindamycin	Dicloxacillin[a]
Carbenicillin	Cloxacillin	Lincomycin
Cephaloridine	Coly-Mycin	Nystatin
Cephalothin	Erythromycin	
Doxycycline	Furadantin	
Gentamicin	Gantrisin	
Mandelamine	Methicillin	
Novobiocin	Nafcillin	
Polymyxin B	Oxacillin	
Rifampin	Oxolinic Acid	
SXT		
Sulfadiazine		
Sulfathiazole		
Triple Sulfa		

[a]One culture of *B. abortus*, biotype 2 was sensitive.

Figure 1. Minimal inhibitory concentrations for dicloxacillin on 49 Brucella cultures


MINIMAL INHIBITORY CONCENTRATIONS FOR DICLOXACILLIN ON 49 BRUCELLA CULTURES
▲ B. abortus, BIOTYPE 1
○ B. abortus, BIOTYPE 2
□ B. abortus, BIOTYPE 4
△ B. abortus, STRAIN 19
■ B. canis
● B. suis, BIOTYPE 1
No. OF BRUCELLA CULTURES
20
18
16
14
12
10
8
6
4
2
0
.25
.50
1
2
4
8
16
32
64
128
>128
μg / ml

cultures showed varying degrees of inhibition. Dicloxacillin inhibited the growth of four cultures of B. abortus, biotype 2 and one culture of B. abortus, biotype 1 at the 2 ug/ml concentration. Appendix, Table 15 lists the complete results.

The MIC results for lincomycin are summarized in Figure 2. All cultures were resistant to the 8 units/ml concentration. The growth of one culture of B. abortus, strain 19 was inhibited at the 16 units/ml concentration. Fourteen cultures were inhibited at 32 units/ml, 30 cultures at 64 units/ml and four cultures at 128 units/ml. The detailed results for the MIC tests for lincomycin are in the Appendix, Table 16.

Sensitivity of non-brucella organisms to antimicrobials

The finding that lincomycin and nystatin did not inhibit the growth of Brucellae and the work of Farrell (23) which reported that bacitracin, polymyxin B and cycloheximide were non-inhibitory gave five antimicrobials that might be useful in a selective medium. The next step was to determine the inhibitory effect of four of these agents on representative strains of non-brucella organisms which would likely be encountered in culturing milk samples for the presence of Brucellae. Disks containing cycloheximide were not available commercially, so it was not tested.

The sensitivity of 12 non-brucella microorganisms to four of the antimicrobial agents was determined by the disk diffusion test. The results are in Table 6. Staphylococcus aureus and Staphylococcus

Figure 2. Minimal inhibitory concentrations for lincomycin on 49 Brucella cultures

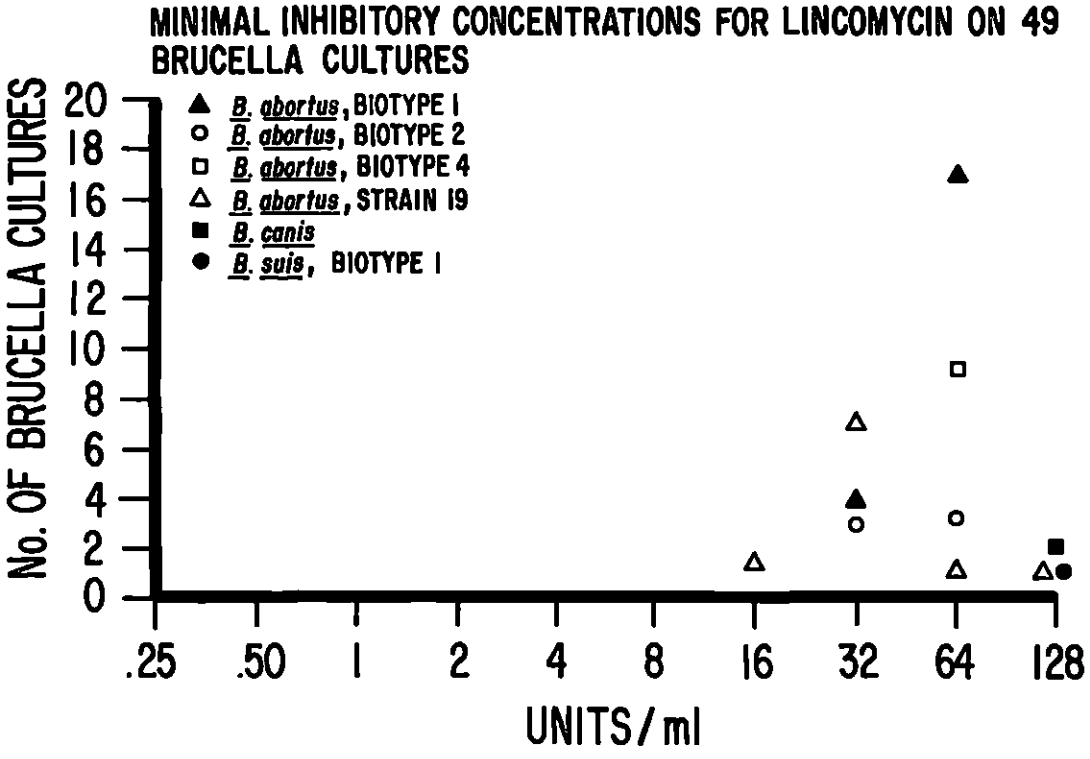

MINIMAL INHIBITORY CONCENTRATIONS FOR LINCOMYCIN ON 49 BRUCELLA CULTURES
No. OF BRUCELLA CULTURES
20
18
16
14
12
10
8
6
4
2
0
B. abortus, BIOTYPE 1
B. abortus, BIOTYPE 2
B. abortus, BIOTYPE 4
B. abortus, STRAIN 19
B. canis
B. suis, BIOTYPE 1
.25
.50
1
2
4
8
16
32
64
128
UNITS/ml

Table 6. Results of the antimicrobial disk diffusion test on non-brucella microorganisms

Microorganism	Bacitracin 10 units Zone of Inhibition (mm)	Polymyxin B 300 units Zone of Inhibition (mm)	Lincomycin 2 mcg Zone of Inhibition (mm)	Nystatin 100 units Zone of Inhibition (mm)
Staphylococcus aureus	16	12	22	0
Staphylococcus epidermidis	16	12	22	0
Enterobacter sp.	0	16	0	0
Proteus mirabilis	0	0	0	0
Escherichia coli 808-1	0	16	0	0
Escherichia coli 25922	0	15	0	0
Klebsiella pneumoniae	0	14	0	0
Pseudomonas aeruginosa	0	15	0	0
Streptococcus dysgalactiae	30	0	27	0
Streptococcus uberis	28	7	27	0
Bacillus subtilis	13	7	17	0
Torulopsis glabrata	0	0	0	27

epidermidis were sensitive to bacitracin, polymyxin B and lincomycin. Enterobacter sp., Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa were sensitive to polymyxin B. Streptococcus dysgalactiae and Streptococcus uberis were sensitive to bacitracin and lincomycin. Bacillus subtilis was sensitive to bacitracin. Torulopsis glabrata was the only one sensitive to nystatin. Proteus mirabilis was resistant to all the antimicrobial agents at the concentrations tested.

Tolerance of Brucella to various antimicrobial agent combinations

The ability of Brucella to grow in the presence of various combinations of antimicrobial agents was determined by inoculating five experimental media with two Brucella strains. When compared to a control, there was no apparent inhibition of growth by any of the media. The growth on the plates inoculated with 0.1 ml of the 10^{-6} dilution was confluent making it impossible to count colonies, therefore, only those plates inoculated with 0.1 ml of the 10^{-7} were counted. The results are given in Table 7.

Inhibition of non-brucella organisms by the experimental media

Table 8 contains the results of the inoculation of each experimental medium with two concentrations of non-brucella organisms. All five experimental media inhibited the growth of Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Streptococcus agalactiae, Streptococcus dysgalactiae, and Streptococcus uberis. Experimental media B, C and E inhibited the growth of

Table 7. The ability of the five experimental media to support the growth of two Brucella strains

Medium	B. abortus, strain 2308		B. abortus, strain 19	
	Colonies per plate 10^{-7} 0.1 ml Dilution	Average per plate	Colonies per plate 10^{-7} 0.1 ml Dilution	Average per plate
Control TS	260 229 250 186	231	267 265 251 282	266
A	240 255 188 199	220	252 325 254 234	266
B	269 231 268 209	244	292 249 272 303	279
C	260 144 333 289	256	241 307 292 337	294
D	255 234 241 227	239	247 269 294 345	288
E	309 270 281 248	277	193 221 246 215	218

Table 8. The ability of five experimental media to control the growth of selected non-brucella organisms

Microorganism	Inoculum Approximate organisms/ml	Medium					
		A	B	C	D	E	TS
Enterobacter sp	9.75×10^6	Conf.[a]	152[b]	136	Conf.	20	Conf.
	9.75×10^2	500	0	0	500	0	500
Staphylococcus epidermidis	9.42×10^6	0	0	0	0	0	TNTC[c]
	9.42×10^2	0	0	0	0	0	1
Klebsiella pneumoniae	1.09×10^6	TNTC	12	7	TNTC	11	TNTC
	1.09×10^3	48	0	0	57	0	58
Escherichia coli	3.58×10^6	TNTC	0	0	Conf.	0	Conf.
	3.58×10^3	287	0	0	290	0	285
Bacillus subtilis	1.75×10^6	0	0	0	0	0	Conf.
	1.75×10^3	0	0	0	0	0	65
Pseudomonas aeruginosa	3.42×10^6	TNTC	TNTC	TNTC	TNTC	TNTC	Conf.
	3.42×10^3	179	37	37	114	0	180
Streptococcus agalactiae	1.13×10^6	0	0	0	0	0	TNTC
	1.13×10^3	0	0	0	0	0	46

Streptococcus dysgalactiae	1.79×10^6	0	0	0	0	0	Conf.
	1.79×10^3	0	0	0	0	0	TNTC
Streptococcus uberis	1.6×10^6	0	0	0	0	0	Conf.
	1.6×10^3	0	0	0	0	0	500
Staphylococcus aureus	8.0×10^6	0	0	0	0	0	Conf.
	8.0×10^2	0	0	0	0	0	18
Listeria monocytogenes	1.5×10^5	2	2	2	0	0	500
	1.5×10^3	0	0	0	0	0	22
Torulopsis glabrata	9.16×10^5	1	0	0	0	0	Conf.
	9.16×10^2	1	1	0	0	0	310
Proteus mirabilis	10^3[d]	2%[e]	2%	2%	15%	1%	25%

[a]Conf.= confluent growth.

[b]Colony count.

[c]TNTC= Too numerous to count.

[d]Approximate dilution.

[e]Percent of surface of plate covered by growth at 24 hours.

Escherichia coli at both concentrations of inoculum and at the low concentration of Enterobacter sp. and Klebsiella pneumoniae. The growth of Listeria monocytogenes and Torulopsis glabrata was either partially or completely inhibited by all the experimental media. The growth from the low concentration of inoculum of Pseudomonas aeruginosa was partially inhibited by media B and C and completely inhibited by the E medium. Proteus mirabilis was not completely inhibited by any medium but was partially controlled by the A, B, C and E media during the first 24 hours.

Isolation of Brucella from milk samples

Table 9 compares the efficiency of seven media for the isolation of Brucella from 277 milk samples representing 100 cows suspected of having a Brucella infection. No medium isolated Brucella from all culture positive cows. Brucella was isolated from 16 of the 18 culture positive cows on the E and TSAEV media, whereas isolation was made from only 12 cows on the B and TSA media. Isolations were made from 14 cows by using the A medium and from 13 cows with the C and D media.

The ability of each medium to inhibit the growth of non-brucella microorganisms is compared in Figure 3. Experimental E medium and the standard TSAEV medium proved the most effective in inhibiting the overgrowth of the agar plate by contaminants. In 17 (6%) milk samples, the E medium failed to control overgrowth and the TSAEV medium failed to do so in 22 (8%) of the samples tested. The experimental B medium was the least effective since it failed to control the

Table 9. A comparison of seven selective media for the isolation of Brucella from milk samples

Medium	Number of Isolations from 18 cows found to be Shedders[a]	Number of Isolations from 34 samples that contained Brucella[b]
A	14	25
B	12	25
C	13	35
D	13	25
E	16	28
TSA	12	25
TSAEV	16	29

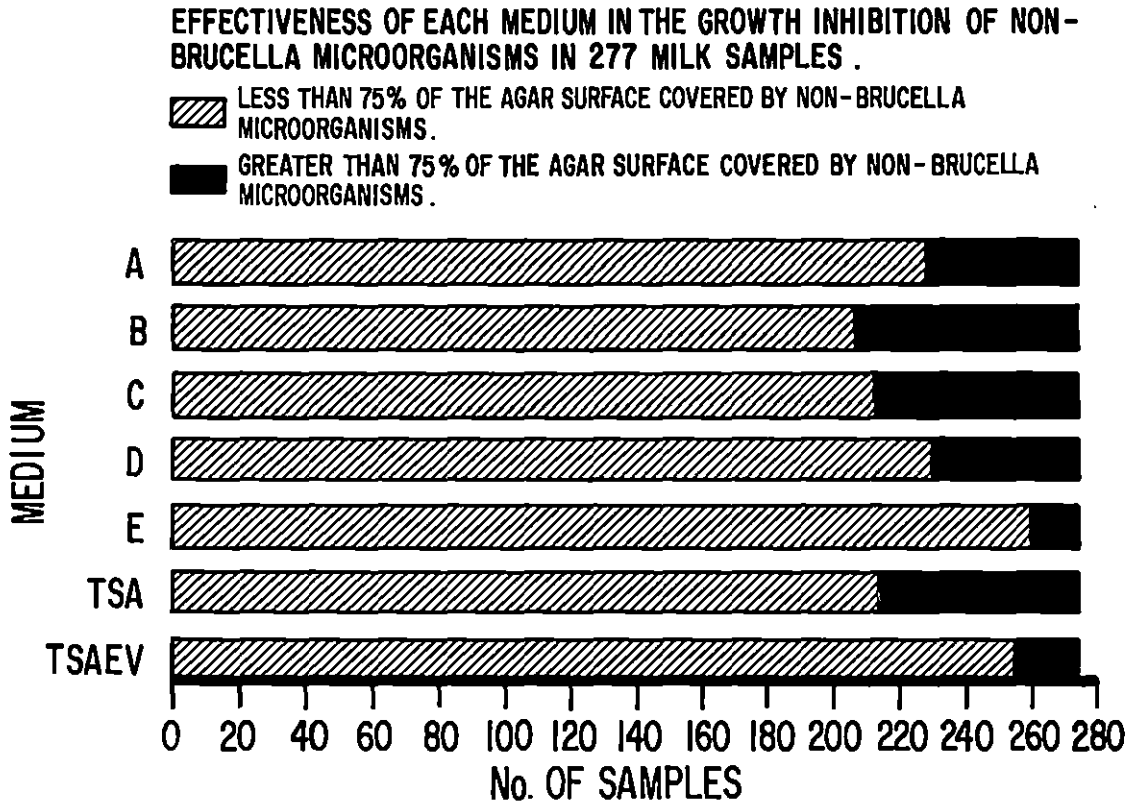
[a] 100 cows were examined.

[b] 277 milk samples were cultured.

overgrowth by contaminants in 69 (25%) of the samples.

A further comparison of the isolation efficiency of the various media was done by devising a scoring system. In this system, a higher score was given to a medium if it was the only medium on which

Figure 3. Effectiveness of each medium in the growth inhibition of non-brucella microorganisms in 277 milk samples


EFFECTIVENESS OF EACH MEDIUM IN THE GROWTH INHIBITION OF NON-BRUCELLA MICROORGANISMS IN 277 MILK SAMPLES .
LESS THAN 75% OF THE AGAR SURFACE COVERED BY NON-BRUCELLA MICROORGANISMS.
GREATER THAN 75% OF THE AGAR SURFACE COVERED BY NON-BRUCELLA MICROORGANISMS.
MEDIUM
A
B
C
D
E
TSA
TSAEV
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280
No. OF SAMPLES

Brucella was isolated. The method of scoring is explained and the results are given in Figure 4. The experimental E medium and the standard TSAEV medium had similar scores of 32 and 31, respectively, followed by TSA--18, A--14, D--13, C--12, and B--10.

Several of the 18 culture positive cows had milk samples which contained large numbers of Brucella; therefore, many colonies grew on all seven media. In other instances of samples with few Brucella organisms or heavy contamination, Brucella colonies only grew on some of the media. Three examples of these variations are displayed in Table 10. Brucella abortus, biotype 1 was isolated from three of the four quarter milk samples from cow 30. All media isolated Brucella from the Y and Z quarters, but media A, E and TSAEV were the only three to fail to isolate from the X quarter. The results obtained from cow 54 illustrates the ability of the E medium to control the contamination present in the milk samples. Although Brucella was isolated on the TSAEV medium from the W and X quarter milk samples, this medium failed to control the contamination present in the Y and Z quarters. The composite milk sample submitted from cow 81 contained B. abortus. Although Brucella was isolated on all seven media, only two, E and TSAEV, supported the growth of a large number of colonies. The estimated number of Brucella colonies developing on each medium from each milk sample was statistically compared using the data in the Appendix, Table 17. Significantly greater numbers of colonies were observed on media E and TSAEV than on the other five media tested ($p < .05$).

Figure 4. Isolation frequency from Brucella infected milk using various media

ISOLATION FREQUENCY FROM BRUCELLA INFECTED MILK USING VARIOUS MEDIA

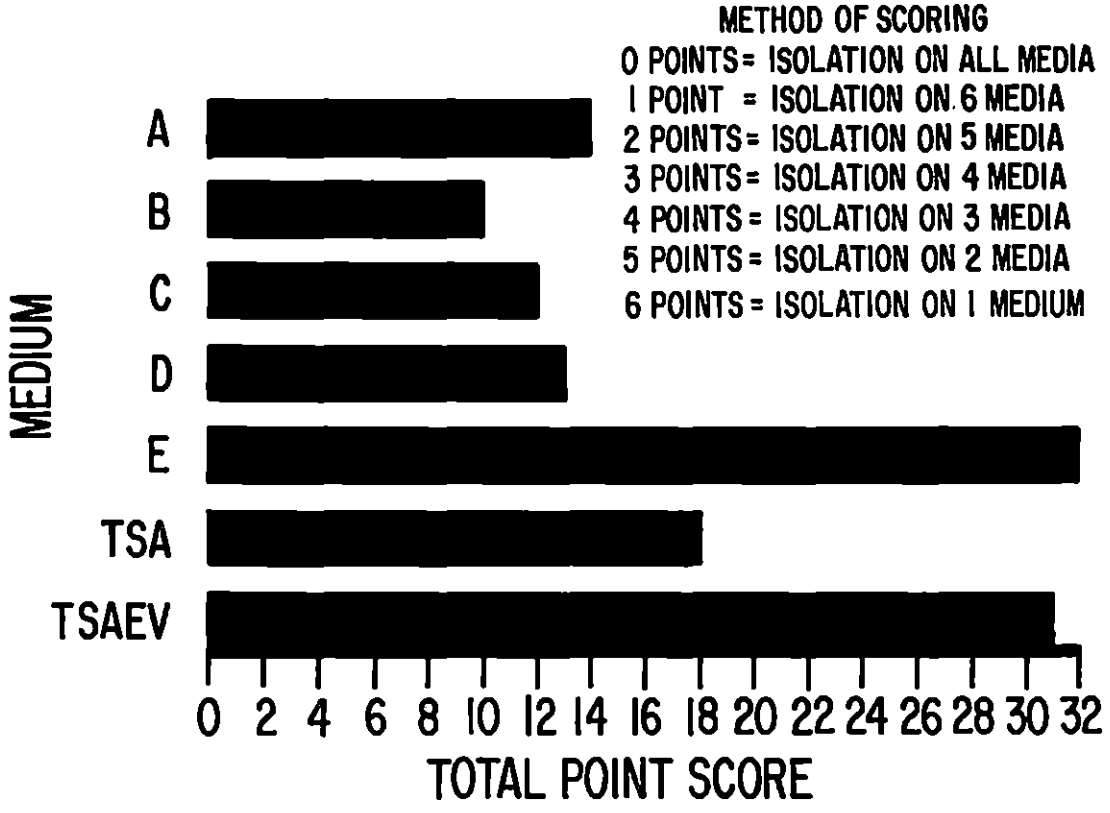


Table 10. Comparison of seven selective media for the isolation of Brucella sp. as illustrated by the results from three cows

Cow Number	Quarter	Medium							Species and biotype
		A	B	C	D	E	TSA	TSAEV	
30	W[a]	-0[b]	-0	-0	-0	-0	-0	-0	
	X	-0	+	+	+2	-0	+2	-0	B. abortus biotype 1
	Y	+3	+3	+3	+3	+3	+3	+4	B. abortus biotype 1
	Z	+2	+2	+2	+2	+2	+2	+3	B. abortus biotype 1
54	W	C	C	C	C	+3	C	+2	B. abortus biotype 1
	X	C	C	C	C	+2	C	+2	B. abortus biotype 1
	Y	C	C	C	C	-0	C	C	
	Z	C	C	C	C	-0	C	C	
81[c]		+	+2	+2	+	+4	+3	+4	B. abortus biotype 1

[a]Udder quarter samples designated W through Z.

[b]-0 = No Brucella isolated.
+ = Single colony of Brucella.
+2 = 2-10 colonies.
+3 = 11-100 colonies.
+4 = 101 or greater colonies.
C = Contaminated.

[c]Composite sample.

DISCUSSION

A new selective medium for the isolation of Brucella from contaminated sources was developed. The combination of antimicrobial agents designated as the E medium proved to be the most efficient of the five experimental media compared. These media were formulated by first screening various antimicrobial agents, selecting possible candidates and then determining the optimum concentration of each to be incorporated into the medium. The experimental media containing various combinations of the five selected antimicrobial agents were compared for their ability to support the growth of known Brucella cultures, inhibit the overgrowth by non-brucella organisms, and to isolate Brucella from milk.

Several differences among the five experimental media were observed in their efficiency to isolate Brucella from naturally infected milk samples and to inhibit the growth of non-brucella microorganisms. The isolation efficiency for the E medium proved to be the best of the five experimental media (Table 9, Figure 4). However, the E medium failed to isolate Brucella from 2 of the 18 culture positive cows. In both cases, less than 11 Brucella colonies were isolated on any of the other media and in several instances only a single colony appeared. This may not reflect a difference in the efficiency of the E medium as much as a matter of probability of any random inoculum of a sample containing a viable Brucella organism except for heavily contaminated samples. Morgan reported the isolation of Brucella on only 4 out of 12 plates which were inoculated with milk that had been

seeded with 15 viable B. abortus cells per 10 ml (46). Overall the E medium supported the growth of more colonies of Brucella per plate than did the other experimental media.

Since some milk samples contain numerous genera of microorganisms other than Brucella, it is important for the selective medium to contol at least the majority of these contaminants. Hunter and Kearns reported the inhibition of Brucella growth by contaminants when culturing milk and vaginal mucus (37). They determined that the isolation frequency was higher for the medium which controlled the majority of the contaminants. Zones of Brucella inhibition surrounding the colonies of some non-brucella microorganisms have been observed in this laboratory. The E medium which contained all five antimicrobial agents proved to be the best in controlling the growth of non-brucella organisms (Figure 3). The B medium was the least effective in controlling contaminants and also had the lowest Brucella isolation frequency. The only difference in the composition of the E and B media is the addition of bacitracin to the E medium. This would indicate that bacitracin is very effective in controlling contaminants in a selective medium for the isolation of Brucella.

Comparison of the ability of the five experimental media to inhibit the growth of 13 common contaminants often found in milk samples showed that the E medium was the most effective (Table 8). The E medium was able to completely or partially inhibit the growth of 12 known non-brucella microorganisms. Although the swarming of Proteus mirabilis was delayed for 24 hours on media A, B, C and E,

none of the five were able to prevent swarming. All of the media plates were completely covered by Proteus mirabilis after 48 hours of incubation. This delay was noted but is of little importance since Brucella requires a minimum of 3 days for colonies to appear on primary isolation.

One of the limitations found in other selective media is the sensitivity of B. abortus, biotype 2 to various antimicrobial agents which may be present in the medium. Morgan (46) reported that B. abortus, biotype 2 was sensitive to ethyl violet and Farrell and Robertson (24) found it was more sensitive to antimicrobial agents than are other Brucellae. In this study, dicloxacillin at low concentrations was found to inhibit the growth of B. abortus, biotype 2 and one culture of B. abortus, biotype 1 (Figure 1). It was determined to be an unsuitable antimicrobial agent for the incorporation into a medium for the isolation of Brucellae.

The basal medium of tryptose agar with 5% bovine serum is a simple medium to prepare and will support the growth of the fastidious strain of B. abortus, biotype 2 (1, 51). Comparison of the number of colonies of B. abortus, strains 19 and 2308 on the TS plates with the number on each of the five experimental media indicated that the growth of Brucella would be supported by all of the media.

The available information on lincomycin states that it inhibits gram positive bacteria which are commonly found in milk samples and has not been previously used for the selective isolation of Brucella. Therefore, lincomycin was considered to be a possible candidate for

the incorporation into a new medium. The antimicrobial disk diffusion test results conducted in this study confirmed the inhibition of gram positive microorganisms by lincomycin and tolerance by Brucella. Kaur and Gupta reported a MIC range of 1.25 to 150.00 ug/ml for 10 strains of B. melitensis by using a disk method (40). In this study, the MIC range of lincomycin for 49 Brucellae cultures was 16 to 128 units/ml. From these results, a concentration of 6 units/ml was determined to be an effective level for growth inhibition of gram positive bacteria without inhibiting the growth of Brucella.

Nystatin is an antifungal agent which affects the growth of yeasts and fungi (64, 65). The findings of this study and Farrell's work show that nystatin does not inhibit Brucella and will control a yeast such as Torulopsis glabrata (23).

The MIC values for polymyxin B, cycloheximide and bacitracin were not determined in this study. Instead, the work of several researchers was used to set the final concentration for each of these three antimicrobial agents to be included in the new isolation medium (1, 23, 39, 42). The colony counts in Table 7 show that the incorporation of these antimicrobials into the media did not affect the growth of B. abortus, strains 19 and 2308.

The lowest concentration of polymyxin B available in the commercial antimicrobial disk was 300 units/ml. This concentration inhibited the growth of all but one of the 49 Brucellae cultures tested. Farrell determined the MIC of polymyxin B for 105 Brucellae strains and found that 5 units/ml would not inhibit Brucellae, including B. abortus,

biotype 2 (23). Kaur and Gupta reported the MIC range of polymyxin B for 10 strains of B. melitensis as 8 to 100 ug/ml (40). Since polymyxin B is one of the few antimicrobials to control Pseudomonas aeruginosa at a concentration of less than 8 ug/ml, it was incorporated into the new selective medium (64, 65). The results from the antimicrobial disk diffusion tests on Escherichia coli and Klebsiella pneumoniae are in agreement with published data (64, 65).

Cycloheximide was incorporated into the new medium at a concentration of 100 ug/ml as suggested by the work of Farrell (23) and others (1, 23, 39, 42).

Likewise, bacitracin was incorporated into the new medium at a concentration of 25 units/ml as recommended by Farrell and others (1, 23, 39, 42). Bacitracin is effective in inhibiting the growth of gram positive bacteria as determined by the antimicrobial disk diffusion test and published data (64, 65).

The E medium which contained all five of the candidate antimicrobial agents proved to be the most effective of the five experimental media. The E medium had the highest isolation efficiency and was the most effective in controlling the growth of contaminants. Comparison of the E medium with three established media which are presently being used for the isolation of Brucella from milk samples is reported in Part II.

PART II COMPARISON OF THE NEW SELECTIVE MEDIUM TO THREE ESTABLISHED MEDIA

MATERIALS AND METHODS

Media

Tryptose agar enriched with 5% bovine serum (TS), TS with antimicrobial agents (TSA), TSA with ethyl violet (TSA EV), and the E medium were described in Part I. Farrell's medium was also used for comparison in this part of the research (23). Table 11 gives the composition of each medium used for comparison of the efficiency of the media in the isolation of Brucella from milk samples.

Milk samples

Milk samples from 224 cows suspected of having an active Brucella infection were cultured onto the four selective media described above. The samples came from two different groups of cows. The first group (vaccination group) consisted of 72 animals used in a vaccination project conducted by the National Veterinary Services Laboratories (NVSL). The second group (field group) contained 152 animals from which samples were taken by cooperative state-federal program personnel and submitted to NVSL for cultural examination.

The animals in the vaccination group were calfhood vaccinated with various concentrations of B. abortus, strain 19. A comparable group of 14 non-vaccinated cows served as controls. Following breeding, each pregnant cow was exposed to 3×10^7 CFU of B. abortus, strain 2308 by the conjunctival route. At the time of parturition or abortion, stomach contents and lung tissue were collected from each aborted fetus and vaginal mucus and quarter milk samples were collected from

Table 11. Composition of the experimental E medium and those used in its evaluation

Media	E	TSA	TSAEV	Farrell
Basal Medium	Tryptose Serum[a]	Tryptose Serum	Tryptose Serum	Serum Dextrose[b]
Bacitracin units/ml	25	7.5	7.5	25
Cycloheximide ug/ml	100	30	30	100
Lincomycin units/ml	6	-	-	-
Nalidixic acid[c] ug/ml	-	-	-	5
Nystatin units/ml	100	-	-	100
Polymyxin B units/ml	5	1.8	1.8	5
Vancomycin[d] ug/ml	-	-	-	20
Ethyl violet ug/l	-	-	1.40	-

[a]Tryptose agar enriched with 5% bovine serum.

[b]Oxoid nutrient agar (K C Biological Inc., Lenexa, KS.) plus 5% horse serum.

[c]Aldrich Chemical Co., Inc., Milwaukee, WI.

[d]Eli Lilly and Co., Indianapolis, IN.

each cow and cultured for Brucella. If Brucella was not isolated from the first collection, then quarter milk samples were collected at 2 and 4 weeks post-parturition or abortion. Tissue was collected at slaughter and cultured from all cows which were previously culture negative. A total of 525 milk samples were cultured from the 72 cows in the project.

The animals in the field group had various histories indicating the possibility of Brucella infection. Some of the milk samples were quarter samples and others were composites. There were 477 samples from 152 cows.

Culture procedures

The milk samples were prepared for inoculation according to the procedure described in Part I. The cream layer and the sediment were inoculated onto the E medium, TSA, TSAEV, and Farrell's medium in the manner previously described. The agar plates were incubated for 7 days at 37°C in 10% CO_2.

Identification of the genus Brucella

Following incubation, colonies which appeared similar to those in the genus Brucella were transferred to a TS plate in order to obtain a pure culture. After 2 days of incubation, those colonies which were confirmed as a Brucella sp. were also biotyped for complete identification by the routine tests previously stated in Part I.

RESULTS

The number of Brucella isolations made on each of the four media from 1002 milk samples representing 224 cows are compared in Table 12. No medium isolated Brucella from all 86 known culture positive cows. The TSAEV medium identified 79 cows as infected whereas the TSA medium only identified 64 cows. Both the Farrell's medium and the E medium were similar to TSAEV in their isolation rates of 78 and 74 respectively. The detailed results for each cow and milk sample on each medium are in Appendix, Tables 18 and 19.

Table 12. A comparison of four selective media for the isolation of Brucella from milk samples

Medium	Number of Isolations from 86 cows found to be shedders[a]	Number of Isolations from 203 samples that contained Brucella[b]
E	74	169
TSA	64	143
TSAEV	79	174
Farrell	78	170

[a] 224 cows were examined.

[b] 1002 milk samples were cultured.

A further comparison of the isolation frequency was made by devising a scoring system. This system gave the highest score to a medium that was the only one on which Brucella was isolated. Figure 5 compares the results and explains the scoring method. There was very little difference among the cumulative scores of three of the media, E (71), TSAEV (80) and Farrell's (74) but the score for the TSA medium (34) was considerably lower.

The presence of non-brucella microorganisms in the milk samples is known to affect the isolation rate for Brucella. Figure 6 compares the ability of each medium to control the growth of contaminants. The E medium, TSAEV and Farrell's was unable to prevent the overgrowth of the agar plate by contaminants in 4%, 3% and 1% respectively of the milk samples. The TSA medium was unable to prevent the overgrowth of 14% of the milk samples.

The estimated number of Brucella colonies developing on each medium from each milk sample was statistically compared from the data in Appendix, Tables 18 and 19. The TSA medium grew significantly lower numbers of Brucella colonies than the other three media ($p<.01$). The isolation of large numbers of Brucella colonies on all four media from several of the culture positive cows was recorded. Other culture positive cows only had isolations on some of the media but not all. Table 13 contains data of four culture positive cows showing varied isolation patterns. Cow 16F is an example in which contamination interfered with the isolation of Brucella on the TSA medium. Cow 59F had a single Brucella colony isolated on the E medium and no colonies

Figure 5. Isolation frequency from Brucella infected milk using various media

ISOLATION FREQUENCY FROM BRUCELLA INFECTED MILK USING VARIOUS MEDIA

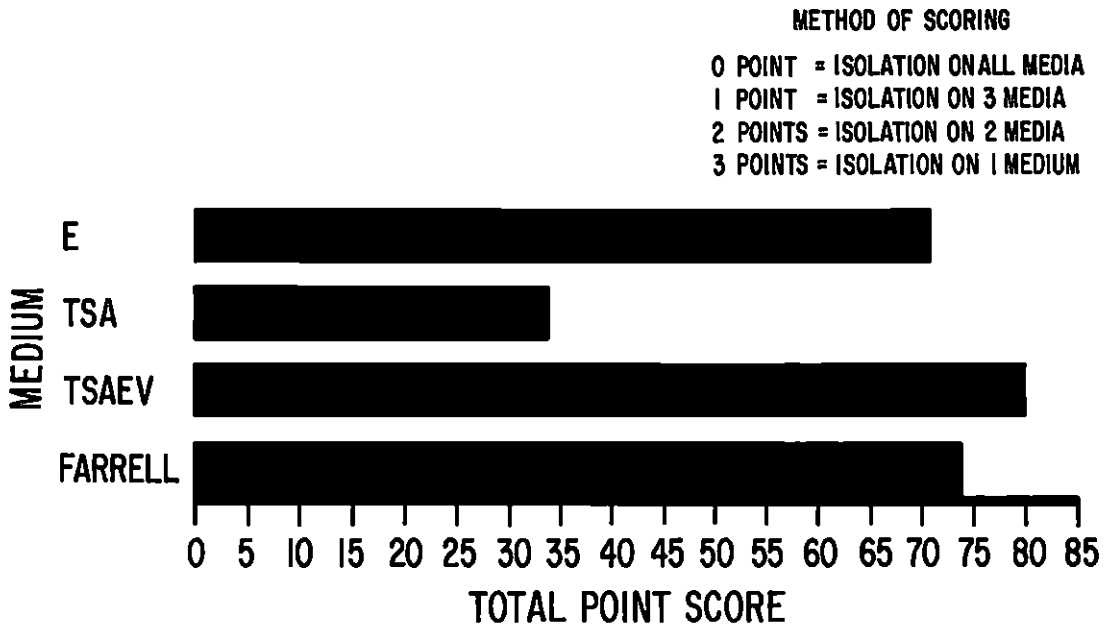


Figure 6. Effectiveness of each medium in the growth inhibition of non-brucella microorganisms in 1,002 milk samples

EFFECTIVENESS OF EACH MEDIUM IN THE GROWTH INHIBITION OF NON-BRUCELLA MICROORGANISMS IN 1,002 MILK SAMPLES

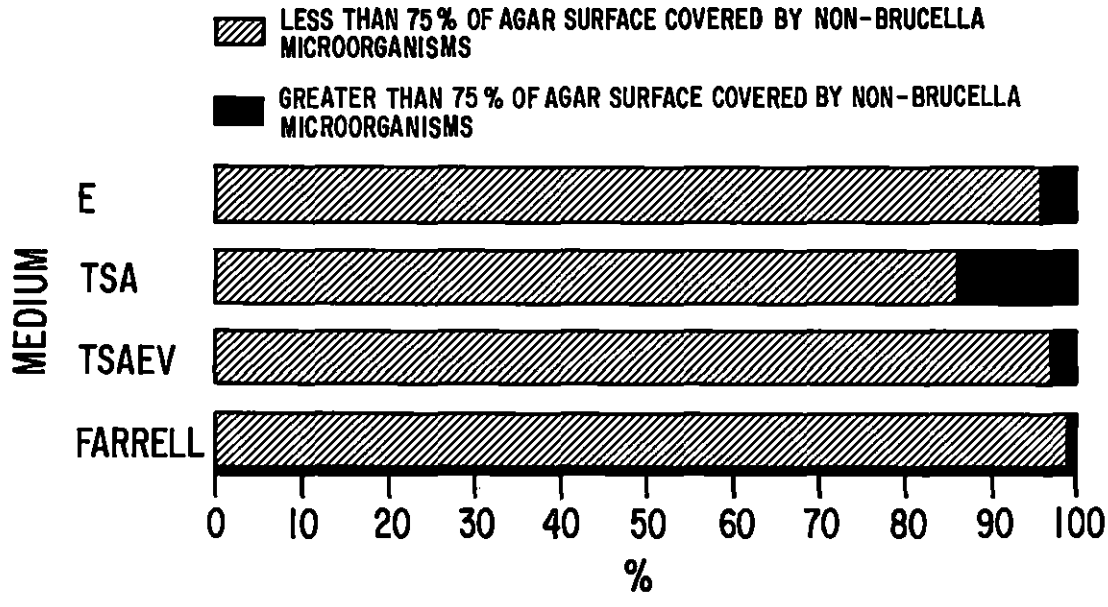


Table 13. Comparison of four selective media for the isolation of Brucella sp. as illustrated by the results from four cows

Cow Number	Quarter	Medium				Species & Biotype
		E	TSA	TSAEV	Farrell	
16 F[a]	Comp[b]	4+[c]	C	4+	4+	B. abortus Biotype 1
59 F	X[d]	+	-0	-0	-0	B. abortus Biotype 1
	Y	-0	-0	-0	-0	
	Z	-0	C	-0	+	B. abortus Biotype 1
13 V	RF	2+	2+	3+	3+	B. abortus Strain 2308
	RR	2+	-0	2+	+	B. abortus Strain 2308
	LF	2+	C	2+	2+	B. abortus Strain 2308
	LR	+	-0	-0	2+	B. abortus Strain 2308
48 V	RF	3+	3+	3+	4+	B. abortus Strain 2308
	RR	2+	-0	C	-0	B. abortus Strain 2308
	LF	-0	-0	-0	-0	
	LR	-0	+	2+	2+	B. abortus Strain 2308

[a] F = Field Group; V = Vaccination Group.

[b] Composite milk sample.

[c] -0 = No Brucella colonies.
+ = Single Brucella colonies.
2+ = 2 - 10 colonies.
3+ = 11 - 100 colonies.
4+ = 101 - 500 colonies.
5+ = 501 colonies.
C = Contaminated.

[d] Unidentified quarter milk samples designated X through Z.

on the other three media from the X quarter milk sample, likewise a single colony was isolated only on Farrell's medium from the Z quarter. Cows 13V and 48V are examples of the isolation pattern where the number of colonies isolated was relatively low except for the RF quarters. The E and Farrell media isolated Brucella from all four quarters of cow 13V whereas the TSAEV medium only isolated from three and the TSA medium from only one quarter. All four media isolated Brucella from the RF quarter of cow 48V. The E medium was the only one to isolate from the RR milk sample and the only one failing to isolate from the LR quarter.

DISCUSSION

A new selective medium, designated as E, was successfully developed for the isolation of Brucella from contaminated milk samples. This medium was supportive of Brucella growth and controlled the growth of the majority of non-brucella microorganisms encountered in the milk samples tested. The E medium was evaluated by comparing it to three established media. Two of these, TSA and TSAEV are media routinely used for the primary isolation of Brucella at NVSL and other laboratories. The third medium was Farrell's which has been reported to give excellent results when compared to a number of other media (25, 37).

The E medium was significantly better in isolating Brucella from milk samples than the TSA and was comparable to TSAEV and Farrell's. It has some advantages over the other media such as supporting a more luxuriant growth of Brucella, contains fewer antimicrobial agents than Farrell's and the basal medium is easy to obtain commercially.

In general, the isolation frequencies for the E, TSAEV and Farrell's media are comparable. There were several instances in which Brucella was isolated on only one of the four media. In each instance, 10 or fewer colonies were observed and in a majority only one colony appeared. This may not reflect differences in the efficiency of the media as much as a matter of probability of any random inoculum of sample containing a viable organism. A similar situation was described by Morgan when he only isolated Brucella on 4 out of 12 plates which were inoculated with milk that had been seeded with 15

viable B. abortus cells per 10 ml (46).

The isolation frequency of the TSA medium was reduced because it was ineffective in controlling contamination. Farrell's medium had the highest isolation frequency and was the most effective in controlling the growth of contaminants (Figures 5 and 6). The main differences between these two media were the number and concentrations of the antimicrobial agents present in each medium. The TSA medium contains three antimicrobial agents at low concentrations and Farrell's contains six at higher concentrations. The inhibition of contaminants by the E and the TSAEV media was similar to Farrell's. The adverse affect of contaminants on the isolation frequency of Brucella has been reported (25, 37, 46). This laboratory has observed zones of Brucella inhibition surrounding colonies of some non-brucella microorganisms. Also, it has been observed that known Brucella colonies on a TS plate can be adversely affected by heavy contamination on the other plates in the same enclosed container. The primary isolation plates were incubated in a closed container into which CO_2 was added. In each container, a TS plate inoculated with B. abortus, biotype 2 was added as an environmental control. Occasionally, the primary isolation plates from a sample were overgrown with a contaminant which produces a volatile end product. In many instances, the Brucella growth on the control plate was either very poor or completely absent.

The growth of Brucella colonies on the E medium was observed to be luxuriant. Single colonies were usually a good size (approximately

2-3 mm) after 7 days of incubation. It has been observed in this laboratory that occasionally the size of the Brucella colonies on Farrell's medium are markedly decreased (approximately 1 mm or less) when compared to the colonies on TSA or TSAEV. In a very few instances, Brucella did not grow on Farrell's when there was confluent Brucella growth on TSA and TSAEV which were inoculated from the same sample. Hunter and Kearns reported diminished colony size on Farrell's medium after 3 days of incubation compared to the other media they tested (37). There may be several reasons for this occurrence, two of which were: 1) the concentration of antimicrobials affects the more sensitive isolates of Brucella or 2) the preparation of the medium was not correct.

The preliminary study in Part I indicated the E medium would support the growth of all biotypes of Brucella found in the United States including the fastidious B. abortus, biotype 2. Morgan (46) reported the inhibition of B. abortus, biotype 2 by a 1:800,000 dilution of ethyl violet but Painter et al. (51) reported it grew on a 1:700,000 dilution. Since TSAEV contains ethyl violet, several researchers recommended its use only in conjunction with other selective media (1, 39, 46, 51). Brucella abortus, biotype 2 was not isolated from any of the milk samples tested. Therefore, a limited study was conducted to determine its viability on the four media. The United States Department of Agriculture viability count procedure was used to inoculate the four media plus a TS control with 10^{-6} and 10^{-7} dilutions of B. abortus, biotype 2 (1). The TSA and TSAEV media showed no inhibition of growth,

Farrell's showed partial inhibition and the E showed complete inhibition. Since lincomycin has never been used before, it was felt that its concentration was too high. A second test was done by inoculating a TS control plate and TS plates containing 6 units/ml, 5 units/ml or 4 units/ml of lincomycin. There was no inhibition of B. abortus, biotype 2 by any of the three concentrations of lincomycin. There may be an interaction occurring among some of the antimicrobial agents in the E medium. This possibility should be studied in more detail. The TSA and Farrell's media have been reported not to inhibit the growth of B. abortus, biotype 2 (1, 23, 25, 51). The limited probe done in this study indicates that Farrell's medium may partially inhibit the growth of B. abortus, biotype 2.

Three of the media, E, TSAEV and Farrell's, were equivalent in their isolation frequency and effectiveness in controlling the growth of contaminants. The TSA medium proved inferior to the other three media in those two criteria. For different reasons, each of the three best media could be used for the isolation of Brucella from contaminated sources. The E medium supported luxuriant growth of Brucella except B. abortus, biotype 2, was highly effective against contaminants, and had a high isolation frequency from naturally infected milk. Farrell's medium had a high isolation frequency, was very effective against contaminants but may adversely affect the growth of B. abortus, biotype 2. The TSAEV medium was also effective in controlling contaminants, had a high isolation frequency, but its affect on the growth of B. abortus, biotype 2 is questionable (1, 39, 46, 51). It

has been reported (46, 51) and observed in this study that the use of several plates increases the isolation chances from samples with low numbers of viable Brucella organisms. The use of the E, TSAEV and Farrell's medium together would increase the isolation frequency from clinical samples. Each medium has different advantages and disadvantages and the three would complement each other.

SUMMARY

A new selective medium (E medium) for the isolation of Brucella from bovine milk was developed. The medium was composed of tryptose agar, bovine serum (5%), bacitracin (25 units/ml), cycloheximide (100 ug/ml), lincomycin (6 units/ml), nystatin (100 units/ml) and polymyxin B sulfate (5 units/ml).

The E medium was compared to three established media (TSA, TSAEV and Farrell's). Brucella abortus was isolated from 86 of the 224 cows which were tested. Of these 86 cows, TSAEV isolated from 79, Farrell's from 78, E medium from 74 and TSA from 64. The TSA medium isolated fewer Brucella colonies than the other three media ($p<.01$). The TSA medium was inferior to the other three media in all comparisons. It is recommended that the E medium replace the TSA medium as a primary isolation medium for contaminated sources.

Although there was little difference among the E, TSAEV and Farrell's media in their isolation frequencies and contamination control, each had some advantages. The sensitivity of B. abortus, biotype 2 to E, TSAEV and Farrell's media tends to vary among the different isolates as indicated by other researchers and further studies done in this laboratory. Therefore, by using a combination of all three media (E, TSAEV and Farrell's) the isolation frequency would be increased and the isolation of B. abortus, biotype 2 would not be missed due to inhibition by one of the media.

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APPENDIX

Table 14. Results of the minimal inhibitory concentraion determination for nystatin on Brucella spp. after 48 hours incubation

NVSL Number	Brucella spp.[a] and biotype	Dilution (units/ml) 128	64	32	16	8	4	2	1	.5	.25	TS[b]
0-1171	BA biotype 2	+[c]	+	+	+	+	+	+	+	+	+	+
0-1288	BA biotype 2	+	+	+	+	+	+	+	+	+	+	+
0-1421	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1422	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1424	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1430	BA strain 19	+	+	+	+	+	+	-	+	+	+	+
0-1457	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1480	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1481	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1485	BA strain 19	±	±	±	-	±	+	+	+	+	±	±
0-1487	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1490	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1492	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1493	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1494	BA biotype 2	+	+	+	+	+	+	+	+	+	+	+
0-1512	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1513	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1516	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1528	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1529	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+

[a] BA = Brucella abortus.
BS = Brucella suis.

[b] TS = Control: Tryptose agar plus 5% bovine serum.

[c] + = Growth.
- = No growth.
± = Light growth.

Table 14. (continued)

NVSL Number	*Brucella* spp. and biotype	Dilution (units/ml)										
		128	64	32	16	8	4	2	1	.5	.25	TS
0-1530	BA strain 19	+	+	+	-	+	+	-	-	+	±	±
0-1533	*B. canis*	+	+	+	+	+	+	+	+	+	+	+
0-1592	BA strain 19	+	+	+	±	±	+	+	+	+	+	+
0-1600	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1608	BA strain 19	+	+	+	+	+	+	+	+	+	+	+
0-1618	BA biotype 2	+	+	+	+	+	+	+	+	+	+	+
0-1647	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1651	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1652	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1653	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1655	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1657	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1733	BA strain 19	+	+	+	+	+	+	+	+	+	+	+
0-1804	BA strain 19	+	+	+	+	+	+	+	+	+	+	+
0-1857	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1858	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1859	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1861	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1862	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1863	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1866	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
Strain 19	BA strain 19	±	+	+	±	±	+	+	+	+	±	+
2308	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
Biotype 2	BA biotype 2	+	+	+	+	+	+	+	+	+	+	+
Suis	BS biotype 1	+	+	+	+	+	+	+	+	+	+	+
Canis	*B. canis*	+	+	+	+	+	+	-	+	+	+	+

Table 15. Results of the minimal inhibitory concentration determination for dicloxacillin on Brucella spp. after 72 hours incubation

NVSL Number	Brucella spp.[a] and biotype	Dilution (ug/ml)										
		128	64	32	16	8	4	2	1	.5	.25	TS[b]
0-1082	BA biotype 2	+[c]	+	+	+	+	+	+	+	+	+	+
0-1171	BA biotype 2	-	-	-	-	-	-	+	+	+	+	+
0-1288	BA biotype 2	-	-	-	-	C	-	-	+	+	+	+
0-1396	BA strain 19	-	+	+	+	C	+	+	+	+	+	+
0-1421	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1422	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1424	BA biotype 1	-	+	+	+	C	+	+	+	+	+	+
0-1430	BA strain 19	-	+	+	+	C	+	+	+	+	+	+
0-1457	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1480	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1481	BA biotype 1	+	+	+	+	C	+	+	+	+	+	+
0-1485	BA strain 19	-	+	+	+	C	+	+	+	+	+	+
0-1487	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1490	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1492	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1493	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1494	BA biotype 2	-	-	-	-	-	-	-	+	+	+	+
0-1512	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1513	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1516	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1528	BA biotype 1	-	-	-	-	-	-	-	+	+	+	+

[a] BA = Brucella abortus.
BS = Brucella suis.

[b] TS = Control: Tryptose agar plus 5% bovine serum.

[c] + = Growth.
- = No growth.
C = Contamination.

Table 15. (continued)

NVSL Number	*Brucella* spp. and biotype	Dilution (ug/ml)										
		128	64	32	16	8	4	2	1	.5	.25	TS
0-1529	BA biotype 1	-	+	+	+	+	+	+	+	+	+	+
0-1530	BA strain 19	-	+	+	+	+	+	+	+	+	+	+
0-1533	*B. canis*	-	-	+	+	+	+	+	+	+	+	+
0-1592	BA strain 19	-	+	+	+	+	+	+	+	+	+	+
0-1600	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1608	BA strain 19	+	+	+	+	+	+	+	+	+	+	+
0-1614	BA strain 19	-	+	+	+	+	+	+	+	+	+	+
0-1618	BA biotype 2	-	-	-	-	-	-	-	+	+	+	+
0-1647	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1651	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1652	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1653	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1655	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
0-1657	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1733	BA strain 19	-	+	+	+	+	+	+	+	+	+	+
0-1804	BA strain 19	+	+	+	+	+	+	+	+	+	+	+
0-1857	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1858	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1859	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1861	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1862	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1863	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
0-1866	BA biotype 4	+	+	+	+	+	+	+	+	+	+	+
Strain 19	BA strain 19	-	+	+	+	+	+	+	+	+	+	+
2308	BA biotype 1	+	+	+	+	+	+	+	+	+	+	+
Biotype 2	BA biotype 2	-	-	-	-	-	-	-	+	+	+	+
Suis	BS biotype 1	-	+	+	+	+	+	+	+	+	+	+
Canis	*B. canis*	-	+	+	+	+	+	+	+	+	+	+

Table 16. Results of the minimal inhibitory concentration determination for lincomycin on Brucella spp. after 72 hours incubation

NVSL Number	Brucella spp.[a] and biotype	Dilution (units/ml)										TS[b]
		128	64	32	16	8	4	3	1	.5	.25	
0-1082	BA biotype 2	-[c]	-	+	+	+	+	+	+	+	+	+
0-1171	BA biotype 2	-	-	-	+	+	+	+	+	+	+	+
0-1288	BA biotype 2	-	-	+	+	+	+	+	+	+	+	+
0-1396	BA strain 19	-	-	-	+	+	+	+	+	+	+	+
0-1421	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1422	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1424	BA biotype 1	-	-	-	+	+	+	+	+	+	+	+
0-1430	BA strain 19	-	-	-	+	+	+	+	+	+	+	+
0-1457	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1480	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1481	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1485	BA strain 19	-	-	-	+	+	+	+	+	+	+	+
0-1487	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1490	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1492	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1493	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1494	BA biotype 2	-	-	-	+	+	+	+	+	+	+	+
0-1512	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1513	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1516	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1528	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1529	BA biotype 1	-	-	-	+	+	+	+	+	+	+	+

[a] BA = Brucella abortus.
BS = Brucella suis.

[b] TS = Control: Tryptose agar plus 5% bovine serum.

[c] + = Growth.
- = No growth.

Table 16. (continued)

NVSL Number	*Brucella* spp. and biotype	Dilution (units/ml)										TS
		128	64	32	16	8	4	2	1	.5	.25	
0-1530	BA strain 19	-	-	-	+	+	+	+	+	+	+	+
0-1533	*B. canis*	-	+	+	+	+	+	+	+	+	+	+
0-1592	BA strain 19	-	-	-	-	+	+	+	+	+	+	+
0-1600	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1608	BA strain 19	-	+	+	+	+	+	+	+	+	+	+
0-1614	BA strain 19	-	-	-	+	+	+	+	+	+	+	+
0-1618	BA biotype 2	-	-	-	+	+	+	+	+	+	+	+
0-1647	BA biotype 4	-	-	+	+	+	+	+	+	+	+	+
0-1651	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1652	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1653	BA biotype 1	-	-	+	+	+	+	+	+	+	+	+
0-1655	BA biotype 1	-	-	-	+	+	+	+	+	+	+	+
0-1657	BA biotype 4	-	-	+	+	+	+	+	+	+	+	+
0-1733	BA strain 19	-	-	-	+	+	+	+	+	+	+	+
0-1804	BA strain 19	-	-	+	+	+	+	+	+	+	+	+
0-1857	BA biotype 4	-	-	+	+	+	+	+	+	+	+	+
0-1858	BA biotype 4	-	-	+	+	+	+	+	+	+	+	+
0-1859	BA biotype 4	-	-	+	+	+	+	+	+	+	+	+
0-1861	BA biotype 4	-	-	+	+	+	+	+	+	+	+	+
0-1862	BA biotype 4	-	-	+	+	+	+	+	+	+	+	+
0-1863	BA biotype 4	-	-	+	+	+	+	+	+	+	+	+
0-1866	BA biotype 4	-	-	+	+	+	+	+	+	+	+	+
Strain 19	BA strain 19	-	-	-	+	+	+	+	+	+	+	+
2308	BA biotype 1	-	-	-	+	+	+	+	+	+	+	+
Biotype 2	BA biotype 2	-	-	+	+	+	+	+	+	+	+	+
Suis	BS biotype 1	-	+	+	+	+	+	+	+	+	+	+
Canis	*B. canis*	-	+	+	+	+	+	+	+	+	+	+

Table 17. The cultural results of 100 cows on seven different media

Cow	Quarter Sample	Medium							*B. abortus* Isolated
		A	B	C	D	E	TSA	TSAEV	
1	RF	0[a]	0	0	0	0	0	0	
	RR	0	0	0	2	+	0	0	strain 2308
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
2	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
3	A[b]	0	0	0	0	0	0	0	
	B	0	0	0	0	0	0	0	
	C	0	0	0	0	0	0	0	
	D	0	0	0	0	0	0	0	
4	A	C	0	C	C	0	C	C	
	B	0	0	0	0	0	0	0	
	C	C	C	0	C	0	C	0	
	D	C	0	0	C	0	C	C	
5	A	0	0	0	C	0	0	0	
	B	0	0	0	0	0	0	0	
	C	C	C	C	C	0	C	C	
	D	0	0	0	0	0	0	0	
6	RF	0	C	C	0	0	0	0	
	RR	0	0	0	C	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	

[a] 0 = No *Brucella* colonies isolated.
+ = Single colony.
2 = 2-10 colonies.
3 = 11-100 colonies.
4 = 101 or more colonies.
C = Contaminated.

[b] Unidentified quarter samples.

Table 17. (continued)

Cow	Quarter Sample	Medium							*B. abortus* Isolated
		A	B	C	D	E	TSA	TSAEV	
7	RF	0	C	C	0	0	C	0	
	RR	C	0	0	C	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
8	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	C	0	
	LR	0	0	0	0	0	0	0	
9	RF	0	0	0	0	0	0	0	
	RR	2	0	0	0	+	0	0	strain 2308
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
10	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
11	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
12	A	0	0	0	0	0	0	0	
	B	0	0	0	0	0	0	0	
	C	0	0	0	0	0	0	0	
	D	0	0	0	0	0	0	0	
13	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
14	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
15	CO[c]	C	C	C	C	0	C	0	

[c]CO = Composite sample.

Table 17. (continued)

Cow	Quarter Sample	Medium							*B. abortus* Isolated
		A	B	C	D	E	TSA	TSAEV	
16	CO	0	C	0	0	0	0	0	
17	A	C	C	C	C	C	C	0	
	B	C	C	C	C	0	C	0	
	C	C	C	C	C	C	C	C	
	D	C	C	C	C	C	C	0	
	E	C	C	C	C	C	C	0	
18	RF	0	0	0	0	0	0	0	
	RR	0	0	0	C	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
19	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
20	RF	0	0	0	0	0	0	0	
21	CO	C	C	C	C	C	C	0	
22	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	C	C	C	C	0	0	0	
23	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
24	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
25	CO	C	C	C	C	0	C	0	
26	RF	3	2	+	3	2	2	2	biotype 4
	RR	C	C	C	0	0	C	C	
	LF	C	C	C	C	C	C	0	
	LR	3	3	3	3	4	3	3	biotype 4

Table 17. (continued)

Cow	Quarter Sample	Medium							*B. abortus* Isolated
		A	B	C	D	E	TSA	TSAEV	
27	A	C	C	C	C	0	C	C	
	B	C	C	C	C	C	C	0	
	C	0	C	0	0	0	C	0	
	D	C	C	C	C	C	C	0	
28	A	2	+	0	0	2	2	2	biotype 1
	B	0	2	+	2	0	0	2	biotype 1
	C	0	0	0	0	0	+	0	biotype 1
	D	0	0	0	0	0	0	0	
29	A	0	0	0	0	0	0	0	
	B	0	+	+	2	0	2	0	biotype 1
	C	3	3	3	3	3	3	4	biotype 1
	D	2	2	2	2	2	2	3	biotype 1
30	A	2	3	2	2	2	0	2	biotype 1
	B	0	0	0	0	0	0	0	
	C	4	4	4	4	4	4	4	biotype 1
	D	4	4	4	4	4	4	4	biotype 1
31	CO	0	0	0	0	0	0	0	
32	A	0	0	0	0	0	0	0	
	B	0	0	0	0	0	0	0	
	C	0	0	0	0	0	0	0	
	D	0	0	0	0	0	0	0	
33	A	4	4	4	4	4	4	4	biotype 1
	B	4	4	4	4	4	4	4	biotype 1
	C	0	0	0	0	0	+	0	biotype 1
	D	0	0	0	0	0	0	0	
34	A	0	0	0	0	0	0	0	
	B	0	0	0	0	0	0	0	
	C	0	0	0	0	0	0	0	
	D	0	0	0	0	0	0	0	
35	CO	C	C	C	C	C	C	C	
36	A	C	C	C	C	C	C	C	
	B	C	C	C	C	C	C	C	
37	CO	C	C	C	C	0	C	0	

Table 17. (continued)

Cow	Quarter Sample	Medium							B. abortus Isolated
		A	B	C	D	E	TSA	TSAEV	
38	RF	0	0	0	0	0	0	0	
	RR	C	C	C	C	0	C	0	
	LF	0	0	0	0	0	C	C	
	LR	C	0	0	C	0	0	0	
39	CO	0	0	0	0	0	C	0	
40	CO	0	C	C	0	0	0	0	
41	CO	C	C	C	C	0	C	C	
42	CO	0	0	0	0	0	C	0	
43	CO	0	0	0	0	0	C	0	
44	LF	0	C	C	0	0	0	0	
45	RF, RR	0	0	0	0	0	0	0	
	LF	0	0	C	0	0	0	0	
	LR	0	0	0	0	0	0	0	
46	RF	0	C	0	0	0	0	C	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	+	+	2	2	0	2	2	strain 19
47	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
48	CO	0	0	0	0	0	0	0	
49	CO	0	0	0	0	0	0	0	
50	CO	0	0	0	0	0	0	0	
51	CO	0	0	0	0	0	0	0	
52	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	

Table 17. (continued)

Cow	Quarter Sample	Medium							*B. abortus* Isolated
		A	B	C	D	E	TSA	TSAEV	
53	A	0	0	0	0	0	0	0	
	B	0	0	0	0	0	0	0	
	C	0	0	0	0	0	0	0	
	D	0	0	0	0	0	0	0	
54	A	C	C	C	C	3	C	2	biotype 1
	B	C	C	C	C	2	C	2	biotype 1
	C	C	C	C	C	0	C	C	
	D	C	C	C	C	0	C	C	
55	RF	C	C	C	0	0	C	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
56	RF	C	C	C	C	C	C	C	
	RR	C	C	C	C	C	C	C	
	LF	C	C	C	C	C	C	C	
	LR	C	C	C	C	C	C	C	
57	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
58	A	0	0	0	0	0	0	0	
	B	0	0	0	0	0	0	0	
59	A	C	0	0	C	0	0	0	
	B	0	0	0	0	0	0	0	
60	CO	C	C	C	0	0	C	C	
61	RF	0	0	0	0	0	0	0	
	RR	0	C	C	0	0	C	0	
	LF	0	C	C	0	0	C	0	
	LR	0	C	C	0	0	C	0	
62	RF	0	0	0	0	0	0	0	
	RR	0	C	C	0	0	C	0	
	LF	0	C	C	0	0	0	0	
	LR	0	C	0	0	0	0	0	

Table 17. (continued)

Cow	Quarter Sample	Medium							*B. abortus* Isolated
		A	B	C	D	E	TSA	TSAEV	
63	RF	C	C	C	C	0	0	0	
	RR	C	C	C	0	0	C	C	
	LF	0	0	0	0	0	0	0	
64	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
65	A	0	0	0	0	0	0	0	
	B	0	0	0	0	0	0	0	
	C	0	0	0	0	0	0	0	
	D	0	0	0	0	0	0	0	
66	CO	0	0	0	0	0	0	0	
67	CO	0	C	C	0	0	C	0	
68	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
69	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	C	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
70	RF	C	C	C	C	C	C	0	
	RR	0	0	C	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	C	C	0	0	C	0	
71	RF	4	4	4	4	4	4	4	biotype 1
	RR	4	4	4	4	4	4	4	biotype 1
	LF	4	4	4	4	4	4	4	biotype 1
	LR	3	4	4	4	4	4	3	biotype 1
72	RR	0	0	0	0	0	0	0	
	LF	4	4	4	4	4	4	4	biotype 1
	LR	4	4	4	4	4	4	4	biotype 1

Table 17. (continued)

Cow	Quarter Sample	Medium							*B. abortus* Isolated
		A	B	C	D	E	TSA	TSAEV	
73	RR	0	C	C	C	0	C	0	
	LR	0	C	C	0	0	C	0	
74	RR	C	C	0	0	0	C	0	
	LR	0	0	0	0	0	0	0	
75	RF	3	3	3	4	4	4	4	biotype 1
	RR	4	4	4	4	4	4	4	biotype 1
	LF	4	4	4	4	4	4	4	biotype 1
76	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
77	RF	0	0	C	0	0	0	0	
78	CO	0	0	0	0	0	0	0	
79	RR	C	0	0	0	0	0	C	
80	CO	0	0	0	0	0	0	0	
81	CO	+	2	2	+	4	3	4	biotype 1
82	CO	3	3	4	3	4	4	4	biotype 1
83	CO	0	0	0	C	0	0	0	
84	CO	C	C	+	C	2	C	2	biotype 1
85	CO	C	C	0	0	0	C	0	
86	CO	4	4	4	4	4	3	4	biotype 1
87	CO	0	C	C	0	0	C	0	
88	CO	+	C	C	0	0	0	2	biotype 1
89	CO	C	C	C	C	0	C	0	

Table 17. (continued)

Cow	Quarter Sample	Medium							*B. abortus* Isolated
		A	B	C	D	E	TSA	TSAEV	
90	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
91	CO	C	C	C	C	2	C	+	biotype 1
92	A	0	0	0	0	0	0	0	
	B	0	0	0	0	0	0	0	
	C	0	0	0	C	0	C	0	
93	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	C	C	0	0	C	0	
	LR	C	C	C	C	C	C	0	
94	RF	0	0	0	0	0	0	0	
	RR	0	C	C	0	0	0	0	
	LF	0	C	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
95	RF	C	C	C	C	0	C	C	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	C	C	0	0	0	0	
96	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	
97	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	0	0	0	0	0	
	LR	0	0	0	0	0	0	0	

Table 17. (continued)

Cow	Quarter Sample	Medium							*B. abortus* Isolated
		A	B	C	D	E	TSA	TSAEV	
98	RF	0	0	0	0	0	0	0	
	RR	0	0	0	0	0	0	0	
	LF	0	0	C	0	0	0	0	
	LR	0	0	0	0	0	0	0	
99	RF	0	0	0	0	0	C	0	
	RR	0	0	0	0	0	0	0	
	LF	0	C	C	0	0	C	0	
	LR	0	0	0	0	0	0	0	
100	RF	0	C	C	0	0	0	0	
	RR	0	C	0	0	0	0	0	
	LF	0	C	C	0	0	C	0	
	LR	0	0	0	0	0	0	0	

Table 18. The culture results of four media using milk samples from 72 cows in the adult vaccination project.

Cow	Sample Dates	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
1	6-28-82	RF	0[a]	0	0	0	
	7-13-82	RR	0	0	0	0	
	7-28-82	LF	0	0	0	0	
		LR	0	0	0	0	
2	2-27-82	RF	2	+	2	3	strain 2308
		RR	2	3	3	3	strain 2308
		LF	2	0	2	2	strain 2308
		LR	5	5	5	5	strain 2308
3	3-31-82	RF	3	3	4	4	strain 2308
		RR	5	5	5	5	strain 2308
		LF	3	4	3	3	strain 2308
		LR	5	5	5	5	strain 2308
4	2-20-82	RF	4	4	4	4	strain 2308
		RR	4	4	4	4	strain 2308
		LF	3	4	3	4	strain 2308
		LR	4	4	3	4	strain 2308
5	3-15-82	RF	2	+	2	2	strain 2308
		RR	3	+	3	3	strain 2308
		LF	0	0	0	0	
		LR	3	3	4	3	strain 2308
6	3-9-82	RF	4	4	4	4	strain 2308
		RR	3	2	3	3	strain 2308
		LF	0	0	0	0	
		LR	4	4	3	4	strain 2308
7	4-20-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	

[a] 0 = No *Brucella* isolated.
+ = Single *Brucella* colony.
2 = 2 to 10 colonies.
3 = 11 to 100 colonies.
4 = 101 to 500 colonies.
5 = 501 or more colonies.
C = Contamination.

Table 18. (continued)

Cow	Sample Dates	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
8	3-9-82	RF	0	0	+	+	strain 2308
		RR	4	3	4	4	strain 2308
		LF	2	+	2	0	strain 2308
		LR	3	3	3	2	strain 2308
9	4-9-82	RF	0	0	0	0	
	4-14-82	RR	0	0	0	0	
	4-28-82	LF	0	0	0	0	
	5-12-82	LR	0	0	0	0	
10	4-1-82	RF	0	0	0	0	
	4-14-82	RR	0	0	0	0	
	4-28-82	LF	0	0	0	0	
		LR	0	0	0	0	
11	3-30-82	RF	0	0	0	0	
	4-14-82	RR	0	0	0	0	
	4-28-82	LF	0	0	0	0	
		LR	0	0	0	0	
12	3-12-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	+	+	strain 2308
13	3-20-82	RF	2	2	3	3	strain 2308
		RR	2	0	2	+	strain 2308
		LF	2	0	2	2	strain 2308
		LR	+	0	0	2	strain 2308
14	3-14-82	RF	+	0	0	0	strain 2308
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	+	strain 2308
15	3-6-82	RF	0	0	0	0	
		RR	2	0	0	0	strain 2308
		LF	5	5	5	5	strain 2308
		LR	4	3	4	4	strain 2308

Table 18. (continued)

Cow	Sample Dates	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
16	4-28-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	C	C	C	0	
17	4-26-82	RF	C	C	C	0	
	5-12-82	RR	0	0	0	0	
	5-24-82	LF	C	C	C	0	
		LR	0	0	0	0	
18	3-12-82	RF	2	2	2	2	strain 2308
		RR	3	3	2	3	strain 2308
		LF	0	+	0	2	strain 2308
		LR	3	3	3	3	strain 2308
19	3-1-82	RF	3	C	3	2	strain 2308
		RR	0	C	0	+	strain 2308
		LF	0	+	+	0	strain 2308
		LR	2	2	2	2	strain 2308
20	4-7-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	3	3	3	3	strain 2308
21	2-24-82	RF	4	4	4	4	strain 2308
		RR	4	4	4	4	strain 2308
		LF	+	0	0	0	strain 2308
		LR	0	0	0	0	
22	3-20-82	RF	0	0	0	0	
	4-7-82	RR	0	0	0	0	
	4-22-82	LF	0	0	0	0	
		LR	0	0	0	0	
23	3-20-82	RF	0	0	0	0	
		RR	0	+	2	0	strain 2308
		LF	3	3	2	3	strain 2308
		LR	0	0	0	+	strain 2308

Table 18. (continued)

Cow	Sample Dates	Quarter Samples	Medium				B. abortus Isolated
			E	TSA	TSAEV	Farrell's	
24	3-20-82	RF	0	0	0	0	
		RR	5	5	5	5	strain 2308
		LF	0	C	0	0	
		LR	5	5	5	5	strain 2308
25	3-30-82	RF	0	C	0	0	
	4-14-82	RR	0	0	0	0	
	4-28-82	LF	0	0	0	0	
		LR	0	0	0	0	
26	3-7-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	+	0	0	strain 2308
		LR	0	0	+	+	strain 2308
27	4-7-82	RF	0	0	0	0	
	4-22-82	RR	0	0	0	0	
	5-5-82	LF	0	0	0	0	
28	2-19-82	RF	3	3	3	4	strain 2308
		RR	0	0	0	0	
		LF	2	2	2	+	strain 2308
		LR	0	0	0	0	
29[b]	4-1-82	RF	0	0	0	0	
	4-14-82	RR	0	0	0	0	
	4-28-82	LF	0	0	0	0	
		LR	0	0	+	0	strain 2308
30	3-18-82	RF	0	0	0	0	
	4-7-82	RR	0	0	0	0	
	4-21-82	LF	0	0	0	0	
		LR	0	0	0	0	
31	4-26-82	RF	0	0	0	0	
	5-12-82	RR	0	0	0	0	
	5-24-82	LF	0	0	0	0	
		LR	0	0	0	0	

[b] *Brucella* isolated only from the milk samples collected on 4-1-82.

Table 18. (continued)

Cow	Sample Dates	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
32	4-21-82	RF	0	0	0	0	
	5-20-82	RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
33	5-3-82	RF	0	0	0	0	
	5-12-82	RR	0	0	0	0	
	5-24-82	LF	0	0	0	0	
		LR	0	0	0	0	
34	3-19-82	RF	0	0	2	+	strain 2308
		RR	3	4	3	3	strain 2308
		LF	0	0	0	0	
		LR	4	4	4	4	strain 2308
35	3-9-82	RF	0	0	2	0	strain 2308
		RR	2	C	2	2	strain 2308
		LF	+	C	0	2	strain 2308
		LR	4	3	4	3	strain 2308
36	3-10-82	RF	+	2	2	0	strain 2308
		RR	0	0	0	0	
		LF	+	0	0	0	strain 2308
		LR	5	4	4	4	strain 2308
37	3-23-82	RF	0	0	0	0	
	4-14-82	RR	0	0	0	0	
	4-28-82	LF	0	0	0	0	
		LR	0	0	0	0	
38	3-27-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	5	5	5	5	strain 2308
39	3-11-82	RF	0	0	2	0	strain 2308
		RR	+	0	2	2	strain 2308
		LF	0	0	0	0	
		LR	5	5	5	5	strain 2308

Table 18. (continued)

Cow	Sample Dates	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
40	4-9-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
41	4-23-82	RF	0	0	0	0	
	5-20-82	RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
42	2-26-82	RF	3	C	3	4	strain 2308
		RR	4	4	5	4	strain 2308
		LF	4	3	4	3	strain 2308
		LR	5	5	5	4	strain 2308
43	2-22-82	RF	2	2	0	+	strain 2308
		RR	4	4	4	4	strain 2308
		LF	4	4	4	4	strain 2308
		LR	3	3	3	3	strain 2308
44	2-25-82	RF	0	0	0	+	strain 2308
		RR	2	+	2	2	strain 2308
		LF	5	C	5	5	strain 2308
		LR	5	3	4	4	strain 2308
45	3-13-82	RF	0	0	0	0	
	4-7-82	RR	0	0	0	0	
	4-21-82	LF	0	0	0	0	
		LR	0	0	0	0	
46	3-3-82	RF	4	4	4	4	strain 2308
		RR	+	+	2	2	strain 2308
		LF	5	5	5	5	strain 2308
		LR	4	4	4	3	strain 2308
47	3-10-82	RF	5	5	5	5	strain 2308
		RR	4	3	4	4	strain 2308
		LF	2	2	2	2	strain 2308
		LR	4	3	4	3	strain 2308

Table 18. (continued)

Cow	Sample Dates	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
48	3-4-82	RF	3	3	3	4	strain 2308
		RR	2	0	C	0	strain 2308
		LF	0	0	0	0	
		LR	0	+	2	2	strain 2308
49	4-7-82	RF	0	0	0	0	
	4-22-82	RR	0	0	0	0	
	5-5-82	LF	0	0	0	0	
		LR	0	0	0	0	
50	3-27-82	RF	0	0	0	0	
	4-14-82	RR	0	0	0	0	
	4-28-82	LF	0	0	0	0	
		LR	0	0	0	0	
51	3-4-82	RF	3	2	2	2	strain 2308
		RR	3	3	3	3	strain 2308
		LF	3	3	3	4	strain 2308
		LR	3	3	3	4	strain 2308
52	3-8-82	RF	2	0	0	+	strain 2308
		RR	0	0	0	0	
		LF	+	2	2	0	strain 2308
		LR	2	+	3	2	strain 2308
53	3-27-82	RF	0	0	0	0	
	4-14-82	RR	0	0	0	0	
	4-28-82	LF	0	0	0	0	
		LR	0	0	0	0	
54	3-8-82	RF	3	3	3	3	strain 2308
		RR	4	3	4	4	strain 2308
		LF	4	C	3	5	strain 2308
		LR	4	2	3	3	strain 2308
55	3-21-82	RF	2	0	0	0	strain 2308
		RR	0	0	2	+	strain 2308
		LF	0	0	0	0	
		LR	0	0	0	0	

Table 18. (continued)

Cow	Sample Dates	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
56	3-24-82	RF	0	0	0	0	
	4-14-82	RR	0	0	0	0	
	4-28-82	LF	0	0	0	0	
		LR	0	0	0	0	
57	3-16-82	RF	0	0	0	+	strain 2308
		RR	+	2	0	2	strain 2308
		LF	0	0	0	0	
		LR	0	+	0	0	strain 2308
58	3-18-82	RF	0	0	0	0	
	4-7-82	RR	0	0	0	0	
	4-21-82	LF	0	0	0	0	
		LR	0	0	0	0	
59	2-26-82	RF	2	2	0	0	strain 2308
		RR	2	2	2	2	strain 2308
		LF	2	0	0	+	strain 2308
		LR	2	2	2	+	strain 2308
60	2-23-82	RF	3	2	3	3	strain 2308
		RR	3	2	3	3	strain 2308
		LF	3	3	3	3	strain 2308
		LR	5	5	5	5	strain 2308
61	3-30-82	RF	0	0	0	0	
	4-14-82	RR	0	0	0	0	
	4-28-82	LF	0	0	0	0	
		LR	0	0	0	0	
62[c]	3-17-82	RF	0	0	0	0	
	4-7-82	RR	0	0	0	0	
	4-21-82	LF	+	0	0	0	strain 2308
		LR	0	0	0	0	
63	3-2-82	RF	0	0	0	0	
		RR	4	C	3	3	strain 2308
		LF	2	0	3	3	strain 2308
		LR	4	4	4	4	strain 2308

[c] *Brucella* isolated only from the milk sample collected on 3-17-82.

Table 18. (continued)

Cow	Sample Dates	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
64	4-21-82	RF	0	C	C	0	
	5-20-82	RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	C	C	0	
65	4-9-82	RF	0	0	0	0	
	4-14-82	RR	0	0	0	0	
	4-23-82	LF	0	0	0	0	
	5-12-82	LR	0	0	0	0	
66	3-20-82	RF	0	0	0	0	
	4-7-82	RR	0	0	0	0	
	4-21-82	LF	0	0	0	0	
		LR	0	0	0	0	
67	2-20-82	RF	0	+	0	0	strain 2308
		RR	4	3	4	4	strain 2308
		LF	4	5	4	4	strain 2308
		LR	4	3	4	4	strain 2308
68	4-2-82	RF	0	0	0	0	
	4-22-82	RR	0	0	0	0	
	5-5-82	LF	0	0	0	0	
		LR	0	0	0	0	
69	3-20-82	RF	0	0	0	0	
	4-7-82	RR	0	0	0	0	
	4-21-82	LF	0	0	0	0	
		LR	0	0	0	0	
70	4-7-82	RF	0	0	0	0	
	4-22-82	RR	0	0	0	0	
	5-5-82	LF	0	0	0	0	
		LR	0	0	0	0	

Table 18. (continued)

Cow	Sample Dates	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
71	3-29-82	RF	0	0	0	0	
	4-14-82	RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	C	0	0	
72	4-2-82	RF	0	0	0	0	
	4-22-82	RR	0	0	0	0	
	5-5-82	LF	0	0	0	0	
		LR	0	0	0	0	

Table 19. The culture results of four media using milk samples from 152 cows submitted from the field for diagnostic purposes

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
1	2-22-82	RF	0[a]	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
2	2-22-82	A[b]	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
3	2-22-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
4	2-23-82	A	0	C	0	0	
		B	4	2	3	4	strain 19
		C	0	0	0	0	
		D	0	0	0	0	
5	3-3-82	RF	0	0	0	0	
		RR	0	C	0	0	
		LF	0	0	0	0	
		LR	0	C	0	0	
6	3-4-82	RF	5	5	5	5	biotype 1
		RR	5	5	5	5	biotype 1
		LF	3	4	3	4	biotype 1
		LR	5	5	5	5	biotype 1

[a] 0 = No *Brucella* isolated.
+ = Single *Brucella* colony.
2 = 2 to 10 colonies.
3 = 11 to 100 colonies.
4 = 101 to 500 colonies.
5 = 501 or more colonies.
C = Contamination.

[b] Unidentified quarter samples designated A through D.

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
7	3-4-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
8	3-11-82	CO[c]	0	C	0	0	
9	3-11-82	CO	0	C	0	0	
10	3-12-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
11	3-12-82	A	0	C	0	0	
		B	0	0	0	0	
12	3-12-82	A	2	2	2	2	strain 19
		B	+	2	2	2	strain 19
13	3-12-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
14	3-16-82	RF	0	C	C	0	
		RR	0	0	0	0	
		LF	0	C	C	0	
		LR	0	C	C	0	
15	3-16-82	RR	0	0	0	0	
		LF	2	2	2	0	strain 19
		LR	0	0	0	0	
16	3-18-82	CO	4	C	4	4	biotype 1

[c]CO = Composite sample.

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
17	3-18-82	CO	2	C	3	3	biotype 1
18	3-18-82	CO	0	C	0	0	
19	3-19-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
20	3-19-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
21	3-19-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
22	3-23-82	RR	0	0	0	0	
		LR	0	0	0	0	
23	3-23-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LR	0	0	0	0	
24	3-23-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
25	3-23-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
26	3-23-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	C	C	0	

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
27	3-23-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
28	3-23-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
29	3-23-82	CO	0	C	C	0	
30	3-23-82	RF	0	C	0	0	
		RR	0	C	0	0	
		LF	0	C	0	0	
		LR	0	0	0	0	
31[d]	3-23-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
		E	0	0	0	0	
32	3-23-82	CO	0	0	0	0	
33	3-25-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
		CO	0	0	0	0	
34	3-25-82	A	0	C	C	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
		CO	C	C	C	0	

[d]Four quarter samples plus a composite sample, unidentified.

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
35	3-25-82	RF	0	0	0	0	
		RR	0	C	0	0	
		LF	0	0	0	0	
		LR	C	C	0	0	
36	3-26-82	RF	5	4	5	5	biotype 1
		RR	3	2	3	3	biotype 1
		LF	2	2	2	2	biotype 1
		LR	5	5	5	5	biotype 1
37	3-26-82	RF	5	5	5	5	biotype 1
		RR	5	5	5	5	biotype 1
		LF	5	5	5	5	biotype 1
		LR	4	3	4	4	biotype 1
38	3-26-82	RF	0	0	0	0	
		RR	0	C	0	0	
		LF	0	0	0	0	
		LR	4	3	4	3	biotype 1
39	3-26-82	RF	0	0	0	0	
		RR	5	5	5	5	biotype 1
		LF	2	0	2	3	biotype 1
		LR	0	C	0	0	
40	3-26-82	RF	0	0	0	0	
		RR	3	2	3	3	biotype 1
		LF	0	0	0	0	
		LR	0	C	0	0	
41	3-30-82	A	0	0	0	0	
		B	0	C	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
42	4-7-82	A	0	C	0	0	
		B	0	C	0	0	
		C	0	C	0	0	
		D	C	C	0	0	

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
43	4-7-82	A	C	C	0	0	
		B	4	3	4	4	biotype 1
		C	2	2	2	0	biotype 1
44	4-7-82	A	0	C	0	0	
		B	0	0	0	0	
		C	0	C	0	0	
		D	0	0	0	0	
45	4-7-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	C	0	0	
46	4-7-82	A	2	2	3	3	biotype 1
		B	3	3	4	4	biotype 1
		C	0	C	0	0	
		D	2	2	2	0	biotype 1
47	4-7-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
48	4-12-83	A	2	C	3	2	biotype 1
		B	0	C	0	0	
		C	0	C	0	0	
		D	C	C	3	2	biotype 1
49	4-12-82	A	C	C	0	0	
		B	C	C	0	0	
50	4-13-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	C	0	0	
		D	0	0	0	0	
51	4-13-82	A	5	5	5	5	biotype 1
		B	3	2	4	3	biotype 1
		C	3	3	3	3	biotype 1
		D	3	3	4	3	biotype 1

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
52	4-13-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
53	4-13-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
54	4-13-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	C	0	0	
55	4-13-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
56	4-13-82	CO	0	C	0	0	
57	4-13-82	A	0	C	0	0	
		B	0	C	0	0	
58	4-15-82	A	2	2	2	3	biotype 1
		B	0	0	0	0	
		C	0	C	0	0	
		D	0	0	0	0	
59	4-15-82	A	+	0	0	0	biotype 1
		B	0	0	0	0	
		C	0	C	0	+	biotype 1
60	4-15-82	A	0	C	0	0	
		B	0	0	0	0	
		C	2	2	2	2	biotype 1
		D	+	0	0	2	biotype 1

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
61	4-15-82	A	3	+	3	3	biotype 1
		B	4	4	4	4	biotype 1
		C	2	2	3	3	biotype 1
		D	3	3	3	3	biotype 1
62	4-15-82	CO	0	0	0	0	
63	4-15-82	CO	0	0	0	3	biotype 1
64	4-15-82	CO	4	4	4	4	biotype 1
65	4-15-82	CO	3	C	3	3	biotype 1
66	4-15-82	CO	5	5	5	5	biotype 1
67	4-15-82	CO	0	0	0	0	
68	4-15-82	CO	0	0	0	0	
69	4-15-82	CO	0	0	0	+	biotype 1
70	4-15-82	CO	0	0	0	0	
71	4-15-82	CO	0	0	0	0	
72	4-15-82	CO	4	3	3	3	biotype 1
73	4-15-82	CO	0	0	0	0	
74	4-15-82	CO	0	0	0	0	
75	4-15-82	CO	2	C	+	3	biotype 1
76	4-15-82	CO	0	0	0	0	
77	4-15-82	CO	0	0	0	0	
78	4-15-82	CO	0	0	0	0	

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
79	4-15-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
80	4-15-82	CO	0	0	0	0	
81	4-15-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	C	C	C	0	
82	4-15-82	RF	0	C	0	0	
		RR	0	C	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
83	4-15-82	CO	0	C	0	0	
84	4-16-82	CO	0	0	0	0	
85	4-20-82	A	0	C	0	0	
		B	C	C	0	0	
		C	0	0	0	0	
		D	C	C	0	0	
86	4-20-82	A	C	C	0	0	
		B	C	C	0	0	
		C	C	C	0	0	
		D	0	C	0	0	
87	4-20-82	A	C	C	0	0	
		B	0	C	0	0	
		C	C	C	0	0	
		D	C	0	0	0	
88	4-20-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
89	4-21-82	A	0	0	0	0	
		B	0	0	0	0	
90	4-21-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
91	4-21-82	RF	0	0	+	0	biotype 1
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
92	4-21-82	RF	0	0	0	0	
		RR	5	5	5	5	biotype 1
		LF	4	5	5	5	biotype 1
		LR	0	0	0	0	
93	4-21-82	CO	0	0	0	0	
94	4-21-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
95	4-22-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	C	0	0	
96	4-23-82	RF	C	C	0	0	
		RR	0	C	0	0	
		LF	0	0	0	0	
		LR	C	C	0	0	
97	4-23-82	RF	0	0	0	0	
		RR	C	C	0	0	
		LF	0	C	0	0	
		LR	0	0	0	0	

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
98	4-23-82	A	0	0	0	0	
		B	0	0	0	0	
99	4-23-82	A	0	0	0	0	
		B	0	0	0	0	
100	4-23-82	A	0	0	0	0	
		B	0	0	0	0	
101	4-23-82	A	5	5	5	5	biotype 1
		B	5	4	5	5	biotype 1
		C	4	4	4	0	biotype 1
		D	4	3	4	0	biotype 1
102	4-27-82	A	C	C	2	3	biotype 1
		B	C	C	C	0	
		C	C	C	0	0	
		D	0	0	0	0	
103	4-27-82	A	0	0	0	0	
		B	0	C	0	0	
		C	C	C	0	0	
		D	0	0	+	0	biotype 1
104	4-27-82	A	0	0	0	0	
		B	0	C	0	0	
		C	0	0	0	0	
		D	3	3	3	3	biotype 1
105	4-27-82	A	3	2	3	3	biotype 1
		B	3	2	3	3	biotype 1
		C	3	2	3	3	biotype 1
		D	0	C	0	0	
106	4-27-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
107	4-27-82	A	2	0	2	0	biotype 1
		B	3	2	3	3	biotype 1
		C	2	2	2	0	biotype 1
		D	0	C	0	0	
108	4-27-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
109	4-29-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
110	4-29-82	A	0	0	0	0	
		B	0	C	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
111	5-4-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	C	C	0	
		D	0	C	0	0	
112	5-4-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
113	5-4-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
114	5-5-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
115	5-5-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
116	5-5-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
117	5-6-82	A	0	C	0	0	
		B	0	C	0	0	
		C	0	0	0	0	
		D	3	C	2	2	biotype 1
118	5-6-82	A	3	C	4	3	biotype 1
		B	0	0	0	0	
		C	C	C	2	2	biotype 1
		D	C	C	3	3	biotype 1
119	5-6-82	A	0	0	2	+	biotype 1
		B	0	0	0	0	
		C	0	0	0	0	
		D	C	0	0	0	
120	5-6-82	A	0	C	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	+	C	3	2	biotype 1
121	5-6-82	A	0	0	C	0	
		B	0	0	0	0	
		C	0	0	0	0	
122	5-7-82	A	0	0	0	0	
		B	0	C	C	0	
		C	0	0	0	0	
		D	0	0	0	0	

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
123	5-7-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
124	5-7-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	C	0	0	
		D	0	0	0	0	
125	5-7-82	A	0	0	0	0	
		B	0	C	C	0	
		C	0	0	0	0	
		D	0	C	0	0	
126	5-7-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
127	5-7-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
128	5-10-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
129	5-11-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
130	5-11-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
131	5-11-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	0	0	0	
132	5-14-82	RF	0	0	0	0	
		RR	0	C	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
133	5-19-82	RF	0	0	0	0	
		RR	0	0	0	0	
		LF	0	0	0	0	
		LR	0	0	0	0	
134	5-20-82	A	0	C	0	0	
		B	0	+	2	+	biotype 1
		C	0	0	0	0	
135	5-20-82	A	0	C	0	0	
		B	0	C	0	0	
		C	0	C	0	0	
		D	C	C	C	0	
136	5-20-82	A	0	C	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	C	C	C	0	
137	5-20-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	+	C	2	2	biotype 1
138	5-20-82	A	0	C	0	0	
		B	0	0	2	0	biotype 1
		C	0	0	0	0	
		D	0	0	0	0	

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
139	5-20-82	A	0	C	0	0	
		B	0	C	0	0	
		C	C	C	0	0	
		D	0	C	0	0	
140	5-20-82	A	0	C	0	0	
		B	0	C	0	0	
		C	0	C	0	0	
		D	0	C	0	0	
141	5-20-82	A	0	C	+	0	biotype 1
		B	0	C	0	0	
		C	4	4	5	5	biotype 1
		D	0	0	0	0	
142	5-20-82	RF	0	C	C	C	
		RR	0	C	C	C	
143	5-20-82	RF	0	C	0	C	
		RR	0	C	C	C	
144	5-20-82	RF	C	C	C	C	
		RR	0	C	0	0	
		LF	0	C	0	0	
		LR	0	C	C	C	
145	5-24-82	CO	5	5	5	5	biotype 1
146	5-24-82	CO	C	C	C	0	
147	5-24-82	CO	0	0	0	0	
148	5-24-82	CO	5	5	5	5	biotype 1
149	5-24-82	CO	0	0	0	0	
150	5-24-82	CO	0	0	0	0	

Table 19. (continued)

Cow	Sample Date	Quarter Samples	Medium				*B. abortus* Isolated
			E	TSA	TSAEV	Farrell's	
151	5-26-82	A	0	0	0	0	
		B	2	2	2	0	biotype 1
		C	0	0	0	0	
152	5-26-82	A	0	0	0	0	
		B	0	0	0	0	
		C	0	0	0	0	
		D	0	2	0	0	biotype 1