Genital Vibriosis In Iowa Cattle

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Vibriosis in cattle was first reported by McFadyean and Stockman in cases of abortion in England in 1913. Later, from 1918 to 1923, Smith and his associates reported on cases of virbriosis associated largely with abortions in New Jersey. Interest in vibriosis as a cause of abortion or genital disease in cattle was eclipsed in the years that followed by the great interest in the study and control of brucellosis. During the thirties it was assumed by many people in the veterinary profession that almost all abortions and genital diseases in cattle were caused by *Brucella abortus*. In 1939, a report from the Iowa State University Veterinary Diagnostic Laboratory by Packer called attention to an aborted bovine fetus of three months development from which *Vibrio fetus* was isolated. It was pointed out that this was a condition that was not caused by *Brucella abortus* and that *Vibrio fetus* infection should be kept in mind when dealing with breeding troubles in cattle. In 1947, Plastridge, Williams and Petrie reported that there was a connection between vibrio infection and reduced breeding efficiency in cattle. These observations were confirmed by similar findings of the Dutch researchers, Sjollema, Stegenga and Terpstra in 1949. Following this report, *Vibrio fetus* was found to be the cause of enzootic infertility that had been observed in some European and Scandanavian countries. During the decade from 1950 to 1960, much work was carried out in many different countries to develop methods of characterizing various strains of *Vibrio fetus* and to develop diagnostic and control measures. Because the serum agglutination test had proven very effective as a diagnostic method in the brucellosis eradication campaign in the United States, much of the *Vibrio fetus* research of this country dealt with the development of a serological test for vibriosis. In Iowa, for instance, during the period from 1945 to 1955 much work was carried out to investigate herds which manifested symptoms of infertility that were suggestive of vibriosis. *Vibrio fetus* was isolated in pure culture on numerous occasions from aborted fetuses submitted to the Veterinary Diagnostic Laboratory, Ames, Iowa. In some instances the agglutination tests showed much promise. The dams of aborted fetuses which yielded *Vibrio fetus* showed higher agglutination titers than did non-aborting cows, but in general there was a great amount of variation and inconsistency in results. The

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difficulties experienced in interpreting the results of herd studies because of false positive tests in herds which were not experiencing abortions or infertility were so great that efforts to perfect this method of diagnosis were abandoned. (Packer 1963)

The problems of herd infertility that were called to the attention of the Veterinary Obstetrics staff of the Iowa State University Clinic were largely herds that were using a breeding program which employed a herd sire, either exclusively or in combination with artificial insemination. The majority of the problems were beef herds using natural service. Many instances of devastating infertility were traced to the use of newly acquired bulls. The symptoms fit the classical description of vibriosis or trichomoniasis. *Trichomonas fetus* was isolated in a few instances, but attempts to demonstrate *Vibrio fetus* from semen or preputial material resulted in negative findings. (Emmerson 1963)

In 1950, Ruebke studied the bacterial flora of the male genital tract using 50 apparently normal bulls and steers from Iowa. He found that many genera of bacteria normally inhabit the prepuce of the bull but did not report finding any bacteria of the genus *Vibrio* in his survey. The preponderance of fast growing types of bacteria in the bull's prepuce presents a serious problem in the isolation of vibrio from suspected bulls. Unless special methods are used, it is impossible to isolate vibrios from the grossly contaminated material of the prepuce. Thus the attempts to demonstrate this organism in suspected bulls failed to yield positive results in many of the investigations that were carried out in Iowa. An additional problem is the method by which the preputial sample is collected. During the decade from 1950 to 1960, the pipette and rubber bulb method was employed in which a small amount of smegma containing epithelial debris and other material in the prepuce was scraped into the end of a plastic pipette. This material was then suspended in saline which was later examined for trichomonads and submitted to bacteriological examination. The bacteriological reports on this material and on semen invariably showed the isolation of *Proteus, Pseudomonas, Escherichia coli* or some other organism that is a frequent preputial inhabitant.

From 1950 to 1960, research workers were very active in the field of vibrio investigations. Much of the work of this period is summarized in the proceedings of a panel of experts on vibriosis organized by the Food and Agriculture Organization of the United Nations. A report of this group was edited by Laing. (1960)

After techniques were developed to isolate the vibrio organism, studies on the pathogenicity, biochemical characterization of various strains and host specificity and relationships remained imminent. Bryner and Frank (1955) reported a method of identification of *Vibrio fetus* based on biochemical characteristics. This work differentiated vibrios on the basis of the reaction to the catalase test and the production of hydrogen sulfide. The catalase positive vibrios were thought to be true *Vibrio fetus* and the catalase negative vibrios that produce large quantities of hydrogen sulfide were not incriminated as causing abortion and infertility in cattle and later were given the name *Vibrio bubulus.*

Florent (1956) explained that *Vibrio fetus* is divided into two types: *Vibrio fetus intestinalis* and *Vibrio fetus venereal is. Vibrio fetus intestinalis* flourishes in media containing 1% glycine and is an inhabitant of the intestinal tract of cattle, sheep and swine. It is not able to survive in the bovine vagina but is the cause of ovine vibrionic abortion and sporadic abortion in cattle.

Vibrio fetus venerealis will not grow in media containing 1% glycine; its predilection site is the prepuce of the bull and genital tract of the cow and heifer. It is able to survive in the bovine vagina, cause infertility and occasionally abortion in cattle. This is the organism that is of greatest interest in the study of vibriosis in cattle.

At the present time, these bases of biochemical characterization are widely accepted as methods for determining the types and pathogenicity of vibrio strains.

There is a great amount of evidence that serological methods using the complement fixation test and other tests related to the antigenic structure of the organism would be a more reliable method of classifying vibrios. (Mitscherlich and Liess, 1958) Additional research is needed to complete our understanding of the various strains of vibrio, their pathogenicity and host specificity.

Epidemiology of Vibriosis in Cattle

Infection with *Vibrio fetus venereaalis* is almost always venereal. Transmission by any other means is very rare. Either the cow or the bull may spread the disease. The infection may be brought into a herd by the introduction of an infected, non-pregnant female or one that aborts after introduction into the herd. The male then becomes infected and exposes all of the females he breeds. More frequently the infection comes into a herd by means of a purchased, borrowed, cooperatively owned or rented bull. No clinical abnormalities can be observed in bulls which are carriers or transmitters of the organism.

The infection may also be transmitted by artificial insemination if semen containing viable *Vibrio fetus* organisms is used. This possibility is highly improbable when semen is produced by artificial insemination establishments that employ rigid health examinations and frequent periodic examinations on bulls before admission to the stud. Most outbreaks of the disease in Iowa occur in herds which are using natural service exclusively or a combined program of natural and artificial service. Once infected, bulls remain carriers for a long time; periods up to six years have been reported.

Symptoms

In the exposed susceptible cow or heifer, infection is first acute but gradually develops into a chronic form. Infertility is usually associated with the acute form of the disease. In the early stages of the disease, vaginal examination may show a catarrhal vaginitis. The cervix and vaginal mucous membrane may be hyperemic and edematous. In a newly infected herd about 15 to 45% of the animals will conceive on the first service. The remainder will return to estrus, often repeatedly with interservice periods being irregular and frequently long. When mating is continued, most animals become pregnant after approximately 90 days or longer. After this, the disease becomes chronic and fertility will be fairly normal. The abortion rate with this disease is usually low. Abortions are most common about the fifth or sixth month and may occur in those cows that become pregnant to the first service. In chronically infected herds, the older cows are largely resistant and the symptoms of infertility are manifest mainly in the heifer. In many areas, chronically infected herds are maintained and the reproductive rate is not as good as it should be, but the condition remains undiagnosed. Breeding bulls from a herd such as this could easily introduce the infection into a vibrio-free herd. In newly infected herds, the delay in conception in a high percentage of the females of the herd upsets the breeding program so that the conception rate is low and many cows that do conceive drop their calves in late summer or fall. This creates an inefficient program. In several instances purebred herds in Iowa have suffered severe financial losses.

Diagnosis, Treatment and Control

Isolation of *Vibrio fetus venerealis* is the most direct method of diagnosis. This may be done by culturing the cervical mucus of suspect cows or by culturing the cervical mucus of suspect cows or by culturing the semen or preputial washings from suspect bulls. There has been a great amount of interest in testing bulls because infected bulls in an artificial insemination center may be a focus for wide distribution of the disease. Bulls may be tested by the direct culturing method or by test-mating of heifers. Test-mating of heifers has been used extensively in Denmark. (Adler 1957) This system involves

depositing the sediment from preputial washings of suspect bulls into the cervix and uterus of non-infected heifers. This method uses the cervix and uterus as a selective culture medium in which the vibrio organism will flourish while the contaminants are eliminated. At a later time a pure culture of vibrio can be recovered from the heifer if the inoculating material came from an infected bull.

The direct culturing method presents the problem of contending with the contaminating organisms that may over-grow the plates and obliterate the vibrio organism.

Indirect methods such as serological tests and cervical mucus agglutination tests are subject to great variation. They are considered by some to have value when used on a herd survey basis but are of very little value in determining whether or not the infection is present in a particular individual.

Controlling the disease by treatment does not seem to be very successful. Antibiotic infusions into the uterus of infected cows has been reported to shorten the convalescent period in some cases. Successful treatment of infected bulls has been reported by Garm *et al.* (1953) and many other investigators but there is some question as to how permanent the elimination of the infection from the prepuce is. Antibiotic treatment of semen to be used for artificial insemination has been reported to be effective in controlling the spread of vibriosis. (Elliott *et al.* 1961) This is a poor substitute for using only vibrio-free bulls in bull studs.

The types of beef cattle programs that are prevalent in Iowa employ natural service to a large extent. Herd sires are frequently transferred from one herd to another on a variety of arrangements such as sale, cooperative ownership, loaning or rental basis. Frequently, even in the sales of young bulls, the individuals are allowed to serve several females before sale to ascertain the bull's capability as a breeder. This type of interchange of bulls between herds provides a natural means for the dissemination and spread of venereal infections including vibriosis.

In designing a control program, the problem of establishing or maintaining a herd of beef cattle that is free of the infection arises. There are three alternatives for preventing the exposure of females: exclusive use of artificial breeding using semen from vibrio-free bulls; purchase of herd sires that have never been previously used for breeding; purchase of older bulls from herds which are known to be free of the disease if suitable diagnostic tests have first been applied to them and have been found to be negative. The need arises for having a test procedure that is suitable for testing bulls that are to be taken into artificial breeding centers and also for testing bulls to be taken into vibrio-free herds as herd sires. The test procedure employed in this survey would be suitable for this purpose. It could also be used for the diagnosis of vibriosis in problem herds.

Because the contaminating organisms present in the bull's prepuce multiply more rapidly than the vibrio organisms, it is necessary to streak the plates containing the selective medium within 6 to 8 hours after the preputial sample is collected. A greater time will allow the contaminating organisms to outgrow and out multiply the vibrio so that the vibrio colonies will not appear after the plates are streaked. Refrigeration of the samples during this time is desirable.

Soderlind (1960) studied 32 cases in which the semen and the washings were examined simultaneously. In 19 cases both materials were found positive, in 7 cases only the semen was positive and in 6 cases *Vibrio fetus* could be determined in the washing only. This indicates that where possible it is best to culture both the semen and the preputial washings.

Material and Methods

The samples in this study were collected from bulls that were brought to the Iowa State University Veterinary Clinic for fertility examination and from bulls that were examined for artificial breeding associations during the years 1961 and 1962.

The method for collecting the preputial samples has been described by Eide (1957) in Norway. The method of cultur-

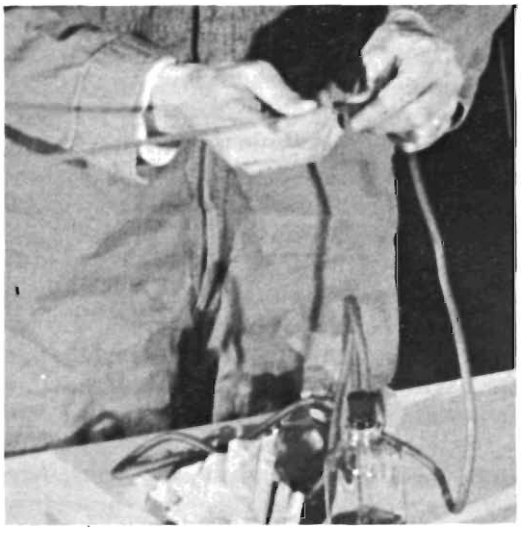

Figure 1

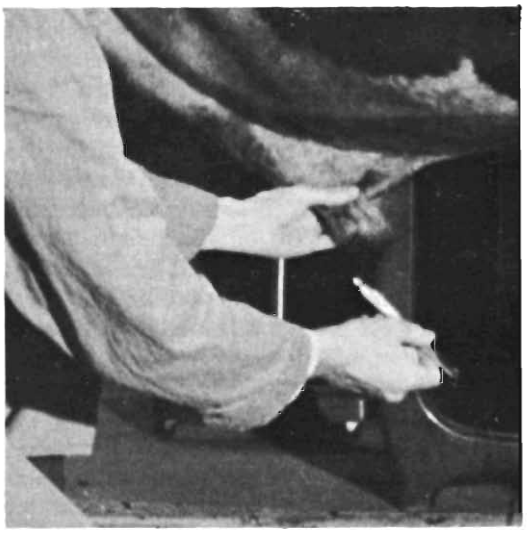

Figure 3

ing *Vibrio fetus* on the special selective medium was originally described by Florent *et al.* (1956) in Belgium and later was modified by Sazegaard (1962) in Norway. These methods will be described in detail because they have not yet appeared in the literature of this country.

The equipment used in collecting the sample is taken from the sterile package and assembled just prior to obtaining the sample (Fig. 1) The external preputial orifice of the bull to be sampled is massaged until the bull urinates. The preputial area is then cleaned and dried. (Fig. 2) A sterile glass tube six inches long and one half inch in diameter is inserted into the preputial orifice. (Fig. 3) A sterile plastic inseminating pipette is attached to a four foot length of sterile rubber tubing and passed through the glass tube into the preputial cavity. (Fig. 4) The six inch glass tube is pulled back and the ex-

Figure 2

Figure 4

ternal preputial orifice compressed around the inseminating tube by hand pressure. The rubber tubing is connected to a bottle containing 125 ml of thiol broth (Difco No. B 434). Thirty grams of the powder and 1000 ml of distilled water are sterilized in the usual way. After cooling 1% bacto suplement B (Difco No. B276) may be added aseptically and the medium is put into sterile bottles in 125 ml amounts.

The thiol medium is allowed to pass into the preputial cavity by gravity flow and prevented from spilling out of the preputial orifice by continued hand pressure around the pastic pipette. (Fig. 5)

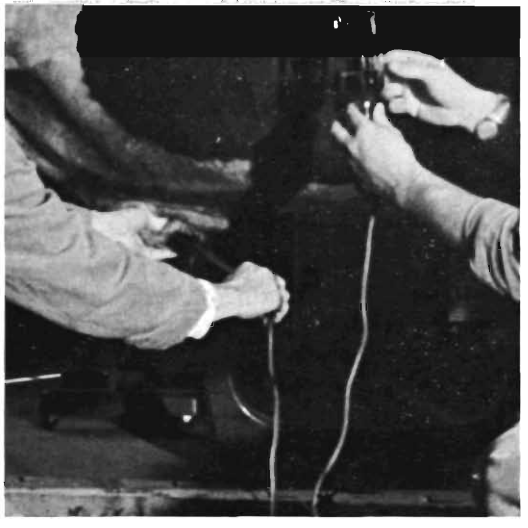

Figure 5

The fluid in the preputial cavity is massaged vigorously for four minutes. At the end of this time the bottle is inverted and lowered and the fluid is drawn back into the bottle by the suction apparatus. (Fig. 6) If good technique is used, a sample containing only organisms present in the preputial cavity can be collected.

Ruebke (1950) studied the bacterial flora of the bovine male genitalia and found that the prepuce commonly contained organisms of the genera *Alcaligenes, Bacillus, Bacterium, Corynebacterium, Escherichia, Micrococcus, Proteus, Pseudomonas* and *Streptococcus*. The members of the genera *Micrococcus* and *Corynebacterum* were the predominant organisms found in the specimens.

The *Proteus, Pseudomonas, Streptococci, Micrococci* and *Escherichia* are the organisms that frequently cause an overgrowth of plates and obliterate the results of cultural studies for virbrio. Because the *Vibrio* genus is a slow growing group, ordinary cultural methods do not yield satisfactory results for the diagnosis of vibriosis from preputial washings.

A special medium is necessary for the primary isolation of vibrios. As a basal medium, thioglycollate medium without dextrose or indicator (Difco No. B 430) is used. To this medium 2% agar agar is added. The medium is prepared according to the usual practice and after sterilization and cooling to 45° C, the following substances are added:

1. 10% defibrinated cattle blood.
2. Brilliant green in concentrations of 1:25,000 and 1:50,000. A stock solution of 0.2% brilliant green is made. To each 110 ml of medium (100 ml thioglycollate medium plus 10 ml blood), 2.2 ml (1:25,000) and 1.1 ml (1:50,000) of the 0.2% brilliant green solution is added, respectively. It has been pointed out by Florent (1956) that the brilliant breen has an inhibitory effect on fecal streptococci.
3. Novobiocin in a concentration of 1:50,000. A stock solution of 0.5% novobiocin is made. To each 110 ml of the medium (100 ml of the thioglycollate medium plus 10 ml blood) 0.55 ml of the 0.4 % novobiocin solution is added. Saxegaard (1962) has pointed out that the novobiocin has an inhibitory effect on *Proteus*.

After thorough mixing the medium is poured into petri dishes at a thickness of five mm.

For inoculation of the plates an appropriate amount of the sample from the bull's prepuce is divided into sediment and supernate either by gravity or centrifuging for five minutes at 3000 RPM. Eight plates are inoculated altogether.

Figure 6

Four plates are inoculated with the sediment and four with the supernate from about five mm. below the surface. To insure that abundant inoculation is made, loops of inoculum are spread over the plate as much as possible. (Fig. 7) One half of the plates will have a concentration of 1:25,000 and one half a concentration of 1:50,000 brilliant green. It is interesting to run concurrent controls of plates of thioglycollate medium without any inhibitors. The plates are incubated at 37° C for six days in closed containers in an atmopshere consisting of 5% oxygen, 10% carbon dioxide and 85% nitrogen. (Fig. 8)

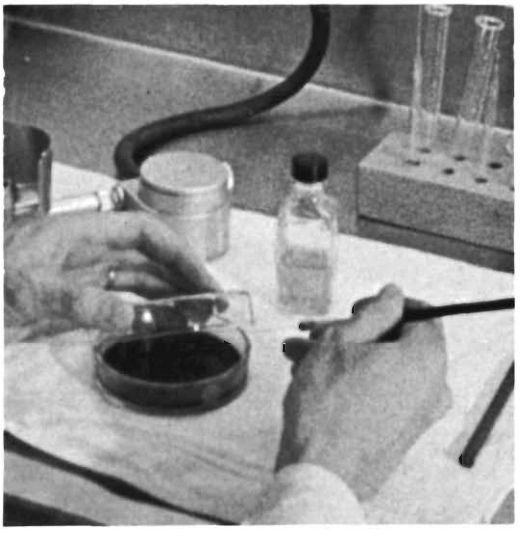

Figure 7

The plates are examined on the third and the sixth days after inoculation. Growth of vibrio colonies is usually not profuse at three days. The colonies of contaminating bacteria are usually restricted by the inhibitors and after six days the vibrio colonies will be visible between the larger colonies if they are present. Many of the plates with additive will have only vibrio colonies growing on them. (Figs. 9 and 10) The plates are examined under a stereomicroscope and suspicious colonies are suspended in saline and examined under the phase contrast microscope. The typical vibrio morphology and motility are easily identified by this method. Colonies of vibrio are inoculated in tubes containing thiol broth (Difco No. B434) to which

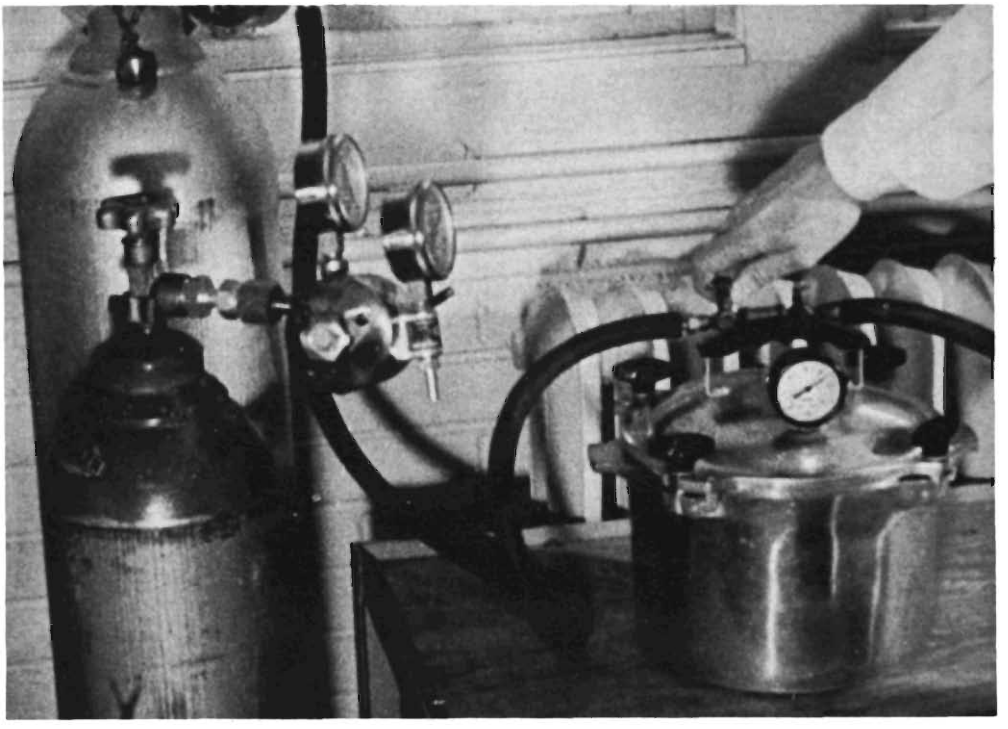

Figure 8

is added 0.1% agar agar and enriched with 1% bacto supplement B. Vibrio will grow in this medium a little below the surface in an ordinary incubator at 37° C in normal air environment. A three day growth in this medium is used to inoculate tubes containing media for biochemical characterization of the vibrio isolated.

Biochemical tests used to characterize the vibrio strains recovered were catalase, H_2S, 3.5% NaCl tolerance and 1% glycine tolerance according to the work of Bryner and Frank (1955) and Frank and O'Berry (1962).

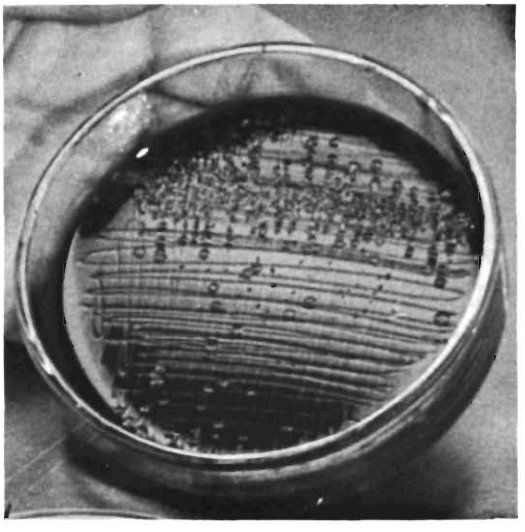

Figure 9

Figure 10

Results

The following is a summary of the bacteriological findings of the seventy-eight bulls studied.

Vibrio fetus venereale	10 bulls
Vibrio bulbus	19 bulls
Pseudomonas sp.	15 bulls—One bull yielded both *Pseudomonas* and *Vibrio bubulus.*
Escherichia coli	32 bulls—6 bulls yielded both *Eschichia coli* and *Vibrio bubulus.*
Corynebacterium sp.	13 bulls
Streptococcus sp.	3 bulls
Proteus vulgaris	9 bulls
Micrococcus	3 bulls
Slime bacteria	1 bull

The selective medium was quite successful in reducing the colonies of *Streptococcus* and *Proteus. Pseudomonas* was the contaminant which spoiled the most plates. Tthe *Pseudomonas* colonies even seemed to spread out over a larger area on those plates containing the additives. *E. coli* was not inhibited in some cases but the colonies seemed to be restricted by the selective medium to the extent that when present, they did not obliterate the vibrio colonies.

Conclusions

1. According to the results of this survey, it is concluded that the use of brilliant green and novobiocin inhibitors in media for the primary isolation of vibrio organisms from preputial washings of bulls is a valuable tool in the diagnosis of bovine genital vibriosis.
2. The method of infusing fluid into the preputial cavity fololwed by massage for four minutes before withdrawal yields a sample which represents the bacterial flora of all parts of the prepuce better than methods previously used.
3. Because *Vibrio fetus venerealis* was isolated from over 10% of the bulls tested in this survey, it is concluded that *Vibrio fetus venerealis* infection is definitely present in Iowa cattle and should be considered a possibility when dealing with problems of infertility in the state.

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