

AN INVESTIGATION OF THE BACTERIAL FLORA OF MAMMARY GLANDS
OF DAIRY CATTLE WITH SPECIAL REFERENCE TO PATHOGENS PRO-

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OF DAIRY CATTLE WITH SPECIAL REFERENCE TO PATHOGENS PRO-
DUCING MAMMITIS.

conducted - September, 1925 - but two or three
cases of mammitis developed in the herd, and these cases
were not found to be of I. FOREWORD. origin. As will be
pointed out later, the absence of mammitis in this herd

may have been. The original problem as outlined was an inves-
tigation of the bacterial flora of the mammary glands of
cattle of a large dairy herd, with special reference to
the pathogenic organisms producing mammitis; and also an
investigation of the possibility of immunizing these
cattle against the incriminating organisms. This problem
was selected because mammitis is a constant source of
annoyance in all large dairy herds, causing loss in both
milk production and in the value of the animals affected,
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consumers of raw milk. The herd to be studied had in par-
ticular realized considerable loss from the effects of the

disease.

II. INTRODUCTION.

However, during the period when this work was conducted - September, 1924 to April, 1925 - but two cases of mammitis developed in the herd, and these cases were not found to be of pathogenic origin. As will be pointed out later, the absence of mammitis in this herd may have been caused by a change in the system of management. This lack of material with which to work necessarily caused a change in the problem, and the bacterial flora of the mammary glands of cows that had been affected with mammitis the previous year was studied to determine the possible presence of carriers of the disease. Due to the absence of pathogenic organisms in the herd, the problem of immunizing the cattle against the organisms was necessarily given up. In fact, not until recent years has the prevalence of mammitis been realized, and the author believes that its

II. INTRODUCTION.

... true importance from an economic standpoint has not yet been fully appreciated. When it does reach its true sta-
Importance of Mammitis. Mammitis is probably one of the most important diseases affecting dairy cattle. With the develop-
... stringent laws regulating its control, traffic, and treat-
ment of the dairy cow through scientific breeding and feeding,
... the one aim in view being to make each individual cow produce
... more milk, she has become what might be termed a specialized
... factory in which two single machines, her digestive and re-
productive systems, are being continually worked to full ca-
pacity. When an organ of any animal is being worked to its
limit, it is the one organ in the whole body that is most
open to disease. Consequently we find that inflammation of
the mammary gland, or mammitis, presents a problem that is
extremely important for the dairyman. Moreover, the higher
production is not temporary, for when the cow recovers she
... the grade of product that he attempts to produce, the more
important it becomes for him to keep mammitis from his herd.
In fact, not until recent years has the prevalence of
mammitis been realized, and the author believes that its

4.

been replaced with connective tissue. Its true importance from an economic standpoint has not yet been fully appreciated. When it does reach its true status as an important disease, there will undoubtedly be stringent laws regulating its control, traffic, and treatment.

Economic Loss Due to Mammitis. Loss due to the presence of mammitis in a dairy herd may be realized in several ways.

When mammitis occurs, milk secretion is suppressed or entirely stopped. If milk is secreted, it is unfit for human consumption. If milk is being produced for certified trade or an inspected market, that from affected cows must be excluded during the progress of the disease, thus causing it to be an entire loss to the producer. This decrease in lactation history of such cow in the herd, the oldest of which had six lactation periods. From the work of Mr. Franklin, the author has arrived at the following figures: From a total of 20 head of purebred milking cows, 13 had shown no signs of being affected with mammitis. Of

5.

been replaced with connective tissue. Moreover, not only is production lessened and the value of the animal as a producer impaired, but the actual sale value of the affected animal is decreased. There is always the possibility that a cow that has once had the disease and recovered, will be a carrier of the microorganisms causing mammitis and thereafter be a menace to the rest of the herd.

The economic importance of mammitis, and the value of efficient management may be realized from the following facts. In 1922 and 1923, Mr. U.D. Franklin (1) studied the history of mammitis in a dairy herd that was producing milk under approved sanitary conditions. He reviewed the complete lactation history of each cow in the herd, the oldest of which had six lactation periods. From the work of Mr. Franklin, the author has arrived at the following figures:

1924, From a total of 20 head of purebred milking cows, 13 had shown no signs of being affected with mammitis. Of

these 13 individuals, however, 7 were in their first lactation period. Of the 7 remaining cows that had been affected with the disease, 1 had apparently recovered, 4 had chronic mammitis with a consequent lessened production, and 2 had been killed because of the disease. In other words, 35% of the herd had been affected. During the winter of 1924, under a different method of management, not a single case of mammitis occurred in the same herd. another. The herd used for the present experiment was likewise conducted under approved sanitary conditions, and while it was situated on the same farm as the herd previously described, the two herds were always handled separately. No history of mammitis for this herd is available farther back than two years, except the statement that the disease had continually been present and had been the cause of a constant source of loss. From September, 1923, to September, 1924, there was a total of 42 cows milked in this herd, 12 covered. All of the cows that had been affected the pre-

7.

of which were in their first lactation period. Nine of these 42 cows were affected with mammitis during this period of a year, and 2 more showed evidences of having had the disease sometime previously and had not recovered from the effects of the disease. This made a total of 11 cows, or 26.8% of the herd that had suffered from the disease during a period of one year. During this time, 3 of the 11 cows died from the effects of mammitis; in another, one quarter of the gland ceased to function and another quarter functioned but partially; 6 of these animals had chronic mammitis during the entire lactation period. So it may be seen that the effects of the disease are not merely temporary, but cause a constant source of loss.

From September, 1924 to April, 1925, under a different system of management, but two cases of mammitis occurred in this same herd, and both animals apparently recovered. All of the cows that had been affected the pre-

this herd because of chronic mammitis doubled the number
reacting to tuberculin. He also states that on another
farm where the general sanitary conditions were excellent
disease.

approximately 10% of the cows in one herd had mammary gland
inflammations
To a person who does not realize the prevalence
of mammitis in dairy herds, the following citations should
serve to show that the disease is widespread and a cause of
great economic loss:

supply, and in some cases it has been stated that the
organisms producing mammitis have been
Yates (2) in describing the effect of mammitis in
certified dairies, states that of 16,000 head examined semi-
annually, mammary gland infections are responsible for 95%
of the rejections.

Dr. G. K. Cooke (4) states that in an epidemic of
septic sore throat in a city of 270,000 population
cases and 22 deaths occurred in 5 days among people
drinking milk from a single cow milk dairy that was rated as
one of the best in the city. On that dairy, however,
streptococci were isolated to almost all cultures from the
inflamed mammary gland of a milking cow in this herd from
a milkery that was the source of the outbreak of septic sore throat
patients. As the strains of streptococci were all of the

this herd because of chronic mammitis doubled the number reacting to tuberculin. He also states that on another farm where the general sanitary conditions were excellent approximately 10% of the cows in one barn had mammary gland inflammations.

Relation of Mammitis to Public Health. Epidemics of septic sore throat have frequently been attributed to the milk supply, and in many cases it has been stated that the organisms producing mammitis have been the causative agents of the sore throat epidemic.

Dr. G.K.Cooke (4) states that in an epidemic of septic sore throat in a city of 270,000 population, 487 cases and 22 deaths occurred in 5 days among people consuming milk from a single raw milk dairy that was rated as one of the best in the city. On investigation, hemolytic streptococci were isolated in almost pure cultures from the inflamed mammary gland of a milking cow in this herd, from a milker's throat, and from the throats of septic sore throat patients. As the strains of streptococci were all of the

human type, he states that it is possible that the milk-
or infected the cow's mammary gland. A.C. Evans (5) states that, "When milk borne
streptococcal infections are discussed, it is generally
streptococcus hemolyticus which is under consideration,
the streptococcus which causes mastitis in cattle and sore
throat in man."

Jones (3a) made examinations of the vaginal dis-
charges, saliva, feces, and skin of cows in a large herd pro-
ducing certified milk. The most frequent types of strep-
tococci which he observed in the market milk from this herd
were identical with those causing mammitis. But during
a period of 2 years, diseases traceable to the milk supply
were not reported, which evidence he considered as elimi-
nating mammitis as a source of severe epidemics of milk
borne sore throat.

Jones (6) states that mammary gland infection
with the human organism is relatively uncommon; neverthe-
less, such infections are liable to occur at any time. In

herds producing raw milk the rejection of milk from all cases of mammary gland disease is essential, until definite proof is furnished that they are not associated with streptococci pathogenic for man.

Investigation, and the most accurate method of differentiating them, a thorough review of all accessible literature pertaining to mammitis has been made, and a comparison chart of these organisms prepared (Table I.) The work that has been done in this subject since Nogard and Heileress made their first study of the etiology of bovine mammitis, in 1884 (7), is voluminous. To attempt to review all of the work would be both impossible and impracticable. Many of these studies have but little bearing on this experiment because recent methods used in the study of microorganisms do not correspond with those used in many of the earlier works. In the course of 40 years, technique used in bacteriology laboratories and methods of differentiation of the various microorganisms have changed, and knowledge of microbiology has ad-

vanced rapidly.

III. REVIEW OF LITERATURE.

Studies of Mammitis in Foreign Countries. Steiger (8)

summarized his bacteriological findings from 46 cases as follows:

In order to determine what organisms might be found in the present investigation, and the most accurate method of differentiating them, a thorough review of all accessible literature pertaining to mammitis has been made, and a comparison chart of these organisms prepared (Table I.) The work that has been done in this subject since Nocard and Mollereau made their first study of the etiology of bovine mammitis, in 1884 (7), is voluminous. To attempt to review all of the work would be both impossible and impracticable. Many of these studies have but little bearing on this experiment because recent methods used in the study of microorganisms do not correspond with those used in many of the earlier works. In the course of 40 years, technique used in bacteriology laboratories and methods of differentiation of the various microorganisms have changed, and knowledge of microbiology has ad-

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Studies of Mammitis in Foreign Countries. Steiger (8)

summarized his bacteriological findings from 46 cases as

follows: contagious mammitis caused by *B. lactis aerogenes*.

Staphylococcus mastitidis *flex* the disease etiology. 6 cases

Galactococci whether it is caused by members of *colou* 10 "

Streptococci paratyphoid or enteritidis groups, 10 " the

B. coli staphylococci or streptococci. 14 "

Mixed infections (*B. coli*, streptococci, mammary gland in-
fections into two *B. necrophorus*) 6 "

Savage (9) recorded examinations of milk from 31
cases of mammitis as follows: acute mastitis of an acute

due to Streptococci character. 21 cases

" " Staphylococci Streptococci. 5 "

" " *B. coli* 2. Staphylococci. 1 "

" " *B. Tuberculosis* *colou bacilli* 1 "

of doubtful origin Interstitial mastitis of a chronic 3 "

Henderson (10) in examining 14 cases of mammitis

1. *B. pyogenes*.

2. *B. tuberculosis*.

14.

believed he was dealing with a mixed infection of streptococci and *B. coli* in 12 of the animals.

Gilruth and Macdonald (11) reported an outbreak of acute contagious mastitis caused by *B. lactis aerogenes*.

Kitt (12) classifies the disease etiologically according to whether it is caused by members of the colon group, of the paratyphoid or enteritidis groups, or of the groups of staphylococci or streptococci.

Wall (13) divides the types of mammary gland infections into two groups:

I. Simple or single mammary gland infection.

A. Parenchymatous mastitis of an acute character.

1. Streptococci.

2. Staphylococci.

3. Colon bacilli

B. Interstitial mastitis of a chronic character.

1. *B. pyogenes*.

2. *B. tuberculosis*.

3. Actinomyces fungus.

4. Necrosis bacillus.

II. Mixed infection.

Includes the same type of organisms as in

simple infections and is usually originally

single infection.

While these studies were conducted in countries other than the United States and afford no positive statement as to the types of organisms that should be found in the present investigation, they do serve to give a general idea of the types of organisms that might be present in cases of mammitis. From the results of these studies, the following organisms might be considered as causing mammitis in France, England, and Germany:

1. Members of the Staphylococcic group.

2. Members of the Streptococcic group.

3. Members of the Colon group.

As the herd under consideration in this work is

an Accredited Tuberculosis Free Herd, the presence of Bact. tuberculosis need not be considered; and since there are no cases of chronic interstitial mammitis with pus formation, B. pyogenes and B. necrophorus may be eliminated. ~~may gain entrance are not alike capable of~~

Early Studies of Mammitis in the United States. The

following reviews are from work that has been carried on in the United States and, the author believes, includes nearly all the work that has been done in this country relative to the etiology of mammitis. ~~of a new short-~~
~~ly after be~~ These reviews are given in chronological order and are divided into two somewhat distinct groups. The first group, dating from 1897 to 1913, contains a brief review of the earlier work done in this country, and is principally a study of the bacterial flora of the mammary glands of dairy cows. The second group contains recent work in which modern technique has been employed.

acidified Dinwiddie (14) states that the species of the organisms that may be found within the teat canal and the ducts, are subject to wide variety in different localities, being dependent on external infection. All species that may gain entrance are not alike capable of maintaining their foothold. inoculation of 0.5 cc. of a

fresh bouill A species which grows and curdles milk equally well with or without the presence of oxygen, and at 37°C. curdles milk in tubes in 12 hours, was injected through a milk tube into the milk cistern of a cow shortly after being milked. It caused no disturbance in function of the gland, and no curdled milk was passed.

Moore (15) examined the milk from 9 cows, taken under aseptic conditions (into sterile open mouthed bottles in the barn). He isolated 20 apparently different species, of which 3 were streptococci, 4 bacilli, and 13 were micrococci. These organisms fermented and

acidified lactose bouillon and lactose milk. Six of the 20 species produced a firm coagulation of milk in 20 hours, and the others precipitated or coagulated the casein in from 4 to 10 days. Eleven of the organisms liquefied gelatine.

The subcutaneous inoculation of 0.5 cc. of a fresh bouillon culture into guinea pigs produced no lesions or sickness. He states that, "in the milk ducts are found peculiar conditions respecting temperature and oxygen, which undoubtedly favor the localization of certain species of microorganisms. These multiply in the ducts, from which they are removed at the subsequent milking."

Ward (16) made examinations of the bacterial flora of the mammary glands of 15 freshly killed milch cows. From agar slant cultures apparently incubated at room temperature he isolated 5 different micrococci, some of which were chromogenic, *B. subtilis* and *B. fluorescens*

liquefaciens. None of these micrococci were capable of rapidly souring milk, and were apparently harmless species.

His important conclusion, as related to this problem, is that the lactiferous ducts of the mammary glands examined harbor bacteria throughout their whole extent, making it appear probable that forms injurious to both the milk and the cow may invade the mammary gland. He states that, "a study of the anatomy of the mammary gland fails to disclose structural features which could prevent invasion by bacteria."

Harding and Wilson (17) made an examination of 1230 samples of milk from the mammary glands of 78 cows. The samples were taken by milking the cows directly into sterile open mouthed test tubes in the barn, with no local disinfection of the mammary gland. The plates were incubated at room temperature. Three times as many bacteria per c.c. were found in the rear quarters as in

the front. Neither the age of the cow nor the period of lactation exerted any marked influence on the germ content of the mammary gland. The organisms, classified by the system of the Society of American Bacteriologists, fell into 71 groups. 75% of the organisms were micrococci, and only 2 streptococci were found.

The works cited thus far have not been described in full here, because they have not attempted to deal with pathogenic organisms. In selecting the cows to be studied in these works, no reference was made to the presence of mammitis in the animals; the organisms were incubated at room temperature instead of at body temperature, so that the organisms isolated are more likely saprophytic; the differential media used except in the work of Harding and Wilson, have been such that definite species of organisms could not be accurately determined.

However, several important conclusions can be

drawn from these works, that have practical bearing on the present study:

1. Normal mammary glands are invaded by a comparatively large number of various species of organisms.
2. Many of these organisms thus far studied are inactive toward milk, and so would not cause the coagulated clotted milk characteristic of mammitis.
3. None of the organisms thus far studied apparently produce any ill effect on the cow, but their mere presence makes it appear probable that pathogens could easily invade the mammary gland.

Recent Studies of Mammitis in the United States. The following reviews are from work conducted in this country in which modern bacteriological technique has been employed and in which attempts to find the pathogenic organisms concerned in the etiology of mammitis have been made:

Rogers and Dahlberg (18) were apparently the first workers in this country to investigate the origin of streptococci in milk. In the isolation of their milk cultures, small portions of the milk were added to lactose bile tubes and incubated at 37°C. Tubes showing streptococci in distinct chains were plated on lactose agar, and the chain forming cocci subcultured. Since they found that the morphology of some organisms varies, little attention was paid to the morphological characteristics beyond determining that it was a coccus apparently dividing.

They found that mammary gland cultures were characterized by the general lack of fermentative ability. This was limited almost entirely to dextrose, saccharose, and lactose, with a comparatively small number utilizing mannite, glycerin and gelatin. On the basis of gelatin liquefaction, two groups were obtained:

one of the milks from individual cows. Of 48 healthy cows, showing no

signs of mammary gland trouble, streptococci were found

1. Possesses fermentative ability, limited to dextrose, saccharose and lactose with an occasional culture fermenting mannite, starch, or inulin. Similar to streptococcus pyogenes.

2. Liquefies gelatin and ferments dextrose, saccharose, lactose, mannite, and usually glycerin.

Sherman and Hastings (19) found streptococci to occur within the mammary glands of about one third of the healthy milch cows which were examined. It was claimed that cows so infected were not suffering from mammary gland trouble nor had they any recent history of such trouble.

They pointed out that the milks from the herds examined were used in the raw state with no ill effects on the consumers as far as could be learned.

Sherman (20) made examinations of the milks from individual cows. Of 48 healthy cows, showing no

signs of mammary gland trouble, streptococci were found in 31.3 per cent of the individuals. Mixed milk from the herd showed streptococci in relatively large numbers. He states that, "it appears that streptococci - indistinguishable from the streptococci of mammitis - are the predominating organisms in milk produced under sanitary conditions. ----- A question in connection with the milk streptococci is whether these apparently harmless types which occur in the mammary glands of cows are a separate group from the type which causes mammitis, or whether they are individuals belonging to the same group but possessed of less virulence." *infrequent in certain cases of* Smith and Brown (21) in their studies of certain milk-borne epidemics of tonsillitis describe ten strains of bovine hemolytic streptococci which they isolated from milk drawn directly from the mammary gland.

Mathers (22) injected human, mastitis, and milk

streptococci into the mammary glands of cows. The human and mastitis strains produced a severe mastitis which became chronic. The non-hemolytic streptococcus lacticus gave rise to an acute mastitis which displayed all the symptoms observed in spontaneous mastitis (swelling, heat, pain , and purulent milk) . The injection of a culture of a hemolytic streptococcus from milk gave much the same result. The inflammation resulting from these injections disappeared within two weeks. The fact remains, however, that both supposedly non-pathogenic cultures produced disease. It is admitted that the inflammation is of short duration, but such is not infrequent in certain cases of spontaneous infection. These results raise the question whether or not many belonging to the so called streptococcus lacticus group may not be identical with mastitis streptococci. *xitross, lactose, succharosa,*

malto Davis (23) examined the market milk from nine

dairies, and found hemolytic streptococci in seven out of eight pasteurized samples. He compared their cultural characteristics with hemolytic streptococci of human origin and from mastitis. He concludes that the milk strains are different from certain strains of hemolytic streptococci found at times in the diseased mammary glands of cows, since the latter are virulent for rabbits and are from human sources.

Jones (3a) found that of 81 animals examined, the majority were suffering from infections of non-hemolytic streptococci, which produced lesions varying from an involvement of the lining of the epithelium of the large milk ducts to severe degeneration and necrosis of the secreting epithelium. They fall into two groups when their fermentative characteristics are considered:

1. Those fermenting dextrose, lactose, saccharose, maltose, and salicin.

2. Those fermenting all the above sugars except salicin.

All the mastitis streptococci failed to act on raffinose, inulin, or mannite. None of the strains proved pathogenic for rabbits. A pig fed on the milk from two typical cases of mastitis remained well.

He found (3b) that hemolytic streptococci produced more or less severe inflammation of the mammary glands of cows, although in a number of cases no gross changes in the mammary gland could be detected. Their fermentative characteristics were identical with the non-hemolytic strains, and possessed but slight pathogenicity for rabbits.

In studying infection of the mammary gland with micrococci and other microorganisms (3c) he found that micrococci produced various types of disease from only a mild catarrh of the milk ducts and cistern, to a more or less severe parenchymatous inflammation, but that

the prognosis is more favorable than with cases associated with streptococci. Staphylococci, however, have caused several cases of considerable severity. Micrococci and streptococci were found to be eliminated from the mammary gland even after an apparent recovery of an attack of mastitis, and in some instances appeared in the normal mammary gland.

Jones (24) studied the streptococci found in the vagina, saliva, skin, and feces. Those from the saliva predominate in fermenting raffinose, inulin, and mannite. The characteristic streptococcus from the skin produces acid in dextrose, lactose, saccharose, maltose, raffinose, mannite, and salicin, but fails to acidulate media containing inulin. The fecal streptococci are characterized by the formation of large amounts of acid in dextrose, lactose, saccharose, maltose, raffinose, inulin, and salicin. Mannite is not fermented.

of cows. Neither the fecal nor the skin streptococci were isolated from the bottled milk with any frequency. Ayers and Mudge (25) found that streptococcus mastitidis is commonly found in the mammary glands of normal cows and also in cases of mammitis, the difference being only in the number of organisms present. They found that its cultural characteristics are identical with those of streptococcus pyogenes, except in the difference in final Ph and the difference in ability to hydrolyze sodium hippurate. Frost and Buchanan (26) examined twelve cows from four herds. Three of these herds were producing certified milk, and the other would be classed as a sanitary dairy. Twenty eight per cent of all the cows examined showed hemolytic streptococci in their milk. They conclude that hemolytic streptococci are to be found quite constantly in the mammary glands of a considerable proportion

of cows, even when kept under the best conditions and with frequent examinations by veterinarians and by the help of physicians. Such milks have been widely used in a raw state without causing disease and may therefore be looked upon as harmless. to be of no value, and in some

Carpenter (27) found that cultures of streptococcus viridans, Bacterium abortum, and Bacterium pyocaneum caused a slight swelling of the quarter infected, with the formation of a few flocculi of pus in the milk which cleared up in a comparatively short time after the injection. Non-hemolytic cultures of staphylococcus aureus caused general symptoms and an acute local condition, followed by destruction of the quarter. B. coli produced an acute mastitis which cleared up eight days after injection. the experiment.

Conclusion: "When it was evident that a mastitis was permanently established, an attempt was made to improve or

cure the affected quarters by injecting subcutaneously suspensions of the dead organisms that had produced the condition. This seemed to be of no value. Milk from the infected quarters was withdrawn and injected subcutaneously. This seemed to be of no value, and in some cases produced large abscesses on the animal. "

It was observed that the age of the cow and the amount of milk given had an effect on the degree of mastitis produced by the organism. As a rule the cows not in milk showed no ill effects from the injection, and the same animals a few months after calving responded to the injection according to the organisms used. The heifers were much more susceptible and suffered more severely from the injection of the different bacteria used in the experiment.

Conclusions Drawn from the Recent Studies. The conclusions to be drawn from the studies cited above and from

Table I may be stated briefly as follows:

1. The organisms most likely to be found associated with mammitis are:
 - a. Streptococci, both hemolytic and non-hemolytic.
 - b. Staphylococci.
2. B. coli or escherichia coli was not found in the mammary gland cultures, and so if present would probably show that contamination had occurred.
3. Streptococci are the predominating organisms associated with mammitis.
4. The streptococci may be differentiated by using sugar media of dextrose, lactose, saccharose, maltose, and salicin; by blood plates; by action on milk; by injection into rabbits.
5. The mammary gland flora may normally contain streptococci and other micrococci, but these are not

necessarily pathogenic even when found associated with mammitis. Object:

6. Due to the variable findings of different investigators, great care should be used in securing samples so that they are free from contamination. Determine the possible presence of animals that are acting as "carriers" of the organisms causing the disease in the herd.

Technique Employed.

Collection of Samples Aseptically. To avoid the possibility of contamination from the air, animal, or utensils, the cement floor was washed down, the cow's mammary gland and flanks curried and then washed with an antiseptic solution. The first few streams of milk were discarded, and the end of the teat was then immersed in tincture of iodine. A sample of the milk was then collected in a flask fitted up as shown in Diagram I. This flask had a

V. THE PRESENT INVESTIGATION.

Object:

The object of the present investigation has been to determine the types of organisms that are causing mammitis in a large dairy herd, and to determine the possible presence of animals that are acting as "carriers" of the organisms causing the disease in the herd.

Technique Employed.

Collection of Samples Aseptically. To avoid the possibility of contamination from the air, animal, or utensils, the cement floor was washed down, the cow's mammary gland and flanks curried and then washed with an antiseptic solution. The first few streams of milk were discarded, and the end of the teat was then immersed in tincture of iodine. A sample of the milk was then collected in a flask fitted up as shown in Diagram I. This flask had a

After 48 hours incubation at 37°C., cultures were made from the plates representing each sample of milk. Several colonies were fished, inoculated into tubes of broth, and examined microscopically for purity and morphology, after 24 hours incubation at 37°C. After examination, the cultures were transferred to agar slants for stock cultures.

closely fitting rubber stopper thru which ran a glass tube. Connecting the glass tube with the milking tube was a short piece of rubber tubing. These were all cleaned, assembled, wrapped in paper, and sterilized in the autoclave at 15 lbs. pressure for 15 minutes. To collect the samples the milking tube was inserted into the teat, a small amount of milk withdrawn. In this way, the possibility of contamination was reduced to the minimum.

Cultural Methods Employed.

Isolation of the Organisms.

The samples were taken to the laboratory immediately after collecting and streaked on plates of Indal Production. Ehrlich's Solution was used, as described in Gilmer's "Microbiology" pp 131 and 399. The solution was added to a 7 day broth culture.

The samples were taken to the laboratory immediately after collecting and streaked on plates of litmus lactose agar. The plates were streaked rather than poured, because the colonies could then be more easily fished for transferring and also because no strictly anaerobes had been reported in the works reviewed.

After 48 hours incubation at 37°C., cultures were made from the plates representing each sample of milk. Several colonies were fished, inoculated into tubes of broth, and examined microscopically, for purity and morphology, after 24 hours incubation at 37°C.

After examination, the cultures were transferred to agar slants for stock cultures.

Cultural Methods Employed.

Gelatin Liquefaction. In this test, a 48 hour culture of the organism was stabbed into a tube of gelatin, and incubated at room temperature for several days.

Indol Production. Ehrlich's Solution was used, as described in Giltner's "Microbiology", pp 131 and 399.

The solution was added to a 7 day broth culture.

Litmus Milk. The milk to be used was titrated and found to be 1.5% acid. Two per cent of a five per cent azolitmin solution was added to the milk, tubed in 8cc. amounts, and sterilized in the Arnold 20 minutes each day for 4 days.

The laboratory work in this investigation was
Fermentation Tests. At first the sugars were added in 1% amounts to Dunham's Peptone Solution, but it was found that some of the cultures would not grow in this medium. So the sugars were then added in 1% amounts to sugar free broth, and tubed in 10cc. amounts. The broth used had the following composition: 500cc. distilled water, 1.5 gms. meat extract, 5 gms. peptone, 2.5 gms. salt. After titrating this to pH 7.2, 1% Andrade's Indicator was added.

Although no indication of mastitis was shown in these cows during the investigation, it was thought
Pathogenicity. One to two c.c. of a 48 hour broth cul-

ture of the organism was injected intra-peritoneally into rabbits. September, 1924, and reports on these animals are included.

CASE REPORTS.

Treatment for Mammitis. When it is stated in the Case

Reports: The laboratory work in this investigation was conducted from September, 1924, to April, 1925. The herdsman then in charge had started working with the herd on September, 1923, and no history of mammitis was available farther back than that date.

b. Milking every two hours.

Animals Studied. Samples of milk from all cows that had been affected with mammitis from September, 1923, to September, 1924, were examined for the presence of microorganisms. Although no indication of mammitis was shown in these cows during the investigation, it was thought that they might be "carriers" of the infection respon-

sible for the disease. Two cows were affected with mammitis after September, 1924, and reports on these animals are included.

Treatment for Mammitis. When it is stated in the Case Reports that the animal received treatment, the following procedure is indicated:

1. External Treatment.
 - a. Hot fomentations, and anodyne liniment.
 - b. Milking every two hours.
2. Internal Treatment.
 - a. Withholding water 6 hours.
 - b. Saline cathartic.
 - c. Withholding water 6 hours more.
 - d. Diuretic.
 - e. Light diet.

Sterile Milk. Milk samples that are reported as being sterile, showed no growth at 37°C. on plates of litmus lactose agar.

Care and Management of the Cows. The cows were stationed at milking time in a modern barn having cement floors. In the winter the cows were housed in an open front shed between milkings, and in the summer they were kept on pasture. Until June, 1924, the posterior portion of the cows was washed by running cold water from a hose directly on them, summer and winter, before each milking. It was thought that this might be a predisposing factor in causing mammitis, so after that date only the floors and feet of the cows were washed with the hose, the mammary glands being washed with warm water and cloths. After November 1, 1924, the hose was disconnected. Ground limestone was spread on the floor

chronic mammitis in the left posterior quarter, but re-

twice daily, the cows were curried and brushed each day, and their mammary glands were washed with warm water containing soap powder, before each milking. Mammary gland greatly congested and swollen, with some blood in the milk. The milk cleared CASE NO. I., and no signs of mammitis were shown during this period. She was milked four times

Holstein Cow -- Age 7½ years.

History of Mammitis from September, 1923, to September,

1924. quarters on January 14, 1925. The cow was perfect-

Parturition occurred on November 3, 1923. The colostrum from all four quarters, appeared to contain much blood, and remained bloody for two weeks after parturition.

The mammary gland was greatly congested and swollen.

Although the cow then showed no signs of mammitis, she was given treatment as a preventive measure. She was milked three times daily throughout her entire lactation period. During this period she had frequent attacks of chronic mammitis in the left posterior quarter, but re-

sponded to treatment each time.

History of Mammitis from September, 1924, to April, 1925.

Parturition occurred on December 9, 1924. Mammary gland greatly congested and swollen, with some blood in the milk. The milk cleared up in 7 days, and no signs of mammitis were shown during this period. She was milked four times daily during the entire period.

Bacteriological Findings. Samples were taken from all

four quarters on January 14, 1925. The cow was perfectly normal. Milk from both anterior quarters and the left posterior quarter was found to be sterile. Milk from the right posterior quarter gave a pure culture of a coccus apparently *Staphylococcus albus*.

CASE NO. 2.

Jersey Cow -- Age $8\frac{1}{2}$ years.

History of Mammitis from September, 1923, to September, 1924.

Parturition occurred on March 9, 1924. Mammary gland was normal with no signs of mammitis, but during this period she had several attacks of chronic mammitis in the left anterior quarter. During this period the cow frequently had convulsions, and once had impaction of the rumen.

History of Mammitis from September, 1924, to April, 1925.

This period was a continuation of the same lactation period as previously described, but no attacks of the disease occurred during this period.

Bacteriological Findings. Sample taken from the left anterior quarter on January 14, 1925. There was no evidence of mammitis. A pure culture of a Streptococcus was isolated.

Parturition occurred on October 9, 1924, and the cow showed no signs of mammitis during the lactation period. She was milked four times daily for three months, and then three times daily.

45.
44.

Bacteriological Findings. Samples taken from both posterior quarters on February 6, 1925, were sterile.

CASE NO. 3.

There was no evidence of mammitis.

Holstein Cow -- Age 9 years.

CASE NO. 4.
History of Mammitis from September, 1923, to September, 1924.

Jersey Cow -- Age 9 years.

Parturition occurred on June 21, 1923. Mammary gland was normal. Three months after parturition the cow suffered

severely from acute tympanites caused by wet alfalfa pasture, and a few days thereafter was attacked with acute parenchymatous mammitis in the right posterior quarter. This was followed by occasional attacks of chronic catarrhal mammitis until the close of the period of lactation. She was attacked with parturient paresis 12 hours after parturition, but quickly recovered after the usual treatment. A month before parturition, the mammary gland on the right posterior quarter was accidentally

cut to the milk duct 2 cm. from the end. This cut had apparently healed over before parturition. On December 14, 1923, a superficial abscess occurred on the right side of the mammary gland, and the cow was attacked

History of Mammitis from September, 1924, to April, 1925.
Parturition occurred on October 9, 1924, and the cow showed no signs of mammitis during the lactation period. She was milked four times daily for three months, and then three times daily.

Bacteriological Findings. Samples taken from both posterior quarters on February 6, 1925, were sterile. There was no evidence of mammitis.

CASE NO. 4.

History of Mammitis from September, 1924, to April, 1925.
This was part of the same lactation period as previous-Jersey Cow -- Age 9 years.

History of Mammitis from September, 1923, to September, 1924.

Parturition occurred on October 29, 1923, with no signs of mammitis. She was attacked with parturient paresis 12 hours after parturition, but quickly recovered after the usual treatment. A month before parturition, the teat on the right posterior quarter was accidentally cut to the milk duct 2 cm. from the end. This cut had apparently healed over before parturition. On December 14, 1923, a superficial abscess occurred on the right side of the mammary gland, and the cow was attacked

with acute interstitial mammitis in the right anterior quarter. She received treatment, but the inflammation persisted for 2 weeks in chronic form, and occurred irregularly for 4 months thereafter.

History of Mammitis from September, 1924, to April, 1925.

This was part of the same lactation period as previously described, but she showed no signs of mammitis.

Bacteriological Findings. Sample taken from the right posterior quarter on February 6, 1925, was sterile.

occurred, and a Streptococcus was isolated.

CASE NO. 5.

CASE NO. 6.

Holstein Cow -- Age $2\frac{1}{2}$ years -- First lactation period.

History of Mammitis from September, 1923, to September, 1924.

Parturition occurred on August 22, 1924. There were no signs of mammitis.

signs of mammitis.

History of Mammitis from September, 1924, to April, 1925.

This was part of the same lactation period previously described. No inflammation, coagulated milk, or other signs of mammitis were noticed, except that gradually posterior quarter occurred on February 8, 1925. The difficulty was experienced in milking the right anterior quarter. On April 6, 1925, the attending veterinarian mammary gland had evidently become chilled. The temperature of the cow was 103.4°F.

Liniment was rubbed in well three times daily and Bacteriological Findings. Samples of milk from the affected quarter were taken as soon as the inflammation occurred, and a Streptococcus was isolated.

Bacteriological Findings. Sample from the right anterior quarter, taken on April 4, 1925, was sterile.

CASE NO. 6.

Holstein Cow -- Age 2½ years -- First lactation period.

History of Mammitis from September, 1923, to September, 1924.

Parturition occurred on July 9, 1922. There were no signs of mammitis.

TABLE - II

Morphological and Cultural Characters

48.

History of Mammitis from September, 1924, to April, 1925.

This was part of the same lactation period previously described. No inflammation, coagulated milk, or other signs of mammitis were noticed, except that gradually difficulty was experienced in milking the right anterior quarter. On April 6, 1925, the attending veterinarian described the case as being chronic catarrhal mammitis. Liniment was rubbed in well three times daily and tincture of iodine applied to the end of the teat each day. In two weeks the quarter again milked normally.

Bacteriological Findings. Sample from the right anterior quarter, taken on April 4, 1925, was sterile.

Case No. 117
 1 RA Sterile
 2 LA Sterile
 3 RP Sterile
 4 LP Sterile
 5 RM Sterile

+ - - - - - + - + - + - + - + -

RP - Right Posterior Quarter of Mammary Gland
 LP - Left " " " "
 RA - Right Anterior " " " "
 LA - Left " " " "

No lsdol was produced by any of the organisms

TABLE-II.

Morphological and Cultural Characters of Organisms Isolated in this Investigation

| Case Number | Quarter Examined [⊙] | Morphology | Gram Stain | Pigmentation | Pathogenicity | Gelatin Liquefaction | Litmus Milk | | | Dextrose Broth | | Lactose Broth | | Maltose Broth | | Saccharose Broth | | Salicin Broth | |
|-------------|-------------------------------|------------|------------|--------------|---------------|----------------------|-------------|-----------|------------|----------------|-----|---------------|-----|---------------|-----|------------------|-----|---------------|-----|
| | | | | | | | acidity | coagulase | peptonized | acid | gas | acid | gas | acid | gas | acid | gas | acid | gas |
| 1 | RP | Staph. | + | - | - | + | + | + | + | - | + | - | + | - | + | - | - | - | |
| 1 | RA | Sterile | | | | | | | | | | | | | | | | | |
| 1 | LA | Sterile | | | | | | | | | | | | | | | | | |
| 2 | LA | Strep. | + | - | - | - | - | - | + | - | + | - | + | - | + | - | - | - | |
| 3 | RP | Sterile | | | | | | | | | | | | | | | | | |
| 3 | LP | Sterile | | | | | | | | | | | | | | | | | |
| 3 | LA | Sterile | | | | | | | | | | | | | | | | | |
| 4 | RP | Sterile | | | | | | | | | | | | | | | | | |
| 4 | LP | Sterile | | | | | | | | | | | | | | | | | |
| 5 | RH | Strep. | + | - | - | - | - | - | + | - | + | - | + | - | + | - | + | - | |

⊙ RP - Right Posterior Quarter of Mammary Gland

LP - Left " " " "

RA - Right Anterior " " " "

LA - Left " " " "

No Indol was produced by any of the organisms

3. A non-chromogenic gram positive

Streptococcus V. SUMMARY OF RESULTS. Culture from an acute

case of mastitis; the organism produced acid in dextrose,

lactose, 1. The bacterial flora of the mammary glands on

of four dairy cattle that had been affected with mastitis
in their previous lactation was studied.

2. From two of these cows, the following two
microorganisms were respectively isolated in pure culture;

a. A non-chromogenic gram positive

Staphylococcus that produced acid in dextrose, lactose,
saccharose, and maltose, but not in salicin; liquefied
gelatin, acidified, coagulated, and peptonized milk; was
not pathogenic to rabbits. Apparently Staphylococcus
albus.

b. A non-chromogenic gram positive

Streptococcus that produced acid in all the above sugars
except salicin; had no action on gelatin or on milk; was
not pathogenic to rabbits.

3. A non-chromogenic gram positive streptococcus was isolated in pure culture from an acute case of mammitis; the coccus produced acid in dextrose, lactose, saccharose, maltose, and had very strong action on salicin; did not liquefy gelatin or ferment milk; was not pathogenic to rabbits.

4. Two of the three cows from which these microorganisms were isolated had been affected with mammitis the previous lactation period, but had not suffered from the disease in the present lactation period. They were undoubtedly carriers of the infection. The third animal was suffering from an acute attack of mammitis at the time the organism was isolated.

5. All the cases of mammitis reported in this work have a history showing that the tissues of the mammary gland had quite probably been made a

52.

51.

favorable medium for the growth of casual organisms of low virulence by some systemic disturbance or traumatic condition.

In this investigation, a dairy herd was studied in which a large per cent of the animals, during the previous years, had been affected with a very serious type of mammitis.

6. Traumatism caused by throwing a stream of cold water by means of a hose directly on the mammary gland was apparently the most important predisposing cause of mammitis in this particular herd previous to September, 1924. While this method was being used, 26.8 per cent of the herd was affected with mammitis in a period of one year. When this single factor was abolished, only 5.2 per cent of the herd was affected with the disease in a period of 8 months.

1. Out of 12 quarters examined, the milk from 7 contained no organisms that would grow upon ordinary media at 37°C.

2. In 2 quarters, streptococci were isolated, and in one a staphylococcus.

3. None of the organisms found were pathogenic to rabbits.

4. Traumatism, due to chilling the mammary

gland by applying cold water directly from a hose was

VI. CONCLUSIONS.

undoubtedly the predisposing factor in causing mammitis,

and was more important in causing the disease than

In this investigation, a dairy herd was studied in which a large per cent of the animals, during the previous years, had been affected with a very serious type of mammitis.

The most important facts brought out in the investigation are that:

1. Out of 12 quarters examined, the milk from 9 contained no organisms that would grow upon ordinary media at 37°C.
2. In 2 quarters, streptococci were isolated, and in one a staphylococcus.
3. None of the organisms found were pathogenic to rabbits.
4. Traumatism, due to chilling the mammary

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