THE PROPHYLACTIC AND THERAPEUTIC EFFECTS OF A
STAPHYLOCOCCIC VACCINE IN BOVINE MASTITIS

by

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INTRODUCTION

Bovine mastitis has been estimated to cost the dairymen of
the United States 226 million dollars yearly - about 100 million
dollars more than any other disease of cattle (Poppensiek, 1960).
Of the many organisms capable of producing mastitis, _Staphylo-
coccus aureus_ (_S. aureus_) appeared to be most significant (Blobel
et al., 1962a). Other authors have verified this fact by bacterio-
logical surveys. McCulloch (1946) found an incidence of Staphylo-
coccus in 64 percent of milk samples from 3,000 cows in northwest-
ern United States. A 50.9 percent incidence of Staphylococcic in-
fection was found in 3,137 milk samples from all over the United
States (Niksch et al., 1960). Galton (1961) in a six month sur-
vey of 1,010 milk samples discovered the presence of coagulase
positive staphylococci in 71 percent of the samples.

The advent of antibiotics not only failed to reduce the in-
fection rate due to _S. aureus_, but in all probability has increased
it by destroying the other bacterial flora in the mammary gland
and allowing the _S. aureus_ to run rampant. The ability of the
staphylococci to develop rapid resistance to the various antibiotics
was illustrated by White et al. 1961. The increasing number of drug
combinations that became available for lay use only increased the
dairyman's expense in his attempt to control this infection. The
loss of milk production was a major factor in the total cost of
mastitis to the dairyman. The greatest loss of milk has been shown
to be the result of subclinical staphylococcic infection. A recent
study (Wilson, 1961) indicated that subclinical infection caused

nearly three times the loss in milk that was seen as a result of
the clinical form of mastitis. It became apparent that in order
to significantly reduce losses from S. aureus mastitis it was im-
portant that protection rather than treatment be considered.

The purpose of this project was to evaluate the effect of
one type of staphylococcic vaccine in the prevention and treat-
ment of infected mammary glands of cattle.

REVIEW OF LITERATURE

The use of vaccines to produce immunity against mastitis was
not new. As early as 1937, observations were made on the effec-
tiveness of vaccination for staphylococcic mastitis in cattle.
Gwatkin (1937) reported no decreases in the incidence of the or-
ganism in infected quarters following intramuscular injections of
a bacterin.

During the same year, Minett (1937b) reported that intra-
mammary and intramuscular injections of toxoid failed to eliminate
the staphylococcic organism from infected mammary glands. He
found no evidence of a toxin in the whey of milk from chronically
infected quarters, although he was able to demonstrate toxins in
the secretions of three cows with a gangrenous mastitis. Minett
(1937a), commenting on Gwatkin's work and his own observations,
asked the question, "If antitoxins are of value, why does a high
incidence of clinical mastitis occur in chronically infected cows
shortly after parturition when the antitoxin titers of colostrum
are high?" Later work by the same author (Minett, 1939) demon-
strated the value of a staphylococcic toxoid in preventing the

systemic effects and reducing the local effects of experimentally
induced attacks of mastitis in ewes. He noted that two injections
of the alum precipitated toxoid at intervals of three weeks gave
better immunity than one dose of the same toxoid, and reported the
alum precipitated vaccine superior to the unprecipitated product.

Richou and Holstein (1941) observed a more rapid recovery from
acute mastitis when a two percent potassium alum toxoid was injec-
ted intramuscularly. They noted the disappearance of staphylo-
cocci from the milk in 16 of 22 cows and an improvement in the
quality of milk in cows with chronic mastitis. Richou and Holstein
(1944) continued their investigation by treating 60 cases of staph-
ylococcic mastitis with a toxoid. They summarized their results
as 45 recovered completely, two improved, and 13 no change. Fail-
ure in 13 cattle was partially attributed to the presence of mixed
infections.

Murphy (1947) observed that, on the basis of evidence thus
far presented, a cow's resistance to infection of the udder could
not be increased by vaccination or other immunization procedures.

Further results of treating bovine staphylococcic mastitis
with a toxoid were summarized by Collinson (1949). Of approxi-
mately 200 affected cows which made clinical recoveries, only 20
remained positive to culture. His method of vaccination involved
the subcutaneous injection of 3, 4, and 5 ml. of a S. aureus toxoid
at five to seven day intervals. The treatment was repeated in any
animal remaining positive at the end of two weeks and the dose in-
creased to 6, 8, and 10 ml. of toxoid. The same time interval was
allowed to elapse between injections. Disposal of the animals

still infected after this second series was recommended. It was Collinson's conclusion that $\underline{S}$. $\underline{aureus}$ toxoid should be administered routinely in the treatment of clinical mastitis.

Brown (1960) has criticized Collinson's work on several points. Brown cautioned that no controls were used, composite samples rather than individual quarter samples were utilized, and the infection was diagnosed from stained smears of incubated milk instead of by bacteriologic isolation and identification of the organisms. The research might also be questioned on the basis that the samples were taken by the owners in sterile vials supplied by the veterinarian, and no serious attempt was made to differentiate between contamination of the sample and actual udder infection. Collinson himself wrote: "This report does not offer conclusive evidence as it is not a completed experiment."

During the early 1950's the French literature abounded with reports of the use of toxoids to produce immunity against mastitis. In general, it was found that subcutaneous or intramuscular injection caused an increase in the blood antitoxin titers. (Ramon et al., 1951; Ramon et al., 1952a; Ramon et al., 1952b; Ramon et al., 1952c; Ramon et al., 1953). Various types of toxoids were used. Ramon et al. (1952b) used a ten percent aluminum hydroxide precipitant. Ramon et al. (1952c) used killed suspensions of staphylococci, and a killed suspension of staphylococci and streptococci. Ramon et al. (1953) repeated the use of the killed suspension of staphylococci, but also added killed coli bacillus and two percent potassium alum to the toxoid. The best antitoxin response occurred when alum or aluminum hydroxide was added to the vaccine.

Preliminary tests of a toxoid-bacterin against bovine staphylococcic mastitis by Spencer et al. (1956) showed that subcutaneous injection of one to three doses would reduce the severity of the subsequent induced disease. No evidence of protection was found in sheep using the same procedure. Only 16 vaccinated quarters and five control quarters were used. Chronic infections subsequently developed in nine of the 16 vaccinated quarters.

A bacterin-toxoid was used by Pearson (1959) for prophylaxis and for treatment. Favorable results were reported for a period of 21 months. The clinical incidence of mastitis and the number of staphylococcic infected quarters were less in the vaccinated animals than in the control group. No significant difference was found between the two groups, however, during the last nine months of the experiment. Pearson concluded that revaccination every six months was not sufficient to maintain immunity. He suggested that the protective value of the vaccine might decrease directly with time, in spite of booster vaccinations.

Slanetz et al. (1959) and Slanetz (1959) presented data on several groups of cattle which were vaccinated with a staphylococcic toxoid and bacterin-toxoid. The 16 percent infected quarter rate dropped to 14.1 percent in the toxoid group at the end of the 18 month test period. The control group's incidence increased from 16.6 percent to 50 percent at the end of the experiment. Seventeen attacks of uncomplicated mastitis occurred in 31 of the vaccinated animals, while nine control animals had 33 attacks. Of eight heifers that were vaccinated with the toxoid before calving, only one developed staphylococcic infection.

Three of four control animals were positive for staphylococcic mastitis. No acute exacerbations were seen in the vaccinated cattle, while ten cases occurred in the four control animals. Similar results were obtained with the bacterin-toxoid although the differences between the control and vaccinated groups were not so marked. Slanetz concluded that vaccination resulted in the production of antibodies and increased resistance of cattle to staphylococcic mastitis. Spread of the infection was almost completely prevented in the vaccinated cattle and there was a marked reduction in the number of acute flare-ups over the 18 month test period. Vaccination did not result in the recovery or elimination of the infection in quarters with well established chronic infections. Following challenge with virulent staphylococci via the teat canal, vaccinated cows developed only mild reactions of short durations while severe acute reactions developed in nonvaccinated cows. Chronic infections often became established in the challenged control cattle.

Attempts to produce immunity against staphylococcic mastitis in sheep by repeated intramuscular injections of mixed bacterin were unsuccessful (Pillet et al., 1959a). The mixed vaccine was then injected into the mammary tissue through the teat duct three or four times at four day intervals. This method protected against intramammary challenge one to three weeks after vaccination. A subsequent report (Pillet et al., 1959b) showed that this protection decreased markedly during the first month and ceased 48 days after the last vaccination. A single booster dose given when immunity had decreased considerably induced high resistance. The effect

was found to be less via the intramuscular route than when the vaccine was injected into the udder.

Further clinical evaluation of the Slanetz vaccine was undertaken at one of the veterinary colleges (Fincher et al., 1960). Two 5 ml. doses of the vaccine were given one month apart to infected and normal cows in five herds. A summary of the first year's results indicated that the product did no harm, produced no change in the incidence of mastitis, and caused the cases that did occur to seem less severe and less damaging to the udder. The vaccine was found to be of no value in cattle with advanced mastitis. Herds that were well managed got along very well after the vaccine was used; however, these herds might also have done well if the vaccine were not used. No control animals were available.

Another survey, involving one herd over a period of three years, revealed a S. aureus incidence of 20 percent the first year, 16 percent the second, and seven percent the third year of vaccination with the Slanetz toxoid (Hodges, 1960). It was not certain whether this reduction occurred as a result of vaccination or because of improved management.

A mixed bacterin containing S. aureus, Streptococcus agalactiae and Escherichia coli was used in six problem mastitis herds by Stevens (1960). A 25 ml. dose was given subcutaneously and repeated in seven to 14 days and thereafter at six month intervals. The udder changes due to mastitis were significantly reduced in the vaccinated animals and the clinical incidence of mastitis was three times greater in the control animals than in the vaccinated

cows. Vaccination was most effective in cattle with little or no udder pathology and in those with infections that had not become well established. Frequent injections of the bacterin proved helpful in non-responsive individuals.

According to Blood et al. (1960) field experience with autogenous bacterins indicated that in some herds there was no apparent protection, while in others, good protection was obtained. Acute flare-ups of mastitis were reported as occurring following vaccination in animals apparently sensitized by previous infection.

Derbyshire (1960) reported that vaccination with an aluminum hydroxide gel precipitated vaccine stimulated higher staphylococcic antibody levels than did the formalized vaccine, the use of cells alone, the toxoid, or whole culture. A higher degree of immunity was also seen with the use of the gel vaccine in goats and cows against intramammary challenge in the living staphylococci. Preliminary results indicated that the immunity against strains of staphylococci other than that used in the vaccine was not as good as that against the homologous strain.

In an excellent review by Brown (1960), the following conclusions were enumerated by the author. Natural antitoxins had little or no value in preventing udder infections. More information was needed to determine the apparent greater protection seen with the injection of toxoid. Blood antitoxins had some value in the prevention of clinical mastitis by reducing the severity of experimental infections and simple attacks in natural infections. Little evidence was available to support the thought that antitoxins had any value in eliminating existing staphylococcic infections.

An extremely interesting point was raised by Derbyshire (1961a) concerning the possible immunity resulting from toxoid administration. He stated that there was no evidence that toxoids have any therapeutic value. Toxoids did stimulate antitoxin function that afforded protection against certain strains of staphylococci. But even with these strains only limited protection was possible because staphylococcic antibodies in healthy cows do not pass through the mammary epithelium. They exerted their activity in the udder only at parturition and at the end of lactation. A later paper (Derbyshire, 1961b) concluded that vaccination of goats with strains of staphylococci resulted in a high level of immunity to mammary challenge with homologous strains, but not against heterologous strains of staphylococci.

During the past two years, interest in vaccination for mastitis has become widespread. Articles have appeared in lay journals, the general attitude of which has been summarized by Guthrie (1961). He has pointed out that research indicated that vaccination softened the impact of organisms capable of infecting the udder. It did not always prevent their activity nor prevent infection from spreading. Vaccination was not recommended until further research was done.

An experiment conducted at the University of Wisconsin showed that cattle vaccinated with a staphylococcus bacterin-toxoid were more resistant to mammary infusion with the vaccine strain of staphylococci (Blobel et al., 1962a). Heterologous strains resulted in a more severe udder reaction, although some protection was also seen against these strains of organisms. It was concluded that antitoxins present in the blood stream appeared to reduce the

severity of clinical mastitis. The results of this experiment
were also reported in a bimonthly professional magazine which in-
correctly stated that the vaccinated cattle "exhibited an increased
resistance to mammary infections with the homologous, but not to
those with the heterologous staphylococcic strains....." (Blobel
et al., 1962b).

In a second article on staphylococcic antitoxins in dairy
cattle, Brown (1962a) showed that the blood antitoxin and agglu-
tinin titers increased as the number of infected quarters in-
reased in cows with chronic staphylococcic mastitis. These
changes seemed to range within a certain level for each individual.
It was found that cows continued to become infected even though
there was an apparent increase in titer with the increased number
of affected quarters. He concluded that more information was
needed and that factors other than blood antitoxins and agglu-
tinins should be considered in studying the resistance of cows to
staphylococcic udder infections. In a later article, Brown (1962b)
reported that 17 of 20 cases of mastitis developed during the
first lactation or during the first two weeks after calving.
While vaccination had shown some promise, he cautioned that it
should not be expected to alleviate all cases. Systemic attacks
and death have occurred even though antitoxins were present in the
blood of the cow. The high incidence of mastitis during the first
lactation and shortly after parturition indicated that that peri-
od should be included in any vaccination program.

A final summation of the "vaccination-for-mastitis" question
has been provided by Gibbons (1962). He stated that the use of

toxoids and bacterins seemed to be a practical method of control, but that they were not effective in treatment. The use of bacterins has definitely reduced the incidence or percentage of cases of mastitis. "The initial vaccination should be repeated in two to three weeks and every three months for the best results, keeping in mind that sanitary milking practices and any other means of mastitis control must be maintained at an effective level."

MATERIALS AND METHODS

Two dairy herds were chosen from the area surrounding Manhattan. Each consisted of a significant number of animals, and each herd had a history of a severe mastitis problem. In both herds S. aureus was by far the most common pathogen isolated from milk samples taken before the experiment was begun.

Herd A consisted of a total of 40 cows during the experimental period. Of these, four were sold during the course of the project because of disease. Two were sold because of chronic attacks of mastitis. One was disposed of because of sterility and the fourth because of an attack of traumatic reticuloperitonitis. Thus, half of the cows sold from Herd A were disposed of primarily because of mastitis.

Herd B had a total of 113 cows on the experiment. Thirty-five were sold during the experimental period because of disease problems or old age. Of these cattle, 12, or 34.3 percent were sold because of mastitis. The remaining cattle were disposed of because of low milk production ("culls"), 15; reproductive problems, four; old age, two; bad disposition, one; and traumatic reticulo-

peritonitis, one. While these 12 cows represented a lower per-
centage of the total cows sold than occurred on Herd A, it was
nevertheless too high a percentage for an efficient operation.

Owners of both herds were contacted and agreed to cooperate
fully in the experimental use of a toxoid vaccine to attempt to
reduce the severity of their mastitis problem. It should be em-
phasized that the use of the vaccine was the only change that was
intentionally made in either herd program. No recommendations or
suggestions were made to help improve the management or milking
procedures. It is difficult, however, to estimate how much effect
the periodic visits to the farms had upon the management. If any-
thing, one could safely say that the owners certainly became more
disease conscious and more aware of their mastitis problem.

Vaccine Used

The product used to immunize Herd A and B against mastitis
was a purified concentrate of whole cultures of S. aureus that had
been inactivated with beta-propiolactone[1]. A total of 101 strains
of the organism isolated from clinical cases of mastitis, includ-
ing phage type 42D, were included in the vaccine. According to
Reid et al. (1959) phage type 42D was one of the strains of
S. aureus most frequently associated with clinical attacks of
mastitis. Fleming (1960) listed phage type 42D as one of the pre-
dominant types seen in mastitis. The vaccine consisted of cellu-
lar antigens from these strains of staphylococcic and standardized
quantities of beta and alpha toxoid. Inclusion of specific con-

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[1] Staphoid A-B, Jensen-Salsbery Laboratories, Inc.

centrations of alpha and beta toxoid was based on a report by
Fleming et al. (1961). Of 101 isolates from clinical cases of
bovine mastitis, all produced alpha toxin, while 56 percent pro-
duced beta toxin. Beta-propiolactone was used as the inactivat-
ing agent as it had several advantages over formaldehyde (Fleming,
1960).

Vaccination Procedure

A minimum of two samples were obtained from each animal be-
fore vaccination was performed in order to establish the "normal"
flora of the udder for each individual. In Herd A, this base pe-
riod before vaccination varied from two to six and one half months,
with the appropriate number of monthly milk samples taken from each
cow. In Herd B, two samples were taken from each cow before vac-
cination was started.

Since Herd A consisted of a relatively small number of cows,
the base period was used as the control period and all cows were
vaccinated. Following the cleansing of the area with an alcoholic
solution of a quaternary-ammonia compound, a dose of 5 ml. of the
vaccine was administered intramuscularly into the gluteal region
of the cows. The procedure was repeated at the end of six weeks
as was recommended by the manufacturer at the time the experiment
was started. (This recommendation has since changed to recommend
booster vaccination at two weeks.) Following the first two in-
oculations the vaccination was repeated at six month intervals.

The records that were available in Herd A made it desirable
to select this herd to test the therapeutic effect of the vaccine.

Thirteen cows from this herd were selected on the basis of chronic and recurring mastitis attacks. These animals also had a high average number of infected quarters per cow. These animals were given multiple injections of therapeutic doses of the vaccine. Doses of 10, 15, and 20 ml. were administered every week for three injections. Because of the quantity of material to be injected, the "hamstring" muscles (semimembranosus, semitendiosus, and biceps femoris) were selected as the area of administration.

Herd B consisted of a larger number of cattle with well documented histories available for each animal. The herd was divided into a control group and a vaccinated group, attempting insofar as was possible to place an equal number of individuals with the same ages, history of mastitis, and previous milk production in each group. The animals were also equally divided on the basis of number of infected quarters, based on the samplings that were taken during the base period (Brown, 1960). The vaccinated animals were injected with 5 ml. of the vaccine in the gluteal muscles, following the preparation of the skin as described in Herd A. This was repeated in six weeks and every six months during the course of the experiment. The control animals were also injected at the same time with a placebo prepared by Jensen-Salsbery Laboratories, Inc. The owner was not advised that a control group was being employed. Positive identification of the cattle was by the use of large aluminum ear tags which could be read from a practical distance. Heifers coming into the milking herd were alternately designated as vaccinates or controls. These animals were vaccinated immediately after the first milk sample was taken, and only

one base period milk sample was available on the newly introduced
animals.

The owners of each herd were supplied with a written form
that was used to record clinical cases of mastitis. The form
contained spaces for the cow's number, date of the attack, and
the quarter affected. All mild attacks were recorded by the own-
ers in this manner. It was requested that the clinician be called
to treat any attacks of mastitis in which the cow was showing
systemic signs. During the monthly sampling visits to the herds,
the forms were collected and new ones supplied.

Sampling Procedure

Milk samples were collected from each cow on the experiment
on a once-a-month basis, as nearly as was practical for the own-
ers and the writer. All animals were sampled except for the rare
cow that was dry and had been turned onto pasture. Cattle that
were not milking but were still in the milking herd were also
sampled.

The owners were instructed to follow their usual milking pro-
cedure. This involved the restraint and feeding of concentrates
to the cattle to be milked, and the washing of the mammary glands
with a sponge and warm water. Herd A employed the use of a
chlorine compound as disinfectant, while Herd B used a soap base
quaternary-ammonia solution. As stated earlier, no conscious
attempt was made to influence the milking procedures.

Following the cleansing of the udder by the owner, samples of
milk were aseptically collected from each quarter in individual

sterile tubes. This was accomplished by the cleansing and wiping
of the teats and teat openings with cotton saturated with an alco-
holic solution of a quaternary-ammonia compound. The cotton was
squeezed slightly before use so that there was little danger of
having a drop of the antiseptic on the teat orifice when the milk
sample was taken. One piece of cotton was used for all four teats.
The teats were cleaned in a systematic fashion, so that the hand
of the operator would not be carried across the teats that had al-
ready been cleansed. This involved cleaning the teats furthest
away first and working toward the milker, then collecting the milk
from the nearest teats first and working away from the milker.
The first milk available was collected, and no more than one or
two strippings of the teat were permitted in order to reduce con-
tamination of the sample to the minimum. One to 5 ml. of milk were
collected. The tubes were identified with the cow's number in order
of milking and the quarter. A master sheet clarifying which quar-
ter from which cow was being collected in what tube was made at
the time of collection.

The milk samples were returned to the laboratory as soon as
practical and placed under refrigeration until the agar plates
were streaked.

Laboratory Examination

All samples were refrigerated before culturing to reduce the
growth of any contaminates that might be present.

Each milk sample was streaked on a sheep blood agar media in
a petri plate. All four quarter samples from one cow were cul-

tured on the same plate. The plate was incubated for 24 hours in an inverted position at 37°C. Following this period the plates were examined and the organisms present identified and recorded. Brown (1960) implies that this is a necessary procedure for the identification of infected quarters. All colonies that were suspicious of being S. aureus were tested for coagulase activity (Fleming et al., 1961; Reid et al., 1959). Organisms that were positive for coagulase were recorded. Plates that were completely negative after 24 hours were incubated for another 24 hours and reexamined.

For a period of time incubated methylene blue stained milk smears of the individual quarters were also examined to determine infected quarters. However, since this examination of smears was not performed all through the experiment its results were unable to be correlated for both the base period and test period. Consequently results of the examination of incubated stained milk smears were not taken into consideration in this thesis.

A total of 19 herd milk samplings and cultures were performed on Herd A over a period of $19\frac{1}{2}$ months. Herd B was surveyed for 14 months during which 11 milk samplings and cultures were conducted.

Recording of Clinical Attacks and Culture Results

A special book was prepared for each herd and a page on this book was assigned to each animal in the experiment. On each page the results of the monthly milk samplings and cultures, and the attacks of clinical mastitis (with the date and quarter affected)

were recorded chronologically. Any treatment or illness that occurred in the herd and on individual animals was also noted.

Cultural results were recorded as they were read in the laboratory. In the recording of the incidence of infected quarters only the quarters infected with S. aureus were tabulated. Since the vaccine being used was specific for this organism, it was felt that its effect against the intended victim should be particularly noted.

RESULTS AND DISCUSSION

The vaccination of all the cows in Herd A complicated the evaluation due to the lack of a control group. Because of this, the effect of vaccination on Herd A was evaluated by comparing the long base period with the test period. Herd B was analyzed by the comparison of the vaccinated and control group of animals. The herds were evaluated on the effect of the vaccination on the clinical incidence of mastitis, the effect of vaccination on the incidence of infected quarters, the therapeutic effect of vaccination on chronically infected cattle, and the side effects of the vaccination procedure.

Effect of Vaccination on the Clinical Incidence of Mastitis

The clinical incidence of mastitis was computed on the basis of attacks per cow month (CM). A cow month was considered as one cow being on the experiment one month.

The clinical incidence of mastitis in the 38 cows of Herd A during the base period and test period is presented in Table 1.

A total of 212 cow months were involved during the base period, while 444 cow months were present in the test period. Fourteen cows had no attacks during the experiment. Eleven cows had attacks of mastitis during both periods.

Table 1. Clinical incidence of mastitis attacks in Herd A during base period and test period.

	Base period			Test period		
	Cows	Attacks	CM per attack	Cows	Attacks	CM per attack
Mastitis	17	40	5.3	19	51	8.7
Mastitis with comp.*	1	1	212.0	2**	3	146.0
No attacks	20			18		

* Mastitis with systemic complications.
** Also had attacks of mastitis.

Although the number of cow months required per attack of mastitis increased for both types of mastitis, this increase was not statistically significant. Thus there was no significant difference in the clinical incidence of mastitis in Herd A following the vaccination.

It was interesting to note that of the 14 cattle that had no attacks of mastitis during the experiment all were four years of age or younger at the start of the test. Of the 11 cattle that had attacks in both the base and test periods only two were under four years of age at the start of the project.

All three of the cows that had attacks of mastitis with systemic complications were older cattle. Two were six and one-half

years old at the start of the experimental period. The other cow was four and one-half years old when the experiment began.

It appeared that even with vaccination, the attacks of mastitis were by far more common in older cattle. As the cow's age increased, so did the incidence of mastitis. In the 24 cows that were four years of age or younger at the start of the experiment, 24 attacks of mastitis were seen. The 16 cattle that were over four years of age at the beginning of the experiment had 67 attacks of mastitis and four attacks of mastitis with systemic complications.

In attempting to evaluate any product against mastitis, the age of the cattle should be taken into consideration, and if a control group is used an equal number of the same aged cows should be placed in the treated and control groups.

The clinical incidence of mastitis in Herd B is presented in Table 2. The base period consisted of one month, while the test period was 13 months. The herd consisted of a total of 113 cows. Forty-six cows had no attacks of mastitis during the experiment.

Table 2. Clinical incidence of mastitis attacks in controls and vaccinates of Herd B during base period and test period.

	Base period					Test period			
CM	:Mas-:titis at- tacks	CM per:C.* at- tack	:M.W. at- tacks	: CM/ :comp. ** at-	CM	:Mas-:titis at- tacks	CM per:C. at- tack	:M.W. at- tacks	CM/comp.attack
Con-trols	54	10	5.4	0	54+	328	52	6.3	3	109.3
Vacci-nates	48	11	4.4	0	48+	363	53	6.8	0	363+
Herd total	102	21	4.9	0	102+	691	105	6.6	3	230.3

  * Mastitis with complications.
 ** CM per complicated attack.

While there was a general increase in the number of cow months required for an attack of mastitis, this increase was present in both control and vaccinated cattle. The difference between the two groups was not statistically significant, although a slightly larger number of cow months per mastitis attack was required in the vaccinated group than in the control group. Vaccination had no significant effect upon the incidence of clinical mastitis in Herd B.

Of interest was the fact that seven of the eight cows in the control group that were sold for mastitis were four years of age or older at the start of the experiment. Nine of the 25 cows in the group with mastitis were four years of age or older when the experiment was begun. Of 11 cows that had two or more mastitis attacks, seven were four years or older at the beginning of the

project. Two of the three attacks of mastitis with systemic complications occurred in cattle over four years of age.

All four of the vaccinated cattle that were sold because of mastitis were over four years of age at the beginning of the project. Seven of the 19 cows that had mastitis attacks were at least four years of age when the experiment began. Seven of the 11 cows with two or more attacks of mastitis were four years of age or older in the beginning of the experiment.

In Herd B 11 of the 12 cows sold because of mastitis were over four years of age. Sixteen of the 44 cows having mastitis attacks were four years or older when the experiment was started, while 14 of the 22 cattle having two or more attacks of mastitis were at least four years of age when the project began.

When it was realized that of the herd of 113 head only 50 cows were four years of age or older when the experiment was started and that 11 of the 12 cattle sold for mastitis were over this age, the effect of age on the incidence of mastitis became more significant. While 63.6 percent of the cattle in the group that had repeated attacks of mastitis was four years of age or older, only 44.2 percent of the entire herd was of this age group.

If protection against mastitis could have been established before infection of the cow had occurred, it would be interesting to speculate what the incidence of mastitis for each age group would have been.

Effect of Vaccination on the Incidence
of Infected Quarters

In order to study the effect of vaccination on the incidence
of infected quarters, the results of bacteriological examination
of milk were recorded. In the analysis only those quarters that
were infected with $\underline{S}$. $\underline{aureus}$ were considered as positive. The
evaluation was based on the average number of infected quarters
per cow for the periodic milk samplings.

The results of repeated milk cultures during the base period
and test period of Herd A are presented in Table 3. The base
period included the sampling that was obtained at the time of the
first vaccination.

Table 3. Average number of infected quarters per cow of Herd A during base
period and test period.

	Base period					Test period				
	No. of monthly samplings:					No. of monthly samplings:				
	3	2	2	2	Av.	3	2	2	2	Av.
No. of cow samples	75	69	71	78		100	51	80	52	
No. inf. qrs.	97	63	71	100		109	73	113	89	
Inf. qrs./cow	1.30	0.91	1.00	1.28	1.12	1.09	1.43	1.41	1.71	1.41

In spite of the vaccination procedure there was a gradual rise in
the average number of infected quarters per cow.

Thirteen of the cows in Herd A were treated for mastitis with
large quantities of vaccine during the process of the experiment
(see the following section). It was, therefore, necessary to com-
pare the treated and untreated animals to be certain that the re-

sults just presented were not affected by this procedure. This comparison is presented in Table 4.

Table 4. Average number of infected quarters per cow of the treated and untreated cattle in Herd A during base period and test period.

	Base period					Test period				
	No. of monthly samplings:					No. of monthly samplings:				
	3	2	2	2	Av.	3	2	2	2	Av.
Treated cows										
No. of cow samples	27	25	24	25		36	23	27	19	
No. inf. qrs.	31	29	35	51		58	39	50	46	
Inf. qrs./cow	1.15	1.16	1.46	1.96	1.43	1.61	1.70	1.85	2.42	1.80
Untreated cows										
No. of cow samples	48	444	47	52		64	28	53	33	
No. inf. qrs.	66	34	36	49		51	34	63	43	
Inf. qrs./cow	1.38	0.77	0.77	0.94	0.97	0.80	1.21	1.19	1.30	1.13

Both treated and untreated groups demonstrated a gradual rise in the average number of infected quarters per cow during the experiment. This was similar to the increase seen throughout the herd. The treated group had a relatively higher average number of infected quarters throughout the experiment because these cattle were selected for a high number of infected quarters.

There appeared to be a wide variation of incidence of infected quarters in this herd and its relationship to the clinical attacks of mastitis.

Cow 45 had only two positive quarters from a total of 17 samplings and a possible 68 infected quarters. Cow 49 had only three positive quarters out of a possible 68. These cattle had 0.12 and 0.18 average infected quarters during the experiment. This was

about one-tenth the herd average of 1.26. It was no surprise that these cattle did not have any attacks of mastitis during the entire 19½ months of the experiment. Nor was it any surprise that cow 5, with only three infected quarters during the testing period, had only one attack of mastitis.

It might be anticipated that cattle with a moderate number of infected quarters should have had an average number of clinical attacks of mastitis. Cows 8 and 44 illustrate some of the average statistics that were found. Both had 11 infected quarters during the experimental period. These cattle had one and three attacks of mastitis, respectively.

As was true of the first cattle cited, cows 12 and 13 also had a low incidence of infected quarters; but in these cattle a high rate of clinical mastitis was seen. Cow 12 had an incidence of 0.75 average infected quarters over the base period and 1.75 during the test period. This is in contrast to her groups average of 1.43 and 1.80 infected quarters per cow for the same periods. Yet cow 12 had four attacks of mastitis during the base period and seven attacks during the test period, for a total of 11 mastitis attacks. The average number of attacks for this cow's group was 4.8. Cow 13 gives a similar pattern. Her average number of infected quarters during the base and test periods were 1.29 and 1.00, respectively. She had a total of seven attacks of mastitis during the two periods.

Cows 10 and 38 are the converse of the first examples cited. Cow 10 had figures of 3.33 and 3.80 for the average number of infected quarters during the base period and test period. Her group's

respective average figures were 0.97 and 1.13. However, cow 10 had only four attacks of mastitis. Cow 38 had 37 infected quarters out of a possible 76, and yet only one simple case of mastitis developed.

There was also the cow that appeared to be resistant to mastitis and yet became severely affected in a short period of time. Of a possible 44 quarters, cow 9 was found infected in only four. Yet over the experimental period she had three attacks of mastitis and one with systemic complications. Three of the attacks took place during the last two months of the test period. She was finally so severely affected with mastitis that one teat had to be amputated. Two of the remaining quarters abscessed and the animal was sold shortly thereafter.

It was extremely difficult to predict the incidence of clinical mastitis in any one cow merely on the basis of that cow's average number of infected quarters.

The average number of infected quarters per cow in Herd B were evaluated on a monthly basis. The sample taken at the time of the first vaccination was considered as part of the base period, the other nine falling into the test period. The monthly and periodic incidence of infected quarters in the control and vaccinated animals in Herd B are presented in Table 5.

Table 5. Average number of infected quarters per cow in the control and vaccinated animals of Herd B during base period and test period.

	Base period			Test period									
	Monthly samplings		Av.	Monthly samplings									Av.
Controls													
No. of cows	54	56		46	41	40	31	31	21	24	22	18	
No. inf. qrs.	35	62		53	52	61	32	30	48	27	58	40	
Inf. qrs./cow	0.63	1.11	0.87	1.15	1.27	1.53	1.03	0.97	2.39	1.13	2.64	2.22	1.46
Vaccinates													
No. of cows	58	49		51	44	44	37	37	29	27	24	21	
No. inf. qrs.	20	55		54	54	54	26	23	33	28	64	45	
Inf. qrs./cow	0.42	1.12	0.77	1.06	1.23	1.23	0.70	0.62	1.14	1.04	1.67	2.13	1.21
Herd total													
No. of cows	102	105		97	85	84	68	68	50	51	46	39	
No. inf. qrs.	55	117		107	106	115	58	53	81	55	122	85	
Inf. qrs./cow	0.54	1.11	0.83	1.10	1.25	1.37	0.85	0.78	1.62	1.06	2.65	2.18	1.32

In both the control animals and the vaccinated group there was an increase in the average number of infected quarters per cow during the course of the experiment. Although both groups started out with no statistically significant difference, at the end of the test period the difference between the control and vaccinated cattle was significant (Chi square = 10.428 with 1 degree of freedom).

The vaccination of cattle in this herd resulted in a statistically significant smaller increase in the average number of infected quarters per cow when compared with that of the control cows.

It was also interesting to observe the wide variation in correlation between the incidence of clinical attacks and the number of infected quarters of certain cows in Herd B. While not quite as striking as the variation seen in Herd A, it nevertheless was difficult to predict a cow's incidence of clinical infection on the basis of her average number of infected quarters.

Only one cow in Herd B did not have any infected quarters during the experimental period. She was sampled on eight occasions during this time. This cow had no clinical attacks of mastitis.

As might be expected, several cattle afflicted with numerous attacks of mastitis also had a high average number of infected quarters. Cow 69 had an average of 2.2 infected quarters during the experiment, and had nine attacks of mastitis. Cow 46 had an infected quarter average of 1.6 and had ten mastitis attacks. Cow 5 had 11 attacks and had an average of 2.1 infected quarters during the course of the experiment. The infected quarter average of the herd was 1.32.

Some cattle had an above average incidence of infected quarters but had few attacks of mastitis. Cow 6 had only one attack but had an infected quarter average of 1.5. Cow 36 had an average of 2.5 and did not have a single attack of mastitis.

There was also cattle that had a low infected quarter average and yet had a severe mastitis problem. Cow 45 had an average incidence of only 0.8 infected quarters, and yet she had three at-

tacks of mastitis and was so severely affected that she had to be
sold. Cow 71 also had a severe mastitis problem and had to be
sold. She had an average incidence of only 0.8 infected quarters,
but had six attacks of mastitis before being disposed of.

The use of large doses of intramammary antibiotics in Herd B
may have been a factor in explaining the closer correlation be-
tween the number of clinical attacks and the incidence of infected
quarters. Herd A did not use any antibiotic therapy. It was
possible that the antibiotic therapy reduced the incidence of in-
fected quarters so it more nearly correlated with the incidence of
clinical attacks.

Clinicians should be aware of the occurrence of these vari-
ations in the clinical and bacteriological incidence of mastitis.
Cattle having a low incidence of clinical mastitis and a high aver-
age number of infected quarters may well be the carrier animals that
have acted as "Typhoid Marys" in the dairy herd. It is hoped that
future clinical research will shed more information on this sub-
ject.

Therapeutic Effect of Vaccination

Following the treatment of 13 selected cows from Herd A with
therapeutic doses of vaccine, the records of these cows were com-
pared with the records of 21[1] untreated cattle in the same herd.
These cattle were evaluated on the basis of clinical attacks of

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[1] Six other untreated cattle in Herd A were not included in
this evaluation because of a lack of complete records during the
base and test period.

mastitis per cow month and average number of infected quarters per cow. The milk sample collected at the time of the first treatment was considered as part of the base period.

The comparison of the treated and untreated cows on the basis of clinical attacks per cow month is presented in Table 6. The average number of infected quarters per cow in the two groups is presented in Table 7.

Table 6. Clinical incidence of mastitis attacks in treated and untreated cows during the base period and test period.

	Base period					Test period				
	CM	Mas-titis at-tacks	CM per at-tack	M.W. at-tacks	CM/ comp. ** attack	CM	Mas-titis at-tacks	CM per at-tack	M.W. at-tacks	CM/ comp. at-tack
Treated	136.0	52	2.6	2	68.0	100.5	10	10.0	1	100.5
Untreat-ed	208.5	14	14.9	1	208.5	178.5	13	13.7	0	208.5+
Herd	344.5	66	5.2	3	114.5	279.0	23	12.1	1	279.0

* Mastitis with complications attacks.
** Cow months per complicated attack.

Table 7. Average number of infected quarters per cow in the treated
and untreated animals during the base and test periods.

| | Base period | | | | | | Test period | | | |
| | No. of samplings | | | | | | No. of samplings: | | | |
	3	2	2	2	3	Av.	2	3	2	Av.
Treated	1.15	1.16	1.46	1.96	1.61	1.47	1.70	1.85	2.42	1.99
Untreated	1.38	0.77	0.77	0.94	0.80	0.93	1.21	1.19	1.30	1.23
Herd	1.30	0.91	1.00	1.28	1.09	1.12	1.43	1.41	1.71	1.52

Following the injection of therapeutic doses of the vaccine an
increase in the number of cow months required for an attack of
mastitis was seen in the treated cows. The untreated animals main-
tained a relatively constant level of cow months per attack of
mastitis. The increase seen in the treated cattle was not sta-
tistically significant however. The average number of infected
quarters per cow increased in both the treated and untreated
groups. There was no significant difference observed.

No significant therapeutic effect from the use of small quan-
tities of the vaccine was noted. The untreated group in Herd A
showed no significant change in the gradual increase in average
number of infected quarters per cow which had been occurring dur-
ing the base period.

The injection of small quantities of the vaccine in Herd B
had no therapeutic effect on the infected quarters. The incidence
of infected quarters per cow continued to increase in the vacci-
nated cattle as well as the control cows. The increase, however,
was smaller in the vaccinated cattle. It was thought that this was

due to a decrease in the rate of spread of the infection, rather
than to the alleviation of previously infected quarters.

Side Effects of the Vaccination Procedure

In a total of 38 cattle in Herd A that were vaccinated
a minimum of three times with an intramuscular dose of 5 ml.
no serious side effects were noted. There was some initial
irritation apparent as expressed by uneasiness on the part of
the patient during the injection. Two cows had a mild swelling
for two to three days at the injection site.

In the 12 cows vaccinated with therapeutic doses of vaccine,
a mild irritant action occurred during administration. There was
swelling in only one animal and this rapidly subsided after three
days. Several days after the injection of 20 ml. of the vaccine,
this cow had a fibrosis at the site of the injection. No clin-
ical lameness or malfunction of the limb was apparent.

No degree of shock or allergic response due to repeated ad-
ministration was noted.

In a total of 113 cattle in Herd B that were vaccinated, no
serious side effects were noticed. A mild thickening and slight
swelling of the injected area was noticed in ten percent of the
cattle, but in all cases this subsided in three to five days.
This reaction was greater in the gluteal region than when the
thigh muscles were utilized. Two cattle showed unusual effects
from the second vaccination. One month after the booster vacci-
nation was administered the owner noticed swelling in the gluteal
region of one cow. It was 10-12 cm. in width when called to our

attention. The swelling was lanced and 100 ml. of a thick yellow material removed. The area was treated locally and healing was uneventful. The other cow had a similar swelling, but it did not appear until two months after the second vaccination. Healing was complete following lancing and local treatment.

It was difficult to explain these reactions without considering the possibility of induced infection at the time of vaccination. No reactions were seen in the animals injected with the placebo. The possibility of the vaccine having been deposited into an area other than the muscle (i.e., into or through the sacrosciatic ligament) must also be considered. The inactivating agent that was used in the preparation of the vaccine could have produced necrosis at the site of injection. Bacteriological culture of the abscesses was not performed.

It is recommended that products similar to that used in this trial be injected into the large muscle masses of the animals body. Large doses are not suggested. Proper injection technique is important to reduce the pain and tissue irritation, as well as to reduce the possibility of abscess formation. Recommended amounts in the "thigh" muscles will result in more rapid absorption, less tissue reaction, and minimal pain to the patient during administration. Sterility of instruments, product and equipment, and general cleanliness aid in securing minimal post-vaccination reactions.

The use of vaccination procedures as a method of controlling mastitis was of insignificant value. The lack of definite information about other factors that influence mastitis control is obvious. Future clinical research in this area is needed, as was well stated

by Blobel and Murphy in the following quotations.

"Until........the factors which play a determinant role in the ability of staphylococci to become established in the udder and cause disease...have been properly identified, particularly with respect to their immunologic significance, attempts at large scale vaccination as a means to control staphylococcic mastitis are likely to remain emperical and probably fruitless." (Blobel et al., 1962a)

"It is to be hoped that...people...will see that only in one of the four disease which comprise mastitis is there sufficient knowledge on which to base a precise, sensible, general control effort....Until we make the tremendous effort needed to correct this deficiency, the control of these other forms will remain in the emperical, trial-and-error area supported at best by testimonials." (Murphy, 1956)

CONCLUSIONS

The vaccination of dairy cattle with a staphylococcus toxoid resulted in a decreased clinical incidence of mastitis. This decrease was not statistically significant.

The clinical incidence of mastitis was shown to increase directly with the age of the cow. The period of four to four and one-half years of age appeared to be critical. A much higher incidence of mastitis was seen in this older group of cattle.

Vaccination had no effect in halting the incidence of infected quarters. A significantly slower rate of spread was noted in the vaccinated cattle of one herd.

The absence of a definite correlation between the incidence of infected quarters and the clinical incidence of mastitis was illustrated.

The treatment of chronically infected cows with large doses of vaccine resulted in a decreased incidence of clinical mastitis. This decrease was not statistically significant. There was no effect on the incidence of infected quarters when cows were treated with large quantities of the vaccine.

No serious side effects were noted from the routine administration or repeated injections of large quantities of the vaccine. The use of the large muscles of the body, proper injection technique, and sterile procedure are recommended.

Staphylococcus toxoid was found to be of limited value in the control of mastitis. The need for further researd into the factors which complicate mastitis control is indicated.

ACKNOWLEDGMENT

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THE PROPHYLACTIC AND THERAPEUTIC EFFECTS OF A
STAPHYLOCOCCIC VACCINE IN BOVINE MASTITIS

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AN ABSTRACT OF A MASTER'S THESIS

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Mastitis is the most costly disease of dairy animals in the United States today. Of the various organisms that are capable of causing mastitis Staphylococcus aureus is, perhaps, the most troublesome. The purpose of this study was to evaluate a staphylococcus toxoid as a prophylactic and therapeutic means of controlling mastitis.

The available literature on the use of vaccination against mastitis was not abundant and the majority was published during the previous twenty years. Clinical reports involving large numbers of cattle and the use of control animals are uncommon. Much of the literature was conflicting.

Two dairy herds were vaccinated with a staphylococcus toxoid. All 38 cows in Herd A were vaccinated, and selected cows with chronic mastitis were treated with therapeutic doses of the product. Herd B was divided into control and vaccinated groups, on the basis of age, previous milk production, history of mastitis, and number of bacteriologically infected quarters. As new additions to the herd were made they were alternately divided among the groups. A total of 113 cows were used in the experiment in Herd B.

The vaccination procedure consisted of the injection of 5 ml. of staphylococcus toxoid in the gluteal muscles. This was repeated in six weeks, and booster injections were given every six months. The cows treated in Herd A were given 10, 15 and 20 ml. of the vaccine at weekly intervals. Monthly milk samples were collected aseptically from both herds for periods of 19½ months in Herd A, and 14 months in Herd B. The samples were cultured on sheep

blood agar media and examined after 24 hours for evidence of bacter-
iological growth. Coagulase determinations were performed on all
organisms that were suspected of being S. aureus. In the absence
of bacterial growth, reincubation for 24 hours and examination was
performed.

Daily records were kept by the owners of both herds of any
clinical attacks of mastitis. These were recorded on a special
form and transferred to the herd test record at the time of
monthly milk sampling. The results of bacteriological determina-
tion were similarly recorded.

Evaluation of the vaccination was based on its effect on the
clinical incidence of mastitis, the effect upon the incidence of
infected quarters per cow, the therapeutic effect upon the chron-
ically affected cows and incidence of infected quarters, and the
side effects that were seen during the vaccination procedure.
Only those quarters infected with S. aureus were considered when
analyzing the incidence of infected quarters.

Vaccination resulted in a slight decrease in the clinical
incidence of mastitis of both herds. This decrease was not sta-
tistically significant. Both herds exhibited a gradual rise in
the incidence of infected quarters following vaccination. The
increase was significantly less in the vaccinated animals of
Herd B when compared with the control animals. No statistically
significant effect was observed due to the treatment of chronically
affected cattle with large doses of the vaccine. Similarly, small
doses of the vaccine had no effect on the bacterial flora of the

udder of cattle in both herds. No severe side effects were seen due to the vaccination procedure.

The use of the vaccine as a means of controlling mastitis was of limited value. The absence of information on factors that would influence the mastitis problem was obvious. Further research work in this area is necessary.