Effect of Vaccination with E. Coli on the Incidence of Acute Mastitis

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Summary

The effect of *Escherichia coli* vaccination on the incidence of acute mastitis in two dairy herds is studied. In Study 1, diagnosis is based on clinical signs. A significant effect is observed in the first lactation. In Study 2, diagnosis is based on culture and clinical signs. An effect is demonstrated, but it was not statistically significant.

Introduction

Acute mastitis can be a serious problem in the dairy herd. Coliform bacteria are a major cause of acute mastitis in dairy cattle.[1,2,3,4,5] Coliforms are a group of bacteria that are lactose-fermenting gram negative rods belonging to the family Enterobacteriaceae. This group includes the genera Escherichia, Klebsiella and Enterobacter. These genera are most commonly represented in mastitis caused by gram negative bacteria.[1,2,3,4,5,6,7,8] Coliforms are widely disseminated in nature. *Escherichia coli (E. coli)* is found in large numbers in manure of cows.[2,6,7]

Incidence of acute coliform mastitis appears to be increasing. Control measures have reduced the level of contagious mastitis caused by *Staphylococcus aureus (S. aureus)* and *Streptococcus agalactiae.* Mammary glands devoid of other infections and having a low somatic cell count are susceptible to new intramammary infections (IMI).[2,5] Housing will have an effect on incidence. Animals concentrated in confinement housing are exposed to increased numbers of environmental bacteria, especially *E. coli.*[3,4,5,6,7,8] Stress due to calving, ketosis, increased production, or concurrent disease has been associated with an increase in acute coliform mastitis.[3,8,9]

Smith et. al.[3] reports 80 to 90% of coliform intramammary infections (IMI) will result in clinical mastitis. Most of these will be subacute and are self-limiting. A small percentage of coliform IMI become acute. Acute mastitis results in economic loss due to death of animals, decreased production or agalactia, lost quarters and treatment costs.

Control measures for contagious mastitis have had little effect on controlling coliform mastitis[3] An alternate approach must be used. Exposure of the teat end to all sources of environmental contamination must be minimized.[3,4,8] Stress must be minimized. Attempts must be made to increase the resistance of the animal. By experiment, vaccination has not been demonstrated to be successful in increasing resistance.[2,3,4] However, veterinarians and dairymen have observed that herds vaccinated for *E. coli* appear to have a decreased incidence of acute mastitis.

In the bovine, both specific and nonspecific immunity play a role in the inflammatory response to mastitis. Immunoglobin levels are low in normal milk. After inflammation has begun, IgG enters the gland from the blood, raising the IgG levels markedly. In the gland, IgG has a role in the opsonization process. Immunoglobins are part of the humoral immune system. They are designed to attack specific antigens. Exposure through natural infections or vaccination is required to stimulate the body to produce specific immunoglobins.[10,11,12]

Effective vaccines have been prepared for *S. aureus* mastitis. Use of a *S. aureus* bacterins reduce the severity of systemic reactions and increase the rate of spontaneous recovery. They will not reduce the incidence of new IMI.[13]

This paper discusses two studies observing the effect of *E. coli* vaccination on the incidence of acute mastitis.

The first study was designed to observe the effect of *E. coli* vaccination on the incidence of acute mastitis. Clinical signs of acute mastitis were used as a measure. An assumption was made that some of the acute mastitis cases observed would not be coliform in origin, and not all coliform mastitis cases that occurred in this herd would be observed.

A herd experiencing an acute mastitis problem was selected. Contagious mastitis was not a problem. Somatic cell count were low (Consistently under 300,000 cell/ml for previous six months). Production was over 19,000 lb per lactation. Cows were kept either in confinement housing or a dirt and grass lot. Cultures taken from previous acutemastitis cases revealed coliforms as well as other environmental organisms (Streptococcus sp.).

The second study was designed to clarify some of the problems encountered in the first study. Milk cultures or

In this paper, acute mastitis means at least acute and includes both acute and paracute mastitis.

effected quarters were down. Clinical signs of mastitis were evaluated. The herd was sorted to minimize bias due to parity and stage of lactation.

Another herd experiencing a high level of acute mastitis was selected. The herd was cultured prior to vaccination to evaluate sublinical mastitis. Monthly somatic cell counts were consistently below 300,000 for the previous six months. Rolling herd average was over 18,000 pounds. Cows were kept in confinement housing and milked in a parlor.

Materials and Methods

Study 1

Animals

A string of 223 holsteins was used. All animals were milking at the time of vaccination and were in their fourth or less lactation. All stages of lactation were represented.

Housing and Management

The animals were housed in a 165 ft. x 96 ft. cold housing building with natural ventilation. During dry weather they had access to a 10 acre grass and dirt lot. One hundred and eighty 4 ft. x 7 ft. concrete free stalls were available to the cows. Straw bedding was added to the stalls every third day. Stalls were cleaned and bedding fluffed twice daily. Alleys were scraped twice daily.

The herd received the same total mixed ration regardless of production level. There was 130 feet of bunk space available.

The milking was done in a double 8 herringbone parlor with a 3 inch low line system. Good milking hygiene was used.

Bacterin

Bovine Pili Shield™* was used.

Bovine Pili Shield™ is a whole cell oil-adjuvanted bacterin containing several strains of *E. coli.*

Vaccination Protocol

Vaccine was given according to manufacturer's instructions. A 2 ml. subcutaneous injection in the area of the dewlap was given.

Ninety four animals were brought, at random, from the milking string and put through the parlor. They were vaccinated and their neck chain numbers were recorded. The vaccination list was not left at the farm.

Records

D.H.I.A. testing was done Aug. 13, 1986. These records were used for individual cow data. Days in milk refers back to D.H.I.A. test day, at the start of the test period, unless cows were dried off between vaccination day and start of vaccination test period. Cows dry at start of test period were recorded as 0 days in milk. Lactation number was taken directly from D.H.I.A. records except those that were dried. They were recorded as in the next lactation.

Test Period and Procedure

Animals were vaccinated Aug. 1, 1986. All cases of acute mastitis that occurred between Aug. 15, 1986 and Dec. 15, 1986 were recorded by the herdsman.

Acute mastitis was defined as one or more hot, hard, swollen quarters with milk from the effected quarters displaying obvious "wateriness." Strings or curds may or may not be present in the milk. The inflammation must be systemic. Milk production should be depressed. A fever may or may not be present. The animal should appear sick (i.e. ears down, depression) and have a decreased appetite.

Study 2

Animals

Herd 2 consisted of 88 animals in two milking groups and one dry group.

Housing and Management

The two milking groups were housed in a cold housing naturally ventilated building. The dry cows were kept in pasture. Eighty one 4 ft. by 7 ft. 6 in. concrete freestalls lined with rubber mats were available to the milk cows. A minimum of oat straw bedding was used. Alleys were scraped once or twice a day.

Milking was done twice a day in a double four herringbone milking parlor using a 3" low line system. Good milking hygiene was used.

Bacterin

Bovine Pili Shield™

Vaccination Protocol

A double blind study was designed. All animals were sorted by parity and stage of lactation. Alternate animals were assigned to one of two groups. Each group was vaccinated using either the *E. coli* bacterin or a placebo. Vaccination technique was the same as in Study 1.

Records

D.H.I.A. records were used as in Study 1.

Test Period and Procedure

Animals were vaccinated May 20th, 1987. A milk sample of all cases of clinical mastitis that occurred between June 3rd, 1987 and Oct. 3rd, 1987 was taken before treatment. The sample was frozen until it could be submitted to our lab. A questionnaire, designed to determine degree ofsystemic involvement, was submitted with each sample. The cow's temperature was recorded. Questions asked were: Is the cow acutely sick? Is the cow off feed? Does the bag have swelling? Is the milk watery? Is the milk chunky?

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Samples were cultured on a blood agar/MaConkey agar biplate. Three or more pink colonies on the MaConkey agar was considered a positive for coliform. Only those cultures that resulted in a positive coliform or in no growth were used in this study.

Results

Study 1

Whole herd results are given in *Table 1*. Using our test criteria, twice the percentage of acute mastitis occurred in the nonvaccinated group (17.1%) as the vaccinated group (8.5%). (P=.029)

TABLE 1

	No. of animals	No. with mastitis	Percent mastitis	P value
Vaccinated animals	94	8	8.5%	.029
Nonvaccinated animals	129	22	17.1%	

There is a possibility that the vaccinations were not totally random. Parity and state of lactation can have an effect on the incidence of coliform IMI. Incidence increases with parity and decreases with stage of lactation.[2,5,9] *Table 2* demonstrates that our vaccination procedure was not truly random. Vaccination percentage increased with parity. The herd was divided by lactation to see what effect this had on the results. (Table 2) Cows in the first lactation demonstrated a significant difference between vaccinates and nonvaccinates. (0% vs 20%, P=.022) The second lactation demonstrated an effect but it is not statistically significant. The third and fourth lactations show little effect from vaccinating.

TABLE 2

	No. Of animals	No. with mastitis	Percent mastitis	Percent vac.	P value
First lactation	71	10	—	30%	
First lac. vac.	21	0	0.0%	—	.022
First lac. nonvac.	50	10	20.0%	—	
2nd lactation	59	7	—	34%	
2nd lac. vac.	20	1	5.0%	—	.193
2nd lac. nonvac.	39	6	15.4%	—	
3rd lactation	18	0	—	50%	
3rd lac. vac.	9	0	0.0%	—	———
3rd lac. nonv.	9	9	0.0%	—	
4th lactation	75	13	—	59%	
4th lac. vac.	44	7	15.9%	—	.222
4th lac. nonv.	31	6	19.4%	—	

Stage of lactation was also considered. Overall, the vaccinated group was farther along in stage of lactation than the nonvaccinated group (125 days in milk vs. 116 days in milk). To minimize any effect this could have, only those animals fresh less than 200 days were considered. *Table 3* describes the effect vaccination had on those animals. The results are similar to the results in Table 2. But, because of diminished numbers of cows, the results are not statistically significant.

TABLE 3: Animals fresh less than 200 days

	No. of animals	No. with mastitis	Percent mastitis
Lac. 1 vac.	12	0	0.0%
Lac. 1 nonv.	40	9	22.5%
Lac. 2 vac.	15	1	6.7%
Lac. 2 nonv.	33	6	18.2%
Lac. 3 vac.	7	0	0.0%
Lac. 3 nonv.	8	0	0.0%
Lactation 4 vac.	44	7	15.9%
Lactation 4 nonv.	31	6	19.4%

Study 2

In this study, cultures were done and clinical signs were recorded. Mastitis was considered to be due to coliforms only if a positive culture was obtained. The mastitis was considered to be acute if the animal was recorded as being acutely sick and/or the animal had a temperature above 103.5 degrees F. and was off feed. Evaluations were done before it was determined which animals received the E. coli vaccine.

Results were compared in three ways. *(Table 4)* Results were based on clinical signs only, culture only, and on clinical signs and culture. When the cows with acute coliform mastitis are considered, a vaccination effect is observed. The vaccinated group had one case, while the nonvaccinated group had five. When just clinical signs are considered, the ratio is two to five. When just culture is considered, the ratio is three to five. The number of cases involved here is too low for any of these results to be statistically significant.

TABLE 4: Study 2 results

	Acute Coliform Mastitis	Acute Mastitis	Coliform Mastitis
Vaccinates	1	2	3
Nonvaccinates	5	5	5

Discussion

Study 1 is based on the diagnosis of acute mastitis based on clinical signs. Milk from affected quarters was not cultured. Based on this criteria, an effect from vaccination was demonstrated. There is some difficulty with the design of this experiment. The test groups were not truly random. Efforts were made to minimize bias. The effect of stage

of lactation is hard to assess because of low numbers in the subgroups. Results in Table 2 and 3 are comparable. Results in the first lactation animals remained significant even after several animals with excessive days in milk were thrown out. Cows vaccinated with *E. coli* had a lower incidence of acute mastitis than those that were not. The effect was significant in the first lactation. The second lactation shows a moderate (but not statistically significant) effect. The third and fourth lactation show little effect.

In study 2, when bacteriological culture is used along with clinical signs, an effect is observed. There is one case of acute coliform mastitis in the vaccinated group and five in the nonvaccinated group. The results are not statistically significant.

The results of these experiments are not conclusive. When test groups with so many variables are used the results are suspect. However, this report has significance in two ways. First, it describes an experimental design based on clinical signs. Effect of vaccination is based on degree of inflammation rather than just whether or not infection occurs. Previous experiments use gland infection as their only criteria.[2,4] Second, it suggests that vaccination with *E. coli* does have an effect on the incidence of acute mastitis.

In theory, immunity does play a role in coliform mastitis.[2,11,12,15]

Gram negative bacteria induce systemic reactions by the release of endotoxins. These endotoxins must enter the vascular system before systemic reactions occur. Endotoxins are part of the cell wall of gram negative bacteria and are released upon death of the bacteria.[2,5,14,15]

Schalm et. al.[2] describes the pathogenesis of coliform mastitis. Multiplication of bacteria in the gland induces local inflammatory response. Leukocytes enter the gland and attack the bacteria. Endotoxin is released as the bacteria are destroyed. Clinical signs are referable to endotoxin release. If local inflammation prevents further multiplication of bacteria, complete recovery can occur. If endotoxin is released to the vascualr system, toxemia and death can occur.

The results that we see in this field trial could be due to specific immune response. IgG specific for antigens contained by the infecting bacteria could enhance local inflammation response and depress release of endotoxin to the systemic circulation. IgG could also have a direct effect on systemic endotoxin reducing the degree of toxemia. Long term tolerance to endotoxin is antibody mediated.[15]

There are few reports studying the effect of vaccination on coliform mastitis. They have not been able to demonstrate an effect on new coliform IMI.[2,4] Effectiveness was based on whether or not coliforms could establish an infection in the mammary gland. Effect on severity of inflammatory response was not condsidered. Perhaps, the rate of new coliform IMI is not effected by vaccination, but the severity of resulting inflammation is reduced. A bacterin that could reduce the severity of acute coliform mastitis would be economically significant.

Studies in other species have shown that vaccination with certain strains of coliforms can result in immunity for a wide range of related coliforms.[14,16]

Animals develop immunity to coliforms due to natural exposure.[14,16] This could explain the decreased effect in later lactations in Study 1. The animals may already have achieved optimum immunity. The increased incidence of acute mastitis in the fourth lactation may be due to increased stress in the older animals. The incidence may also be due to the older animals decreased ability to handle stress. These factors may override the protection provided by immunity. Or, the acute mastitis observed may be due to some other organism.

In these herds, vaccination apparently reduced the incidence of acute mastitis. In Study 1, the effect was statistically significant in the first lactation.

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