Summary

It was found that all of the commercially available vaccines tested in this field trial increased colostral and serum antibodies against ETEC. Calf performance or challenge studies were not done because of the sheer numbers of animals and that was beyond the scope of this trial.

It also appears that a single vaccination using Coli-bovis at 2-3 weeks pre-partum will produce protective antibody levels in colostrum.

References


Infectious Bovine Keratoconjunctivitis: Comparison of Immunological Response and Disease Reproduction in Vaccinated and Non-Vaccinated Calves

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Introduction

Moraxella bovis is considered to be the main causative agent of infectious bovine keratoconjunctivitis (IBK), commonly known as pinkeye. IBK has been reproduced with M. bovis organisms alone or in combination with other enhancing factors.

Numerous attempts have been made to produce a M. bovis vaccine utilizing viable and nonviable organisms in both experimental and natural environmental conditions. In most cases these vaccines consisted of a heat-killed, formalin-killed, or viable autogenous M. bovis bacteria injected at weekly intervals intramuscularly or into the third eyelid. While in many cases M. bovis antibodies were produced, fewer positive cultures were obtained, and the severity of lesions were frequently reduced, vaccinations did not produce practical protection against the disease.

Other factors such as age, vaccination schedule, and the use of homologous strains of M. bovis have been studied. M. bovis may exist in either a smooth or rough colony form, with rough colonies exhibiting pili extending from the cell walls. These pili are delicate elongated unbranched filaments which contain no central pore and have a peritrichous distribution. Pili appear to provide additional extra-cellular antigens, which may be of importance in development of resistance to the organism. Studies using a M. bovis pilus vaccine indicated the stimulation of immune response to M. bovis which may provide a more protective immunity than previous vaccines.

Local resistance to bacterial infection of corneal and conjunctival surfaces is a complex system involving several antibacterial substances, including secretory IgA, lysozyme, beta-lysin, and lactoferrin. M. bovis antibodies have...
been found in cattle tears and, while increased during active infection, resistance is not necessarily associated with high antibody levels.\textsuperscript{24, 27}

Recently we were able to reproduce IBK in calves in 66% of inoculated eyes using virulent \textit{M. bovis} organisms alone\textsuperscript{3}. The study did not reveal demonstrable alterations in tear or serum levels of IgA, IgG or IgM. Tear antibodies were produced in both affected and non-affected calves, but did not appear to provide any protection from the disease. Lysozyme, a potent antibacterial agent in human tears, was not found in any calves.

The purpose of this paper is to examine the clinical and immunological responses of calves administered a non-virulent bacterial vaccine prior to challenge with a virulent \textit{M. bovis} and to compare these results to those obtained from non-vaccinated calves challenged with the same organism.

**Materials and Methods**

**Subjects:** Twelve week old Holstein calves, free from any clinical disease or infection, were used. Prior to use, a thorough examination of the anterior segment was performed. All calves were kept inside a building in a fly-free environment with no exposure to sunlight.

**Test Group:** Calves were divided into two groups: Control Group: Six calves (12 eyes) were used as positive controls and received virulent \textit{M. bovis} in an effort to produce disease.

Vaccinated Group: Twelve calves (24 eyes) were vaccinated with a live non-attenuated bacteria on day 0 and revaccinated on day 14. On day 21 these calves were challenged with the virulent \textit{M. bovis} in a manner identical to the control group.

**Collection of Tears:** Tear samples were collected with nonheparinized capillary tubes placed in the lower cul-de-sac of the eye following physical restraint with a halter and ropes. Approximately 0.5 ml of tears was collected from each eye and placed in the micro-centrifuge vials and stored at \(-40^\circ\text{C}\).

**Determination of Disease:** All animals were examined daily for the presence of significant eye diseases, symptoms of which included blepharospasms, increased lacrimation, conjunctivitis, corneal opacification, ulceration and rupture. Affected eyes were sequentially photographed on a weekly basis.

**\textit{M. bovis} and Neisseria Vaccine Antibody Production:** Four healthy New Zealand white rabbits, weighing 8-10 pounds, were used for antibody production against the \textit{M. bovis} and Neisseria vaccine organisms (2 rabbits each). Each rabbit received six separate subcutaneous injections of 0.5 ml of live bacteria in tryptcase soy broth (TSB) (8 x 10\textsuperscript{6} organisms/ml). The injections were repeated in five days and blood was taken two weeks after the second injections, and then at two week intervals. Blood samples were centrifuged for ten minutes and the serum removed and frozen at \(-40^\circ\text{C}\).

**Bacterial Vaccine Production and Inoculation:** A Neisseria bacteria was isolated and grown on 5% BBA for 48 hours at 37\degree\text{C}. Bacterial growth was scraped from plates and suspended in TSB to a concentration of 5 x 10\textsuperscript{6} organisms/ml as determined from BBA plate counts and chamber count. This suspension of live bacteria was the vaccine, and was applied topically to the eye (0.5 ml/eye) on days 0 and 14 in 12 calves (24 eyes).

**Challenge with Virulent \textit{M. bovis}:** Both the positive control group (challenge only) and the vaccinated group were challenged in an identical fashion with the same \textit{M. bovis} organism. A virulent hemolytic \textit{M. bovis} was grown on 5% BBA for 48 hours, removed from the plates and suspended in TSB with a concentration of 8 x 10\textsuperscript{6} organisms/ml. This inoculum was applied directly to the eye in one-half of all the calves.

Calves not challenged with the TSB inoculum were challenged with a pure bacterial paste. In these calves, the virulent \textit{M. bovis} was grown for 48 hours on BBA. The bacterial growth from one plate was then removed and applied directly to the eye.

The vaccinated group was challenged on day 21, one week following the second vaccination.

**Determination of Tear and Serum Antibody Titers to \textit{M. bovis} and Neisseria by Bacterial Agglutination:** Lyophilized samples of \textit{M. bovis} and Neisseria were reconstituted with 0.3 ml TSB and streaked on three 5% BBA plates. Bacterial organisms (0.5 ml) were removed after 48 hours and suspended in 5 ml phosphate buffer solution (PBS) at a pH of 7.2. This suspension was washed three times with PBS. One ml of this bacterial stock solution was diluted with 3 ml of PBS for a 1:4 dilution.

Ten microliters of PBS was placed in each well of a Falcon plate. Ten microliters of the sample to be tested were then placed in the first well and a serial twofold dilution was performed. Following this, 10 microliters of either the diluted \textit{M. bovis} or Neisseria bacterial stock solution were placed in all wells. After 24 hours the plates were read for positive agglutination of bacteria.

**Determination of Anti-\textit{M. bovis} Immunoglobulins in Tears:** The positive bacterial agglutination reactions for four affected controls and four vaccinated calves were collected and washed three times with PBS and the bacteria removed. The bacteria were divided into three separate tubes, to which fluorescein conjugated anti-bovine IgA, IgG and IgM were added\textsuperscript{28, 29}. Following one hour incubation at room temperature, the bacteria were washed three times in PBS, placed on a slide and examined for fluorescence with an Olympus U-V microscope. The reaction was graded on a scale of 0 to +4, depending on the degree of fluorescence by two independent examiners. In cases of discrepancy, the lower of the two values was used.

**Determination of General Immunoglobulin Levels in Tears:** A single radial immunodiffusion method was employed for the quantitative determination of tear immu-
noglobulins IgA, IgM and IgG. Tear samples and control standards were placed in wells and incubated at room temperature for 24 hours. After incubation, zones of precipitation were measured and the concentration determined from a standard curve.

**Terminology**

The word *infected* is defined as “to contaminate with a disease-producing substance or agent.” All calves were infected with *Moraxella bovis*. The control calves were infected (challenged) at day 0, while the vaccinated group was infected (challenged) at day 21 following two vaccinations with Neisseria at day 0 and day 14.

**Results**

**Reproduction of IBK**

*Positive Controls:* Six calves (12 eyes) were directly inoculated with 0.5 ml virulent *M. bovis* organisms. While all six calves developed pinkeye, 58% (7 eyes) of the total number of eyes developed significant disease (Figure 1). The onset of disease was rapid, with most calves developing significant pinkeye by day 12. The lesions appeared identical to the natural disease with progressive corneal ulceration, etc. Both the bacterial paste and TSB suspension were capable of producing disease, with the paste producing a more acute and severe process than the TSB suspension.

**Neisseria Vaccine Group:** Twelve calves (24 eyes) were challenged with virulent *M. bovis* organisms in a manner identical to the positive controls, following Neisseria vaccination. None of the calves developed any sign of IBK for the 42 days of post challenge observation.

**Bacterial Agglutination Titers to M. bovis**

*Tears:* In the positive control group, both affected and non-affected eyes developed a similar antibody response which rose to a high of approximately 1:75 at three weeks post challenge, then dropped off precipitously (Figure 2). In the vaccinated group, the titers to *M. bovis* rose above 1:150 before the challenge (day 21) and peaked at over 1:200 by day 33 (Figure 2). As in the positive control group, the titer then dropped off quickly.

**Serum:** Titers in both the challenge and control groups remained low for the first three weeks post challenge (Figure 3). At that time an increasing titer was observed in both groups. In the control calves the titer leveled off after reaching a high of slightly over 1:900. The vaccinated calves developed a tremendous rise in titer to *M. bovis*, which was nearly 1:6000 in the final sample studied.

**Tear Immunoglobulins**

Three classes of immunoglobulins were studied (IgA, IgG and IgM). Tear IgM levels were consistently too low to measure and are not included.

Low levels of IgG were present in baseline tears of both groups (Figure 4). In control calves a slow three-to-fivefold increase in tear IgG was observed post challenge with little variation between affected and non-affected eyes. In the vaccinated group, however, tear values were near 1:225 at the time of challenge. This titer peaked seven days following challenge at over 1:325 and then dropped off rapidly. This

*Miles Laboratories, Elkhart, Indiana.*
represented a thirteenthl fold increase in IgG.

Baseline levels of tear IgA were higher in the control calves than the vaccinated group (Figure 5). In the control group, both affected and non-affected eyes demonstrated a slight rise in tear IgA two weeks following challenge, which then fell to below baseline levels.

In vaccinated calves, tear IgA levels increased threefold during the vaccination phase, demonstrated a leveling off phase for three weeks post challenge, then dropped off rapidly.

IgG classes were present, peaking out between two and three weeks post challenge, after which they were essentially non-detectable (Figure 6). This was comparable to the mild tear titer response seen in the same four calves at days 15 and 21 (Figure 6).

Neisseria Vaccine Group: In the four vaccinated calves studied, a greater and more sustained response was seen. By day 21, just prior to challenge, tear titers slightly over 1:600 were associated with high levels of IgA and IgG bound to the bacteria (Figure 7). Following the challenge there was a marked drop in tear titers, although the fluorescent antibody test demonstrated continued high levels for over three weeks post challenge.

Classification of Anti-M. bovis Antibodies

Positive Controls: In tear samples collected from four affected control calves, antibodies from both the IgA and
In this study we were readily able to reproduce clinical IBK with the use of virulent *M. bovis* organisms alone, as we had previously reported. The disease was indistinguishable from the naturally occurring disease in clinical development and progression. The Neisseria vaccine provided complete protection under the controlled environment of this study.

*M. bovis* does not appear to be an effective immune stimulator. The slight rise in tear antibodies was transient and minimal. The lack of antibody variation in affected and non-affected calf tears suggests the absence of any meaningful protection against the disease. The minor immune response was associated with IgA and IgG antibody production; however, these were virtually nondetectable after three weeks post challenge, and were associated with slight changes in gross IgA and IgG tear levels.

The Neisseria vaccine was in comparison a far superior stimulator of the immune system against *M. bovis* antigens. Prior to challenge, the vaccine stimulated a marked increase in tear *M. bovis* antibodies, which was concomitant to increasing levels of tear IgA and IgG. These levels were substantially greater than the control group in either affected or non-affected calves throughout the study.

Fluorescein antibody studies confirmed that the increased levels of IgA and IgG in tears were, in fact, associated with *M. bovis* antibody production. While no antibody was detected on day 0, significant levels were already detectable three days following initial vaccination. At the time of challenge with *M. bovis*, these values were much higher than at any time in affected control calves, and remained high for over three weeks post challenge. In control affected calves, prominent fluorescein antibody tagging of bacteria was found on days 15 and 21 post challenge, after which they were essentially absent.

Preliminary studies have indicated several cross antigens between our Neisseria vaccine and *M. bovis*. This apparent cross antigenicity appears to be the basis for the induced protective immunity to *M. bovis*. A vaccine utilizing the concept of cross-antigenicity is currently used in inducing resistance against canine distemper with a modified live measles virus. 

Why *M. bovis* does not produce a similar immunity to itself is unclear. This may be the key to both the pathogenicity of *M. bovis* in bovine cornea and the difficulty of previous investigators in using *M. bovis* as an effective vaccine against itself.

**References**


Editor’s Note: Several papers which were presented at the various seminars will appear in the 1984 BOVINE PRACTITIONER.
Lutalyse® Sterile Solution
(dinoprost tromethamine)

VETERINARY - For intramuscular use in cattle when regression of the corpus luteum is desired. This includes estrus synchronization, treatment of unobserved (silent) estrus and abortion of feedlot and other non-lactating cattle.

INDICATIONS AND INSTRUCTIONS FOR USE
Lutalyse (dinoprost tromethamine) sterile solution is indicated as a luteolytic agent. Lutalyse is effective only in those cattle having a corpus luteum, i.e., those which ovulated at least five days prior to treatment. Future reproductive performance of animals that are not cycling will be unaffected by Lutalyse injection.

1. For Intramuscular Use for Estrus Synchronization in Beef Cattle and Non-Lactating Dairy Heifers. Lutalyse is used to control the timing of estrus and ovulation in estrus cycling cattle that have a corpus luteum. Inject a dose of 5 ml Lutalyse (25 mg PGF2α) intramuscularly either once or twice at a 10 to 12 day interval. With the single injection, cattle should be bred at the usual time relative to estrus. With the two injections cattle can be bred after the second injection either at the usual time relative to detected estrus or at about 80 hours after the second Lutalyse injection. Estrus is expected to occur 1 to 3 days after injection if a corpus luteum was present. Cattle that do not become pregnant to breeding at estrus on days 1 to 5 after injection will be expected to return to estrus in about 18 to 24 days.

2. For Intramuscular Use for Unobserved (Silent) Estrus in Lactating Dairy Cows with a Corpus Luteum. Inject a dose of 5 ml Lutalyse (25 mg PGF2α) intramuscularly. Breed cows as they are detected in estrus. If estrus has not been observed by 80 hours after injection, breed at 80 hours. If the cow returns to estrus breed at the usual time relative to estrus.

3. For Intramuscular Use for Abortion of Feedlot and Other Non-Lactating Cattle. Lutalyse is indicated for its abortifacient effect in feedlot and other non-lactating cattle during the first 100 days of gestation. Inject a dose of 25 mg intramuscularly. Cattle that abort will abort within 3 days of injection.

WARNINGS
Not for human use.
Women of child-bearing age, asthematics, and persons with bronchial and other respiratory problems should exercise extreme caution when handling this product. In the early stages, women may be unaware of their pregnancies. Dinoprost tromethamine is readily absorbed through the skin and can cause abortion and/or bronchospasms. Direct contact with the skin should, therefore, be avoided. Accidental spillage on the skin should be washed off immediately with soap and water. Use of this product in excess of the approved dose may result in drug residues.

PRECAUTIONS
Do not administer to pregnant cattle unless abortion is desired. Do not administer intravenously (I. V.), as this route might potentiate adverse reactions. Cattle administered a progestogen would be expected to have a reduced response to Lutalyse. Aggressive antibiotic therapy should be employed at the first sign of infection at the injection site whether localized or diffuse. As with all parenteral products careful aseptic techniques should be employed to decrease the possibility of post injection bacterial infections.

ADVERSE REACTIONS
1. The most frequently observed side effect is increased rectal temperature at a 5x or 10x overdose. However, rectal temperature change has been transient in all cases observed and has not been detrimental to the animal.
2. Limited salivation has been reported in some instances.
3. Intravenous administration might increase heart rate.
4. Localized post injection bacterial infections that may become generalized have been reported. In rare instances such infections have terminated fatally. See PRECAUTIONS.

IMPORTANT
No milk discard or pre-slaughter drug withdrawal period is required for labeled uses.

DOSAGE AND ADMINISTRATION
Lutalyse is supplied at a concentration of 5 mg dinoprost per ml. Lutalyse is luteolytic in cattle at 25 mg (5 ml) administered intramuscularly. As with any multidose vial, practice aseptic techniques in withdrawing each dose. Adequately clean and disinfect the vial closure prior to entry with a sterile needle.

HOW SUPPLIED
Lutalyse Sterile Solution is available in 10 and 30 ml vials.

Caution: Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian.

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