Coliform Mastitis Study Designs:
Veterinarian Supplement to Hygieia Biological Laboratories' J-5 Escherichia Coli Bacterin Data Presentation.

Field Efficacy Study
This study was performed on a commercial 500 cow Holstein dairy in Sonoma County, California. The study design compared the incidence rates of coliform mastitis cases in each of three cohorts over approximately two year’s time; field exposure served as the source for the challenge. The three cohorts were established using a random numbers table to provide approximately 50% of animals assigned to the Upjohn (Zoetis) vaccinate cohort, 25% of animals assigned to the Hygieia vaccinate cohort, and 25% of animals serving as unvaccinated controls.

Cows were enrolled on a rolling basis at dry-off (seven months gestation) and followed until they were again dried off at the end of the subject lactation. Cows culled from the dairy for reasons other than coliform mastitis were excluded from the analysis. Cows which developed coliform mastitis (whether or not they remained in the herd until the end of their lactation) and all animals completing the lactation were included in the data analysis. Vaccines were administered at dry-off (7 months gestation), one month later, and after freshening by the herd veterinarian. Other than adding the J-5 vaccinations, all other management practices remained unchanged.

Identification of mastitis was per usual herd practice (clinically abnormal udder and/or abnormal milk), with milk samples obtained aseptically by the herd veterinarian and culture performed independently by a commercial milk quality laboratory. Coliform mastitis was defined as a clinical finding of mastitis where the milk quality laboratory also identified coliform bacteria as the causative agent (defined as a culture plate exhibiting typical coliform colonies in a pure to nearly pure culture).

A total of 219 Upjohn (Zoetis) vaccines, 122 Hygieia vaccines and 121 unvaccinated controls completed the study. Forty-seven cases of clinical coliform mastitis were observed during the study. Repeated cases were not counted; each cow could only have a single case of clinical coliform mastitis scored.

The historical incidence (average of three prior years) of clinical coliform mastitis at the study dairy was 25% per lactation. In this investigation, unvaccinated controls experienced a 22.1% (27/121) rate of clinical coliform mastitis. This was not significantly different than the historical incidence rate (when none were vaccinated).

Study Results:
Among the vaccinated animals, the Upjohn (Zoetis) cohort experienced a clinical coliform mastitis incidence of 7.8% (17/219), for a reduction of 65% compared to the control incidence. The Hygieia cohort experienced a clinical coliform mastitis incidence of 2.5% (3/122) representing a reduction of 89% compared to the control incidence. Both of these results were statistically significantly different from the control cohort incidence. Moreover, the Hygieia vaccinates were also statistically significantly different from the Upjohn (Zoetis) vaccinates, with less than one-third of the clinical coliform mastitis that was seen in the Upjohn (Zoetis) vaccinate cohort.

Intra-mammary Challenge Study
This placebo controlled study was performed by Hygieia personnel on a new commercial 450 cow Holstein dairy in Stanislaus County, California. Eighty-six first-calf heifers were enrolled in this trial based upon 1), negative core antigen vaccination history confirmed by negative serology for J-5 E. coli, 2), absence of mastitis (normal SCC and CMT in each quarter), 3), absence of micro-organisms in the milk on quarter culture, and 4), being less than nine weeks post-calving at the time of the first dose of vaccine. All animals selected had calved within a five week period.

A random numbers table was used to randomly allocate animals into cohorts. A total of 53 heifers were assigned to the vaccinate cohort and 33 were assigned to the control cohort.

A total of three doses of Hygieia’s Escherichia Coli Bacterin were administered to each heifer in the vaccinate cohort. At the same time periods, a total of three doses of placebo were administered to each heifer in the control cohort. Four weeks elapsed between the first and second dose, and six weeks elapsed between the second and third dose; this schedule was intended to mimic the timing of dry-off, close
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up, and two weeks fresh vaccination schedule specified by the product instructions.

Ten controls were lost to the challenge study before challenge: six were used in two pilot studies to ascertain the virulence of the two candidate challenge strains, and four were culled by the herd owner (1 LDA, 1 trampling death, 2 ill-thrift due to respiratory illness). Four vaccinates were lost to the challenge study before challenge: all four were utilized in a pilot study to confirm the virulence of the selected challenge strain.

The challenge portion of the study was blinded: personnel performing the challenge were not aware of the vaccination status of the animals challenged. Post-challenge scoring of mastitis and other parameters were also blinded; personnel performing the evaluations were not aware of the vaccination status of the animals.

Eighteen vaccinates and eight controls were challenged by intra-mammary infusion with a tiered dose of a virulent field strain of Escherichia coli bacteria derived from a lethal case of coliform mastitis.

The challenge was inadvertently made more severe by the dairy owner’s changing from 3x milking to 2x milking on the day of challenge; thus, instead of an 8–9 hour delay between challenge and first milking, the trial was forced to wait over 13 hours between challenge and first milking. This resulted in a much more severe challenge than was planned based upon the pilot studies.

A Composite Udder Score was computed for each animal at each milking during the study. The composite score ranged from 0 to 16, and included assessments of udder swelling, udder color, udder texture, number of quarters involved, and time/duration of mastitis. The Peak Severity Udder Score was the highest composite score seen in each animal post-challenge, and was used to determine the severity of mastitis.

All eight control animals developed mastitis post-challenge. Two cases were mild (score of 2–6)1, five cases were moderate (score of 7–11), and one case was severe (score of 12–16). Of the eighteen vaccinates, three cows (16.7%) remained symptom-free (maximum score of zero) following challenge. Thirteen cows developed mild mastitis (score of 2–6), one developed moderate mastitis (score of 7–11), and one developed severe mastitis (score of 12–16). In percentage terms, 89% of vaccinates experienced mild or no mastitis, while 75% of controls experienced moderate to severe mastitis.

At the time of challenge, twelve of the eighteen vaccinates and five of the eight controls were pregnant. None of the vaccinates aborted; one control aborted and the challenge strain of E. coli was recovered from the lungs and spleen of the fetus. In addition, none of the vaccinates developed dry quarters following challenge, while five of the eight controls developed dry quarters.

There were statistically significant differences between vaccinates and control animals in peak udder score, challenged quarter milk score2, abortions, dry quarters, and duration and morbidity of clinical mastitis.

Study Results:
In this fully blinded, randomized, placebo-controlled, prospective-cohort, vaccination-challenge study, Hygieia’s J-5 Escherichia Coli Bacterin demonstrated statistically significant differences in the level of protection afforded by the vaccine against a highly virulent challenge strain and markedly severe challenge model compared to placebo-vaccinated control animals. Vaccinates experienced statistically significantly less mastitis, less severe peak mastitis, shorter duration of mastitis, fewer complications (abortions, dry quarters), less culling, and quicker return to normal milk than control animals.

Learn more at: hygieialabs.com/j-5.html.

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1. It was not possible to have a score of "1"; if any sign showed up in the quarter, the score would include a composite value made up of the number of quarters plus a score for the abnormality. The minimum score for any sign of mastitis is thus "2".

2. Data not shown, but parallels the udder scores.