Efficacy- Challenge Study for *PASTEURELLA MULTOCIDA* BACTERIN for use in Rabbits.

**Introduction and OBJECTIVE(S):**

This study was designed to test efficacy of an experimental *Pasteurella multocida* Bacterin in laboratory rabbits by vaccination and challenge. Colorado Serum Company (CSC) has two currently licensed products containing *Pasteurella multocida*: 1. *Pasteurella multocida* Bacterin, Bovine and Porcine isolates (code# 2701.22), and 2. *Mannheimia haemolytica*- *Pasteurella multocida* Bacterin (code # 2700.00). The firm intends to use the currently licensed Bovine isolate (strain 1062) only, for a rabbit use license.

The study was conducted according to the approved efficacy protocol from a letter dated October 4, 2010, including recommended modifications by the firm’s reviewer.

The study was collaborated between Colorado Serum Company and Pan American Veterinary Laboratories (PAVL). PAVL has used CSC’s *Mannheimia haemolytica*- *Pasteurella multocida* Bacterin (code # 2700.00) off label in their laboratory rabbits and there is anecdotal evidence that the vaccine appears to decrease morbidity and mortality caused by *Pasteurella multocida*. The *Mannheimia haemolytica*- *Pasteurella multocida* Bacterin also contains the same Bovine isolate (strain 1062) as in the license code # 2701.22. Colorado Serum Company provided the experimental *Pasteurella multocida* Bacterin, while the study was conducted at PAVL.

The goal of this study was to show that this experimental *Pasteurella multocida* Bacterin decreased morbidity and mortality associated with *Pasteurella multocida* infection in a minor species (rabbits) by vaccination and challenge.

**STUDY PARTICIPANTS**

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**MATERIALS AND METHODS**

 **study Schedule**

Date of Initiation: March 21, 2011

 Two vaccinations four weeks apart with challenge 3 weeks after last vaccination. See **Table 1.** Schedule of Events

Date of Completion: May 23, 2011

 **Table 1. Schedule of Events**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Study Day | Blood Collection | AdministerVaccines | AdministerChallenge | Monitor for clinical signs of Disease/ Mortality |
| -7 days  | X  |  |  |  |
| Day 0 (V1) |  | X |  |  |
| Day 28 (V2) |  | X |  |  |
| Day 49 | X |  | X |  |
| Day 50-64 |  |  |  | X |
| Day 64 | X | End Study |  |  |

**Study Design**

**Treatment Groups:**

 Thirty (30) New Zealand White rabbits at 12 weeks of age and mixed sex were used for this study and separated into three treatment groups. Inclusion criteria included healthy rabbits that were sero-negative to Pasteurella multocida.

 Blood was drawn according to the schedule noted in Table 1 to evaluate sero- negative animals at the beginning of study and antibody production response after vaccination. An ELISA screen was performed at PAVL. See **Attachment 1** for ELISA protocol.

**Experimental Design:**

The study was a blinded study containing three treatment groups. Three vaccines were prepared at Colorado Serum Company and sent to PAVL- Vaccine A, Vaccine B, and Vaccine C. Two of the vaccines (Vaccine A and B) contained *Pasteurella multocida* Bacterin, and the third (Vaccine C) was a placebo. PAVL did not know which of the three vaccines contained the placebo thus providing a blinded study model. Record of the receipt and use of the vaccine at PAVL and also the return of the unused vaccine and disposal at CSC is included in **Attachment 2.** Additionally, a copy of the letter received from the Texas Animal Health Commission granting approval for CSC to ship the unlicensed Bacterin to PAVL for use in the efficacy study is included in **Attachment 3**.

PAVL vaccinated ten rabbits per vaccine at Day 0 of the study. The rabbits received a second dose of vaccine at day 28. At day 49, PAV administered the challenge to all of the rabbits in each of the three Vaccine groups. All three Vaccine groups were monitored during days 50-64 for clinical signs of disease, duration of disease, and death.

**Vaccine:**

The vaccine used in this study was Experimental *Pasteurella multocida* Bacterin Code# 2701.02, unlicensed (Colorado Serum Company Lot # 570, Harvest Date- 4- 8 10, Serial 101A). A Serial Mix Overview and Bacterial Count sheet is located in **Attachment 5**. The bacterial count per dose in the final vaccine is within the range set forth in the proposed Production Outline Section IV. D.

**Vaccine and Challenge Administration:**

**Vaccination Regimen:**

There were three groups of treatment rabbits. Animals were vaccinated as follows: Groups A, B, and C (vaccination and challenge)- 10 rabbits per group for a total of 30 rabbits. See Table 1 for Schedule of events.

V1: Day 0 with 0.5 ml of vaccine.

V2: Day 28 of the study with 0.5 ml of vaccine.

All vaccinations were given subcutaneously (SC) above the shoulders, using sterile needle and syringe for each rabbit.

**Challenge Regimen[[1]](#footnote-1)**

All three groups of rabbits were challenged as follows:

Each group was challenged on Day 49 of study with live *Pasteurella multocida* Strain 1062 (Bovine origin) Type A.[[2]](#footnote-2) Each rabbit received 0.25ml of live challenge culture in each nostril for a total of 0.5ml challenge dose.

**Challenge culture**

Prior to conducting the actual Efficacy Study, proof of concept studies for the challenge culture were conducted on several groups of unvaccinated, sero-negative rabbits, in order to evaluate the proper challenge dose. The challenge dose stated in the submitted protocol and referenced article by Chengappa et al, was 1.0- 2.0 x 1010 organisms/ 0.5 dose intranasal. This dose was proven to be too high of a challenge in our model as it resulted in death of 100% of the rabbits challenged. The study was designed to find a dose that would result in disease symptoms in 100% of the rabbits challenged and a mortality rate of +/- 50%.

The studies concluded that a challenge dose of 0.5ml (0.25/ nostril) containing approximately 1x107 organisms total, achieved the type of challenge desired for the study. See **Attachment 6** for Pasteurella count Protocol/Verification.

**Observations:**

**Daily health**

Study investigator or designated qualified caretaker at PAVL observed the rabbits daily from Day 0 to day 49 of study (before challenge) for general health. Rabbits were also weighed weekly throughout the study.

**Observation of rabbits after challenge**

Rabbits were observed daily from Day 50 to Day 64 of study (for 15 days post challenge) for clinical signs of disease. Observations included: nasal discharge, sneezing, rales (audible snuffles), anorexia, and death. These were based on a graded system for each disease symptom. The daily clinical signs scores were totaled in order to conclude illness severity which was determined as no disease, mild, moderate, or severe. The numbers were statistically analyzed for submission and analysis includes disease severity and duration of disease symptoms. Additionally, post mortem exams were performed and recorded by the Study Investigator.

**Sample Collections Schedule, volumes, and storage conditions.**

**Blood Collection Schedules:**

Blood was collected at days -7, 49, and 64 (total of three bleeds). Sample volumes were adequate for ELISA test requirements. Blood samples were collected from an ear vein on Day -7 and 49, and ear vein or intracardiac, under anesthesia, for day 64.

**Storage Conditions**

Serum was separated from the clot by refrigeration overnight or centrifugation and stored in 3 aliquots of 1ml each at -20°C.

**Concurrent/Concomitant Medications/Treatments:**

At the discretion of the Study Investigator at PAVL, animals could be treated for infectious diseases or other illnesses. Injured animals may also have been treated.

**RESULTS**

 **Daily Health**

Each of the 30 rabbits total were observed daily for general health during the 49 day vaccination period before challenge. The rabbits remained healthy without sickness either related or un-related to the vaccine. There were not any treatments given for sickness or injuries to any of the rabbits throughout the observation period. The only entries were from a rabbit from Vaccinate Group A (#1) that had issues with mal-occluded incisors which had to be trimmed on several occasions

 **Weekly Weight Record**

The rabbits were weighed weekly during the study. Weight differences were not significant between the three Vaccine Groups throughout the study.

 **Post Challenge Observation**

The rabbits were observed for 14 days post challenge. The clinical signs were rated and recorded for every individual rabbit each day based on a grading guide listed on the Post- Challenge Observation Form. The grading guide contains the scoring system recorded for clinical signs including nasal discharge, sneeze frequency, rales, and anorexia. These clinical signs were analyzed for both severity and duration of disease. Death after challenge was also analyzed.

 **Duration of Clinical Signs.**  The median number of days animals in the placebo control group were observed with disease was 15. In the vaccinated group, the median number of days with disease was 9. The duration of disease was significantly lower in the vaccinated animals as compared to the controls (P<0.0001).

 **Severity of Clinical Signs**. There were only mild to moderate (not severe) symptoms observed according to the grading system in place. The severity of disease was lower in the vaccinated animals as compared to the placebo animals (P=0.0577 in Fisher’s exact test).

 **Mortality.** Mortality was observed in 60% of the unvaccinated placebo group while 0% was observed in the vaccinated groups. Therefore, mortality was significantly lower in the vaccinated animals as compared to the placebo animals (P<0.0001).

 **ELISA.** The grouped by day interaction was significant (P<0.05); therefore within day group effects were investigated. Sample titers collected from vaccinated animals on May 9 and May 24 were significantly higher than those in the control group (P<0.01). See electronic data for ELISA values and statistical report for ELISA summary. The firm believes that this test has the potential to be used as the potency test for serial release and may be pursued once licensing is obtained.

**SUMMARY**

The goal of this study was to show that this experimental *Pasteurella multocida* Bacterin decreased morbidity and mortality associated with *Pasteurella multocida* infection in a lesser species (rabbits) by vaccination and challenge. The vaccine significantly lowered the duration of the disease from a median of 15 days to 9 days. The severity of disease in the vaccinated animals was also lower. Mortality was observed in 60% of the un- vaccinated control group, with none observed in the vaccinated group, therefore the vaccine prevented mortality in 100% of the vaccinated animals. The data from this study significantly supports this vaccine and its decreased morbidity and mortality claim.

1. Challenge model referenced from M M Chengappa, et al (1980) “A Streptomycin Dependent Live Pasteurella multocida Vaccine for the Prevention of Rabbit Pasteurellosis.” [↑](#footnote-ref-1)
2. Referenced from Z. S. Al-Lebban, et al (1989) “Rabbit pasteurellosis: Respiratory and renal pathology of control and immunized rabbits after challenge with Pasteurella multocida.” [↑](#footnote-ref-2)