Grant Expenditure Responsibility Reports

2008
USEF Equine Health
Research Fund

$25,000
For Research by
the University of
Florida into Ethyl
Pyruvate in Lactated
Ringer's Solution
and whether It's an
Inexpensive and
Effective Treatment Solution for
Endotoxemia in Horses

FINAL REPORT

1. **Institution:** *University of Florida College of Veterinary Medicine*
2. **Date of report:** *Sept 11, 2012*
3. **Title of project:** *Ethyl pyruvate in intravenous fluids: An inexpensive and effective treatment for endotoxemia in horses.*
4. **Names of investigators:** *Robert J. MacKay*
5. **Start date:** *April 1, 2009*
6. **End date (note any no-cost extensions):** *November 1, 2011, by no-cost extension*

Lay language update - This portion of the report will be shared with donors and study sponsors

7. Summary statement.

A. Significance of project and how this project sought to address the identified health issue for animals

One of the main reasons horses with severe colic and severe diarrhea get sick is because a part of the bacteria gets released into the blood stream. This part, called endotoxin, is very damaging to the tissues of the affected horse. Unfortunately, there are no good treatments for endotoxin, so we are investigating a substance called ethyl pyruvate, which has had promising results in experimental animals.

B. Method(s) undertaken during the project

The problem with endotoxin in the blood (called endotoxemia) can be recreated humanely by giving small amounts of endotoxin into the blood of healthy horses. We did this to 3 groups of 6 horses each. One group got endotoxin and ethyl pyruvate (EP) at the same time, another group got EP 2 hours after endotoxin, and the third group got endotoxin without EP. We measured clinical signs and tested the blood of treated horses to find whether or not EP had an effect.

C. Brief description of the study's major findings.

Horses given endotoxin got the expected mild signs of fever and increased heart and respiratory rates. Unfortunately, EP had no effect on these signs of endotoxin.

D. Description of this applied research will impact veterinary professionals/wildlife managers in a way that improves patient care, diagnosis or treatment of disease

This was applied research. Veterinary professionals will learn from this project that ethyl pyruvate, although highly promoted as a miracle treatment on the basis of lab animal studies, likely has no role in the treatment of sick horses.

8. **Hypothesis & Objectives**. Summary of the hypothesis and objectives of project and completion of proposed objectives.

The hypothesis of the research was that ethyl pyruvate would reduce the adverse signs of endotoxin in the blood. The objectives were to examine clinical signs and blood markers in groups of 6 horses that had been given intravenous endotoxin and either (1) EP for 2 hours intravenously, beginning at the same time as endotoxin; (2) EP for 2 hours intravenously, beginning 2 hours after endotoxin, and; (3) saline as a control. Treatments 1 and 2 were expected to reduce the adverse effects of endotoxin, compared to what was found in group 3, the treatment control group.

9. **Species/Breed.**

Horses of various breeds were used. The results are applicable only to horses and other equids.

10. **Problems**. Summary of problems encountered and solutions to those problems.

Experiments were not begun until November 2009 for the reasons enumerated in my previous

progress reports. At the beginning of December 2010 I was assigned a new lab technician after the death of my previous technician. This new technician underwent training in the techniques required and has completed the analyses of stored samples.

11. **Animal Involvement.** Summary of animal care protocols and animal experiments.

    These experiments involved the intravenous infusion of endotoxin to simulate the condition found in many sick horses. The dose that we used was designed, on the basis of previous experiments, to induce a mild syndrome of malaise (a feeling of illness), fever, and increased heart and respiratory rates that persisted for several hours. Within 2 hours of endotoxin all horses resumed drinking and eating and, 24 hours after endotoxin, all horses were given a dose of flunixin (Banamine) to treat any remaining signs. No horse met any of the treatment triggers that would have required Banamine treatment during the 24 hours of the experiments. All experiments were approved by the IACUC committee of the University of Florida.

12. **Training component.**

    This project involved one resident, one technician, and several OPS student workers. No veterinary students or graduate students were involved in the project.

13. **Study Success.** Summary of study successes.

    Unfortunately, the results of the study did not support the use of ethyl pyruvate as a treatment for endotoxemia. While negative results have value, we were disappointed that this agent did not live up to its original potential.

14. **Publications.** List of all past, currently accepted and future planned publications.

    A publication for the American Journal of Veterinary Research is currently in preparation.

15. **Presentations.** List of all past, currently accepted and future planned presentations..

    These data will be presented at the ACVIM Annual Convention 2014.

16. **Patents.**

    No patents will be submitted as a result of this work.

17. **Scientific Summary**. Summary of progress and results as related to each of the original objectives:

    The hypothesis was that EP would improve the signs of endotoxemia in horses. This hypothesis was addressed by examining the effects of Magnesium-rich Normosol containing EP (Norm-EP-Mg) in 3 groups of healthy horses (6/group) given LPS according to the following specific aims: first, to record clinical and metabolic responses of horses to IV LPS (Group 1); second, to evaluate the ability of Norm-EP-Mg to prevent effects of LPS by beginning Norm-EP-Mg infusion at the time of LPS administration (Group 2), and; third, to evaluate the ability of Norm-EP-Mg to treat effects of LPS by beginning infusion 2 h after LPS administration (Group 3). Clinical signs, CBC, blood lactate, tumor necrosis factor, HMGB1, thromboxane, prostacyclin, nitric oxide, and 8-isoprostanol (indicator of oxidative damage) were to be measured at intervals for 24 h in each group. The expectation on which the work was based was that early EP infusion will substantially or completely prevent all of the signs of endotoxemia.

    The 3 objectives have been completed. All animal work has been completed including infusions (LPS and EP), recording of clinical signs, and collection of samples. Analysis of samples is complete with the exception of HMGB1 and 8-isoprostane, for which there was insufficient sample volume remaining for assay.

18. **Major Findings.**

Endotoxin induced the expected clinical and metabolic changes that were expected; however, there was no significant effect of ethyl pyruvate infusion, either early (first 2 hours) or late (beginning 2 hours after endotoxin).

19. **Project Goals.**

We accomplished our goals of testing the hypothesis that ethyl pyruvate would ameliorate the signs of endotoxemia. The hypothesis was not supported, which is a useful, albeit disappointing, result.