Project 1: Monitoring Caribbean donkey population by applying PZP and rPZP

Abstract: Donkey populations continue to increase on Caribbean islands while resources become more limited and the donkeys' welfare continues to be compromised. Recent studies at Ross University have shown promising results for administering porcine zona pellucida (PZP) and recombinant porcine zona pellucida (rPZP). PZP works by blocking sperm cells from penetrating the outer covering of the egg by producing an antibody. The vaccine can be administered once a year and prevent pregnancy. One time application has shown as high as 75 percent efficacy rates in preventing pregnancy and 95% with a booster. The vaccine is fairly inexpensive and does not alter natural behavior or cause abortions if the jenny is already in foal. Considering the environment where the donkeys are living, on an island it is feasible and realistic that the jennies could be captured and then released after vaccination and monitored for a years' time for pregnancy. Future application of the vaccine could be accomplished by darting. The study protocol will be tested on two islands Nevis and Bonaire. Ross University in St. Kitts has a strong relationship with Nevis regarding working with the donkey population for teaching and research. Bonaire will be the second test island where PVDR has assisted in the past with the donkey population and has established collaborations. Both PZP and rPZP have shown in clinical trials by Ross University Veterinary Teaching Hospital to work well in donkeys and could help reduce populations greatly in as short as one year.

Study Protocol: A pilot study with 125 jennies will be trapped using protocols successfully developed by PVDR and Ross University on each island. Donkeys will be trapped on two test islands Bonaire and Nevis. A onetime 10 mL blood sample will be taken from the jugular vein and analyzed for serum progesterone to detect pregnancy. The jennies will be divided into 3 groups, control, PZP and rPZP. Each jenny will be fitted with a neck collar and microchipped. Neck collars will be three different colors, red for control, green for PZP and yellow for rPZP. Groups will be randomly assigned. Fifty jennies will be treated with PZP and fifty with rPZP. Twenty-five jennies will not receive a vaccine. Donkeys will be monitored by Ross University veterinary personnel and/or principal investigator every three months for one year after the administration of the vaccines. Monitoring will include recording signs of estrus and jennies that foal. Foaling records will be compared with serum progesterone results taken at the beginning of the study. If positive results are seen and pregnancy rates are reduced then a continuation of the study should continue for an additional year. Research protocol will be approved by the UC Davis Institutional Animal Care and Use Committee prior to starting the study to ensure the best welfare possible for the donkeys in the study.

Expected outcome: PZP and rPZP will prevent pregnancy in jennies for at least one year. Similar low to zero conception rates will be seen with both vaccines. A continuation of the study will be necessary for one additional year adding a fourth treatment group. The fourth treatment group will include a combination of PZP and rPZP treated jennies plus a booster at 2-6 weeks. Additional blood sample will be taken to detect pregnancy at the time of vaccination. After two years of monitoring a decision can be made if PZP needs to be booster at 1 year or if 2 years is adequate. Then population will likely be controlled with the use of PZP and/or rPZP

Budget: $39,670

Expense	Description	Number	Total
Supplies			
PZP	Porcine zona pellucida vaccine + adjuvant ($1/dose)	50	1200
rPZP	Recombinant Porcine Zona Pellucida vaccine + adjuvant ($1/dose)	50	1200
Hypodermic Needles	14 x 1” (100/box)	3 boxes plus shipping	1300
Hypodermic Needles	18 x 1 ½ “ (100/box)	3 boxes plus shipping	140
Syringes	12 mL (100/box)	4 boxes plus shipping	100
Test Tubes	Purple top tubes (100/ flat)	3 boxes plus shipping	100
Betadine solution	1 bottle		20
Latex gloves	3 boxes (100/box, Small, Medium, Large)	3 plus shipping	60
Sharps container	Pack of 3	3 plus shipping	60
Lab fees	Progesterone serum assay (to detect pregnancy in blood)	125	1560
Neck collars	Neck collars $4/collar	125	500
		Total	**6,240**
Travel expenses			

Airfare	SMF-SKB x 1 person (Researcher from Sacramento to St Kitts) x 2 trips (1 trip for survey, 1 trip to conduct study and 6 month check)	2	2800
Airfare	SKB-BON x 2 people)	2	1000
Airfare	SKB-BON x 2 people for 3 month surveys x 4	2 x 4	4,000
Lodging-Bonaire	2 people for 2 weeks, AirBnB house/ apartment loft	1 apartment/house for 2weeks in Bonaire	1800
Lodging-St Kitts	1 person AirBnB house/loft/apartment	1 apartment/house for 2 weeks in St Kitts	1500
Per Diem	Food and incidentals St Kitts x 14 days	2 people x 14 days	3800
Per Diem	Food and incidentals Bonaire x 14 days	2 people x 14 days	3700
		Total	**18,600**
Research Stipends			
Principal Investigator	Researcher stipend to develop study, implement design, analysis data and write findings for scientific report/ journal	1 month	11,330
Ross University Research Assistant 1	Researcher fee $500/ data collection point	5	2500
		Total	**13,830**
		Total Budget	**39,670**

Project 2: Developing a grimace scale for mules

Abstract: We continue to see a growing interest and increase in the mule population in the U.S. On a global level mules, hinnies and donkeys are still used in many developing countries as draft animals but here in the US and other industrial nations the desire to trail ride and compete with mules is increasing. However, very little information is reported in scientific literature about mules. For the first time the Vet Clinics of North America, a reference text for most veterinarians devoted a special edition to Donkey and Mule Medicine but still a majority of the chapters focused on donkeys. Mules are not horses nor are they donkeys. From pharmacokinetic studies we know they respond and metabolize medication different than horses or donkeys. In some cases, mules are more hardy than horses, faster and often bigger than donkeys but what do we truly know about their needs? From a behavior stand point we often see combined traits from both parents but in some situations such as restraint we have found in our studies on mule and hinny behavior that certain applications of learning theory and early foal handling are essential for shaping adult mule behavior. From studying what little we do know about mule behavior how do we identify pain in mules? Some mules seem to be more sensitive and show signs of acute pain while others maybe more similar to their sire and express fewer traditional signs of pain that have been documented in horses with similar painful conditions. We know from a few studies in donkeys that their stoic nature may mask or cover signs of pain and for example when a donkey

is feeling pain such as abdominal pain from colic the disease may be more advanced by the donkey not expressing signs of pain early on and therefore it becomes more difficult to treat the condition. This study begins to define signs of pain in mules that could share as an educational tool for recognizing pain hopefully early on so proper treatment can be applied. The use of new technology through a smart halter may also help identify early physiological signs of pain such as increased heart rate and respiration and or recumbency behavior that could suggest the mule is not comfortable.

Study Protocol: Ten healthy male mules will be photographed, videoed, and equipped with a smart halter pre and post castration. Photographs will be taken 48 hours, 24 hours, 12 hours, 6 hours and 1 hour prior to castration. Post castration photographs will be taken 1-hour post castration, 6 hours, 12 hours, 24 hours and 48 hours. Videos will also be been taken at the same time point for 5 mins. All mules will be equipped with the smart halter (NightWatch, Austin, Tx) 48 hours prior to castration and kept on during the procedure, then after the procedure for 48 hours. The halters will record information every 15 seconds. An ethogram displaying key body language often used to evaluate pain in other animals will be developed to train observers to evaluate body language for signs of change. The training module will include photographs and videos with written descriptions, and a voice-over to describe each descriptor, photograph and video. Changes in tension, posture, or position will be displayed in the training material and described by written and voice descriptions. Signs of discomfort or pain that has been documented in other animals including most recently donkeys, such as orbital tightening of eyes, muzzle tension, along with ear and body language will be illustrated and described. Each observer will be required to complete the mule ethogram training module before completing the study survey. From the ethogram training materials, nine facial and body language markers (ear frontal and side position, eye description -shape and tension-, muzzle and nostril description -shape and tension- and stance) along with behaviors such as tail swishing will be identified as possible pain indicators per findings from other studies and will be utilized for the development of the mule grimace scale tested in the survey. The study will be carried out at the American Fonduk Veterinary Clinic, Fes, Morrocco. All castration surgeries will be conducted by the Fonduk's team of veterinary surgeons. The castrations will not be conducted for this study, we will be observing scheduled castrations requested by mule owners. All procedures will be reviewed by the UC Davis Institutional Animal Care and Use Committee and owner consistent for us to monitor the mules physiological signs with a smart halter and to video/photograph mules pre and post castration will be obtatined before proceeding with the study.

Expected Outcome: The development of a mule grimace scale will create more training tools for those who work with mules from owners to veterinarians. The additional educational tool will hopefully improve people's ability to detect pain and or discomfort in mules and therefore can identify any slight discomfort and provide treatment immediately. We hope one of the outcomes is improved welfare for mules

by identifying discomfort quicker. A second expected outcome is to learn more about physiological standards and reference values for mules using the smart halters. In a fast-paced world we hope the halter will provide additional insight on recognizing signs of stress or discomfort by adding to our knowledge about reference ranges for basic vital signs such as heart rate and respiration. We will know more about sleep patterns in mules as well and how much time they spend standing, rolling or lying down. All this information will serve as great teaching tools and educational material that has great potential to improve our care and understanding of mules.

Budget: $11,330

Expense	Description	Individual Cost	Total
Supplies, Equipment, travel and other expenses			
Nightwatch (Smart Halters)	Smart halter to record physiological data	6	$3600
Video Cameras	Cameras to monitor overnight behavior of mules pre/post castration	6	1,200
Travel costs to Vet Clinic	Flight to American Fonduk, Fes, Morocco		1800
Publication Costs	Print research article	1600	1600
		Total	**8,200**
Personnel:			
Principal Investigator	Researcher stipend- to carry out the study, implement design, analysis data, and write scientific journal article and reports on the findings so educational tools can be created.	1 month	11,130

Student researcher	Research assistant- to assist with data collection and organizing data for analysis	1,500.00	1500
		Total	**20,830**
		Total supported by Morris Animal Foundation Equine Behavior Grant	**9,500**
		Total requested	**11,330**
		TOTAL 2 PROJECTS	**50,000**

Total 2 projects: $50,000 one year