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Veterinary Research Manpower Development for Defense

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The long-term goal of this training program is to increase veterinary research manpower by providing research training to veterinary students. The proposed training program included joint degree programs (DVM/MPH and DVM/MS-LAM) and the summer research program. The three trainees recruited in the joint degree program are continuing their training and the 7 trainees, recruited in the summer research program, have completed their research projects. One trainee joined the US Army. No publication has resulted from the research conducted by the trainees. No major problem was encountered with recruiting or maintaining trainee interests in the training program during the first year of the program and hence we plan to continue the program as originally proposed.

Veterinary Research Manpower, Joint-degree program, Summer Research Program

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INTRODUCTION:

The long-term goal of this training program is to increase veterinary research manpower by providing research training to veterinary students. The program is expected to develop a pipeline of research ready veterinarians to solve biodefense and public health problems facing our country, in general, and our armed forces in particular. The following training programs offered by Cummings school of veterinary medicine were proposed for this purpose.

1. Summer Research Program
2. Four-year joint DVM/Master’s degree in Public Health degree (DVM/MPH) program
3. Four-year joint DVM/Master of Science degree in Laboratory Animal Medicine (DVM/MS-LAM)
4. Five-year joint DVM/MS in Biomedical Science (DVM/MS-BS)

During the summer research program, veterinary students are trained to conduct mentored research under the guidance of a faculty. Veterinary students are also recruited in joint degree programs that allow more specialized training in public health and laboratory animal medicine and more in-depth research training biomedical sciences.

This is a multi-year program with the exception of the Summer Research Program. Because of the lead time needed to recruit trainees in the five-year joint DVM/MS in Biomedical sciences, this program was not included in the first year of this multi-year program.

Body:

This is a training grant with training opportunities in multiple programs, and this progress report includes accomplishment during the first year of this multi-year program.

Program Implementation: As proposed in the application, veterinary students were informed of the training program soon after receiving the award notice. A website as well as a brochure (see appendix) was created to inform veterinary students and faculty (http://www.tufts.edu/vet/researchtraining/defense.html). The website included the program description, responsibilities of trainees and mentors, list of program faculty and individual program directors and an application package. In addition, students and faculty were informed of the program via email with reference to the website and a seminar was held to explain the program to students. Prospective trainees were asked to contact program faculty for summer research projects and program directors for the joint-degree programs.

Recruitment: The goal of the program during the reporting period was to recruit 7 trainees in the Summer Research Program and 2 trainees each in two of the joint-degree programs (DVM/MPH and DVM/MSLAM). Fourteen students applied for the 7 trainee positions for the Summer Research Program, 2 students applied for the DVM/MPH and one student applied for the DVM/MSLAM program. Since students applying for the joint-degree programs met the eligibility criteria, they were recruited in the program. Because of limited positions for the Summer Research Program, 7 trainees out of 14 applicants were selected based on the scientific merit of the proposal, relevance to biodefense and stated interest in an army career. All recruited trainees in various programs are listed in table 1.

Progress to date: All trainees in the joint degree programs are still in the program, are in good academic standings and are expected to complete their training in 2009 or 2010 (see table 1). All 7 trainees in the summer research program have completed their research projects (Table 2) and are in the process of preparing research reports due by the middle of September, 2007. These trainees will present their research work at the annual veterinary student research day on October 17, 2007. They will also compete for three
awards based on the written report and the oral presentation, and will receive the award at the end of the annual veterinary research day.

Each trainee in the Summer Research Program is required to complete a survey to provide feedback on their research experience and future plan. The survey data will be available after the due date of this progress report and will be included in future progress reports. However, an informal get together with the trainees revealed that all trainees are happy with the program and most of them plan to seek further research training in the future.

All trainees were required to attend special information session on career opportunities in the US Armed forces for veterinarians. Please note that one of the trainees (Ms. Jessica Connolly) has joined the Army during the academic year.

**Table 1: Trainees recruited in various programs**

<table>
<thead>
<tr>
<th>Name of Trainee</th>
<th>Training program</th>
<th>Training completion year</th>
<th>DVM graduation year</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jessica Connolly</td>
<td>DVM/MS-LAM</td>
<td>2009</td>
<td>2009</td>
<td>Joined Army in 2007</td>
</tr>
<tr>
<td>Jennifer McRobbie</td>
<td>DVM/MPH</td>
<td>2010</td>
<td>2010</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Summer Research Program</td>
<td>2007</td>
<td>2010</td>
<td></td>
</tr>
<tr>
<td>Tammy Han</td>
<td>DVM/MPH</td>
<td>2009</td>
<td>2009</td>
<td></td>
</tr>
<tr>
<td>Victoria Fields</td>
<td>Summer Research Program</td>
<td>2007</td>
<td>2010</td>
<td></td>
</tr>
<tr>
<td>Miranda Hillyard</td>
<td>Summer Research Program</td>
<td>2007</td>
<td>2010</td>
<td></td>
</tr>
<tr>
<td>Shannabeth Minior</td>
<td>Summer Research Program</td>
<td>2007</td>
<td>2009</td>
<td></td>
</tr>
<tr>
<td>Misha Park Robyn</td>
<td>Summer Research Program</td>
<td>2007</td>
<td>2009</td>
<td></td>
</tr>
<tr>
<td>Rebecca Steers</td>
<td>Summer Research Program</td>
<td>2007</td>
<td>2010</td>
<td></td>
</tr>
<tr>
<td>Tierra Wilson</td>
<td>Summer Research Program</td>
<td>2007</td>
<td>2010</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: 2007 Summer Research Projects**

<table>
<thead>
<tr>
<th>Name of Trainee</th>
<th>Name of Mentor</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jennifer McRobbie</td>
<td>Dr. Lindenmeyer</td>
<td>The Serological Surveillance of Rhinolophus Megaphyllus For Hendra Virus and Australian Bat Lyssavirus</td>
</tr>
<tr>
<td>Victoria Fields</td>
<td>Dr. Lindenmeyer</td>
<td>A Study of <em>Desmodus Rotundus</em>-Mediated Bovine Rabies in Ometepec, Mexico</td>
</tr>
<tr>
<td>Miranda Hillyard</td>
<td>Dr. Epstein</td>
<td>Investigating the Risk of Food-Borne Transmission of Nipah Virus in Bangladesh</td>
</tr>
</tbody>
</table>
Shannabeth Minior  Dr. Miranda  Prevalence of Brucellosis in Zimbabwean Caprines
Misha Park Robyn  Dr. Jost  Determining the Sensitivity of the Participatory Disease Surveillance Method for Detection of Highly Pathogenic Avian Influenza in Java, Indonesia
Rebecca Steers  Dr. Lindenmeyer  Detection of Mycobacterium bovis in Nasal Mucous and Milk Samples Using Polymerase Chain Reactions
Tierra Wilson  Dr. Kaufman  Differentiating M. tuberculosis, M. bovis, and M. avium in Captive Elephants in Nepal

Key Research Accomplishments
The goal of this program is to provide research training to veterinary students by allowing them to participate in a research project over the summer months (June-August). This short term research training usually does not result in the publication of a manuscript, as the trainees are still learning the research methods. However, minor advances are usually made. Since the final report of the research accomplishments is not due until the middle of September, 2007, i.e., after the due date of this progress report, key research accomplishment will be reported in the next annual progress report. However, the breadth of research conducted by the trainees can be found in table 2 and in the abstract of the proposal listed in appendix.

Reportable Outcomes:
1. Two trainees were recruited in the DVM/MPH joint degree program
2. One trainee was recruited in the DVM/MS-LAM joint degree program.
3. Seven trainees were recruited in the summer research program

Conclusion
The first year of this multi-year program is considered to be successful, as evidenced by the number of trainees recruited in the program. All trainees in the combined degree program are making satisfactory progress and no one has dropped out of the program. No publication has resulted from the research conducted by the trainees. No major problem was encountered with recruiting or maintaining trainee interests in the training program during the first year of the program and hence we plan to continue the program as originally proposed.

References: No peer-reviewed publications resulted from the Summer Research activities of the trainees.

Appendices: The following documents have been appended:

1. Program brochure
2. Abstracts of summer research projects
Program Brochure:

**APPLICATION PROCESS FOR TUITION REMISSION:**
Applications should include a letter of interest and the completed application form. Please click on one of the following links:
- Application for DVM/MS-Comparative Biomedical Sciences
- Application for DVM/Master of Public Health
- Application for DVM/MS-Laboratory Animal Medicine

**Send your completed application form along with your letter of interest to:**
MaryAnn Miller, Graduate Program Manager
Tufts Cummings School of Veterinary Medicine
Administration Building, Room 110
200 Westboro Rd.
North Grafton, MA 01536

Once selected for support, students are expected to pursue their academic program (DVM and MS or MPP) and remain in good academic standing at TCSVM. During the funding period, they will make themselves available on campus at least once annually, to discuss further and explore careers in the Army Veterinary Corps, the Air Force Environmental Service, and the Uniformed Branch of the U.S. Public Health Service.

**Program Director:**
M.S. Anwer, PhD, DMVH
Associate Dean for Research
508-839-0700
sawkat.anwer@tufts.edu

**TCSVM**
- has been awarded a four-year (2006-2010) grant from the U.S. Army Medical Command for veterinary research manpower development for national defense. The goal of this program is to increase the number of veterinarians trained in research generally, and in public health related research for planning and implementation of a response to bioterrorism and in assessment of environmental threats.
- The programs eligible for these funds include:
  - DVM/MS in Comparative Biomedical Sciences
  - DVM/Master of Public Health
  - DVM/MS in Laboratory Animal Medicine
  - Summer Research Training Program

**Eligibility Requirements:**
1. Good academic standing for the TCSVM DVM degree program
2. No record of felony conviction
3. Interest in or willingness to explore military veterinary careers
4. Evidence of leadership ability
5. Must be a U.S. citizen or permanent resident
6. Candidates demonstrating a military commitment or prior service will be given preference

**NOTE:** There is no requirement for students funded through this program to enlist in the military.
DVM/MS in Comparative Biomedical Sciences

Program Director: Robert S. Bridges, PhD

The grant will provide funds for the following:
- Tuition for the MS in Comparative Biomedical Sciences
- One year Stipend plus health insurance
- Tuition for half year of DVM program

This combined 5-year DVM/MS program offers veterinary students the opportunity to gain intensive research training in biomedical research within one of the laboratories of program faculty. Students take a year out from the veterinary curriculum to train intensively in biomedical research with a faculty mentor. This program is also supported by an NIH training grant from the National Center for Research Resources as well as by Cummings School of Veterinary Medicine at Tufts University.

DVM/Master of Public Health

(Offered in conjunction with Tufts University School of Medicine)

Program Directors: Anthony Schlauf, MD, MPH, FACPM & Joann Lindenmayer, DVM, MPH

The grant will provide funds for the following:
- Tuition for the Master of Public Health program
- Research-related expenses for 3 months - Stipend and Supplies

The DVM/MPH program is designed to prepare veterinary students for a career in public health, or to better apply the principles of epidemiology and public health to more traditional forms of practice or research. With its emphasis on human and animal diseases, accuracy in research, and rigorous application of statistical methods, the combined DVM/MPH degree would provide an excellent foundation for students wishing to pursue public health positions in government, Army Veterinary Corps, research, international health, private industry, and academia.

DVM/MS in Laboratory Animal Medicine

Program Director: Angeline Warner, DVM, DSc

The grant will provide funds for the following:
- Tuition for the MS in Laboratory Animal Medicine
- Research related expenses for 3 months - Stipend and Supplies

This program is designed to provide bright and highly motivated veterinary students an opportunity to study laboratory animal medicine and animal resource management in conjunction with their quality DVM training at Tufts’ Cummings School of Veterinary Medicine. Students in the combined degree program earn both an MS in Laboratory Animal Medicine and a DVM in the same four years.

The program emphasizes education in quality humane care and use of research animal with a focus on ethics in research and environmental enrichment programs.

Summer Research Training Program

Program Director: M. Sawkit Assem, PhD, DVM/PhD

The grant will provide funds for the following:
- Stipend for 3 months
- Research supplies

This program is intended to help students crystallize their interest in research, to make them aware of their research potential, and to familiarize themselves with the opportunities inherent in a research career by exposing them to an active biomedical research environment. Students will learn how to evaluate a scientific article, design an experiment, organize and critically analyze data, translate data into hypothesis, and transmit research information in writing and speech. Use Summer Research Training application form for this program.
Abstracts of summer research projects

Trainee Name: Victoria Fields V’10
Mentor: Dr. Joann Lindenmayer
Title: A Study of Desmodus Rotundus-Mediated Bovine Rabies in Ometepec, Mexico

Summary: Bat-mediated bovine rabies is a concern in many parts of the world, including Latin America, where bats transmit rabies to humans and to domestic animals. This study will describe the situation of bat-mediated bovine rabies in southern Mexico. A map will be developed that will show an inventory of the farms, including their location, size, herd number, and presence of vampire bats. An estimate of rabies virus prevalence will be made by capturing bats, testing them for the virus, and establishing which strains they are carrying. Feeding habits will also be analyzed, specifically by looking at stomach contents to establish origin of the meal. There has been innumerable loss of valuable cattle in Mexico as the rabies problem persists, therefore the project will focus on studying bat-mediated bovine rabies in southern Mexico to develop a better picture of the situation.

Trainee Name: Miranda Hillyard V’10
Mentor: Dr. Jonathan Epstein
Title: Investigating the Risk of Food-Borne Transmission of Nipah Virus in Bangladesh

Summary: My project aims to investigate the risk of zoonotic transmission of Nipah virus as a food-borne pathogen in Bangladesh. My specific aim is to determine how common a practice it is to pick and eat fruit that has been dropped or partially eaten by fruit bats, which may be a risk factor for Nipah virus transmission. Under the supervision of Dr. Jonathan Epstein, I will collect data on the prevalence of Nipah virus in its putative natural reservoir, the Indian flying fox (Pteropus giganteus), which is the only species of pteropid bat found in Bangladesh. We will develop and conduct a survey of people who collect and sell fruit in two field sites: one village that is within 20km of a past Nipah virus outbreak, and one that is greater than 100km from any reported cases of Nipah virus. We will collect samples of fruit partially eaten by bats and determine whether Nipah virus is present on the surface.

This project will be in collaboration with the Henipavirus Ecology Collaborative Research Group (HERG - www.henipavirus.net). This group, created by the Consortium for Conservation Medicine, is conducting an extensive study of Henipaviruses in Pteropus species, the presumptive reservoir.

Trainee Name: Jennifer McRobbie V’10
Mentor: Dr. Joann Lindenmayer
Title: The Serological Surveillance of Rhinolophus Megaphyllus for Hendra Virus and Australian Bat Lyssavirus

Summary: Bats are being increasingly recognized as a public health threat by serving as reservoir hosts for viruses which can cross species barriers and infect humans. The recent appearance of Hendra virus (HeV) and Australian bat lyssavirus (ABL) in fruit bats in Australia has led to greater surveillance of certain families of bats such as Pteropodidae, which are frequently associated with known human pathogens (Halpin et al. 2000). It is reasonable to assume that other Australian bats may be carriers of these new viruses, yet there has been limited surveillance of many genera of bats which inhabit the same areas where known carriers are found. These species might serve as potential reservoirs for HeV and ABL. Furthermore, studies have suggested that cross-infection between and among bat species may generate new viruses which are able to jump species barriers more efficiently (Wong et al. 2006), making increased bat surveillance even more crucial to the identification, prevention and control of emerging viral diseases. Rhinolophus megaphyllus is a species that now has increased interest due to SARS surveillance but has limited previous HeV or ABL surveillance. A survey of R. megaphyllus is warranted to further our understanding of the ecology of these viruses and identify new reservoir species.

In this study, I will identify the presence or absence of HeV and ABL antibodies in Rhinolophus megaphyllus. In collaboration with ongoing surveillance of R. megaphyllus I will capture and bleed 60 specimens using a harp trap and test for the presence of antibodies to HeV and ABL using available ELISAs. I will also capture and bleed other wildlife (i.e., rodents and small marsupials) in the proximity of the R. megaphyllus roosts using Elliot traps. Wildlife found within 500 meters of the roosts might come into contact with bats or bat excrement and thus may be exposed to HeV or ABL through such routes if the bats can carry the virus and transmit it. These wildlife blood samples will also be tested for the presence of antibodies to HeV and ABL.

Using my results, I will evaluate the data and uncertainties concerning ABL and HeV in order to understand the impact of R. megaphyllus on the current risk for human ABL and HeV exposure. The data will be used to estimate the potential of these viruses to spread from R. megaphyllus and
wildlife to human populations. This analysis could be used to determine control measures and management strategies to minimize risk of exposure to HeV and ABL. I hypothesize that the presence of HeV and ABL will be detected in Rhinolophus megaphyllus and some species of wildlife. The detection of HeV and ABL in serum from a species with limited surveillance could indicate the possibility of a new reservoir and warrant further investigation.

Trainee Name: Shannabeth Minior V’09  
Mentor: Dr. Louise Miranda  
Title: Prevalence of Brucellosis in Zimbabwean Caprines

Summary: Brucellosis is a disease caused by the genus Brucella that affects animals and humans. It is known to cause contagious abortions in livestock, thus decreasing the reproductive efficiency and milk output of farm animals. This not only creates monetary loss for the farmer, but also has negative impact on livelihoods. The aim of this study is to determine seroprevalence rates of Brucellosis melitensis in resident and community groups of goats at the Africa Center for Holistic Management. If the prevalence rates are high, it could justify further testing of wildlife to determine if the wildlife population is at risk for the disease, given the close interaction between livestock and wildlife in the ecosystem. If the prevalence rates are low, funds for wildlife medical research can be directed towards more appropriate problems.

Trainee Name: Misha Park Robyn V’09  
Mentor: Dr. Christine Jost  
Title: Determining the Sensitivity and Specificity of the Participatory Disease Surveillance Method for Detection of Highly Pathogenic Avian Influenza in Java, Indonesia

Summary: Two epidemics of H5N1 Highly Pathogenic Avian Influenza (HPAI) occurred between 2003 and 2004 in Indonesia. It is now endemic on the islands of Java, Bali, and Sumatra. The first human case was reported in 2005, and as of 25 January 2007 there have been 80 human cases confirmed, 62 of which have been fatal. According to the United Nations' Food and Agriculture Organization (FAO), Indonesia remains one of the countries in which HPAI poses serious risk to animal and human health, as millions of backyard chickens (classified as Sector 4 by the FAO) live in close contact with people and other livestock species. There are approximately 300,000,000 sector 4 poultry in Indonesia, with over half found on the island of Java. Sector 4 poultry are an important part of an Indonesian household. They contribute to a family's economic capital, and are socially important due to the key roles that they play in cultural and religious activities.

Participatory Disease Surveillance (PDS) is case finding methodology used by the Government of Indonesia (GOI), in partnership with the FAO, to detect active HPAI. The advantage of the methodology is that it allows for a timely response. The FAO advocates the use of PDS as a sensitive method for the early detection of active HPAI. The purpose of this project is to determine the sensitivity and specificity of the PDS method for detecting HPAI in Indonesia. The sensitivity and specificity of the PDS method will be determined at three levels: the clinical case definition used to diagnose HPAI, the combined clinical outbreak definition and rapid Anigen® test when the rapid test is used to confirm a finding of active HPAI using the case definition, and the rapid Anigen® test regardless of clinical case definition results. Reverse-transcriptase polymerase chain reaction (RT-PCR) will be used to test chicken tracheal and cloacal samples taken from every PDS investigation, regardless of outcome, for H5N1. It is hypothesized that both the sensitivity and specificity of the PDS method of clinical definition confirmed by rapid Anigen® test will be 95% or above.

Trainee Name: Rebecca Steers V’10  
Mentor: Dr. Joann Lindenmayer  
Title: Detection of Mycobacterium bovis in Nasal Mucous and Milk Samples Using Polymerase Chain Reactions

Summary: Mycobacterium bovis is a zoonotic disease that has multiple impacts on society, including public health and economics, and is therefore important to control. M. bovis continues to be of great concern in Mexico, since it continues to persist in the dairy cattle industry. This study aims to compare the test results of polymerase chain reactions (PCR) on nasal mucous and milk samples of animals with these same animals' known true states of infection. The results will be used to calculate specificity, sensitivity, and predictive values to validate this antemortem detection test.
Trainee Name: Tierra Wilson V’10
Mentor: Dr. Gretchen Kaufman
Title: Differentiating *M. tuberculosis*, *M. bovis*, and *M. avium* in Captive Elephants in Nepal

Summary: Captive elephants play an historically important and integral socioeconomic role in Nepalese society. Their working roles place them in contact with humans and cattle on a daily basis. *Mycobacterium tuberculosis* and *Mycobacterium bovis* are endemic to humans [18] and cattle [2] in Nepal and present a significant risk of infection to elephants. In Nepal, captive elephants are also in regular contact with wild elephants during grazing and breeding placing the wild population at risk for contracting tuberculosis. Differentiating *M. tuberculosis* from *M. bovis* infections and selecting appropriate therapy is essential for effective treatment of the captive elephant population, for prevention of multi-drug resistant infections, and for developing effective management strategies to limit the spread of tuberculosis between humans, cattle and elephants in Nepal.

This study will focus on testing elephants previously identified as TB positive or suspected TB positive based on a study conducted in January 2006 by Elephant Care International [7]. Trunk wash samples will be collected using USDA standard operating procedures [15]. Bacterial DNA will be isolated at the IAAS Veterinary School in Chitwan District, Nepal and DNA will be analyzed either at IAAS or the Veterinary Services Dept. in Kathmandu. PCR will be used to amplify the gyrB gene. *M. tuberculosis*, *M. bovis*, and *M. avium* will be differentiated by restriction fragment analysis of the gyrB gene. Prevalence of *M. tuberculosis* vs. *M. bovis* vs. *M. avium* will be calculated. Comparison of PCR data with previous tests will be made on a subjective, case by case basis. Data will be given to ECI, Nepal and compiled with previous diagnostic information on these elephants.

This direct PCR method is expected to be a sensitive and effective technique that will improve the IAAS Veterinary School’s capacity to continue diagnostic studies in elephants, cattle and other animals. A higher prevalence of *M. bovis* is expected in TB positive elephants than found in the United States due to the higher prevalence of the disease in developing countries [2,13]. The results of this study will be shared with Elephant Care International and the Government of Nepal to assist in developing effective treatment and management strategies. Extracted DNA from positive samples will be saved for future fingerprinting studies (along with samples from a similar study being conducted in cattle) to determine modes of transmission between humans, cattle and elephants in Nepal.