

**BVA OVERSEAS TRAVEL GRANT REPORT 2010
KATIE WU – EGYPT**

***A STUDY OF THE PREVALENCE OF EQUINE PIROPLASMOSIS
THROUGH BLOOD SMEARS FROM HEALTHY WORKING HORSES
IN EGYPT***

This summer the BVA overseas travel grant supported my summer extra mural studies at the Animal Care in Egypt (ACE) (picture 1). This placement is a UK registered charity and they provide free veterinary services to the local animals in Luxor. The main animals that are treated at the hospital are horses, donkeys and mules that are worked by the Egyptians to earn money from tourists. These horses are used to pull carriages containing tourists around Egypt. The worked horses are mostly ill-shod, malnourished and are fitted with poor harness that causes skin lacerations and damage. Julie Wartenberg and Kim Taylor are the founders of Animal Care in Egypt and they realized the need for veterinary help once they saw the use of carriage horses there. The hospital purpose is to provide washing and grooming facilities and clean fresh water to working horses and a clinical exam by their three resident veterinarians Dr. David Machelles Saweris E.V.M., Dr. Assma Abd El Mowgood E.V.M.S and Dr Hannah Gamal AbdAllah E.V.M.S. (Picture 7).

I began my journey arriving at Cairo to meet the distributor of Andover Healthcare. They had generously donated six boxes of Powerflex bandage to Animal Care in Egypt (ACE). My first impression of Cairo was that the city was very bright and busy and most stores were open 24 hours a day. Since I am from Edinburgh where stores close at 5:00 p.m., it was pretty amazing to still buy food at 3:00 in the morning after coming back from a boat ride. The most memorable experience in Cairo was riding a camel during my trip to the Pyramid of Giza (picture 2). I made sure that the camel I used was in good body condition and looked healthy, as I do not support the use of ill-treated animals.

After meeting with the distributors, I flew into Luxor and arrived at around 11:00 p.m. I was greeted outside the airport by one of the volunteering veterinarians, Rachel O'Higgins who graduated at University of Glasgow and has been working for three months.

The next day, I started my first day with their morning rounds of in-patients (Picture 2). Rachel showed me around the hospital and we performed clinical exams on all the admitted donkeys and horses. We took their temperature, heart rate, and respiratory rate. We also checked for any abnormalities and gave them their medication. After rounds started around 8:00 a.m., the attending veterinarians would discuss each in-patient and their clinical findings and treatment plan for the day (Picture 3). After the morning rounds, David would spend his time treating any incoming patients (Picture 4) and the rest of us would treat the in-house patients (Picture 5). At around noon, I did my first flank spay on a stray cat with Assma. It was challenging to spay an animal with the temperature at 45 degrees C. The sweat dripped profusely down my face, while I tried very hard to avoid breaking aseptic techniques. After lunch, I visited their Egyptian tortoises and had the pleasure of

bathing each and every one of them (Picture 6). By the end of the day, I was overstimulated by the new practice and exhausted from the summer heat, but I was excited for the next day's adventure to start as every day in ACE, there were new and exciting things to do.

Picture 1. The front door of the ACE hospital



Picture 2. My camel ride up to see the Pyramid of Giza



Picture 3. Rachel discussing the in-house patients.



Picture 4. David treating a laceration wound on a donkey.



Picture 5. Katie doing a dental health check on a in-patient horse.



Picture 6. The Egyptian tortoises being bathed.



Picture 7. The ACE veterinarians and one veterinary student.



Top: Assma, Hana, Bottom: Katie, David, Rachel

Abstract

Theileria equi and *Babesia caballi* are tick-borne hemoprotozoan parasites that can cause a disease called equine piroplasmiasis. Currently, there are no studies conducted on the prevalence of equine piroplasmiasis in Egypt. This research is a study of the incidence of the *Babesia* parasite in healthy working horses and also an overview of recent trends in the diagnosis of babesiosis. These methods include a microscopic exam, a polymerase chain reaction (PCR), an immunofluorescent antibody test (IFAT) and an enzyme-linked immunosorbent assay (ELISA). The data was conducted in Luxor, which has a large population of working horses. Whole blood samples were collected from fifty healthy working horses and a microscopic exam was used in screening for the *Babesia* species in red blood cells. A Giemsa-stained blood smear was used to stain the slides. The result has shown that seven out of the fifty slides were positive for *Babesia* species. Future studies will involve the use of PCR as whole blood samples were collected on FTA card. The PCR will be used to assess the true prevalence of piroplasmiasis in this population and determine the sensitivity of blood smear examination for diagnosis of piroplasmiasis.

Introduction

Theileria equi (formerly known as *Babesia equi*) and *Babesia caballi* are tick-borne hemoprotozoan parasites of horses that can cause the clinical disease known as equine piroplasmiasis or babesiosis. They are transmitted by ticks of several genera including *Boophilus*, *Hyalomma*, *Dermacentor* and *Rhipicephalus*. The parasite can affect a wide group of species, including humans, baboons, dogs and donkeys. Equine piroplasmiasis is a disease that can be a major concern in tropical and subtropical countries (Bose et al., 1995) and it also has an economic impact to

the horse industry as importation regulations restrict movements of infected animals into a number of countries that are considered equine proplasmiosis-free (Friedhoff, 1982). Clinical signs are variable and often include icterus (jaundice), haemoglobinuria and fever. The horses can be chronically or acutely affected or can be carriers of this disease. The main aims of this project are twofold. The first part is to screen the incidence of parasites present in healthy horses through examining their blood smears. Part two of the project, which will be carried out on a later date, will test the sensitivity and specificity of the blood smears compared with polymerase chain reaction analysis as gold standard; PCR is expected to have a higher sensitivity in identifying the presence of babesia (Bose et al., 1995).

Materials and Methods

Whole blood samples were taken from 50 healthy adult horses between Animal Care Egypt Hospital and Hod. Hod is an Arabian horse breeding facility. Samples were collected by jugular venipuncture and 1 ml of blood was transferred into tripotassium ethylenediamine tetraacetate (EDTA) tubes. Blood smears were made within one hour of blood sampling and fixed with alcohol for two minutes and two drops of whole blood were applied to Whatman FTA cards for the PCR study. Data collected from each horse included the reason for the hospital visit, an assessment of the horse's temperature to screen out horses with fever and examination of the mucous membrane color to screen out jaundiced horses. To minimize sampling the same horses twice, the Egyptian veterinarian received consent from owners for blood sampling and instructed them to inform us if we had already taken blood for the same test. The method for staining the slide was Giemsa, but unfortunately their Giemsa solution had expired; therefore, the slides had to be stained by a pathology technician at the University of Edinburgh. Microscopic examination with a 100x oil immersion lens was undertaken, scanning each slide for 20 minutes.

Statistical Analysis

As this was a health screen for equine piroplasmiosis, there were no statistical analyses conducted. The only data recording was of positive and negative results for the presence of parasites from blood smears via microscopic examination.

Results

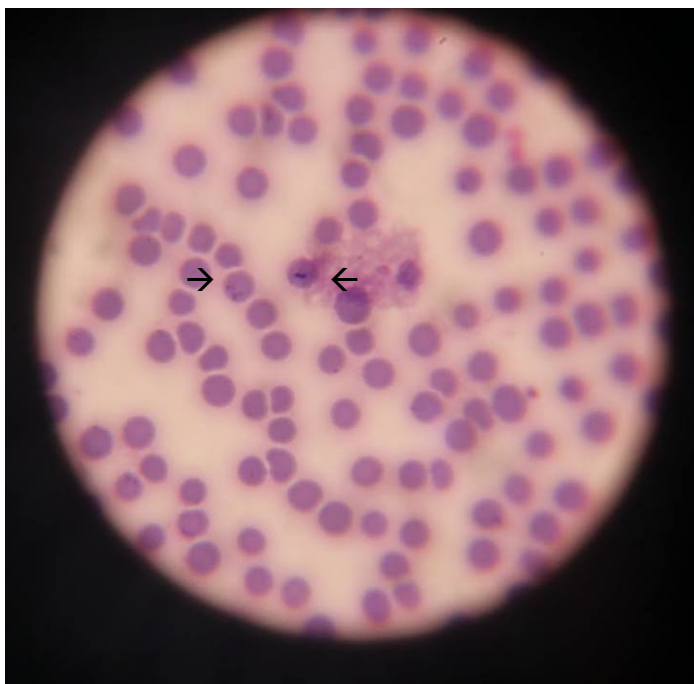
Table 1. Results of the Giemsa-stained blood smears and recorded reason for visit.

Number	Patient ID	Reason for visit	Temperature	Slide
1	20	Deworming	39C	positive
2	27	Skin Laceration	38C	negative
3	4	Deworming	38C	negative
4	7	Deworming	38C	negative
5	9	Mange	37.5C	positive
6	10	Hoof abscess	38.7C	positive
7	11	Skin Laceration	38C	negative
8	12	Lameness	38.4C	negative
9	13	Lameness	38C	negative
10	14	Firing*	38C	negative
11	15	Ticks	38C	negative
12	18	Skin Laceration	38C	negative
13	8	Skin Laceration	37.7C	negative
14	19	Skin Laceration	38C	negative

15	21	Ticks	37.5C	positive
16	23	Allergy from Flies	39C	negative
17	26	Deworming	38.2C	negative
18	25	Deworming	38C	negative
19	24	Skin Laceration	38C	negative
20	22	Sore back	38C	negative
21	1	Lameness	39C	negative
22	16	Lameness	38C	negative
23	64	Breeding	38C	negative
24	28	Skin Laceration	38C	positive
25	29	Skin Laceration	38C	negative
26	30	Skin Laceration	38.3C	negative
27	31	Allergy from Flies	37.5C	negative
28	32	Deworming	39C	negative
29	33	Deworming	39C	positive
30	34	Skin Laceration	38.9C	negative
31	35	Nursing mare	38C	negative
32	36	Lameness	38C	negative
33	37	Mange	38C	negative
34	38	Deworming	38C	negative
35	39	Deworming	38.5C	negative
36	40	Breeding	38C	negative
37	41	Breeding	38C	negative
38	42	Breeding	38C	negative
39	43	Breeding	38C	negative
40	44	Breeding	38C	negative
41	45	Breeding	38C	negative
42	47	Breeding	38C	negative
43	49	Breeding	38C	negative
44	50	Breeding	38C	negative
45	52	Breeding	38C	negative
46	53	Breeding	38C	negative
47	46	Breeding	38C	positive
48	51	Breeding	38C	negative
49	49	Breeding	38C	negative
50	57	Breeding	38.3C	negative

*Firing is an old tradition, in which owners burn their horses' legs, which is supposed to help with keeping the horse strong and healthy.

Figure 2. Picture of *Babesia* species inside a red blood cell.



Of the fifty horses sampled, seven were positive for the presence of parasites in their red blood cells (Figure 1). The temperature of these horses ranged from 37.5 degrees C to 39 degrees C and none presented with clinical signs or histories indicative of piroplasmosis. The reasons these horses visited the hospital ranged from deworming (9), skin laceration (10), breeding (16) lameness (5), mange (2), allergy from flies (2), sore back (1), nursing mare (1), firing (1), hoof abscess (1) and tick (2). Of the seven positive cases, only one owner came in for complaints of a tick problem.

Discussion

As suspected, healthy working horses in Egypt can be carriers, with no clinical signs or histories indicative of equine piroplasmosis. This is an important finding because there are currently no studies published on the prevalence of equine piroplasmosis in Egypt. Only detection of *Babesia* via PCR in cattle has been conducted in Egypt (Adham et al., 2009). Currently, the treatment of choice for horses presenting with clinical signs of equine piroplasmosis is imidocarb. Treatment can become expensive considering most of the horses that visit the Animal Care in Egypt practice seek free veterinary services. Despite this, seven cases of the presence of *Babesia* were found in the present study. It is not uncommon for horses which are *Babesia equi* carriers to have low circulating parasitemias that would not be detected in a single blood smear (Maurer, 1962). The extremely low circulating parasitemias makes it difficult to positively identify carrier animals or to obtain adequate numbers of parasites from samples from the hospital; (Patricia, 1998) therefore, future tests via PCR should more accurately identify positive, negative and false positive results (Bose et al., 1995).

There are different methods for diagnosing babesiosis and microscopic techniques are the cheapest and most appropriate technique to diagnose acute disease (Bose et al., 1995). Other techniques involve DNA probes for post-mortem

identification of parasites in certain organs. Equine piroplasmosis can also be detected through specific antibodies by using the immunofluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA); these tests are currently more widely used. The advantage of using IFAT is that cost per test is low; however, it requires low sample throughput and creates operator fatigue when processing more than 90 samples per day (Bose et al., 1995). The advantage of using ELISA is that it can be a very sensitive and efficient test, but the quality of the antigen is crucial in developing the test as some may lead to false positive results (Bose, 1995). Lastly, the gold standard in detecting babesia in blood samples is PCR technique as it can detect carrier animals. The disadvantages, however, is that PCR tests are expensive and only certain laboratories in the United Kingdom have the materials to conduct the test, and dead parasites will be detected.

As stated earlier, further study will compare the results of a microscopic exam with PCR. This would give us a better understanding of whether or not there were more than seven positive results showing the presence of parasites. Because the facility in Luxor had expired Giemsa stain, it was necessary to fix the slides with alcohol, which should have been methanol (Bose et al., 1995). This may or may not have affected the results as the slides were restained once they were brought back to Edinburgh, although it was considered unlikely. The future trends in testing for babesiosis should be cheap and convenient, as facilities in third world countries may not have access to do PCR. As for microscopic examinations, they are more reliable with a skilled technician but false negative results have been detected (Maurer, 1962; Holman et al., 1998).

Acknowledgement

Many thanks to Animal Care in Egypt, located in Luxor, where I conducted my research and to the Egyptian veterinarians who worked there and helped me collect data for my project. This project could not have happened without the generous funding from BVA travel grant, EVZS travel scholarship and Andover Healthcare for donating bandages to the practice. Lastly, my gratitude to Neil MacIntyre of the Veterinary Pathology Unit for staining my slides and my supervisor, Elspeth Milne, for her advice and helping me obtain licensing to bring my blood smear samples back to Edinburgh.

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Personal comments

My experience at ACE has been wonderful and I fully enjoyed the variety of animals that are brought to the hospital to seek medical attention. The main focus of the charity hospital is to provide free veterinary services to the local working equines. They also provided educational tours to tourist visiting the hospital and to the local Egyptian children. During the summer, many Egyptian children would accompany their parents to ACE with their donkey to seek veterinary care. The staff and I would spend time educating their children about taking care of their donkeys and explaining to them how not to hit their donkeys on the head or rump region with a stick, as injuries may occur. We also promote the use of proper head collars as most of the equine owners use harsh material to make their head collars which leads to skin trauma and laceration. I believe it is very important to educate the younger generation about equine welfare, as many injuries can be prevented if they are more aware of improving husbandry care for their animals.

My short one month experience at ACE has increased my confidence as a final year veterinary student. I was able to educate the public via giving tours around the hospital to the visiting tourist, spend time with the local children and lastly helping the animals, who are in need of veterinary care. This is an excellent charity placement and I would recommend any veterinary students who would like some equine experience to come and spend some time at ACE.