



Due Diligence Review

Performing Animal Welfare Society
San Andreas, California

2012-11-18

Introduction:

The Performing Animal Welfare Society (PAWS) opened in 1984 and is a private not-for-profit organization governed by a Board. PAWS is located in San Andreas California, in the foothills of the Sierra Nevada mountains approximately 200 km east of San Francisco. It is a member of the Global Federation of Animal Sanctuaries. It is not an Association of Zoos & Aquariums (AZA) affiliated institution.

The due diligence process was set out in the Council motion of October 25, 2011 and in the Zoo Board of Management motion of November 24, 2011 and recognizes the professional standards that govern the Toronto Zoo (TZ) as established by Canada's Accredited Zoos and Aquariums (CAZA) and the Association of Zoos and Aquariums (AZA). Fact-finding and discussions included the following topics: PAWS site, financial, animal management and training, facilities, climate, nutrition, staffing, transportation, education and advocacy, and medical issues.

Site Visit-December 19-20, 2011 – General Comments:

- Five Toronto Zoo staff travelled to PAWS for the site visit: Dr. William Rapley – Executive Director of Conservation, Education & Wildlife; Dr. Graham Crawshaw – Senior Veterinarian; Maria Franke – Curator of Mammals; Chris Dulong – Wildlife Care Supervisor of African Savanna and Elephants; and Vernon Presley – Senior Elephant Keeper.
- TZ staff were hosted for two days by Ms. Pat Derby – Founder and President of PAWS, and Mr. Ed Stewart – Co-Founder and Secretary-Treasurer. TZ also met Ms. Margaret Whittaker (trainer with Active Environments), Dr. Jackie Gai (contract veterinarian) and other keeping staff.
- PAWS has 930 hectares (2,300 acres) at the San Andreas site of which approximately 30 hectares (80 acres) is devoted to African elephant facilities
- PAWS has permits associated with having exotic animals in the state of California.
- They have six bears, five lions and 26 tigers also in the collection.
- Time was spent viewing the African elephant facilities – both indoor and out. They have 3 female African elephants. TZ staff were only allowed to view two of the five elephant barns on the site. The presence of PAWS staff carrying or wearing facemasks suggested that some sort of quarantine was in place. Since that time, it has been learned that tuberculosis is an ongoing issue there.
- TZ was also shown one of the Asian bulls “Nicholas” who is in his own area.
- TZ asked to see the other Asian facilities and was advised, that on the advice of PAWS lawyer, they could not give access to the three other facilities.
- PAWS elephant collection also included (at the time) three female Asian elephants and three bull Asian elephants. Asian bull “Sabu” died January 12, 2012 after the site visit.
- The African cows are 21-year-old “Mara,” 24-year-old “Maggie,” and 43-year-old “Lulu.”. Previously PAWS had accommodated two other African cows: “71” and “Ruby.” “Ruby” died in March 2011 at the age of 50 and “71” in 2008 at the age of 26.
- Discussions covered facilities, ability to integrate TZ elephants, managing multiple herds, etc.

- An invitation was put forward for key PAWS staff to visit the Toronto Zoo (which occurred in March 2012).

Financial:

- PAWS is a 501(c)(3) not-for-profit organization (NGO) in the United States.
- PAWS financial statements are available on their website at www.pawsweb.org
- Toronto Zoo finance staff completed a financial review of the 2010 Audited Financial Statements and Form 990 (charitable income tax return) for the Performing Animal Welfare Society (PAWS). The review concluded that the financial well being of the organization is good, with the balance sheet indicating relative stability that could reasonably support the projected operating costs of the organization for the next two - three years. The only significant question related to the long term sustainability plan for the sanctuary with regard to the indefinite renewal of the land lease scheduled for 2016.

Animal Management, Training & Enrichment:

- Active Environments, a professional animal training organization, works extensively with PAWS elephants and training. PAWS uses protected contact to manage their elephants. It was mentioned that PAWS staff no longer go in with their elephants but they do use distance as a barrier in large enclosures.
- They have a training program in place and training guidelines are documented. They do not work actively with the Africans on a daily basis.
- TZ observed a training session with two of their African elephants including a blood draw.
- PAWS does not have an elephant fitness program. Elephants are put out in the paddock for the day and brought in for the night.
- They keep all elephants separated in the barn at night, and they are put out in paddocks during the day when the temperature is above 5° Celsius.
- They do not have a formal behavioural enrichment program for the African elephants. They are not against an enrichment program and will look at the Toronto Zoo enrichment program. This is important since animals are held indoors for about 12 hours at night.
- The African elephants appeared to be generally in good physical health, although in our estimation they were heavy. Obesity can result in health complications in elephants as in other species.
- Daily logs are kept on all of PAWS' elephants.
- An Emergency Protocol is posted in the barns. The veterinarian is doing new protocols based on state requirements and this should be completed in 2012. They have firearms on site for any animal escapes/emergencies.

Facilities:

- The African elephant house is an 1850 m² (19,900 sq. ft.) indoor facility. Outdoor space for the African elephants includes a 30 ha (80 acre) outside area. The elephants use of space in the outdoor areas is very seasonal. A significant portion of this space is a steep grade or treed ravine which is not used by the animals.

- The African elephant house is separated by a distance of approximately 40 m from the Asian female elephant house (Attachment 1). The Asian paddocks are adjacent to the African paddocks, and there is a shared waterway that fills up seasonally during rainfalls. There is a service road approximately 12 m wide at the closest point, separating the outdoor areas of the two species. This proximity is significant in the control of disease and is discussed further under Disease Concerns below.
- In the African barn there are 22 hydraulic gates and 9-10 individual stalls with a long hallway that can be used to introduce/socialize elephants.
- The floor substrate is a thick rubberized coating over concrete, with some give, and they have heaters and heated floors.
- A moveable chute is available to assist in the care and treatment of animals.
- The elephant indoor pool or “spa” consists of a deep concrete pool with no jets or hydro pump in the African elephant house. TZ staff were told the pool is not used much by the animals as they do not like to go in it. The pool was empty during the visit.
- The outdoor paddocks have elephant fences but, like many other elephant facilities in North America, are not predator proof. It is possible for mule deer, other wildlife, cats, pigeons, wild turkeys, and vermin to move freely between elephant paddocks. Wild deer, domestic cats, and pigeons were observed on the site during the visit. This point becomes significant in the control of disease, as discussed below under Disease Concerns.
- A perimeter fence is in place around the entire property.

Climate:

- The elephants are kept in the barns if the temperature is below 5° C.
- In the winter months, the site is often near or below freezing at night. During the site visit on December 19, the temperature at night was 0° C. On December 20, the temperature was below freezing and the ground was covered in frost. The elephants did not go out until 9:30 a.m. when the temperature reached 5° C and they were brought in by 5:00 p.m. Occasionally, significant snowfall occurs at San Andreas.
- The San Andreas site can be very hot and dry in the summer, so adequate shade and the ability for the elephants to cool off in ponds or mud wallows is important. A pond in the African paddock was completely dry at the time of the December site visit, as the San Andreas area had not had the usual seasonal rainfall.

Nutrition:

- Although the elephants have access to large paddocks, natural forage is limited by the season and type of grass. Much of the native vegetation at this time of year was dried up, and TZ did not see foraging by the elephants. Feed is provided with grass and oat hay, and commercial elephant pellets and a small amount of bran and other items.

Staffing:

- All elephant keeping staff work only with elephants, no other species.
- Two full time elephant supervisors and the two directors, work in excess of 40 hours per week, observing elephants, working directly with the elephants in foot care and other

training, overseeing all elephant care. All four monitor behaviour, assist in veterinary care and develop protocols for care of new arrivals.

- Five senior keepers work regular shifts, supervising cleaning, feeding, shifting, and veterinary care. They are assisted by three senior assistant keepers and three apprentice keepers. This total of 15 caregivers is almost a ratio of 2 keepers to 1 elephant.
- One Director has over 40 years of experience working with elephants; the other has over 35 years of experience. One elephant supervisor has over 15 years working with elephants, including Asian bulls, with experience in protected contact training, transporting, foot care, blood draws, and all aspects of husbandry.
- A night keeper does check elephants on rounds, and TZ was told there is someone present onsite 24/7.
- TZ was told that no volunteers work directly with the elephants.
- Veterinary support is provided by a contract zoo/exotic animal veterinarian who is based approximately 150 km away, a distance that is of some concern to TZ. The veterinarian indicated she attends the facility approximately 2-3 days a week or as needed. Additional veterinary support is provided through the School of Veterinary Medicine, University of California at Davis, or other consultants arranged by the veterinarian.

Education and Advocacy:

- PAWS is not routinely open to the public but has monthly “seeing the elephant” getaways and open houses several times a year for a fee.
- PAWS has run special sessions such as training programs for foot care and elephant training sessions in conjunction with Active Environments and the Detroit Zoo.
- PAWS hosts and participates in conferences such as the “Summit for the Elephants, 2012” hosted at the Oakland Zoo from March 28-30 with numerous attendees and participants.
- PAWS promotes education and conservation programs on their website and has partnerships with many field conservation experts in conservation.
- PAWS educates the entertainment industry, public officials, enforcement agencies, the media, and the general public on critical issues related to captive wildlife, performing animals and protection of wild species and habitat with newsletters, PAWS publications, PAWS in the media, conferences, and other affiliations.
- PAWS’ Wildlife Classrooms to the World serves as an educational and research centre.
- PAWS works in partnership with the University of California, Davis to collaborate on various humane studies relating to biological sciences, animal science, animal behaviour, zoology, animal welfare, and veterinary science. This cooperative program also provides opportunities for PAWS to participate in veterinary students’ training in the humane care of captive wild animals.

Transportation:

- Under the TZ/PAWS legal agreement, PAWS is responsible to make all the arrangements for and cover all the costs relating to the safe and humane transport of the elephants.
- At the site visit, TZ mentioned a pros/cons list in regards to truck or air transport. A four to five day (or longer) journey by road was a concern expressed by TZ for the older Toronto

elephants. Discussions took place and PAWS said they would look into flight/truck options and availability.

- When PAWS visited the Toronto Zoo in March 2012, travel arrangements were discussed in detail. Concerns expressed by the TZ included the shipping distance, as the land travel of 4-5 days was again proposed by PAWS.
- In April 2012 PAWS proposed to move the elephants by an AN124 air carrier. Later, TZ and PAWS both agreed that this was not suitable because the cargo area was not sufficiently pressurized to ensure safe transport for the elephants. Also, there were additional concerns about noise and vibrations which could cause unacceptable stress to the elephants.
- TZ requested that PAWS obtain confirmation from the US Fish & Wildlife Service that their transportation plan meets the animal welfare requirements as required by the International Air Transportation Association worldwide standard for shipping live animals.
- At the time of writing, PAWS has been unable to organize transportation for the three elephants that would meet these animal welfare standards.

Quarantine & Medical:

- At the site visit, TZ asked about the tuberculosis (TB) status of the PAWS elephants.
- It should be noted that the USDA and the US Public Health authorities require that all elephants in the United States be part of a tuberculosis control program that was developed in response to an increasing number of cases of tuberculosis in elephants and their handlers. In most cases the infection was traced back to circus animals but some animals were distributed in zoos and sanctuaries. All animals are tested with a trunk wash culture and a STAT-PAK blood test annually. Positive TB results on the trunk wash indicate that the animal is infected but negative results are not necessarily indicative of an uninfected animal. False positive reactions to the STAT-PAK may be seen occasionally. Animals reacting to the STAT-PAK are tested in the laboratory with the MAPIA and DPP confirmatory tests. Reactions to the latter tests strongly suggest that the animal is TB infected.
- PAWS staff indicated that the African elephants were negative on both trunk wash culture and blood tests (STAT-PAK) but that the existing (or some) Asian elephants were reactive on blood test(s) but negative on trunk wash cultures. However, they did not indicate the TB status of the elephants, how many, which animals or to which tests the animals were reactive. It should be noted that USDA procedures place elephant herds into categories depending on test results and the history of any previous exposure to a known infected animal. Each category carries a different requirement in regard to treatment and on-going monitoring.
- At the site visit, TZ was not given details on the quarantine status for the Asian elephants and not allowed to visit all of the Asian elephant barns. It is possible that some animals were being treated for tuberculosis but this was not disclosed. The fact that TZ was not invited to see the Asian elephants and that staff members caring for the barn containing elephant cows were wearing facemasks, strongly suggested that a quarantine was in operation as a result of either confirmed or suspicious cases of TB in a USDA quarantine.
- At the site visit, there was no mention of the Asian elephant "Rebecca" who TZ later found out had died on January 8, 2011 with significant TB lung lesions that cultured positive for

tuberculosis. Later TZ also found that Rebecca harboured two different strains of human tuberculosis (information obtained through the Freedom of Information Act – FOIA).

- PAWS do not keep a separate quarantine barn at their facility to separate new animals on arrival.
- There is some ability to manage sick animals given the limitations of the species and protected contact, but no ability to quarantine disease-risk elephants separately on the site.
- USDA inspection reports under the auspices of the US Animal Welfare Act were shown for years 2007, 2008, 2009 and 2011, and indicated that the facility was in compliance under those guidelines. No details of interim reports or other recommendations were provided, nor was the 2010 report provided (it was not requested at the time of the site visit). There were no detailed USDA tuberculosis control reports provided.
- At the site visit, PAWS requested that the Toronto Zoo elephants be tested using the Stat Pak and trunk wash culture tests. (This was completed by March 2012 and all TZ elephants were negative for TB).
- As determined from the site visit, on February 16, 2012 TZ requested further medical information as follows:
 - Medical records of the three African elephants and six Asian elephants for the past 5 years.
 - Reports of other significant disease conditions e.g. Salmonella and elephant herpesvirus occurring in the past 5 years.
 - Necropsy reports of elephants that have died at the facility in the past 5 years, including culture and histology results for tuberculosis.
 - Results of USDA tuberculosis testing (trunk wash and blood test results) for all elephants.
 - A declaration of the USDA status of quarantine for the premises.
 - Clarification of the tuberculosis control program category for both species and all groups of elephants.
 - Reports of any tuberculosis treatment program instituted for animals.
 - TB status for the entire premises including deer, wildlife, and domestic cats and vermin.
 - Details of the health management program to prevent the spread of infectious disease from Asian to African elephants.
 - Reports of any staff cases or treatment for tuberculosis in humans at PAWS.
- When there was no response from PAWS on the above medical information, five more requests were sent by TZ staff or City Legal on the following dates: March 26, 2012; April 2, 2012; April 11, 2012; April 30, 2012; and May 23, 2012.
- Some of the records were eventually provided in June and July, however some of the key information was not up to date and additional information and clarification was requested. Review of the clinical and post-mortem records that were provided, as well as information obtained from other sources, confirmed the presence of tuberculosis to be a significant concern.
- More complete and updated records for 2012 were not received. PAWS' legal advisors indicated in September that no more records would be made available.

Tuberculosis and Disease Concerns:

- Six elephants have died at PAWS in the past five years (eight in the past nine years). The two most recent animals euthanized were: a female, “Rebecca” in January 2011 and a bull, “Sabu” in January 2012 and both were confirmed to be infected with tuberculosis (Attachment 2).
- “Rebecca” was known to have been infected when she went to PAWS and, in fact, was treated there (presumably unsuccessfully). “Sabu” was believed to be positive for tuberculosis upon arrival in 2010 and was euthanized in January 2012 due to arthritis, but he also had lesions of active tuberculosis. Recent reports received from third parties accessed through the Freedom of Information Act have shown some positive tests for TB in the joint fluids of “Sabu”.
- Of the three living Asian cows, one is an active case of TB by a positive trunk culture, and the two in-contact animals must be considered at high risk of infection. Results of an initial screening blood test for TB (Stat-Pak) indicated that two of these three females and one bull at PAWS were reactive even in June 2011, and those same three animals were also reactive on the confirmatory test (MAPIA) which has been shown to have a high degree of accuracy. In the opinion of the Toronto Zoo’s veterinary staff, and that of other elephant disease experts, those animals would likely become active cases at some stage in the future. One of those three elephants, “Annie”, did indeed become culture-positive in June 2012, and “Sabu” was shown to be infected on post-mortem early this year. Records and testing results of the other Asian elephants were suggestive of exposure to the disease and at this stage were considered to be latently infected (Attachment 2).
- In the opinion of the Toronto Zoo’s veterinary staff, the prevalence of active and latent TB in the Asian elephants at PAWS is many times higher than that in the overall North American zoo elephant population. This is not at all surprising since many of the animals were acquired from herds known to be infected.
- The presence of infected and potentially infected Asian elephants is of serious concern for Toronto Zoo’s disease-free animals. The opportunity for direct or indirect contact is present. This could occur from infected personnel, food or water, or vectors such as other animals. The presence of deer, stray cats and other wildlife and rodents moving freely through the elephant paddocks is a concern both for the potential transmission from one group to the other as well as for the potential spread into the native deer population. Cats were present around the barns and staff moved freely from the Asian barn into the African barn. Several staff members who left the Asian elephant quarantine were carrying a face mask and using a foot bath, but without appearing to take any other specific precautions.
- For at least a five-year period, prior to the construction of the African barn, PAWS African elephants were housed in the same barn and in adjacent paddocks to Asian elephants that developed tuberculosis. The incubation period of TB in elephants is prolonged, from 2 to 20 years, so previously exposed African elephants may develop infection later in life.
- Treatment of TB in elephants is a long (1-2 years), difficult and expensive process. For multiple-drug resistant cases, the costs for one animal may exceed \$100,000, and even then it may not be effective. Clearly, prevention of exposure to the disease is a preferable option.
- As noted in the Quarantine section above, TZ’s veterinary staff did not receive the additional information to demonstrate that the disease has been contained. As well, TZ

veterinary staff did not receive sufficient assurance that the many aspects of the biosecurity program at PAWS were adequate to prevent possible spread to our own animals. In three recently published reports of cases in zoological facilities around the world, unrelated animals up to 100 m away became infected with the same strain of TB found in the elephants. In a similar situation at another elephant facility in 2012, a TB-infected animal originating from the same source as two of PAWS' elephants was considered by disease experts to have transmitted that same strain of bacterium to another elephant nearly 300 m away. At PAWS, the same staff appears to care for and train both the African elephants and the infected Asian elephants, and the facilities are adjacent.

- TZ has consulted with experts in bacteriology, epidemiology, TB in elephants and veterinary science. Dr. Michele Miller, DVM, PhD, and Veterinary Advisor to the Elephant Advisory Group of AZA, has indicated that "relocation to a facility where there is no history of tuberculosis presents a better option when considering risk for disease transmission" (Attachment 3). She further states "there is a risk of transmission from culture-positive and even seropositive elephants that have a strong probability of becoming culture positive, to other animals that are in contact, and even those that are not in direct contact but on the same premise as these animals." Dr. Ramiro Isaza, DVM and Associate Professor of Zoological Medicine at the University of Florida states "If these elephants were under my care, I would make the clinical and managerial decision to not send them into a situation where they might be infected with tuberculosis" (Attachment 4).
- Dr. Ian Duncan, retired Chair in Animal Welfare at the University Guelph, provided his opinion regarding disease and transportation concerns and writes "Tuberculosis is a debilitating illness that causes suffering and really reduces welfare. No animal should be exposed to it if alternative arrangements can be made" (Attachment 5). Dr. Dale Smith, a Professor in the Department of Pathology at the University of Guelph, advises it is "unethical" to mix elephants that are TB-free with those infected with TB (Attachment 6).
- TZ staff were able to meet with senior USDA/APHIS officials to review the TB situation. TZ asked USDA about meeting with their vet, who is familiar with the PAWS situation and TB treatment on site. USDA replied that PAWS, as a private facility, would need to give permission. TZ asked PAWS, but this request was denied by the USDA vet involved with the PAWS site.
- Once TB information was received from PAWS, TZ intended to consult with a veterinary epidemiologist to properly assess risk. However, almost one year later, this information to complete a thorough review has not been provided.
- In the event that PAWS ceases operations, or otherwise is unable to provide for the continued care of the TZ's elephants, the ownership of the elephants reverts to the Toronto Zoo Board of Management. If the elephants are exposed to TB at PAWS, it is unlikely that another suitable home could be found in an uninfected herd. The elephants could also be quarantined at PAWS and not be able to be removed from quarantine.

Summary of Major Points and Conclusions:

The Performing Animal Welfare Society has many positive attributes including the size of the outdoor paddocks, a modern indoor barn, professional and compassionate animal and veterinary care, and a healthy financial situation. However, there are several significant concerns that lead the Toronto Zoo to conclude that PAWS is not an acceptable or suitable facility for the Toronto Zoo's elephants. The following summarizes the Toronto Zoo's findings:

1. Six elephants have died in the last five years and at least two have had active tuberculosis that cultured positive. There is a reasonable concern of a tuberculosis outbreak at the PAWS sanctuary at this time that is affecting the Asian elephants.
2. The PAWS sanctuary has not provided all required information. TB testing and culture results were requested repeatedly since February 2012, to be able to complete the medical due diligence.
3. Based on old test results provided by PAWS, the three PAWS African elephants were testing negative to tuberculosis in 2011. However, these animals have had significant direct or indirect exposure to TB in the past and there is a risk of TB infections showing up in this herd in the future.
4. The Toronto Zoo elephants have tested negative for TB and the overall site history for tuberculosis at Toronto Zoo is clean.
5. The Toronto Zoo elephants will need to share the same barn and paddocks with the existing three African elephants.
6. There appears to be inadequate separation and quarantine practices in place with the Asian elephants to ensure that African elephants are not exposed.
7. PAWS is responsible for all transportation arrangements. PAWS has been unable to organize suitable transportation that meets International Air Transport Association and welfare standards to transport Toronto Zoo's elephants.
8. Much of the information on the TB status of elephants at PAWS came through the Freedom of Information Act by the public. In AZA or CAZA accredited zoos, information sharing is transparent and free flowing, resulting in a timely and simple decision making process.

Toronto Zoo Staff Involved in Due Diligence Review:

Dr. William Rapley Executive Director of Conservation, Education & Wildlife	Robin Hale Chief Operating Officer
Dr. Graham Crawshaw Senior Veterinarian	Sue Gunton Director of Corporate Planning
Maria Franke Curator of Mammals	Paul Whittam Manager of Financial Services
Dr. Dave Barney Acting Director of Wildlife	
Chris Dulong Supervisor of African Savanna	
Vernon Presley Senior Elephant Keeper	

Experts Consulted or Contacted in Completing the Due Diligence Process:

Dr. Chester Gipson USDA, APHIS	Dr. Yvonne Nadler Lincoln Park Zoo
Dr. Barbara Kohn USDA, APHIS	Emeritus Dr. Kay Mehren - retired Former Toronto Zoo Senior Veterinarian
Dr. John Prescott University of Guelph	Martha Fischer AZA Elephant SSP
Dr. Dale Smith University of Guelph	Charlie Gray AZA/CAZA Elephant SSP
Dr. Ian Duncan University of Guelph	Dr. Michele Miller Veterinary Advisory to AZA Elephant Group
Dr. Ramiro Isaza University of Florida	

List of Attachments:

1. PAWS Aerial Site
2. USDA Reports for “Rebecca” and “Sabu” and PAWS' other elephants
3. Letter from Dr. Michele Miller, DVM, PhD, Veterinary Advisory to the AZA Elephant Advisory Group
4. Letter from Dr. Ramiro Isaza, DVM, MS, MPH, DACZM, Associate Professor of Zoological Medicine, University of Florida
5. Letter from Dr. Ian Duncan, Professor Emeritus, Emeritus Chair in Animal Welfare, University of Guelph
6. Letter from Dr. Dale Smith, DVM, DVSc, Professor, Ontario Veterinary College, University of Guelph

Publications Used For Due Diligence Review:

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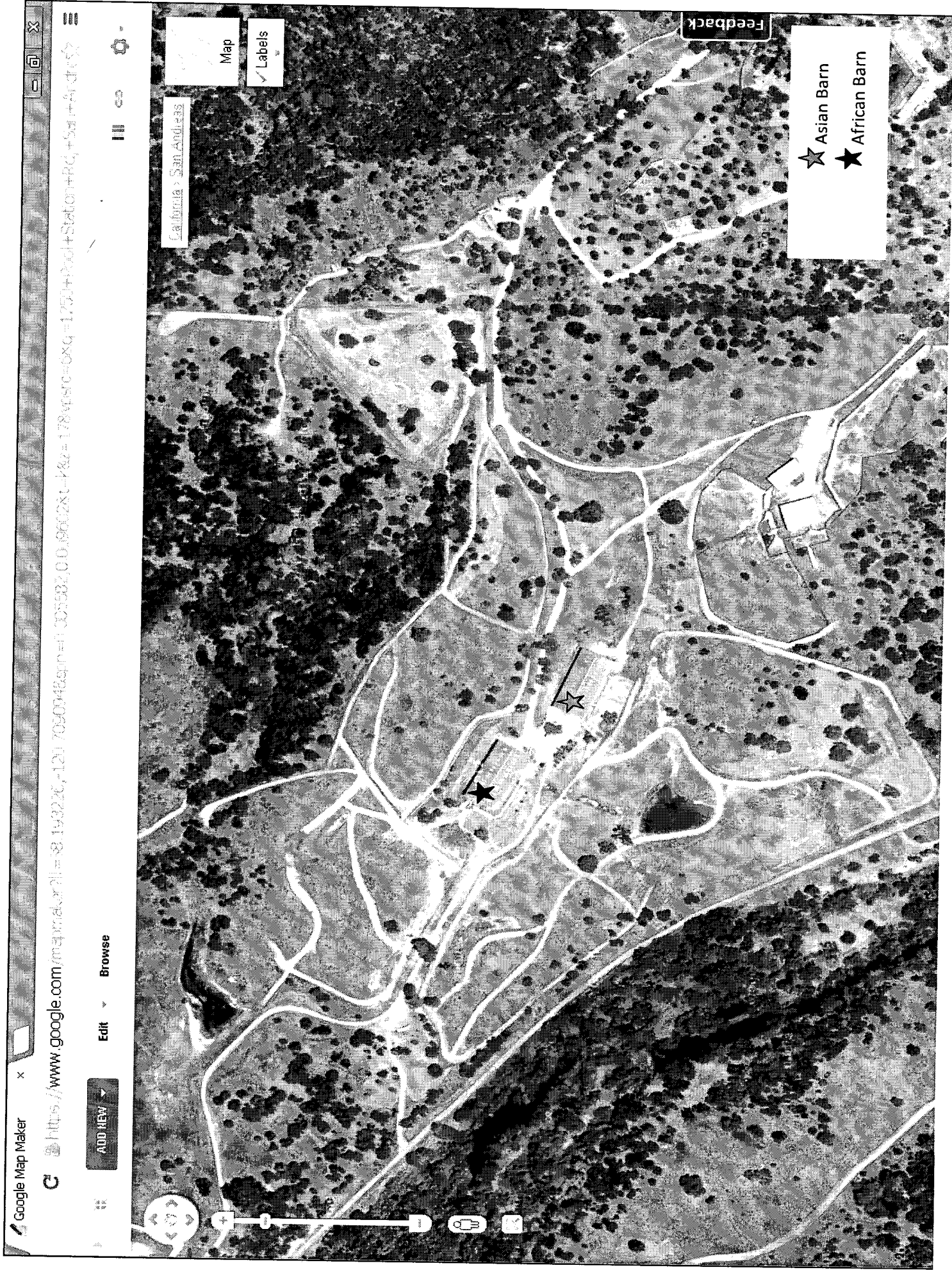
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ATTACHMENT 1
PAWS AERIAL SITE





United States Department of Agriculture
Animal and Plant Health Inspection Service

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Phone (515) 337-7388; Fax (515) 337-7315

Mycobacterium tuberculosis Final Genotyping Report

February 15, 2012

This is a genotyping report for a *M. tuberculosis* isolate (NVSL Accession No. 12-001688, TB No. 12-02772) obtained in February, 2012 from necropsied lung tissue from Sabu (aka 'Look Chal'), a deceased Asian male elephant. Sabu (b. 1982) was diagnosed with acute arthritis and euthanized on January 11, 2012, in his stall at the Performing Animal Welfare Society's ARK 2000 sanctuary in San Andreas.

M. tuberculosis had been cultured in December 1999 at the NVSL from a trunk wash from Sabu collected at the Ringling Bros. facility in Williston, FL in November, 1999. Accordingly, PAWS and the UC-Davis necropsy team excised lung tissue and forwarded it to the Mycobacteria and Brucella Section of the National Veterinary Services Laboratory, where it was received on January 17, 2012; culture of the lung tissue was initiated on January 18. Acid-fast bacteria were observed in a liquid media culture on January 31 and subjected to genotyping the weeks of February 5 and February 12.

[In addition to the lung tissue, swabs of lung tissue were obtained by Jackie Gal, of PAWS, and received at the NVSL on January 13; as of the time of writing, acid-fast bacilli have been observed in the associated liquid and solid media cultures and are scheduled for genotyping later this month.]

Two techniques, widely used by laboratories working on *M. tuberculosis*, were used to genotype the isolate. The first method is spoligotyping, and relies on the presence or absence of so-called 'direct repeats' in the *M. tuberculosis* genome to differentiate between isolates. It is considered a 'medium resolution' genotyping technique. The second method is variable number tandem repeat (VNTR), and this examines the genome of a given *M. tuberculosis* isolate for the presence of 'tandem repeats'. Differentiation between isolates can be mediated by differences in the numbers of tandem repeats observed at 11 or more loci in the genome. The VNTR method is considered to be a 'high resolution' genotyping technique.

The NVSL used a VNTR assay targeting 11 loci ('VNTR-11') from 2009 – October 2011. In November 2011 the NVSL adopted an expanded version of the VNTR method, targeting 24 loci ('VNTR-24'); this expanded panel is equivalent to the one used by the CDC, and its contracting state laboratories in California and Michigan. As of February 2012, at least one *M. tuberculosis* isolate from every positive elephants in the NVSL database has received the upgraded VNTR-24 assay. (For some elephants with multiple isolates, if every isolate shares the same spoligotype and VNTR-11 profiles, then only one of these isolates has been upgraded to VNTR-24).

Spoligotype and VNTR-24 profiles for the February 2012 isolate of *M. tuberculosis* from Sabu were compared to profiles for all elephant isolates in the NVSL BioNumerics database, including the isolate made from Sabu's trunk wash from November 1999.

The spoligotype for the *M. tuberculosis* strain from Sabu's lung tissue possessed octal code 407777777760771. This is different from that observed for the *M. tuberculosis* strain recovered from Sabu's trunk wash in 1999 (octal code 47777777760731). To confirm that this observation was reproducible, the culture from Sabu from 1999 / 2000 was retrieved from the freezer, re-extracted, and subjected to spoligotyping the week of February 12, 2012; it generated the same spoligotype that was observed originally, ie, 47777777760731.



United States Department of Agriculture
Animal and Plant Health Inspection Service

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***Mycobacterium tuberculosis* Final Genotyping Report**

The spoligotype generated from the February 2012 Sabu isolate was identical to the spoligotype observed for isolates from one other elephant in the NVSL database, Tb Nos. 99-3867 and 00-3206 (Figure 1, below). During 1999 this elephant was a herd mate to Sabu.

The VNTR-11 and (where present) VNTR-24 profiles for the *M. tuberculosis* isolates from Sabu were subjected to a statistical analysis using the unweighted pair-group method with arithmetic mean (UPGMA) technique; the resultant dendrogram is shown in Figure 2 (below). The VNTR-11 / VNTR-24 profile for the February 2012 isolate from Sabu is very different from that observed for the 1999 isolate, and in fact segregates into a cluster occupied by the same isolate, TB No. 99-3867, from the elephant which was a herd mate to Sabu.

There is a difference in VNTR-24 profile between the 2012 Sabu isolate and the 1999 herd mate isolate of one repeat, at one locus (VNTR 2163 aka QUB11b); this may reflect a minor, host-related genetic difference in this lineage of *M. tuberculosis*.¹

Taken together, the spoligotype and VNTR profiles for the February 2012 isolate from Sabu indicate that this elephant was infected with a second strain of *M. tuberculosis*. This second strain possesses a genotype (spoligotype + VNTR profile) identical to that recorded for an isolate (TB No. 99-3867) made in 1999 from a herd mate to Sabu. This herd mate was euthanized in October of 1999 due to arthritis and tuberculosis.

It could be hypothesized that Sabu acquired this second strain from his herd mate prior to the latter's death, but that, at the time of the trunk washing obtained from Sabu in November 1999, bacteria from this second strain either chanced not to be present in the wash, or had not replicated to numbers sufficient to be detected in the wash.

Jim Higgins
Mycobacteria and Brucella Section
NVSL / APHIS / USDA
1920 Dayton Ave
Ames, IA 50010
515-337-7034
515-337-7315 (fax)

[As of the writing of this report, the VNTR-24 assay has not yet been subjected to ISO17025 certification by the NVSL.]

¹ Readers are invited to submit the spoligotype (as the octal code) and VNTR data (as 12, or 15, or even 24 loci) for Sabu to the MIRU-VNTRplus website, <http://www.miru-vntrplus.org/MIRU/index.faces>, to learn if any *M. tuberculosis* isolates of human origin share this genotype



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Mycobacterium tuberculosis Final Genotyping Report

March 14, 2012

NVSL Accession # 11-002174, TB No. 11-2528, 'Rebecca', PAWS, Galt, CA

This is an updated version of a genotyping report originally distributed to VS Staff and Oklahoma State Health Lab staff on February 18, 2011, for a *Mycobacterium tuberculosis* isolate recovered from Rebecca, a 50 year-old Asian elephant, owned by the Performing Animal Welfare Society (PAWS), who died on January 8, 2011. Lung and lymph node specimens were received at the NVSL on January 12, 2011.

The *M. tuberculosis* isolate generated from Rebecca's tissues was subjected to genotyping analysis (i.e., spoligotyping and variable number tandem repeat, or VNTR) during February 2 – 8, 2011.

A prior trunk wash submission from Rebecca, delivered to the NVSL in 2002 (when Rebecca was owned housed at PAWS), generated a *M. tuberculosis* isolate, Accession No. 154146, TB No. 02-2450. This isolate was not subjected to VNTR until March 2011; accordingly, information about the 2002 isolate of Rebecca was not included in the original genotyping report for her February, 2011 isolate. Accordingly, information about the 2002 isolate is included in this updated genotyping report.

Because the spoligotype profile for the 2002 isolate from Rebecca (performed in 2005) was faint, another aliquot of DNA was extracted in the Fall of 2011 for the purpose of obtaining an expanded VNTR-24 panel for the 2002 isolate— was subjected to spoligotyping the week of February 19, 2012. A more satisfactory spoligotype (albeit one with the same profile as that performed in 2005, i.e., octal code 000000007760771) was observed, and is used in this report.

The 2002 and 2011 isolates differ in both their spoligotype (octal code 000000007760771 Vs 776377774020771) and VNTR profiles, indicating that Rebecca was infected with two different strains of *M. tuberculosis* (Figure 1, below). Neither spoligotype has previously been observed in the NVSL database, which makes it unlikely that these isolates represent contaminants that have been circulating in the laboratory.

The spoligotypes for both isolates has previously been recorded at the online, open-access SITVIT and MIRU-VNTplus websites: the 2002 isolate is Shared Type (ST) 4 (171 matches with SITVIT entries), while the 2011 isolate, ST383 (8 matches with SITVIT entries). Neither isolate's VNTR-24 profile is recorded in either the SITVIT, or MIRU-VNTplus, websites. Lauren Cowan at the CDC kindly agreed to a request from the NVSL to see what other isolates in the CDC's proprietary internal database matched the spoligotype for the 2011 Rebecca isolate. Cowan indicated that the spoligotype for the 2011 isolate had characteristics of the EuroAmerican S and EuroAmerican Haarlem *M. tuberculosis* lineages.¹ However, none of the *M. tuberculosis* isolates in the CDC internal database possessed a spoligotype matching that for the 2011 isolate from Rebecca.

When compared with the 186 *Mycobacterium* strains in the MIRU-VNTplus website, the 2002 Rebecca isolate segregated into a cluster with a 1999 (human) isolate from Uganda (No. 2224). The 2011 Rebecca isolate segregated into a larger cluster shared with (human) isolates, of the 'Haarlem' lineage of *M. tuberculosis*, from human patients in the interval 2002 – 2003 (see the accompanying pdf file of the MIRU-VNTplus dendrogram containing the two Rebecca isolates, highlighted in yellow).

A comparison of the two Rebecca isolates with all other elephant *M. tuberculosis* isolates (for which complete VNTR-24 profiles are available) in the NVSL database is presented in Figure 2. This analysis was generated using the Unweighted Pair Group

¹ www.biomedcentral.com/1471-2180/6/23



United States Department of Agriculture
Animal and Plant Health Inspection Service

National Veterinary Services Laboratories
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Mycobacterium tuberculosis Final Genotyping Report

Method with Arithmetic Mean (UPGMA) algorithm from VNTR-24 profiles. A dendrogram, presented on the left side of the VNTR number matrix, demonstrates the clustering of identical and / or closely related isolates. Based on the dendrogram, the two Rebecca isolates, and the two isolates from Sabu, another former Ringling Bros. elephant later housed at PAWS / ARK 2000, have no similarity to each other.

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Figure 1. Comparison of genotypes for 2002 (TB No. 02-2450) and 2011 (TB No. 11-2528) isolates of *M. tuberculosis* from Rebecca

Spoligotyping	VNTR																								TB No	Spoligo Octal Code
	VNTR 0024	VNTR 0577	VNTR 1644 (HRLU16)	VNTR 1856	VNTR 2185 (ETR A)	VNTR 2401	VNTR 2461	VNTR 2607 (HRLU24)	VNTR 2608 (HRLU28)	VNTR 3102 (HRLU51)	VNTR 4032 (CLB-26)	VNTR 3007 (HRLU27)	VNTR 0630 (HRLU4)	VNTR 0602 (HRLU46)	VNTR 0630 (HRLU10)	VNTR 2153b (CLB-118)	VNTR 3000	VNTR 4100	VNTR 0164 (HRLU02)	VNTR 2039 (HRLU23)	VNTR 2047	VNTR 2001 (HRLU22)	VNTR 3171	VNTR 4348 (HRLU09)		
	3	4	3	1	4	2	1	1	5	3	1	3	3	4	3	4	4	1	1	2	3	5	3	2	02-2450	000000007760771
	2	3	2	3	3	4	2	1	5	4	10	3	2	3	5	3	3	3	2	2	4	5	2	2	11-2528	776377774020771



Elephant TB StatPak Official Annual Test

License #
 Customer #
 Name of Facility
 Site Name

93-0-0074
 3551
 PAWS
 002 San Andreas

Date

6/28/11

Blood draw 6/27/11 (except late-6/28/11) Tests run 6/28/11 (Stat-Paks)

Signature of Attending Vet
 Printed Name of Attending Vet

Pamela Smith

Signature of Attending Vet
 Printed Name of Attending Vet

28

Page 1 of 2

Animal	Photo taken	Age	Sex	Species	Comments	Results	Initials
1 Mara	Previously TB positive	31 yrs	—	—	African ♀ 31 yrs.	—	—
2 Maggie	—	—	—	—	African ♀ 31 yrs.	—	—
3 Annie	—	—	—	—	Asian ♀ 51 yrs	Reactive	FO 12-08299
4 Gypsy	—	—	—	—	Asian ♀ 44 yrs	Reactive	FO 12-08299
5 Wanda	—	—	—	—	Asian ♀ 52 yrs	—	—



Assurance

Customer #

Name of Facility

Site Name

2010

93-6-0074

1553

Stairways - EOD-415

Plains

6/27/11

Signature Federal Vet

Printed Name of Federal Ver

Signature of Attending Vet

Printed Name of Attending ve 66

Page 2 of 2

(S)(a)

14-00000

Signature of Attending Vet

Printed Name of Attending ve 66

Animal	Photo taken	SP-2 Scribble	Sex	Distant 185091510	* 185091510	Age	Coverage	ADWA Number	Initials
Nick	Pennedy	1850916	Distant 185091510	*	—	Asian ♂ 18 yrs	—	—	—
Sabu	Yes				*	Asian ♂ 29 yrs	Reactive	(b)(6)	
Lulu	Pennedy	→	→	*	—	African ♀ 45 yrs	—	—	FOIA b7-D b7-E

ATTACHMENT 3

19 November 2012

Dear Dr. Crawshaw:

This letter is to follow-up on the information provided in the correspondence dated August 11, 2012. There have been a number of peer-reviewed scientific publications that have examined the results of exposure of elephants to TB. One paper published this year prospectively studied 14 elephants that had been exposed to other culture-positive TB elephants and/or became seropositive by STAT-PAK and confirmatory tests (Lyashchenko, et al., 2012). All of these elephants eventually were diagnosed to have TB by confirmed culture, although it took up to 15 years in one case. Two elephants housed with a confirmed TB elephant diagnosed in 1996, became seropositive in 1996 and 1997. Although tested by trunk wash culture annually, they remained negative. However when daily pooled trunk wash samples were started in 2011, both animals were diagnosed positive by culture within 2 and 3 ½ weeks.

Similar studies have been published investigating transmission of TB among elephants and other exposed animals in zoos that were NOT in direct contact, yet became infected (see Lewerin, et al., 2005; Oh, et al., 2002). Currently, the Taronga Zoo in Australia is dealing with TB in imported elephants from Thailand that had been quarantined for several years, yet still started shedding when one of the elephants became pregnant and calved. This facility now has another seropositive elephant as well as TB in their chimp troop (Vogelnest, personal communication).

These scientific publications demonstrate that there is a risk of transmission from culture-positive and even seropositive elephants that have a strong probability of becoming culture positive, to other animals that are in contact, and even those that are not in direct contact but on the same premise as these animals.

As I've previously stated, based on this information there is a risk of transmission of tuberculosis from an infected elephant whether there or not there is direct contact with other elephants including potentially your elephants that may be relocated to a facility with a history of TB. At this time, it is difficult to provide a quantifiable risk since many factors will impact this. However, it is obvious that relocation to a facility where there is no history of tuberculosis presents a better option when considering risk for disease transmission.

Please feel free to contact me if you have any questions or wish to discuss this matter further.

Sincerely,



Michele Miller, DVM, PhD
AZA Elephant Taxon Advisory Group Veterinary Advisor
Palm Beach Zoo, Director of Animal Care and Conservation
1301 Summit Blvd., West Palm Beach, FL 33405
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mmiller@palmbeachzoo.org

August 11, 2012

Dear Dr. Crawshaw:

In consideration of your questions regarding risks of transmission of tuberculosis between elephants, there is still a lot of debate among experts in the field of mycobacteriology, epidemiology, and infectious disease as to the factors contributing to the risks. As you are aware a number of workshops have been held recently, and I would refer you to some of the presentations available at the website [http://www.aphis.usda.gov/animal_welfare/pg.php?pg=Tuberculosis in Elephants](http://www.aphis.usda.gov/animal_welfare/pg.php?pg=Tuberculosis%20in%20Elephants), in particular the "Epidemiology of Tuberculosis in Elephants, 1994-2011, United States" by Dr. Kathleen Orlowski and the presentation by Dr. Rendi Murphree, which shows indirect exposure through aerosolization of *M. tuberculosis* at an elephant sanctuary.

Since the currently available diagnostic tests for detecting tuberculosis in elephants are still less than ideal for identifying early infection, it is only after an infected elephant is shedding the organisms and presents a risk to other animals and people that it is typically diagnosed. By this time, the likelihood of contamination of the environment and exposure of animals and people has already occurred.

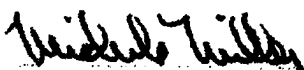
In other cases, tuberculosis has occurred in facilities in which there has been no direct contact between animals. Currently there is an epidemiological investigation underway at the Taronga Zoo in Australia in which tuberculosis in elephants has spread to the chimpanzee troop (L. Vogelstein, pers. comm.). Other documented cases have also included outbreaks at the Los Angeles Zoo in which mountain goats, black rhinoceros and elephants were infected with *M. tuberculosis* without having any direct contact (Oh et al., 2002) and in elephants, tapir, giraffe, and rhinoceros in a Swedish zoo (Lewerin et al., 2005).

These examples demonstrate that the risk of transmission of tuberculosis from an infected animal at a facility to other animals, even those not in direct contact, is a documented risk. The duration of contact is probably associated with risk of transmission although those studies have not been done in elephants. As the examples above demonstrate, the time from diagnosis in an infected animal to identification of infection in a secondary case can be as short as 2 years but could be decades.

Therefore, based on this information there is a risk of transmission of tuberculosis from an infected elephant whether there or not there is direct contact with other elephants including potentially your elephants that may be relocated to a facility with a history of TB. At this time, it is difficult to provide a quantifiable risk since many factors will impact this. However, it is obvious that relocation to a facility where there is no history of tuberculosis presents a better option when considering risk for disease transmission.

Please feel free to contact me if you have any questions or wish to discuss this matter further.

Sincerely,



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ATTACHMENT 4

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Senior Veterinarian Toronto Zoo
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Canada

November 19, 2012

Dear Dr. Crawshaw,

This letter is written in reference to the two phone conversations we had pertaining to the elephants in your care at the Toronto Zoo, and their potential move to another facility. As a clinical zoological specialist and researcher studying elephant tuberculosis, I find your situation very interesting.

I generally agree with your assessments and understanding of elephant tuberculosis in the United States. Furthermore, I understand and absolutely agree with your position to err on the side of precaution for the Toronto Zoo elephants.

From what you told me about your elephants, there is no evidence of tuberculosis exposure in the Toronto Zoo elephant collection. If this is correct and the results of this testing are consistent, you have good evidence that the Toronto Zoo elephants have not been exposed to tuberculosis in the past and probably do not have latent or active tuberculosis. In contrast, from the information you shared with me, the institution that wants to accept the elephants had elephants that cultured positive for tuberculosis, and serological evidence of exposure in several of the elephants currently housed at the facility.

If these elephants were under my care, I would make the clinical and managerial decision to not send them into a situation where they might be infected with tuberculosis. Such an exposure may result in disease with significant welfare implications. The exposure would also complicate future transfers of the elephants to other collections. As clinicians for zoological collections, our responsibility is to make decisions that protect the interest and health of the animals in our care.

Sincerely,

A handwritten signature in black ink, appearing to be 'R. Isaza', written over a horizontal line.

Ramiro Isaza, DVM, MS, MPH, DACZM
Associate Professor of Zoological Medicine

The Foundation for The Gator Nation



ONTARIO AGRICULTURAL COLLEGE
Department of Animal and Poultry Science

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Toronto City Council

15th November 2012

Dear Councillors,

I am writing this letter as an expert on animal welfare and as Chair of the Toronto Zoo Animal Care and Research Committee (ACARC). I have been a member of this Committee for 10 years and have been Chair for just over a year.

In my opinion, the decision by Toronto City Council to send the three elephants, Thika, Toka and Iringa to the PAWS Sanctuary in northern California is not in the animals' best interests. I think that everyone, including ACARC, agrees that, in order to ensure their welfare, these aging elephants should be moved. The facilities at Toronto Zoo no longer meet their requirements particularly during the winter months. However, I firmly believe that the decision by Council to send the elephants to the PAWs Sanctuary is misguided. I base my belief on three pieces of evidence:

1. There is overwhelming evidence that there is active tuberculosis infection amongst the elephants recently and currently at the PAWS Sanctuary. In my opinion, it is completely unethical to send animals which are free from tuberculosis, and are known to have been free from tuberculosis since they were brought to Toronto Zoo many years ago, to a facility where that infection is known to exist. The fact that the Toronto Zoo elephants have not been exposed to tuberculosis means that they will be very susceptible to the infection and are likely to become very ill.

The fact that no elephants at PAWS have *died* from tuberculosis is irrelevant to the argument; several elephants have been culled at PAWS that have been shown at *post mortem* examination to be infected with tuberculosis. Tuberculosis is a debilitating illness that causes suffering and really reduces welfare. No animal should be exposed to it if alternative arrangements can be made.

These remarks should not be seen as a criticism of the PAWS Sanctuary. From pictures and television clips that I have seen of PAWS, it looks like a very nice retirement home for elephants. I think it is wonderful that such a Sanctuary exists – a Sanctuary that will accept abused and injured elephants and elephants suffering from tuberculosis. However, I strongly believe that it is not an appropriate sanctuary for the Toronto Zoo elephants because they are known to be free from tuberculosis.

2. Transporting three elephants from Toronto Zoo to the PAWS Sanctuary in northern California is a huge problem and poses a very real risk to their survival. By road, this journey of about 4,300 km will take four days to complete. In my opinion, it is doubtful if Thika, Toka and Iringa would survive such a stressful journey. Two of the elephants are already trained to enter a crate while the third elephant is two-thirds trained (awaiting a final crate modification). However, it will take very little to disrupt this training. Thus, once crated, the journey to the final destination must be completed. The journey cannot be broken to allow the elephants out of the crates to exercise, feed and drink, and rest; there is a big risk that they would not enter the crates again. These are wild animals, not trained circus elephants that can be manipulated at will. It is unknown if the Toronto elephants will eat and drink during a long road journey. If they do not, they will not have the stamina to survive.

This leaves air transport as the only alternative means of getting the elephants to PAWS. To my knowledge, a plane with proper pressure and temperature control that would be capable of air-lifting three elephants has not yet been identified.

3. Although the winter climate at the PAWS Sanctuary is much milder than at the Toronto Zoo, it is not ideal for elephants. There are still many days and nights through the winter when the temperature falls to and below 0°C. This means that the elephants at PAWS spend a considerable amount of time indoors. Although the floors of the barns have a thick rubberized coating and are heated, there is no indoor enrichment for the elephants.

For all these reasons given above, I recommend strongly that the Toronto Zoo elephants should not go to the PAWS Sanctuary in northern California and that the decision on their final home should be left to the Toronto Zoo staff.

There is a new sanctuary currently being built in Florida which will be AZA accredited and which looks very promising as a retirement home for the Toronto elephants. It will be free from tuberculosis, will have an ideal climate for elephants and will be reachable in 22 hours by road from Toronto. This will still be a stressful journey for the elephants but one which they should survive.

Yours sincerely,

Ian J.H. Duncan
Professor Emeritus
Emeritus Chair in Animal Welfare