



MEMORANDUM: ELEPHANT TRANSFER STATUS

DISTRIBUTION: TORONTO ZOO BOARD OF MANAGEMENT

Dear Board of Management members:

Purpose:

The purpose of this memorandum is to provide Board members with the current status as it relates to the Elephant transfer (Iringa, Toka, Thika) from the Toronto Zoo to PAWS in northern California.

Background:

The Toronto Zoo has been and continues to be committed to following the City of Toronto Council and the Toronto Zoo Board of Management's directives for the safe and humane transfer of the three elephants to PAWS complying with all applicable laws and standards, and to undertake due diligence prior to any transfer of the elephants to PAWS.

The Toronto Zoo and PAWS have entered into a legal agreement that also sets out their respective obligations.

Current Status:

There are a number of complexities involved with transferring three elephants over an international border, and the associated technical (permits, crates, training, transportation plan, health records etc.) elements that a privately owned/operated sanctuary and a public institution (Toronto Zoo) need to coordinate as part of due diligence. In partnership with the PAWS team we have achieved a number of key components necessary for the move, please refer to attachment (key dates), there are several outstanding items to complete the transaction along with new information that we are now aware of that may impact the health of our elephants at the PAWS sanctuary.

As of September 18, 2012:

Permits:

PAWS is responsible for obtaining the required permits to import the elephants into the USA. The Toronto Zoo is responsible for the export permits from Canada. The Toronto Zoo applied for the export permits on December 6, 2011 and Environment Canada has been prepared to issue the permits as soon as they received confirmation of the USA import permits.

On September 18, 2012 the Toronto Zoo was advised that all required import permits had been obtained and PAWS provided a copy of the final import permit (see attached import permit received Sept. 18th, 2012). The permit was apparently issued on July 26, 2012 and it will expire October 1, 2012. This is the first time we were made aware of the issuance of the final import permit. The Toronto Zoo forwarded this import permit on September 19, 2012 to Environment Canada so that the export permit can be issued.

Final Transportation Plan:

Under our agreement, PAWS is responsible to make all of the arrangements for, and cover all costs relating to, the safe and humane transport of the elephants to their facility. PAWS and the Toronto Zoo mutually agreed that air transportation would be best for the welfare of the elephants. We have requested that PAWS obtain a confirmation from the US Fish & Wildlife Service (USFWS) that PAWS' transportation plan meets the animal welfare requirements as required by the International Air Transportation Association (IATA) worldwide standard for shipping live animals. PAWS agreed that the air carrier (AN124) originally proposed by them was not suitable to transfer the elephants to California because the cargo area was not sufficiently pressurized to ensure the safe transport of the elephants and accompanying personnel. There may also be additional concerns regarding noise and vibrations which could cause unacceptable stress to the elephants. See attached letter from EMA.

We do not currently have a transportation plan from PAWS for the safe and humane transport of the three elephants. It is PAWS' obligation to provide the transportation plan. PAWS has experience in transferring elephants by air, for example, when they flew African elephant Maggie in November 2007 from Alaska to California on a special US airforce C17 cargo plane that was fully pressurized.

Crates and Training:

PAWS is responsible for providing transportation crates that take into account the safe and humane welfare of all three elephants. As of September 24, 2012, Thika will walk into her crate, present her front foot and allow keepers to attach her restraint. The side door on the crate is opened at which time keepers walk up behind her. A hind bar is slid in behind her, she is then asked to present her hind foot onto the bar and at which time the hind restraint is attached. She will stand comfortably at which time four additional bars are being put in behind her, all of which are above the foot present bar. The last front bar is then put into place. She will then stand in her crate for 1.5 minutes at which time it is then removed. The four bars behind her are then removed in the reverse order and she is asked for a hind foot present and the hind restraint is removed. Keepers will continue to increase her duration in the crate while on restraints with bars in place.

Iringa has had some regression in her sessions since the last update. She was walking into her crate, presenting her front foot and allowing her restraint to be attached. The side door on the crate was opened at which time keepers were walking up behind her. A hind bar had been slid in place behind her and the apparatus to prevent the bar from moving was put into place. Keepers were then asking for a foot present onto this bar. A hind restraint was being attached to this leg and she was released. Unfortunately, she has regressed and was slow to come back into her crate for a few sessions. She will now walk into her crate and present her front foot and allow for mock hook ups with her restraint. Please note: This regression is fairly normal as well as expected during the training process. Keepers will work through this regression and be back on track as soon as possible.

Toka continues to walk into her crate, present her front foot and allow her restraint to be attached. Keepers have also increased the movement behind her, as well as the movement along the side of her crate. Keepers are also inserting a bar into a collar on one side of the crate in order to mimic the sounds associated with the bar being inserted. Keepers cannot move forward with her hind bar training until modifications can be done on her crate. The crate needs to be extended in order to provide enough space for adequate movement and allow enough room for Toka to present her hind foot. We have requested PAWS to modify the crate on numerous occasions.

Medical and Health Records:

The due diligence process is ongoing between our experienced and qualified zoo senior veterinarians and PAWS representatives. It was only in late June 2012 that the qualified staff from the Toronto Zoo (Dr. Crawshaw and Dr. Rapley) were permitted by PAWS to inspect and take notes of medical records and information from PAWS with respect to the elephants housed at their facility. There are some public accounts that point to the presence of tuberculosis (TB) in the elephants at PAWS. See attached medical records. Zoo staff, during their site visit to PAWS, December 2011, were not provided details on the health status on the Asian elephants and were only allowed to visit two of the five elephant barns under direction from the PAWS lawyer. The fact that they were only invited to see one Asian elephant and three African elephants, that PAWS staff caring for the other barns were wearing face masks, strongly suggested that quarantine was in operation as a result of either a confirmed or suspected case of tuberculosis. The confirmation of TB at PAWS and the threat, if any, it may pose to our elephants, is part of the due diligence review.

Dr. Graham Crawshaw has provided a list of questions/issues that he needs to be supplied or addressed by PAWS that are most pertinent outstanding medical record related questions, and that need to be answered in order to permit Dr. Crawshaw to finish his due diligence review. PAWS has been provided with Dr. Crawshaw's request for information.

Other Documents:

There are a couple of other minor administrative items we are currently taking care of and will provide to PAWS in a timely fashion.

Summary:

The Toronto Zoo has been and continues to be committed to performing, in good faith, its obligations under the Elephant Transfer agreement. The delays to date in the safe, humane, and compliant (with all applicable laws and standards) transfer of Iringa, Toka, and Thika can be primarily attributed to a number of factors under the responsibility of PAWS including: USA permits/documents, supply of right-sized crates, sound transportation plan, and provision of medical/information as it relates to PAWS' elephant collection.

Prepared by:

John Tracogna
Chief Executive Officer

Dr. Graham Crawshaw
Senior Veterinarian

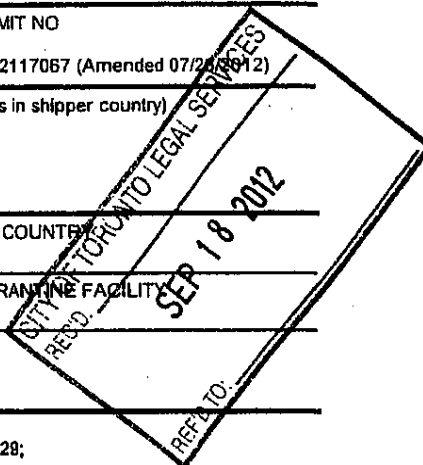
Date: 2012-09-25

Toronto Zoo – Transfer of Elephants to PAWS

Key Dates (up to 2012-09-17)

- **December 6, 2011** – Toronto Zoo submitted the application for CITES export permits for the three elephants to Environment Canada.
- **December 18-19, 2011** - Five Toronto Zoo staff made a site visit to PAWS.
- **February 9, 2012** – Legal agreement finalized between Toronto City legal and PAWS lawyers and agreement signed by both parties.
- **February 27, 2012** – PAWS crate (first crate) arrives and is put in place to start crate training.
- **March 14, 2012** – Two of PAWS staff made a site visit to the Toronto Zoo. Medical records for 0.3 Toronto Zoo elephants were provided and Dr. Gai was shown Iringa's foot x-rays.
- **March 14, 2012** - Blood draws were completed with 0.3 TZ elephants. * STAT PAC tests were performed by PAWS veterinarian and all TZ elephants were negative for TB.
- **March 23, 2012** - PAWS announces via media that Bob Barker will pay to fly TZ 0.3 elephants to the PAWS Sanctuary.
- **March 28, 2012** - Toronto Zoo elephant Trunk Wash cultures back from lab and results are negative for TB.
- **April 17, 2012** – Transporter arrives and two elephant crates are unloaded in the lower Toronto Zoo elephant paddock. Elephant crate training ongoing.
- **April 25, 2012** – Toronto Zoo staff contact PAWS transporter and inform him that the two additional elephant crates are too short for TZ elephants.
- **June 20, 2012** – Graham Crawshaw and Bill Rapley met with Zoocheck lawyer and Julie Woodyer from Zoocheck to review PAWS medical records. No copying was permitted although copies of several records were requested by TZ staff (none of the requested records have been supplied). Hand written notes were taken as much as possible within the time allowed. Many of the records were not up to date (2011).
- **July 27, 2012** – Graham Crawshaw, John Tracogna, Bill Rapley and City Legal met with Zoocheck lawyer and Julie Woodyer to discuss several outstanding issues (transportation, permits & health records). Additional but limited health records were made available under the same protocols as the previous meeting. A conference call took place with Graham Crawshaw, Bill Rapley and Dr. Gai (PAWS vet) during this meeting.
- **August 23, 2012** – Blood samples taken from 0.3 Toronto Zoo elephants for pre shipment testing as per the request by PAWS.

UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE VETERINARY SERVICES WASHINGTON, D.C. 20250		DATE ISSUED 26-JUL-2012	PERMIT NO RIV12117067 (Amended 07/26/2012)
UNITED STATES PERMIT TO IMPORT IMPORTANT: Upon arrival at the U.S. Port of Entry the animals must pass veterinarian inspection and if accompanied by this Permit and a Health Certificate will be quarantined as indicated herein. The Importing Carrier shall not stop at any port except as provided in item 7.		1. SHIPPER (Name and address in shipper country) The Toronto Zoo 381A Old Finch Avenue Scarborough, Ontario M1B5K7 Canada	
2. PERMIT FOR (Name & Address of Importer - include zip code) Performing Animal Welfare Society 125 Pool Station Road San Andreas, California, 95632 United States TEL: 209-745-2606		3. PORT OF EMBARKATION & COUNTRY Any, Canada	
		4. NAME OF APPROVED QUARANTINE FACILITY (If Applicable) TRANS CLASS - AIR, chartered airplane	



6. Permit to Import

1 Number N/A / Elephant: African Bush Elephant (*Loxodonta africana*) - ID: Female; 31; Thalka; T2 ISIS 14029; 00-06F2-A015.

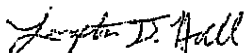
1 Number N/A / Elephant: African Bush Elephant (*Loxodonta africana*) - ID: Female; 42; IRINGA; T2 ISIS 5402; 00-06F2-9COA.

continued on next page...

PERMISSION GRANTED TO IMPORT THE ABOVE ELEPHANTS FROM CANADA INTO THE UNITED STATES. NOTE ROUTING: Toronto, Canada - San Francisco, CA - San Andreas, CA

Permission granted to import the Elephants listed on the permit through the route indicated. No deviations are permitted.

continued on next page...

7. Route of Travel from Port of Embarkation (including carrier stops enroute) Any, Canada / San Francisco, CA, United States No other stops permitted		8. Proposed Shipping Date 09/01/2012	10. Valid only at this United States Port of Entry San Francisco, CA
		9. Proposed Arrival Date 09/01/2012	
11. TOTAL NUMBER THIS PERMIT AUTHORIZES 3	12. THIS PERMIT VOID AFTER 01-OCT-2012	14. QUARANTINE AT PORT OF ENTRY (Only Federal Charges are shown; Public Charges could apply)	
13. Address of Port Veterinarian at USDA-APHIS-VS 389 Oyster Point Blvd, Suite 2 San Francisco, California 94080 (650) 876 9358 / (650) 876 0915		14. Minimum No. Days 15. Charge Per Animal Per Day \$	
17. Signature Langston Hull 	18. Title National Center - Import - Export	16. Minimum Charge Each Importation (if any) \$	

NOTE: Health Certificates must be attached. Failure to do so could necessitate rejection at the Port of Entry.

RESPONSIBILITIES

The U.S. IMPORTER must forward the Original and Carrier's Copy to the shipper in the country of origin; and must make arrangements at the U.S. port of entry for Customs broker's service if desired, necessary quarantine space and transportation of the shipment to and from quarantine. The Importer's Copy to be retained.

THE SHIPPER in the country of origin must make certain that a Health Certificate covering requirements in Item 6 is completed by a salaried veterinary officer of the National Government of the country from which the animals are shipped; and deliver the Health Certificate, Original and Carrier's Copy to the Initial transporting carrier.

THE SALARIED VETERINARY OFFICER of the National Government of the country of origin is being forwarded a copy of this permit and is responsible for insuring that the necessary inspections and Health Certificate are completed and attached to the Original, provided circumstances are such that the certification can be properly issued.

THE INITIAL CARRIER must make certain that the Health Certificate is attached to the Original and has been completed and signed by a salaried veterinary officer of the National Government of the country from which the animals are shipped; make sure that the Original and Health Certificate accompany the animals to the U.S. port of entry; and make certain that the Carrier's Copy is available for the final carrier who will transport the shipment to the U.S. port of entry.

VS VETERINARIAN AT PORT OF ENTRY will hold the Customs Copy until the shipment is received, then forward it to the U.S. Collector of Customs. The Original and the signed Health Certificate will be retained by the VS Veterinarian at the U.S. port of entry.

Permit Number: RIV12117067 (Amended 07/26/2012)

Permit To Import (continued)

Additional Animals

1 Number N/A / Elephant: African Bush Elephant (*Loxodonta africana*) - ID: Female; 41; Toka; T2 ISIS 4771;
00-0713-7987.

Additional Restrictions

An entry inspection and ectoparasite treatment(s) will be performed by APHIS personnel at the destination facility

Transportation to the destination facility will be made under APHIS supervision and USDA seal.

The shipping containers in which the animals arrive in the United States will be wrapped at the port of arrival underneath and up the side to a minimum height of two feet with impermeable plastic to prevent contact of bedding or wastes with the environment.

All disposable wastes will be incinerated after arrival at the destination facility and the shipping containers will be properly cleaned and disinfected.

The elephants must be accompanied by a valid official health certificate with the following statements

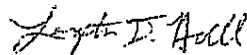
The elephants were inspected by the individual signing the health certificate and found free of any ectoparasites and clinical signs of diseases not more than 72 hours before being loaded on the means of conveyance which transported the animal to the United States.

A user fee will be collected at the port of entry for services provided in accordance with entry inspection. A premium user fee will be charged for services performed outside the normal tour of duty or on federal holidays. Also, an extra fee can be charged for a change in date/arrival time without adequate prior notification to the port veterinarian.

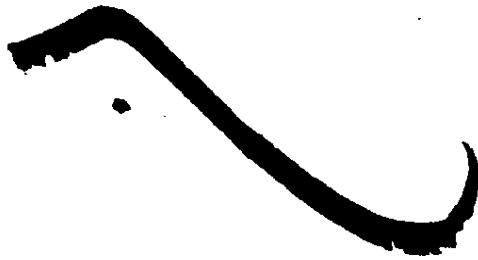
At least 72 hrs prior to arrival, the importer must notify the VS port veterinarian of the confirmed time and date of arrival.

A \$145.00 import permit application fee has been collected by staff.

Signature:



Langston Hull



THE ELEPHANT MANAGERS ASSOCIATION
1513 Cambridge Street, Houston TX 77030

July 30, 2012

Mr. Tim van Norman
Chief, Branch of Permits
US Fish and Wildlife Service
4401 North Fairfax Drive
Arlington, VA 22203

Dear Mr. Van Norman,

The Elephant Managers Association (EMA) is writing you to respectfully oppose the planned transport of three African elephants from the Toronto Zoo in Canada to the Performing Animals Welfare Society (PAWS) in Galt, California. The EMA is an international nonprofit organization of professional elephant handlers, administrators, veterinarians, researchers, and elephant enthusiasts.

It has come to our attention that this transport may be in conflict with CITES Guidelines on Live Animal Transport. We wish to express our concerns regarding this planned transport and encourage the USFWS Management Authority to review the arrangements to ensure the safe and humane transport of these three elephants.

It has been reported the mode of transport of these three elephants is an unpressurized Russian cargo aircraft flying at a maximum height of 10,000 feet necessitating a circuitous route around the Rocky Mountains to the southern border of the United States then to northern California. It is well documented that this type of aircraft produces an extreme level of noise and vibrations from which the animals cannot be habituated prior to the flight. This is unacceptable stress for the elephants as there is no way to adequately replicate the extreme vibrations and noise associated with this transport. Moreover, it is unnecessary. There are other options that would be more appropriate and less stressful for the elephants while significantly reducing travel time. It is our understanding that this aircraft was chosen for transport as it was the only piece of equipment large enough to transport all three elephants at the same time. We would like to point out that the Pittsburgh Zoo recently transported three similar sized African elephants via a pressurized aircraft, so it appears this planned transport has been predicated on saving money instead of doing what is best for the individual animals.

CITES Articles III, IV, V and VII requires the Management Authorities to be satisfied, before granting export permits, or re-export or traveling exhibition certificates, that specimens will be so prepared and shipped as to minimize the risk of injury, damage to animal's health, or cruel treatment. Furthermore, transport of a CITES-listed species requires compliance with the CITES Guidelines on Live Animal Transportation. CITES Resolution Conf. 10.21 (rev COP14) recommends that "suitable measures be taken by the Parties to promote the full and effective use by Management Authorities of the Live Animals Regulations (for animals)." Under the Resolution, "applicants for export permits or re-export or traveling exhibition certificates are to be notified that, as a condition of issuance, they are required to prepare and ship live specimens in accordance with the Live Animals Regulations."

We believe that the planned transport fails to meet CITES humane transport requirements and does not take into consideration the best interests of the animals. Therefore, we strenuously object to the transport arrangements currently in place for the import of these animals to the United States, and urge the Service to ensure that alternative arrangements are made, and to suspend the permit until such time as the issue is appropriately resolved.

Thank you for your time and consideration. We would be glad to have a representative from our organization speak to you about captive elephant management issues. Please contact us if you require additional information on this or other important matters of concern to the elephant care community.

Sincerely,

A handwritten signature in black ink, appearing to read 'A. Smith', with a long, sweeping horizontal line extending to the right.

Andrew Smith
President

EMA Board of Directors:
Mike McClure, Baltimore, MD
Joanne Smith, Perris, CA
Ellen Wiedner, Gainesville, FL
Trudy Williams, Polk City, FL
Daryl Hoffman, Houston, TX - Executive Director

CC: Canadian Wildlife Service, CITES Management Authority



National Elephant TB StatPak Official Annual Test

Form 1 (USDA)
received Sept 2012

Page 1 of 2

License #
Customer #
Name of Facility
Site Name

93-C-10074
3551
PAWS
002 - San Andreas

Signature Federal Vet
Printed Name of Federal Vet
Signature of Attending Vet
Printed Name of Attending Vet

Pamela Smith

Signature of Attending Vet
Printed Name of Attending Vet

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

6/28/11

Animal	Photo taken	Kill #	Serial	Lab	Result	Comments	MAHA Results	Initials
1 Mara	Previously	TB VN 810	Diluent TBS D091510	*	*	African ♀ 31 yrs.	7/5/11	
2 Maggie				*	*	African ♀ 31 yrs.		
3 Annie				*	*	Asian ♀ 51 yrs	Reactive	(b)(6)
4 Gypsy				*	*	Asian ♀ 44 yrs	Reactive	(b)(6)
5 Wanda				*	*	Asian ♀ 52 yrs		



page 2 of 2

11/22/9

Printed Name of Attending ve [66]

Animal	Photo taken	Kit # Serial	Results		Comments	MAPIA Results	Initials
			Diluent TBSD091570	Non-reactive Linear			
Nick	Premortally	1801110	*	—	Asexual ♂ 18 yrs.		
Sabu	Yes			*	Asexual ♂ 29 yrs.	Reactive	(b)(6)
Lulu	Premortally		→	*	Asexual ♀ 45 yrs.		



United States Department of Agriculture
Animal and Plant Health Inspection Service

National Veterinary Services Laboratories
1920 Dayton Ave., Ames, IA 50010
Phone (515) 337-7388; Fax (515) 337-7315

***Mycobacterium tuberculosis* Final Genotyping Report**

February 16, 2012

This is a genotyping report for a *M. tuberculosis* isolate (NVSL: Accession No. 12-001666, TB No. 12-02772) obtained in February, 2012 from necropsied lung tissue from Sabu (aka 'Look Chai'), 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 Gai, 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 477777777760731). 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, 477777777760731.



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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 – 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



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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 0424	VNTR 0577	VNTR 1844 (MIRU16)	VNTR 1955	VNTR 2165 (ETR A)	VNTR 2401	VNTR 2461	VNTR 2587 (MIRU24)	VNTR 2998 (MIRU26)	VNTR 3192 (MIRU31)	VNTR 4062 (QUB-26)	VNTR 3007 (MIRU27)	VNTR 0580 (MIRU04)	VNTR 0802 (MIRU40)	VNTR 0960 (MIRU10)	VNTR 2163b (QUB-11B)	VNTR 3690	VNTR 4156	VNTR 0154 (MIRU02)	VNTR 2059 (MIRU20)	VNTR 2347	VNTR 2531 (MIRU23)	VNTR 3171	VNTR 4346 (MIRU39)		
	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