ATTACHMENT 3
EX24.30.85

Councillor Michelle Berardinetti

TORONTO

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Date: November 27, 2012

To: Mayor & Members of Council

From: Councillor Michelle Berardinetti

Re: Executive Committee Report No. 24.30

I am submitting copy of the report entitled *'Review of current elephant tuberculosis control measures used at the Performing Animal Welfare Society (PAWS) Sanctuary, San Andres, California* – Report written by Dr. Susan Catherine Cork & Dr. David Abraham' for consideration by City Council for Executive Committee Report No. 24.30.

Enclsoed are also authorization letters from Dr. Susan Catherine Cork and Dr. David Abraham authorizing me to release the report along with their Curriculum Vitaes to become a public document for Toronto City Council.

Should you have any questions, please contact me at 416-392-0213.

Councillor Michelle Berardinetti
Ward 35 - Scarborough Southwest

Attachments

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November 27, 2012

To Whom It May Concern,

On behalf of Dr. Susan Cork, David Abraham, & University of Calgary Faculty of Veterinary Medicine, I hereby authorize Toronto City Council to release the 2012 PAWS report prepared by Dr. Cork on behalf of Zoocheck Canada Inc. This includes the body of the report, all appendices, and the CV's of Dr. Cork & Mr. Abraham.

Regards,

Alastair Cribb, DVM, PhD, FCAHS
Dean
Professor, Clinical Pharmacology
University of Calgary Faculty of Veterinary Medicine
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Attachments:
David Abraham CV
Susan Cork CV
Cover Letter to Zoocheck
2012 Zoocheck Report
Appendix 1: Definitions
Appendix 2A: Summary of trunk wash cultures of *currently alive* elephants at PAWS sanctuary
Appendix 2B: Summary of trunk wash cultures of *previously dead* elephants at PAWS sanctuary
Appendix 3A: Summary of Elephant TB STAT-PAK® and MAPIA® testing of *currently alive* elephants at PAWS sanctuary
Appendix 3B: Summary of Elephant TB STAT-PAK® and MAPIA® testing of *previously dead* elephants at PAWS sanctuary
Appendix 4: Details of post-mortem examination of elephants conducted at the PAWS sanctuary
Appendix 5: Location map of PAWS ARK2000 facility at San Andreas, California
Appendix6: Biosecurity protocols at the PAWS ARK2000 facility at San Andreas, California
PAWS Zoocheck UCVM Release Authorization

Notes on the Report

Review of current elephant tuberculosis control measures used at the Performing Animal Welfare Society (PAWS) Sanctuary, San Andreas, California

(Report written by Dr Susan Catherine Cork & Dr David Abraham)

- PAWS ARK 2000 is a well-managed animal sanctuary with a high standard of animal care.

- The elephants observed during the visit, including the quarantined group are in good health and had access to large outdoor enclosures and spacious naturally ventilated barns.

- Biosecurity (*a set of preventive measures designed to reduce the risk of transmission of infectious diseases-HMR*) and quarantine protocols are in place to control the aerosol spread of tuberculosis.

- The facility follows local health authority recommendations to mitigate the primary risks for the aerosol transmission of tuberculosis from the quarantined elephants to staff working at the facility.

- The current facility veterinarian has specific experience with the test protocols used to detect and monitor tuberculosis in elephants due to her work at PAWS and her experience as a veterinarian with the United States Department of Agriculture (USDA).

- The testing and management protocols used at the PAWS facility are based on the USDA endorsed guidelines for the control of tuberculosis in elephants.

- The facility was considered USDA compliant at the last inspection in April 2012 and is accredited by the Global Federation of Animal Sanctuaries November, 2012.

- PAWS veterinarian follows the USDA endorsed guidelines for the control of tuberculosis in elephants and exceeds those guidelines.

- The layout of the enclosures at PAWS provides the opportunity for effective mitigation of disease exposure risk when compared to smaller facilities (*such as Toronto and most AZA facilities-HMR*) where separation of infected and non-infected groups might not be possible.

- The female Asian elephants and one of the male elephants are currently under quarantine for tuberculosis and are being managed according to current USDA endorsed guidelines for the control of tuberculosis in elephants. These guidelines are currently considered by experts in this field to be best practice for the control of tuberculosis in elephants.

- Tuberculosis, caused by Mtb has now become fairly widespread in captive elephants in North America and has been reported in elephants held in both zoological gardens and private facilities in several states.

- The elephants at PAWS have come from a number of different facilities across the USA and may have been exposed to tuberculosis from other elephants, other animal species, humans or environmental sources prior to arrival at the PAWS facility. This is a situation that is common to the many facilities, including many zoological gardens, currently holding elephants in North America (Anon 2011c).

- The PAWS veterinarian has considerable experience with these (TB) tests, through her work at PAWS and as a veterinarian for the USDA, and works closely with her veterinary colleagues at the USDA to ensure that best practice is followed with regard to collecting and handling samples.

- Numerous variables such as age, genetics, nutritional condition, immune status, concurrent health problems (e.g. arthritis)(*Both elephants with TB at necropsy were euthanized due to severe crippling arthritis-HMR*).), and other factors influence the development of disease in an individual animal following exposure to a pathogenic agent (Anon, 2011a, Anon 2011b).

- PAWS facility has developed elephant testing and management protocols that adhere to, or exceed, the 2008 USDA elephant tuberculosis control guidelines.

- In any setting where elephants develop tuberculosis it is hard to quantify the extent of the risk. The infectious dose *(which is not known in elephants—HMR)*, rates and routes of shedding Mtb in infected elephants are likely to vary from case to case. Transmission risk can be mitigated by separating infected and non-infected animals and implementing strict biosecurity and quarantine protocols, which PAWS is doing.

- At the present time the facility management and the attending veterinarian are applying the USDA endorsed guidelines for the control of tuberculosis in elephants and are adhering to best practice guidelines.

- Now that tuberculosis is established in a number of captive elephants and facilities in North America, and given the scientific uncertainty with regard to infectious dose, host susceptibility and disease transmission pathways for Mtb in elephants, a determination of the acceptable level of risk would also need to take into account factors other than tuberculosis such as other potential costs or benefits of moving elephants to this or another facility. *(In other words quality of life, habitat, social groups, space, and not the least—choice—HMR)*

DISCUSSION POINTS

1. The PAWS facility has a high standard of animal care, and complies with or exceeds United States Department of Agriculture (USDA) guidelines in regards to control of tuberculosis. In fact, PAWS is following what experts in the field consider to be best practise. Their testing protocol is based on endorsed USDA guidelines from 2008 and the more recent 2011 recommendations.

2. Tuberculosis is not unique to PAWS, but is fairly widespread in captive North American elephants (both in zoos and private facilities). Since PAWS is a sanctuary and the elephants have come from a number of different facilities, some may have been exposed to tuberculosis prior to arrival. This siutation is common to many captive situations, including zoos. One elephant at PAWS is shedding Mtb, so the hazard of tuberculosis is present in that quarantined group. PAWS is taking every effort to minimize risk of exposure to other elephants and to humans. Most outbreaks are associated with aerosol transmission in indoor setting - this scenario is unlikely to occur at PAWS because if its design, location, etc. The residual risk depends on whether infected elephants start to shed large numbers of Mtb and if the Mtb is likely to be transmitted via routes other than aerosal. This is <u>not</u> currently the case at PAWS. The risk of current or new elephants developing tuberculosis does exist, but it is low and is not the only factor to take into account when considering moving an elephant to PAWS.

3. Because tuberculosis is established in captive elephant facilities (not just PAWS, but other zoos and facilities as well), other factors (such as quality of life, habitat, space, social groups, etc.) besides the presence of tuberculosis must be taken in to account when deciding to move an elephant to or from the facility. It is important to consider the other potential benefits.

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Curriculum Vitae (short version)

Name: Dr Susan Catherine Cork

Qualifications: BVSc ,B.Phil (vet)., PhD (Massey University);
PG Diploma Public Policy (Victoria University)

Affiliations: MSB, CBiol. MRCVS

Contact address:

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Summary of employment history

Current: Head of Department & Professor of Ecosystem & Public Health
Faculty of Veterinary Medicine (UCVM), University of Calgary (from September 2008)

Research Interests

Wildlife Diseases, Public Health, Animal Health Policy, Risk Assessment,

Previous employment:

Team Manager, Virology, Animal Health Laboratory, Wallaceville, Investigation and Diagnostic Centre, Biosecurity New Zealand, Ministry of Agriculture and Forestry, New Zealand (May, 2006-September, 2008)

Senior Science Advisor, Strategic Science Team, Policy and Risk Directorate, Biosecurity New Zealand, Ministry of Agriculture and Forestry, New Zealand (February, 2005 - May, 2006)

Senior Advisor Risk Analysis, Animal Biosecurity Team, Policy and Risk Directorate, Biosecurity New Zealand, Ministry of Agriculture and Forestry, New Zealand (May, 2003 – April, 2005)

Veterinary Inspector (Bioethics and Animal Welfare),Senior Civil Service Grade, Animal Scientific Procedures Inspectorate, Community Policy Directorate, British Home Office, London, England (Dundee Office) (February, 2002-May, 2003)

Horizon Lecturer in Organic Livestock Production (Industry Chair), Institute of Rural Studies (now IBERS), University of Wales, Llanbadarn, Aberystwyth, Wales (March, 2000-February, 2002)

Senior lecturer in Animal Health (Associate Professor), Harper Adams University College, Newport, Shropshire, England (February, 1998- March, 2000)

Consultant, Clinical Trials, Pfizer Animal Health Product Development, Sandwich, Kent, England (responsible for Northern United Kingdom, located in Edinburgh) (August, 1997-February, 1998)

Veterinary Laboratory Manager & Livestock Extension Advisor (Animal Health) working for the Ministry of Agriculture, Royal Government of Bhutan under the

auspices of Volunteer Services Overseas (NGO) London. (June 1995- February 1997)

Wildlife Health Advisor /Pathologist and research associate
New Zealand Department of Conservation (DoC) /Massey University,
(June 1990- July 1994)

Clinical appointments, Small Animal and Exotic Animal practice, UK (1987-1990)

Academic Qualifications

Post Graduate Diploma in Public Policy: 2004-2007 (part time study), Victoria University, School of Government, Wellington, New Zealand.

Papers in Public Health Policy, Economics, Political Science and Strategic Analysis/Management

PhD (Veterinary Science): 1991-1994 Department of Veterinary Pathology and Public Health, Faculty of Veterinary Science, Massey University, Palmerston North, New Zealand

Thesis: Cork, S.C (1994) *Yersinia pseudotuberculosis*, iron and disease in birds. Published in a series of five papers (see refereed publications. Copies available on request)

B.V.Sc: Degree in Veterinary Science: 1981 - 1986, Faculty of Veterinary Science, Massey University, Palmerston North, New Zealand

B.Phil (Vet) in Veterinary Anatomy: 1983-1984, Department of Physiology and Anatomy, Faculty of Veterinary Science, Massey University, Palmerston North, New Zealand

[redacted]

Published Books or Monographs *(as author or editor; include accepted or in-press)*

Cork, S.C. & Halliwell, R (2012) The Veterinary Field and Laboratory Manual, Second Edition (ISBN987-1-908062-09-3) A technical handbook for veterinary field staff and laboratory technicians in developing countries. Nottingham University Press. Publication launch expected June 2012.

Published Contributions to a Collective Work and Book Chapters *(include chapters written on invitation or collective works derived from conferences or symposiums; include accepted or in-press)*

Cork, S.C. & Checkley, S (2010) Globalization of the food supply and the spread of disease (20 pp), Chapter 1, in Krause, D., Hendrick, S (Eds) Zoonotic Pathogens in the Food Chain, CABI, UK

Cork, S.C (2010) Epidemiology of pathogens in the food supply (30 pp), Chapter 2, in Krause, D., Hendrick, S (Eds) Zoonotic Pathogens in the Food Chain, CABI, UK.

Relevant recent conference abstracts

Abraham, D., Mundayoor, S., Madhavilatha G.K., Whiteside, D., **Cork, S.C** (2012) *'Mycobacterium tuberculosis* in captive Asian elephants *(Elephas maximus)* and other species in Southern India' International Exchange Symposium, ISID, Bangkok, Thailand, June 2012..

Abraham, D., **Cork, S.C.**,Venugopal, K.P.,Shah, A., Madhavilatha, G.K. Anusree, B.S., and Mundayoor, S.(2012) Strain identification of Mycobacterium complex isolates from rescued Sloth bears (*Melursus ursinus*) in Southern India. International Wildlife Tuberculosis Conference. Pretoria, South Africa, Sept. 2012.

Abraham, D., **Cork, S.C** (2010) *Mycobacterium tuberculosis* Complex Infection in Asian Elephants (*Elephas maximus*): Implications for Policy Guidelines in Range Countries. John Waters Zoonoses workshop, Calgary, November 18/19th 2010.

Abraham, D., **Cork, S.C.,** Mundayoor, S., Paul, L., Madhavi Latha, G.K., Alex, P.C., Ganguly, S., Cheeran, J.V., Mikota, S. K (2010) Identification of Mycobacteria identified from captive elephants and their Mahouts. American Association of Zoo Veterinarians Meeting. USA. November 2010.

Le Ba, Q., Hall, D.C., **Cork, S.C** (2012) Vietnam National Rural Clean Water Supply and Sanitation Strategy: Implications for Reduction of Risk Factors for Waterborne Diseases. GRF One Health Summit, February 2012. Davos, Switzerland.

Zuliani, A., Lysyk, T.J., Johnson, G., Massolo, A., Waeckerlin, R., Cully, A.S., **Cork, S.C** (2012) Working towards a risk-based surveillance system for *Culicoides*-borne diseases in Southern Alberta (Canada) and Montana (U.S.A.).International Society for Veterinary Epidemiology & Economics (ISVEE), Maastricht, August 2012 – Accepted as an oral presentation.

Selected Published Refereed Papers *(in chronological order, including authors, year, title, journal, volume, complete page numbers; include accepted or in-press papers)*

Rawdon, T.G., Tana, T., Thornton, R.N., McKenzie, J.S., Stanislawek, W.L., Kittelberger, R., Geale, D., Stevenson, M.A., Gerber, N., **Cork, S.C** (2010) Surveillance for avian influenza virus subtypes H5 and H7 in chickens and turkeys farmed commercially in New Zealand. New Zealand Veterinary Journal. 58(6), 292-298.

T Zheng, B Adlam, TG Rawdon, WL Stanislawek, **SC Cork,** V Hope, BM Buddle, K Grimwood, MG Baker, JS O'Keefe and QS Huang (2010) A Cross sectional survey of influenza A, and management practices in small scale backyard poultry flocks in two regions of New Zealand. New Zealand Veterinary Journal. 58(2), 74-80.

Langstaff, I., McKenzie, J.S., Stanislawek, W.L., Reed, C.E.M., Poland, R., **Cork, S.C** (2009) Surveillance for highly pathogenic influenza in migratory shorebirds at the terminus of the East Asian-Australasian Flyway. New Zealand Veterinary Journal. 57(3), 160-165.

McFadden AMJ, Rawdon TG, Bingham P, Mackereth GM, Stanislawek, WL, **Cork SC**, Clough R, King C (2007) Managing the risk of equine influenza in horses imported from Australia during the 2007 Australian epidemic. Surveillance. 34(4), 4 - 8.

Cork, S.C., Geale, D., Thornton, R., Pharo, H (2006) Response Policy Options and Compartmentalisation for Avian Influenza Surveillance in New Zealand. Schudel, A., Lombard, M (Eds). OIE/FAO International Scientific Conference on Avian Influenza. Dev. Biol (Basel), Karger, vol 124. p228.

Consent for public distribution given ✓

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Personal Particulars

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1 September 2009 till date: Graduate Student
Supervisor: Dr. Susan C. Cork, Professor and Head, Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary,
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1 August 2006 to 31 August 2009: Research Associate & Wildlife Veterinarian
Asian Elephant Research and Conservation Centre
A Division of Asian Nature Conservation Foundation
C/o Centre for Ecological Sciences
Indian Institute of Science
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1November 2002 to 30 June 2004: Research Assistant
Asian Elephant Research and Conservation Centre
A Division of Asian Nature Conservation Foundation
C/o Centre for Ecological Sciences
Indian Institute of Science
Bangalore 560012, India

Education

Master of Science in Wildlife Biology and Conservation, 2004 to 2006
Wildlife Conservation Society – India Program
National Centre for Biological Sciences (Affiliated to Manipal Academy of Higher Education), GKVK Post, Bangalore 560 065, India

Bachelor of Veterinary Science and Animal Husbandry, 1995 to 2001
College of Veterinary and Animal Sciences
Kerala Agricultural University
Thrissur, Kerala, India

Awards

1. American Association of Zoo Veterinarians (AAZV), 'International Conference Scholarship' to attend the 2010 AAZV Annual Conference, 23-29 October 2010, South Padre Island, Texas, USA.
2. University of Calgary, 'Veterinary Medicine Graduate Studentship Entrance Award 2009-10.
3. Student Scholarship (Travel Grant and Registration Waiver) to attend the 12th International Symposium on Veterinary Epidemiology and Economics, 10-14 August 2009, Durban, South Africa.
4. Wildlife Conservation Society, 2009 Animal and Human Health for the Environment and Development (AHEAD) 'AHEAD Beyond Boundaries Journal Award'.

Training/ Summer Institute/ Dissertation/ Project.

1. Training program on 'Field Epidemiology and Communicable Disease Control', 12-16 December 2011, by the Public Health Foundation of India, at the Indian Institute of Public Health, Delhi.
2. Hands on training on 'DNA Fingerprinting Following Random Amplified Polymorphic DNA (RAPD) and Microsatellite Techniques', 1-18 February 2011, at the Regional Facility for DNA Fingerprinting, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India.
3. Co-Chair for the session 'The Elephant in the Room, Pachyderm Medicine',AAZV Annual Conference, 23-29 October 2010, South Padre Island, Texas, USA.
4. Envirovet 2010 Summer Institute, Seven Week Lecture, Laboratory and Field Sessions on Terrestrial and Aquatic Animal Ecosystem Health in Developed and Developing Country Contexts, 16 June to 10 August 2010, Department of Veterinary Biosciences, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, USA.
5. Identification of mycobacteria isolated from captive elephants and mahouts (2010)Winter Semester Project, Part of graduate study at the University of Calgary, Funded By SeaWorld and Busch Gardens Conservation Fund, 2009 Grant Cycle.
6. Health assessment of captive Asian elephants in India with special reference to tuberculosis (2008) One year project, Research Associate with Asian Nature Conservation Foundation, Bangalore. Funded by Elephant Care International.

7. Studies on prevalence of tuberculosis in wild Asian elephants in southern India (2006)M. Sc. Dissertation, Thesis can be accessed online at http://msc.wcsindia.org/DAVIDABRAHAM_THESIS.PDF

Publications

1. Verma-Kumar, S., **Abraham, D.**, Dendukuri, N., Cheeran, J.V., Sukumar, S., Balaji, K.N. (2012) Serodiagnosis of tuberculosis in Asian elephants (*Elephas maximus*) in southern India: A latent class analysis, PLoS ONE 7(11): e49548.
2. Ajitkumar, G., Naryanan, H.P.M., Radhakrishnan, S., **Abraham, D.** and Alex, P.C. (2010) Prevalence of ocular problems among captive Asian elephants of Kerala, *Zoos' Print Magazine*, 25 (10): 27.
3. Ajitkumar, G., **Abraham, D.**, Cheeran, J.V. and Chandrasekharan, K. (Eds.) (2009) *The Captive Asian Elephant: Proceedings of the International Workshop on Captive Elephant Management*, Pub.: Elephant Welfare Association.
4. **Abraham, D.** and Davis, J. (2008) Revised trunk wash collection procedure for captive elephants in a range country setting,*Gajah, Newsletter- Asian Elephant Specialist Group of the IUCN*, 28:53-54.
5. **Abraham, D.** (2003) Training Program in Conservation Biology, *Journal of the Indian Veterinary Association Kerala Chapter*, 1 (1): 56.

Presentations

1. **Abraham, D.**, Cork, S.C., Sha, A., Alex, P.C., Venugopal, K.P., Anusree, S.B. and Mundayoor, S. (2012) Strain identification of Mycobacterium tuberculosis complex isolates from rescued sloth bears in southern India, ***Oral Presentation***, International Wildlife Tuberculosis Conference, University of Pretoria, 9-12 September 2012, Kruger National Park, South Africa.
2. **Abraham, D.**, Cork, S.C., Mundayoor, S., Paul, L., Madhavilatha, G.K., Alex, P.C., Ganguly, S., Cheeran, J.V. and Mikota, S.K. (2010) Identification of mycobacteria isolated from captive elephants and mahouts, ***Poster Presentation***, Annual Conference of the American Association of Zoo Veterinarians, 23-29 October, South Padre Island, Texas, USA.
3. **Abraham, D.** & Cork, S.C. (2010) Implications for policy guidelines: *Mycobacterium tuberculosis* complex infection in Asian elephants (*Elephas maximus*), ***Poster Presentation***, John Waters Zoonotic Disease Workshop, 18-19 November 2010, University of Calgary, Calgary, Canada.
4. **Abraham, D.**, Cork, S.C., Cheeran, J.V. and Mikota, S.K. (2010) Health assessment of captive Asian elephants with special reference to tuberculosis – Experiences from southern India, ***Oral Presentation***, Asian Elephant Range Country Workshop – Strategies for Tuberculosis Control and Prevention in Asian Elephants, Organised by the Department of National Parks and Wildlife Conservation (DNPWC), World Wildlife Fund (WWF) and National Trust for Nature Conservation (NTNC), 20-21 May, Kathmandu, Nepal.
5. **Abraham, D.**, Cork, S.C., Cheeran, J.V. and Mikota, S.K. (2010) Study of health determinants among captive Asian elephants (*Elephasmaximus*) in southern India with special reference to tuberculosis, ***Poster Presentation***, Third National Conference on Infectious Diseases, Organised by the Infectious Diseases Society of India, All India Institute of Medical Sciences, 17-18 April, New Delhi, India.
6. **Abraham, D.**, Ramnath, V., Cheeran, J.V. and Mikota, S.K. (2010) Comparing tuberculosis seroprevalence and serum biochemistry in captive Asian elephants (*Elephas maximus*), ***Poster Presentation***, International Conference on 'Wildlife Conservation, Health and Disease Management – A Post-Millennium Approach', Organised by the Madras Veterinary College,3-5February, Chennai, India.
7. **Abraham, D.** (2009) Seropositivity to human/bovine strains of tuberculosis among captive Asian elephants (*Elephas maximus*) in southern India: Conservation and zoonotic implications, ***Poster Presentation***, 12th International Symposium on Veterinary Epidemiology and Economics, 10-14 August, Durban, South Africa.
8. **Abraham, D.**, Cheeran, J.V., Alex, P.C. (presenting author), Ramnath, V., Ajitkumar, G., and Rajiv, T. S. (2008) Population health assessment of captive Asian elephants – Experiences from southern India, ***Oral Presentation***, International Elephant Conservation and Research Symposium, Organised by the International Elephant Foundation,24-26 November, Bangkok, Thailand.
9. **Abraham, D.** (2007) Screening wild Asian elephants (*Elephas maximus*) in southern India for human strain of tuberculosis, ***Poster Presentation***, Annual Conference of the Wildlife Disease Association, 12-17 August, Estes Park, Colorado, USA.
10. Mikota, S.K. (Presenting author), Dumonceaux, G., Miller, M., Gairhe, K., Giri, K., Cheeran, J.V., **Abraham, D.**, Lyashchenko, K., Larsen, S., Payeur, J., Waters, R., Kaufman, G. (2006) Tuberculosis in elephants: An update on diagnosis and treatment; implications for control in range countries.***Oral Presentation***, International Elephant Conservation and Research Symposium,Organised by the International Elephant Foundation, 21-22 October, Copenhagen, Denmark.

consent for
Public distribution given ✓

Review of current elephant tuberculosis control measures used at the Performing Animal Welfare Society (PAWS) Sanctuary, San Andreas, California

Dr Susan Catherine Cork, BVSc, PhD, PG Dip. Public Policy

&

Dr David Abraham BVS&AH, MSc

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Review of current elephant tuberculosis control measures used at the Performing Animal Welfare Society (PAWS) Sanctuary, San Andreas, California

Table of contents:

Executive summary

This report is based on examination of elephant health records, and supplementary information, provided by the current PAWS veterinarian and a subsequent site visit, and meeting, with the PAWS veterinarian and the facility manager of ARK 2000 on the 12th November, 2012. The purpose of the visit was to assess the current risks, and risk mitigation measures in place, with respect to the transmission of tuberculosis from the quarantined Asian elephants to elephants that might be transported to the facility.

In summary, PAWS ARK 2000 appears to be a well-managed animal sanctuary with a high standard of animal care. The elephants observed during the visit, including the quarantined group appeared to be in good health and had access to large outdoor enclosures and spacious naturally ventilated barns. Biosecurity and quarantine protocols are in place to control the aerosol spread of tuberculosis and the facility follows local health authority recommendations to mitigate the primary risks for the aerosol transmission of tuberculosis from the quarantined elephants to staff working at the facility. The current facility veterinarian has specific experience with the test protocols used to detect and monitor tuberculosis in elephants due to her work at PAWS and her experience as a veterinarian with the United States Department of Agriculture (USDA). The testing and management protocols used at the PAWS facility are based on the USDA endorsed guidelines for the control of tuberculosis in elephants. The current veterinarian keeps comprehensive health records for the elephants under her care. The facility was considered USDA compliant at the last inspection in April 2012 and is accredited by the Global Federation of Animal Sanctuaries http://www.sanctuaryfederation.org/gfas/for-sanctuaries/accreditation/

From the visit and examination of the records provided it is evident that the PAWS veterinarian has made every effort to follow the USDA endorsed guidelines for the control of tuberculosis in elephants and has explored options to exceed the guidelines. The layout of the enclosures at PAWS provides the opportunity for effective mitigation of disease exposure risk when compared to smaller facilities where separation of infected and non infected groups might not be possible. However, there are some additional biosecurity measures that could be considered in the event that elephants in the quarantine group begin to shed a significant number of Mtb organisms or if transmission of Mtb by fomites or the oro-fecal route was considered to be a significant risk. In any setting where elephants develop tuberculosis it is hard to quantify the transmission risk or to predict the consequences of exposure. Now that tuberculosis is established in a number of captive elephants and facilities in North America, and given the scientific uncertainty with regard to infectious dose, host susceptibility and disease transmission pathways for Mtb in elephants, a determination of the acceptable level of risk would also need to take into account factors other than tuberculosis such as other potential costs or benefits of moving elephants to this or another facility.

1.0 Background:

The Performing Animal Welfare Society (PAWS), founded in 1984, is comprised of 30 Acres at Galt, California and the Ark 2000 facility which includes 2,300 acres of land in San Andreas. The San Andreas facility, Ark 2000, is currently home to eight elephants as well as lions, bears and tigers. The elephants do not have direct contact with other species held at the park although wildlife such as block tailed deer, mountain lions, lynx, turkeys and other birds occur have been

seen in the area and goats are periodically brought onto the property to clear vegetation as part of a fire prevention plan. The PAWS captive wildlife sanctuaries were originally established by Pat Derby, a former Hollywood animal trainer, and her partner Ed Stewart, to provide a home for abandoned, abused or retired performing animals. PAWS is accredited by the Global Federation of Animal Sanctuaries http://www.sanctuaryfederation.org/gfas/for-sanctuaries/accreditation/

The PAWS facilities are animal sanctuaries with a mandate to provide a long term care for elephants and other animals requiring a new home and, unlike zoological gardens, PAWS ARK 2000 is not generally open to the public. The entire facility is enclosed behind an 8 ft high perimeter fence as required by the regulatory authorities. The PAWS ARK 2000 facility does not intend to breed elephants and incoming elephants are tested for tuberculosis and other infectious diseases before their arrival to the sanctuary. On arrival, new elephants currently undergo quarantine for a period of 30 days on site prior to introduction to the resident elephants. Pat Derby and Ed Stewart, the facility owners, have more than 70 years of experience in elephant care between them and the elephant supervisor, and ARK 2000 sanctuary manager, has more than 16 years of experience with elephants. PAWS uses 'protected contact' methods for training elephants.

PAWS currently maintains five Asian (*Elephus maximus*) and three African (*Loxodonta africana*) elephants. The two species are managed separately although some animals from the two groups did share a barn in the early days of the establishment of PAWS ARK 2000 prior to the confirmation of tuberculosis at the facility. All eight elephants currently held at PAWS have access to outdoor enclosures with water features and natural vegetation. Each group of three female Asian elephants and three female African elephants has access to a large purpose built elephant barn (one Asian and one African) see maps in Appendix 5. There are currently two male Asian elephants which are managed separately. The barns and outdoor enclosures can be viewed on the PAWS website http://www.pawsweb.org/ along with information on the animals at both the GALT and ARK 2000 facilities. As part of routine health monitoring the PAWS veterinarian undertakes general screening of all elephants for tuberculosis at least once a year. The female Asian elephants and one of the male elephants are currently under quarantine for tuberculosis and are being managed according to current USDA endorsed guidelines for the control of tuberculosis in elephants. These guidelines, along with recent amendments, are currently considered by experts in this field to be best practice for the control of tuberculosis in elephants.

The following report is based on examination of elephant health records, necropsy reports and supplementary information provided by the current PAWS veterinarian and a subsequent site visit, and meeting with the PAWS veterinarian and the facility manager of ARK 2000, on 12th November, 2012. The purpose of the visit was to assess the current risks, and risk mitigation measures in place, with respect to the transmission of tuberculosis from the quarantined Asian elephants to other elephants that might be transported to the facility.

2.0 Introduction

Tuberculosis (TB) is a chronic disease caused by intracellular bacteria in the genus *Mycobacterium*. Mycobacteria infect a broad range of species including humans, non-human

primates, carnivores, marine mammals, birds, domestic and non-domestic ungulates and elephants (LoBue *et al.*, 2010., Mikota & Maslow, 2011). Species susceptibility to specific mycobacteria varies and some Mycobacteria are considered host specific. In mammals, the term "tuberculosis" is usually used for disease caused by Mycobacteria in the *Mycobacterium tuberculosis* (*M. tb*) complex. Some Mycobacteria are saprophytes and live in soil or water, these are not considered to be primary pathogens although may cause disease under some circumstances (Smith *et al.*, 2009). These non-pathogenic (or non tuberculous bacteria (NTM) might occasionally interfere with diagnostic screening for the presence of *Mycobacterium tuberculosis* (Mtb) complex organisms by causing false positive (+ve) test results.

The M tb complex includes *M. tuberculosis, M. bovis, M. caprae, M. africanum, M. microti, M. canetti, and M. pinnipedii. Mycobacterium tuberculosis* is the predominant disease-causing agent in elephants although cases caused by *M. bovis* have been reported (Payeur, 2002). Although M tb complex bacteria have been isolated from African elephants most reported cases of tuberculosis have been in Asian elephants (Montali *et al*,2001). *Mycobacterium avium* and other Mycobacteria have also been isolated from elephants but have not, in most cases, been associated with clinical disease (Lacasse *et al.*,, 2007). *M.tuberculosis* is generally thought to be spread via aerosol although, like other Mycobacteria, it can potentially also be spread via fomites such as contaminated bedding, food or water sources or feces (Jackson *et al*, 1995., Johnson *et al*..2000). The infectious dose of Mtb for elephants is not known and will most likely depend on the general health status of the elephant, presence of concurrent diseases, immunity and husbandry (Anon 2011a, Anon 2011b). Most reported cases of tuberculosis have been in older elephants but the pathogenesis of the disease and the time frame for the development of lesions is not well understood (Anon 2011b., Montali *et al*., 2001).

Due to potential zoonotic transmission of Mtb complex organisms in addition to concerns over animal health and welfare for captive and wild animals there has been a significant amount of research undertaken to improve diagnostic testing for tuberculosis as well as to understand disease transmission mechanisms and risk (Mikota & Maslow, 2011). Despite this, there remains a lot that is unknown with regard to the transmission and pathogenesis of tuberculosis in elephants and other species. Tuberculosis, caused by Mtb has now become fairly widespread in captive elephants in North America and has been reported in elephants held in both zoological gardens and private facilities in several states (Mikota & Maslow, 2011). Many of the infected animals share a common contact history although it is often difficult to trace back to the original source of infection because some elephants have changed ownership several times and not all of these animals, even those in close contact, share the same strain (Anon 2011c).. Tuberculosis, caused by Mtb, is also widespread in elephant range countries such as India, Nepal and Thailand (Angkawanish *et al.*, 2012) with up to 25% of animals being reactive in screening tests for tuberculosis in Southern India (Abraham *et al.*, 2010a & b). However, although tuberculosis is relatively common in captive elephants in range countries it is rarely the primary cause of death (Abraham *et al.*, 2010a., Mikota, 2008). In many cases the presence of tuberculosis is only confirmed at necropsy and elephants may not have demonstrated any clinical signs suggestive of the disease ante-mortem (Mikota & Maslow, 2011). Thorough assessment of disease transmission risks has been difficult due to the lack of reliable diagnostic tests. Trunk wash sampling has been considered to be the 'gold standard' but is not a sensitive test and bacterial shedding may be intermittent resulting in false negative results. The development and ready availability of new blood tests (Lyashchenko *et al.*, 2006., 2012) has allowed better screening although these tests (STAT-PAK ® and MAPIA ™) have the potential to yield both false positive and false negative results. At the present time, however, the Elephant TB STAT-PAK ® test is currently the screening test recommended by the USDA for tuberculosis in elephants and is thought to have a high degree of sensitivity (100%) and

specificity (95%). To further enhance specificity it is recommended that samples that are reactive in this test are then screened using a second test (MultiAntigen Print ImmunoAssay (MAPIA ™), animals that test positive in both of these tests are thought likely to be infected with TB (Lyashchenko *et al.*, 2012). However, other diseases such as arthritis or infection with other Mycobacteria may result in false positive results in the MAPIA.™ and the use of a blood test to detect infection with Mtb remains controversial (Anon, 2008)..Due to the complexity of the subject of tuberculosis control in elephants and the need for more information on disease transmission and transmission risk, it is likely that the current elephant TB guidelines will continue to evolve in response to new findings (Anon, 2011c).

3.0 The USDA endorsed guidelines for the control of tuberculosis in elephants

In the United States of America (USA) Mtb in elephants emerged as an issue in 1996 with the death of two circus elephants. .Tuberculosis (TB) was subsequently identified in five additional elephant herds and prompted a collaborative initiative by the United States Department of Agriculture (USDA), the American Association of Zoo Veterinarians (AAZV), zoos, circuses and experts from the veterinary and human health care communities to develop guidelines for the control of tuberculosis in elephants (Mikota & Maslow, 2011).

The National Tuberculosis Working Group for Zoo and Wildlife Species has been monitoring TB in elephants since 1996. The original Guidelines for the Control of Tuberculosis in Elephants were released in 1997 and modified in 2000, 2003 & 2008. The Guidelines include recommendations for the testing, treatment, and surveillance of TB in elephants and are revised as new information becomes available. In 2009 the Elephant Tuberculosis Subcommittee of the Tuberculosis Committee of the United States Animal Health Association (USAHA) assumed responsibility for updating the Guidelines based on current scientific information.

http://www.aphis.usda.gov/animal_welfare/downloads/elephant/elephant_tb.pdf (accessed 14th November 2012)

The 2008 guidelines were updated in 2010 and reviewed again in 2011 in response to a meeting of experts organized by the Animal and Plant Inspection Service (APHIS) of the USDA (see below).

http://www.aphis.usda.gov/animal_welfare/pg.php?pg=Tuberculosis_in_Elephants (accessed 14th November 2012) Summary of scientific meeting in Kansas, April 2011, 'Tuberculosis in elephants, science myths and beyond'.

http://www.aphis.usda.gov/animal_welfare/downloads/policy/Policy%201%20Final.pdf (accessed 14th November 2012) Revised Policy on the control of Tuberculosis in elephants March 25th, 2011.

Additional elephant health guidelines are produced by the Association of Zoos & Aquariums Elephant Taxon Advisory Group this includes a section on tuberculosis and general health management and quarantine procedures. These guidelines state that USDA guidelines must be adhered to for the testing and treatment of tuberculosis in zoo elephants. In this respect, all facilities, including zoos and sanctuaries are expected to follow the USDA endorsed guidelines for the control of tuberculosis in elephants.
http://www.elephanttag.org/Professional/Elephant_Routine_Health_Procotol_2012.pdf

http://www.elephanttag.org/Professional/professional_Medical_Health_Care.html (accessed 14th November 2012)

The USDA endorsed guidelines were developed by experts and based on the best available scientific data. Most policy documents still refer to the 2008 guidelines although many establishments are also following additional recommendations which were accepted, although not implemented, in 2010. A summary of recommended revisions to the 2008 guidelines are summarized in one of the presentations available on line from the scientific meeting in Kansas, April 2011. Most of the revisions related to the availability and performance of screening tests and options for treatment.
http://www.aphis.usda.gov/animal_welfare/downloads/elephant/2008%20and%202010%20Elephant%20TB%20Guidelines.pdf (accessed 21st November, 2012)

4.0 The PAWS elephants & tuberculosis

PAWS currently cares for five Asian (*Elephus maximus*) and three African (*Loxodonta africana*) elephants. The two species are managed separately with three female Asian elephants (AsF1,AsF2 & AsF3) and one male Asian elephant (AsM2) under quarantine due to reactive tuberculosis STAT-PAK ® test results. Another male Asian elephant (AsM1) is also managed separately away from the Asian females. The African elephants, all females, (Af1F, AfF2, AfF3) are managed together as a separate group and are not under quarantine (see Table 1).

A map of the PAWS ARK 2000 facility layout can be seen in Appendix 5. The closest point between the outdoor enclosure of the quarantined Asian female herd and the African female elephant herd is 40 feet where there is a road between the two enclosures. All of the elephant barns (5) are physically separated and rely on natural ventilation. The closest distance between the barns for the African and Asian female elephants is180 feet. Biosecurity protocols are in place for all barns (see Appendix 6) with separate tools used for the quarantine and non quarantine barns. Tools and footwear are disinfected after use in the quarantine barn areas.

Staff are trained by the facility manager on elephant care and handling as well as the biosecurity and health and safety precautions required as determined by the Medical Adviser (MD) for the facility in accordance with the local health authority. The current medical advice has been focused on preventing aerosol transmission risks for Mtb and, the risk of transmission of *M.tuberculosis* from elephants to humans by fomites and the oro-fecal route has not been considered significant by medical advisors to the facility. Expert opinion on the significance of oro-fecal or fomite transmission of Mtb in elephants remains divided (Anon, 2011c., Murphee *et al.*, 2011).

As part of routine health monitoring the PAWS veterinarian undertakes general screening for all of the elephants with trunk wash ('triple samples method') for bacterial culture and blood samples (STAT-PAK ® and if reactive, followed by MAPIA ™) done at least once a year for detection of tuberculosis. STAT-PAK ® reactive elephants are monitored more frequently. To date, none of the three African elephants have been reactive in any of the blood tests or positive on trunk wash. Two of the Asian female elephants have been reactive in STAT-PAK ® and MAPIA ™ blood tests (AsF2&3) and one of these animals tested positive in trunk wash cultures earlier this year (AsF3). One female Asian elephant (AsF1) remains negative in all tests. PAWS has separate enclosures for Asian bulls away from the female herds. One of the males (AsM2) was reactive in one of two blood tests (STAT-PAK ®) but the other male elephant remains

negative in both blood tests (AsM1). Full details of testing and test results are provided in Appendices 2 & 3 and summarized in Table 1.

Table (1) Overview of the PAWS elephants and test results

ID*	Age	Year of arrival	Additional information	Test results
African female elephants				
AfF1	32	1990	Currently in good condition	STAT-PAK ® unreactive
AfF2	32	2007		STAT-PAK ® unreactive
AfF3	46	2005		STAT-PAK ® unreactive
Asian female elephants				
AsF1	54	2005	The older females have arthritis and are managed to minimize joint problems	STAT-PAK ® unreactive
AsF2	45	2007		Reactive STAT-PAK ® & MAPIA ™
AsF3	52	1995		Reactive STAT-PAK ®, MAPIA ™ & Mtb isolated from trunk wash sample (TB), Mtb strain the same as that found in AsFX1
Asian male elephants				
AsM1	18	2007	Currently in good condition	STAT-PAK ® unreactive
AsM2	25	2011		Reactive STAT-PAK ®
Asian elephants – necropsy reports where tuberculosis confirmed**				
AsFX1	50	2001	Had been in contact with AsF3, AsMX2	Tuberculosis confirmed at necropsy, Mtb cultured,
AsMX2	30	2010	AsMX2 had a different strain of Mtb to AsFX1	Tuberculosis confirmed at necropsy – Mtb cultured

*In order to protect confidentiality elephants have been an ID rather than using their given names

**Necropsies are performed on all elephants that die or need to be euthanized at PAWS. To date, necropsies have been completed on seven elephants (1 African male, 1 Asian male and 5 female) by pathologists at the University of California Davis. Tuberculosis was only detected in the two animals listed in the table above, an Asian female (AsFX1) and an Asian male (AsMX2). A summary of necropsy results is given in Appendix 4.

Two elephants that were previously kept at PAWS and are now deceased had evidence of TB at necropsy. One was a female Asian elephant (AsFX1) who was housed with the current Asian female herd. The other was an Asian bull (AsMX2) that was housed alone and away from the females

AsFX1 was trunk washed annually from 2002-2010. All trunk washes were negative accept one dated 2/11/02. AsFX1 underwent treatment in line with the USDA endorsed guidelines. This elephant was reactive in both the STAT –PAK ® and MAPIA ™ tests prior to death and had extensive pulmonary lesions at the time of necropsy in November 2011. Polymerase chain reaction (PCR) and culture results confirmed the presence of Mtb.

AsMX2 was consistently trunk wash negative since his arrival at the PAWS facility in 2010 but he was reactive in the STAT-PAK ® and MAPIA ™ tests in 2011. This elephant was euthanized earlier this year and a necropsy was performed. Mtb was cultured from fresh lung tissue although the lung lesions were not the predominant necropsy finding.

Two strains of Mtb were isolated from the male elephant (AsMX2), and, on different occasions, from the female elephant (AsFX1), Genotyping was done at the USDA National Veterinary Services Laboratory, Ames, Iowa, using VNTR-24 and spoligotype and indicated that one strain isolated from AsMX2 did not match the strain found in the deceased female elephant (AsFX1). Although it is not uncommon to find more than one strain of Mtb in an infected host (Braden *et al.*, 2001) it isn't clear exactly when and where AsMX2 became infected with this strain. The strain found in AsFX1 appears to be the same as that isolated from the trunk wash sample of the current shedding elephant (AsF3), Given previous contact history and the origin of these elephants it is likely that all three became infected prior to arriving at PAWS but the NVSL report suggests that further infection may also have occurred during contact at the facility. However, this is not known. Trace back, identification of infected elephants and contact history are hard to determine in such cases and many facilities, including zoos that hold captive elephants have been faced with this challenge especially before the availability of screening tests such as the STAT-PAK ®. This is because the trunk wash culture lacks sensitivity as a diagnostic test and false negative results are common which made identification of elephants requiring quarantine difficult (Mikota & Maslow, 2011).

The elephants at PAWS have come from a number of different facilities across the USA and may have been exposed to tuberculosis from other elephants, other animal species, humans or environmental sources prior to arrival at the PAWS facility. This is a situation that is common to the many facilities, including many zoological gardens, currently holding elephants in North America (Anon 2011c).

4.1 Testing for tuberculosis

The rationale for the PAWS tuberculosis testing protocols is based on the USDA endorsed guidelines (2008) and the recommendations outlined in the 2010 revisions. The current PAWS veterinarian has considerable experience, with these tests, through her work at PAWS and as a veterinarian for the USDA, and works closely with her veterinary colleagues at the USDA to ensure that best practice is followed with regard to collecting and handling samples. A summary of samples collected and test results for all current PAWS elephants is provided in Appendix 2 & 3.

In summary:

-All elephants are tested annually by culture (using the "triple sample method.") and with the Elephant TB STAT-PAK ® Assay (a blood test).

-All samples required for official testing are collected under the supervision of a USDA, Animal Care employee and the sample(s) are sent to a certified laboratory.

-Elephants with a reactive Elephant TB STAT-PAK ® Assay results are tested using the confirmatory MultiAntigen Print ImmunoAssay (MAPIA ™).

See http://www.surecheck.com/pdfs/ElephantTBSellSheet02.pdf for information on the Elephant TB STAT-PAK R® Assay as well as Lyashchenko et al., 2006, Greenwald et al., 2009 for more information on both the STAT-PAK ® screening test and the MultiAntigen Print ImmunoAssay (MAPIA ™. The latter is recommended as a confirmatory test to the ElephantTB STAT-PAK ® Assay for detection of antibodies to *M. tuberculosis* and *M. bovis* in elephant sera or plasma

-More frequent testing is undertaken on elephants under quarantine i.e. those that have reactive blood test (STAT-PAK ®/MAPIA ™) results and/or are trunk wash positive.

In the guidelines it is recommended that positive cultures from laboratories that do not have the capability to differentiate *M. tuberculosis* complex organisms are forwarded to the USDA National Veterinary Services Laboratory (NVSL) for sequencing. Determination of both species and strain characteristics provides additional information which can be used to see if animals (and people) share the same strain and can help to trace the origin of the infection. However, although there is now a growing data base of Mtb strains isolated from elephants it isn't always easy to identify the origin of some strains many of which appear to be unique (Anon 2011c).

Aside from culture, which is still considered to be the 'gold standard' for identifying infected elephants there are now three available blood tests (STAT-PAK ®, MAPIA ™ and DPP ®) to determine whether or not elephants have been exposed to tuberculosis. The DPP (Dual Path Platform (DPP®) VetTB Assay) is a new generation screening kit for the rapid detection of IgG antibodies to *M. tuberculosis* or *M. bovis* in elephant serum, plasma, or whole blood and has shown 100% correlation with MAPIA ™ (Greenwald *et al.*, 2009). The MAPIA ™ and the DPP ® can be used as confirmatory tests for samples that are reactive in the STAT-PAK ® screening tests. As mentioned previously, the problem with trunk wash culture is that this method is insensitive and positive cases can be missed. The blood tests are useful to detect presumptive positive cases (Lyashchenko *et al.*, 2012) although a positive blood test does not confirm that the elephant is currently infected or shedding bacteria.

The USDA endorsed guidelines recognize that the interpretation of any test results can be complicated because of false positives and false negatives. It has been found that elephants can develop antibodies to mycobacterial antigens months to years prior to detection by culture, however, the time interval between exposure, seroconversion, and shedding of Mycobacteria is not known (Mikota & Maslow, 2011). Numerous variables such as age, genetics, nutritional condition, immune status, concurrent health problems (e.g. arthritis), and other factors influence the development of disease in an individual animal following exposure to a pathogenic agent (Anon, 2011a; Anon 2011b). The result of a second blood test i.e. MAPIA ™ testing STAT-PAK ® reactive samples is thought to be helpful in determining which elephants should remain under more frequent surveillance or undergo prophylactic antibiotic treatment. The guidelines suggest that elephants that are culture negative but ElephantTB STAT-PAK ® positive and MAPIA ™ positive should be considered to be at increased risk of having TB. Factors to consider in the decision to administer treatment rather than increased monitoring include exposure history, age, travel history, potential exposure of personnel or public, concurrent health problems, side effects of treatment, etc. Increased monitoring by culture ('triple sample method') is recommended every 2–3 months. The PAWS veterinarian has taken all of this into consideration in the development of the facility testing and quarantine protocols.

4.2 Management Groups for the control of Tuberculosis in elephants

As stated earlier, the PAWS facility has developed elephant testing and management protocols that adhere to, or exceed, the 2008 USDA elephant tuberculosis control guidelines http://www.aphis.usda.gov/animal_welfare/downloads/elephant/elephant_tb.pdf

Based upon the trunk wash culture results (for *M. tuberculosis complex*), and blood test (i.e. Elephant TB STAT-PAK ® & MAPIA ™) results, elephants fall into one of four management groups (1-4) or untested (group 5). A culture positive elephant is defined as an elephant from which *Mycobacterium tuberculosis* or *Mycobacterium bovis* has been isolated from any specimen collected. Any culture positive elephant is considered positive until it has met the treatment requirements as outlined in the USDA endorsed guidelines

Summary of the four management groups outlined in the guidelines (2008):

Group 1: Culture negative; ElephantTB STAT-PAK R® negative; no exposure to culture positive elephant in past 12 months.

In this group the elephants should be monitored annually by culture (triple sample method) and ElephantTB STAT-PAK ® (single serum sample collected concurrently). No treatment or travel restrictions are recommended, No elephant should move into a facility where there is an untested elephant. If an elephant has had exposure to other untested elephants in the previous 3 months, then a negative STAT-PAK® test should be repeated in 3 months time to confirm. If the ElephantTB STAT-PAK® remains negative, the elephant continues in Group 1.

Group 2: Culture negative; Elephant TB STAT-PAK R®negative; exposure to culture positive animal within the last 12 months.

Monitor by culture (triple sample method) and ElephantTB STAT-PAK ® every 3 months for one year post-exposure, then every 6 months for two years then annually thereafter if all cultures and ElephantTB STAT-PAK ® remain negative. No treatment or travel restrictions. If the results during any of the follow-up testing become positive, the individual elephant will change category. No elephant should move into a facility where there is an untested elephant.

Group 3: Culture negative; ElephantTB STAT-PAK R® positive

The USDA guidelines recommend that elephants are monitored by culture (triple sample method) every 3 months for the first year after becoming ElephantTB STAT-PAK ® reactive, then every 4 months for the next 3 years. If all cultures remain negative during this period, annual testing may resume. However, continuing more frequent testing by culture may be advisable. It is required that blood from elephants with positive ElephantTB STAT-PAK ® results be submitted for MAPIA ™ testing. No treatment or travel restrictions are required.

If, during any of the follow-up testing, the culture results become positive, the individual elephant will change category. No elephant should move into a facility where there is an untested elephant. Culture positive elephants that have completed a course of anti-tuberculosis therapy may remain ElephantTB STAT-PAK ® reactive and fall into this category. If culture results

during any of the follow-up testing become positive, the individual elephant will move to Category 4.

Group 4: *M. tuberculosis* complex positive culture

Animals that have had *Mycobacterium tuberculosis* complex isolated from any sample (trunk wash/sputum, stool, tissue, etc.) are considered to be culture positive for TB i.e.. 'A culture positive elephant is defined as an elephant from which *Mycobacterium tuberculosis* complex organism has been isolated from any body site or specimen'. A culture positive elephant is considered positive until it has met the treatment requirements as outlined below (see sections 9-11 in the 2008 guidelines for the control of tuberculosis in elephants for examples of treatment options).

The above management guidelines have been adopted by most zoological gardens and sanctuary facilities in North America as best practice. Currently the three female African elephants at PAWS and one of the Asian males (AsM1) would be in Group 1. The other Asian male (AsM2) would be in Group 3. The three Asian females are managed as one group. Due to the fact that one elephant has been trunk wash positive, the group is considered as one 'Biosecurity group.' However, because not all of the female Asian elephants in the group are STAT-PAK ® culture positive individual elephants can be classified in different Management groups under the guidelines (see Table 2). In effect, all of the Asian females are managed as one group but only AsF3 is shedding and currently considered to require treatment.

Table (2) Summary of PAWS most recent elephant test results and management grouping

Elephant	USDA Management Group #	Trunk wash result	STAT-PAK ® result	Frequency of testing	MAPIA TM ***	Comments
African females (managed as one group)						
AfF1	1	-ve **	Unreactive	Annual	Not required	
AfF2	1	-ve	Unreactive	Annual		
AfF3	1	-ve	Unreactive	Annual		
Asian females (managed as one group)						
AsF1	1 ##	-ve	Unreactive	3 monthly	Not required	Quarantined, consider all in same group
AsF2	3 (##)	-ve	Reactive		Reactive	
AsF3	4 (##)	+ve/-ve ###	Reactive		Reactive	
Asian males – managed separately						
AsM1	1	-ve	Unreactive	Annual	Not required	
AsM2	3	-ve	Reactive	3 monthly	Reactive	Quarantined

see above for description of management groups

All of these animals are considered as one 'biosecurity group' as all are managed together and exposure risk is considered higher for AsF1 and 2 who are 'in contact' with AsF3.

###Trunk wash positive in April sample/negative in most recent sample. Shedding can be intermittent therefore could be a false –ve.

** -ve = negative on 'triple' sample trunk wash culture after 8 weeks of culture

*** only required on STAT-PAK ® reactive samples

4.3 Veterinary care

The animals at the PAWS facility are under the care of an experienced veterinarian with experience in dealing with elephant tuberculosis through both her work with the USDA and at PAWS in helping validate the current blood screening tests for TB in elephants (Lyaschenko *et al.*, 2012). The attending veterinarian is responsible for the animals at both the Galt and ARK 2000 facilities and is routinely on location at the ARK facility two days a week. The day to day running of the facility is supervised by the facility manager who has over 16 year of experience with elephants. He is responsible for selecting and training staff. Elephants are checked regularly throughout the day and brought inside during the evening or in inclement weather. The purpose built elephant barns have been designed to facilitate elephant training for veterinary procedures such as blood sample collection general examination and medication using 'protected contact' methods. The preventative medicine program for the PAWS elephants is outlined below.

-Vaccinations- Tetanus toxoid every three years
-Fecal flotation and direct examination for parasites annually
-Routine blood work (CBC/Chemistry panel) at least annually with extra serum banked frozen
-Full examination and weight check at least annually
-Serum Vit. E annually
-Foot radiographs, urinalysis, leptospirosis titres and fecal cultures as appropriate
-Tuberculosis testing in compliance with USDA guidelines to include:
    a) Trunk wash ('triple sample method') at least annually
    b) STAT-PAK ® at least annually
Frequency of trunk washes and STAT-PAK ® testing is determined by each individual elephant's TB Management group classification as defined in the most current version of the USDA guidelines

At the current time the female Asian elephant (AsF3) that has a positive trunk wash culture is undergoing training to facilitate treatment for tuberculosis. There are a number of treatment options. The best option will be selected by the PAWS veterinarian with reference to the USDA endorsed guidelines and the health of the elephant. The subject of treatment for elephant tuberculosis remains highly contentious due to the difficulty in administering the correct does, variability in effective dose and potential side effects. There is also the concern about the potential to induce antibiotic resistance if effective doses are not maintained.(Mikota & Maslow, 2011., Anon 2011c)

There are also specific preventive medicine protocols for other species at both the PAWS Galt facility and the ARK 2000 facility including tuberculosis screening for primates and hoof stock. At the present time, tuberculosis has not been reported in species other than Asian elephants at the ARK 2000 facility. The ARK 2000 facility does not currently hold hoof stock or primates.

Necropsies are performed on all animals that die at the PAWS facility. Most necropsies are performed by the U.C. Davis pathologists either on site at PAWS or at the UC Davis Veterinary Teaching Hospital. Feral/Wild animals found dead on the PAWS property are examined by the attending veterinarian when appropriate. Necropsy reports for elephants that have died or been euthanized at PAWS are summarized in Appendix 4.

Necropsy reports from seven tigers that had died at the ARK 2000 site were also examined. The causes of death were varied and largely related to old age. Tuberculosis was not considered to be the cause of death in any of these animals and Mtb was not isolated at necropsy. At the time of writing this report, to the best of our knowledge, no bears or lions at the ARK 2000 facility have died.

4.4 Biosecurity & Biosafety

As recommended in the USDA endorsed guidelines the PAWS facility has developed a program to protect employees from TB exposure. This includes the use of appropriate face masks (e.g. N95) certified by the National Institute for Occupational Safety and Health to protect against TB), protective clothing, disinfection procedures, and the use of separate implements for infected animals. The guidelines suggest that facilities should base best practices for the safe conduct of work in the presence of Mtb on the guidelines outlined for biomedical and clinical laboratories and animal facilities in regards to *Mycobacterium tuberculosis* listed in the 5th Edition of Biosafety in Microbiological and Biomedical Laboratories published by the U.S. Department of Health and Human Services in 2007. http://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf accessed 15th November). An updated assessment of elephant to human Mtb transmission risks is discussed in Murphee *et al.*, (2001) in response to an incident in a facility with Mtb in an elephant in Tennessee.

The biosecurity guidelines developed by the PAWS veterinarian are presented in Appendix 6. These guidelines have been developed to prevent the transmission of tuberculosis from elephants in quarantine to non quarantined elephants, and other animals in the facility, and to protect human health. It was observed that separate tools and equipment are used for the quarantine and non-quarantine areas and that staff are required to use protective clothing in quarantine barns and foot baths and foot spray between barns. As part of the biosecurity protocol in the quarantine barns and the non-quarantine elephants barns there are foot baths containing a solution of disinfectant (TB Quat 3M). This product is recommended for use in facilities that might have Mtb in human patients http://solutions.3m.com/wps/portal/3M/en_US/Commercial/Care/Solutions-for/Infection-Control/3M-TB-Quat-Disinfectant-Cleaner-Ready-to-Use/

The efficacy of foot baths in killing Mtb and other pathogens can be negatively impacted by the presence of organic matter so regular replenishment of the disinfectant is required. There are rubber mats at each foot bath station to remove excessive organic material from the foot wear prior to stepping in the foot bath. The soles of foot wear are sprayed with additional bleach based disinfectant after using the foot bath. Designated coveralls for quarantine areas are not currently used at the facility.

The guidelines state that all employees that are in direct contact with elephants should be tested for TB annually following established human testing guidelines and that new employees should be tested prior to contact with elephants. At the PAWS facility staff are tested prior to employment and are monitored for TB in accordance with the local health authority under the advisement of the facility Medical Advisor (MD). To the best of our knowledge, there have not been any cases of TB reported in staff at the PAWS facility.

All elephant barns are cleaned daily using a standard hose and brushes. Separate equipment is used for each barn. Staff are required to wear fit tested N95 masks and disposable gloves when cleaning the quarantine barns. High pressure hoses are not used at this facility.

The flooring of the African barn which houses the African female elephants has a yielding artificial surface which is easy to clean. In the Asian barn, which houses the quarantined female Asian elephants,, there is an area of concrete flooring and also an area of dirt floor. The barns for the two Asian male elephants also have both concrete and dirt flooring. The flooring options in the Asian elephant barns were selected to maintain elephant foot health but do have the disadvantage of being difficult to disinfect. The current barns are spacious and have natural ventilation.

Fecal waste and contaminated biodegradable materials from the barns are taken to a site away from the animal enclosures to be composted. The compost piles are exposed to sunlight. This method of composting is used by other facilities although 'best practice' for waste disposal is not outlined specifically in the guidelines for the control of tuberculosis in elephants. Waste material from the quarantine barns is kept separate from material collected from the non quarantine barns. Staff wear protective clothing including masks when handling material from the quarantine barns and also if they are within 30 ft from either of the quarantine barns. Waste disposal should be done in accordance to regional authority guidelines and the PAWS facility veterinarian has considered this in the development of the PAWS protocols.

5.0 Risk Assessment

Risk is generally defined as the likelihood x consequence of an event occurring. Based on the OIE guidelines*, a standard approach to risk assessment based on http://www.oie.int/doc/ged/D6586.PDF 'volume one -a guide to qualitative risk assessment' is outlined in the steps below:

A) Hazard identification

The hazard(s) under consideration are Mycobacteria in the Mtb complex with specific reference to *Mycobacterium tuberculosis*. The 'at risk' population under consideration includes captive elephants currently at the PAWS sanctuary, San Andreas and elephants that might be introduced in future. The hazard (Mtb) is primarily transmitted from animal to animal by aerosol but other MTB complex organisms can also be transmitted by the oral-fecal route and by fomites (Montali *et al.*,2001). Infection can be transmitted within animal and human populations and can also be transmitted between species. A brief overview of what is currently known about *Mycobacterium tuberculosis* and MTB complex in animals is provided below.

Tuberculosis (TB) is a disease of global importance, and one-third of the world's human population is infected with *Mycobacterium tuberculosis* (Anon., WHO Report 2008., Anon, 2011a). Over the past decade there has been a growing awareness that *M.tuberculosis* is not restricted to the human population. There are numerous reports of *M.tuberculosis* in both domestic and wild animals (Michalak *et al.*, 1998., Ocepek *et al.*, 2005). In most cases it is thought that this reflects a 'spillover' of infection from the human population although there have been cases of zoonotic transmission from infected animals to humans reported (Whelan *et al.*, 2010). Within the 120 or more described species in the genus Mycobacterium is a small, closely

related group known as the *Mycobacterium tuberculosis* complex (MTB complex). This group includes *M.tuberculosis* along with *M.canettii, M.africanum* (clades 1 and 2), *M.pinnipedii, M. microti, M.bovis supsp. Caprae* and *M.bovis* (Smith *et al.,* 2009). This group of organisms shows greater than 99.95% sequence similarity at the nucleotide level. All members of the MTB complex appear to have a distinct host preference but although *M.tuberculosis* is generally associated with disease in humans this is not always the case and more recent studies have shown that *M.tuberculosis* is not uncommon in species kept in close proximity to human populations where the prevalence of TB is high (Ocepek *et al.,* Whelan *et al.*, 2010). As molecular techniques have become more widely available, mycobacteria such as the dassie bacillus, *M. mungi* and *M. orygis* are now suggested as members of MTB complex (Alexander 2002, Alexander *et al.,* 2010b). These additions followed the first reports of tuberculosis in free-ranging wild animal species like the cape hyrax (*Procavia capensis*), banded mongoose (*Mungos mungo*) and oryx (*Oryx lecoryx*) (Alexander 2002). Elephants in captivity are known to be susceptible to *Mycobacterium tuberculosis* (Mtb) and *M.bovis*. Studies have confirmed that there is a potential for transmission of TB (Mtb) between elephants, and humans (Michalak *et al.* 1998). Captive elephants are potentially exposed to Mtb through direct and indirect contact with infected members of public or their handlers as well as from other infected animals. In India, there are reports of TB in elephants in ancient Ayurvedic literature over 2000 years ago (Montali *et al.*, 2001). Although humans are considered to be the main source of infection in captive elephants in India and other elephant range countries where the prevalence of tuberculosis is high in the human population there is a lot that is unknown about the risk factors leading to infection in elephants (Abraham *et al.,* 2010a). In the past few decades, TB in elephants has emerged as a disease of concern in captive elephants in the United States (Mikota & Maslow, 2011). It is now estimated that over 12 per cent of Asian elephants in the United States are infected with TB (Murphee *et al.,* 2011) see also www.elephantcare.org

B) Release assessment

There is evidence that one elephant at the PAWS facility (AsF3) has been shedding *Mycobacterium tuberculosis.* Two other elephants have tested positive in the STAT-PAK ® TB screening test and one of these other elephants is also positive in the MAPIA ™ blood test. There is a history of contact between the shedding elephant and other Asian elephants at the PAWS facility. There is also a history of contact between the Asian elephant group at PAWS and one deceased elephant which had tuberculosis.

From review of the records provided it can be concluded that the hazard is present in at least one of the quarantined group of female elephants and that this could serve as a source of infection for other elephants, humans and the environment.

C) Exposure assessment

The likelihood that other elephants on, or introduced to, the facility are exposed to tuberculosis depends on whether or not there is contact between the infected and un infected elephants and also whether or not there is exposure to contaminated fomites, infected humans that are shedding Mycobacteria or other sources of Mycobacteria such as infected wildlife, environmental sources, food or water supplies.

There is potential for exposure of other elephants to the hazard especially those managed in the same group as the shedding female as well as humans handling the shedding elephant. However, this depends on the amount of Mycobacteria that are being shed, the frequency and

duration of contact, likelihood of aerosolization (i.e. use of high pressure hoses etc.) and persistence, facility design (i.e. sunlight and ventilation etc.) and management practices. (Anon, 2011c)

D) Consequence assessment

Exposure to Mycobacteria in the Mtb complex may or may not result in infection in humans or elephants. The outcome of exposure depends on the infective dose, transmission route, concurrent health problems, genetics, immune status, husbandry etc. The consequence of exposure to *Mycobacterium tuberculosis* (or other MTB complex organisms) might be no infection, active disease or latency (Murphee *et al.*, 2011., Anon, 2011c).

There is a lot that is still unknown about the pathogenesis and transmission of tuberculosis in elephants. However, taking the precautionary principle means that every effort should be made to mitigate any risks that are identified.

5.1 Risk mitigation measures in place

At the PAWS facility risk mitigation measures are in place to reduce the likelihood of elephant to elephant transmission of Mtb. This includes the quarantine of infected elephants and the close monitoring of elephants that are reactive in the elephant TB STAT-PAK ® screening test. Biosecurity protocols have been developed to mitigate the risk of spreading the hazard between barns by using separate equipment for each barn, staff training, use of disinfectants and use of protective clothing. Additional biosecurity measures such as the use of designated or disposable protective coveralls and boots for quarantine areas and/or restricting access to specific staff only might be recommended to further reduced the risk of hazard release and subsequent exposure risk. General guidelines for the prevention of the aerosol transmission of tuberculosis to humans are followed in accordance with local health authority guidelines and the USDA endorsed recommendations for preventing infection in humans. These guidelines also require testing of staff prior to working with elephants which largely mitigates the potential risk of infected humans coming into contact with the elephants. The general public is generally not allowed access to the facility therefore the risk of exposure to tuberculosis through that source is unlikely. Groups can tour the facility by appointment and all visitors and required to adhere to the biosecurity guidelines. Risks to and from other species and the environment are harder to assess but the location of the facility, the climate, low stocking rate and the free range design of the enclosures facilitates a good degree of separation between different groups of elephants. Most outbreaks of tuberculosis have been associated with the generation of infectious material in aerosol in indoor settings (Murphee *et al.*, 2011). This scenario is unlikely to occur at PAWS while current management practices and risk mitigation measures are in place. The presence of the 8 ft high perimeter fence and sturdy enclosure fencing precludes significant interaction between captive animals on the property and larger wildlife such as deer. Wildlife that dies on the property under goes necropsy by the facility veterinarian. To date, to the best of our knowledge, tuberculosis (*M.bovis* or *M.tuberculosis*) has not been reported in dead wildlife on the property. Waste from the quarantine barns is composted in the open and exposed to sunlight. The facility was inspected by the USDA in April this year and was considered compliant.

5.2 Risk Estimation

The presence of at least one elephant with active tuberculosis at the PAWS facility and a history of contact between several of the current Asian elephants and an elephant that recently died and was found to have tuberculosis indicates that the risk of current and new elephants being exposed to *Mycobacterium tuberculosis* is not negligible. In any setting where elephants develop tuberculosis it is hard to quantify the extent of the risk (Anon, 2011b,c, Murphee *et al.*, 2011). The infectious dose, rates and routes of shedding Mtb in infected elephants are likely to vary from case to case (Anon, 2011c). Transmission risk can be mitigated by separating infected and non-infected animals and implementing strict biosecurity and quarantine protocols. At the present time the facility management and the attending veterinarian are applying the USDA endorsed guidelines for the control of tuberculosis in elephants and have sought to implement recent revisions to the guidelines and as such are adhering to best practice.

Many factors need to be considered with regard to estimating the residual risk remaining after risk mitigation measures have been put in place. In this case it will depend, to some extent, on whether or not infected elephants begin to shed large numbers of Mtb and if Mtb is likely to be transmitted from elephant to elephant or to human handlers via routes other than aerosol. At the present time, given available evidence, this does not appear to be the case at the PAWS facility.

The likely consequences of exposure to Mtb are also hard to assess given the wide range of factors that determine whether or not an exposed elephant goes on to develop clinical disease (Anon 2011b). Risk is defined as the likelihood x the consequence of an event occurring. In this case it could be considered that the likelihood of exposure to Mtb is not negligible at PAWS but that the consequences are hard to predict. This would be the case at any facility holding, or likely to hold, infected elephants. The decision as to whether or not the level of risk is acceptable depends on a number of variables. Now that tuberculosis is established in a number of captive elephants and facilities in North America the determination of the acceptable level of risk would also need to take into account factors other than tuberculosis such as other potential costs or benefits of moving elephants to this or another facility.

6.0 Summary

PAWS ARK 2000 appears to be a well-managed animal sanctuary with a high standard of elephant care. The elephants observed during the visit, including the quarantined group and the intermittently shedding female Asian elephant (AsF3) appeared to be in good health and had access to large outdoor enclosures and spacious naturally ventilated barns. Biosecurity and quarantine protocols have been implemented. The facility manager and veterinarian have experience in dealing with elephants and the facility veterinarian has considerable experience with the test protocols used to detect and monitor tuberculosis in elephants. The testing and management protocols used at the PAWS facility are based on the current USDA endorsed guidelines for the control of tuberculosis in elephants. The facility veterinarian keeps comprehensive health records for the elephants under her care. PAWS ARK 2000 was considered USDA compliant at the last inspection in April 2012 and is accredited by the Global Federation of Animal Sanctuaries.

Due to the complexity of tuberculosis control in elephants and the need for more information on disease transmission and transmission risk, it is likely that elephant TB control guidelines will continue to evolve in response to new findings. However, for the current time, the USDA endorsed guidelines for the control of tuberculosis in elephants are considered to be best

practice for any facility holding elephants in North America. The PAWS veterinarian has made every effort to follow the guidelines and has explored options to exceed the guidelines. There are some additional biosecurity measures that could be considered in the event that elephants in the quarantine group begin to shed a significant number of Mtb organisms and if transmission of Mtb by fomites or the oro-fecal route was considered likely. At the present time, there is no evidence to suggest that this is the case at PAWS. The last trunk wash cultures from AsF3 were negative. In any setting where elephants develop tuberculosis it is hard to quantify the transmission risk or to predict the consequences of exposure (Murphee *et al.*,2011., Anon, 2011c).. Now that tuberculosis is established in a number of captive elephants and facilities in North America, and given the scientific uncertainty with regard to infectious dose, host susceptibility and disease transmission pathways for Mtb in elephants, a determination of the acceptable level of risk would also need to take into account factors other than tuberculosis such as other potential costs or benefits of moving elephants to this or another facility.

Acknowledgments

The following experts were contacted during the preparation of this report. We would like to acknowledge their contributions but note that the final conclusions represent those of the primary authors.

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Appendix 1. DEFINITIONS (derived from the USDA endorsed guidelines for the control of tuberculosis in elephants, 2008/2010)

Aerosol transmission: Aerosol transmission occurs when pathogens travel through the air to enter a host. Aerosols may be large droplets that are deposited on the mucous membranes or smaller particles that are inhaled. For most pathogens transmitted by this route, specific data defining risk of infection are limited; in general, risk of aerosol transmission increases with proximity to the source and duration of exposure. Aerosols can contain environmentally persistent pathogens that serve as a source for indirect contact transmission (NASPHV, 2010).

Attending veterinarian: A person that is licensed in the state in which they practice and has graduated from a veterinary school accredited by the American Veterinary Medical Association's Council on Education, or has a certificate issued by the American Veterinary Medical Association's Council on Education Commission for Foreign Veterinary Graduates; has received training and/or experience in the care and management of the species being attended; and who has direct or delegated authority for activities involving animals at a facility subject to the jurisdiction of the Secretary (i.e. a USDA licensed facility).

Culture positive (*M.tb* complex) elephant: An elephant from which a *M. tuberculosis* complex organism has been isolated from any bodily specimen. A culture positive elephant is considered positive until it has met the treatment requirements as outlined in the current Guidelines.

Disease: A disordered or incorrectly functioning organ, part, structure, or system of the body resulting from the effect of infection; any abnormal condition that interferes with its vital physiological processes, caused by pathogenic microorganisms.

Dual Path Platform (DPP®) VetTB Assay: A new generation screening kit for the rapid detection of IgG antibodies to *M. tuberculosis* or *M. bovis* in elephant serum, plasma, or whole blood. The DPP® has shown 100% correlation with MAPIA™ (Greenwald et al. 2009).

ElephantTB STAT-PAK® Assay: A qualitative screening kit for the detection of antibodies to *M. tuberculosis* and *M. bovis* in elephant sera, plasma, or whole blood (Lyashchenko 2005, 2006, Greenwald 2009).

Exposure to *Mycobacterium tuberculosis* complex – Any situation in which an individual is in direct or indirect contact with *Mycobacterium tuberculosis* complex organisms, or an *M. tuberculosis*-infected animal (e.g., *M. tuberculosis* infected elephant, human, or other animal).

Fomite: An inanimate object or material on which infection-producing agents may be conveyed.

Genotyping assay: A technique for the identification and analysis of polymorphism in certain types of repeat units in DNA. Restriction fragment length polymorphism (RFLP) and variable number tandem repeat (VNTR) are examples of genotyping techniques.

Infected elephant: an elephant from which *Mycobacterium tuberculosis* complex has been identified through culture, polymerase chain reaction (PCR) or other molecular techniques.

Infection: Invasion and multiplication of microorganisms in body tissues, causing local cellular injury.

Intradermal tuberculin test (skin test): The injection of purified protein derivative (PPD) tuberculin into the skin for the purpose of detecting exposure to tuberculosis. In cattle, the test site is either the caudal fold (CFT) or cervical region (e.g. comparative cervical test, CCT) and the test is read by observation and palpation at 72 hours (plus or minus 6 hours) following injection. In humans, the test site is the forearm and the test is read at 48-72 hours. The intradermal tuberculin test is not a reliable test in elephants (Mikota 2001, Lewerin 2005).

MultiAntigen Print ImmunoAssay (MAPIA™): A confirmatory test to the ElephantTB STAT-PAK® Assay for detection of antibodies to *M. tuberculosis* and *M. bovis* in elephant sera or plasma (Lyashchenko 2000, 2006, Greenwald 2009).

Mycobacterium bovis (M. bovis): The primary causative agent of tuberculosis in cattle, bison, and cervids; may also affect a variety of mammals including pigs, humans, primates, and non-domestic ungulates.

Mycobacterium tuberculosis (M.tb): The primary causative agent of tuberculosis in humans; may also affect a variety of animals, including primates, pigs, cattle, dogs, parrots, elephants, and rhinos.

***Mycobacterium tuberculosis* complex (*MTB* complex):** A group of mycobacteria which includes *M. tuberculosis, M. bovis, M. africanum, M. microti, M. canetti, M. caprae, and M. pinnipedii.* A vaccine strain derived from *M. bovis* (*M. bovis BCG*) is sometimes listed as a separate member of this complex.

No isolation: Absence of growth of *M. tb* complex organisms from trunk wash, feces, tissue or other samples using standard mycobacterial culture methods. Failure to isolate organisms may be due to the following reasons:

1. The animal is not infected
2. The animal was not shedding at the time of sample collection
3. Sampling error (culture overgrowth by contaminating organisms, inadequate sample, or laboratory error)
4. Improperly handled or shipped sample

Non-reactive: Absence of response; in the context of serological testing for TB in elephants, a non-reactive result indicates that an antigen-antibody reaction has not occurred in the presence of an appropriate positive control response.

Non-tuberculous mycobacteria (NTM): Mycobacteria that generally do not cause the formation of granulomas. Most NTM are saprophytes found in soil or water. They are typically non-pathogenic but may occasionally cause infection in humans and animals, including elephants. Also referred to as "atypical" mycobacteria or "Mycobacteria Other Than TB" (MOTT).

Reactive: Presence of response; in the context of serological testing for TB in elephants, a reactive result indicates that an antigen-antibody reaction has occurred.

Spoligotyping: A genotyping assay, see Gori et al., 2005 Emerging infectious diseases 11 (8), 1242-1248. http://wwwnc.cdc.gov/eid/article/11/8/pdfs/04-0982.pdf

Variable number of tandem repeats (VNTR): A genotyping assay see Savine et al., (2002) Journal of Clinical Microbiology. 40(12),4561-4566
http://www.ncbi.nlm.nih.gov/pubmed/12454152

Triple sample method: A method of culture collection whereby 3 samples are obtained on separate days.
Trunk wash: A procedure used in elephants to obtain a sputum sample using one of the approved methods outlined in Section 4 – Culture Collection Procedure.in the 2008 USDA endorsed guidelines for the control of tuberculosis in elephants.
http://www.aphis.usda.gov/animal_welfare/downloads/elephant/elephant_tb.pdf

Sensitivity (Diagnostic – Dse) : A measure of the ability of a test to identify infected animals. Sensitivity is the frequency of a positive or abnormal test result (e.g. a test that is outside of the reference interval) when infection is present (i.e. the percentage of true positive results). Sensitivity = [TP + (TP + FN)] X 100 where TP = true positive; FN = false-negative).

Specificity: (Diagnostic – Dsp) A measure of the ability of a test to identify non-infected animals. Specificity is the frequency of a negative or "normal" test result when infection is absent (i.e. the percentage of true-negative (TN) test results. Specificity = [TN ÷ (TN + FP)] X 100.
Untested elephant: An elephant is considered "untested" if it has not had three trunk washes obtained by the method outlined in this protocol within a 12 month period; or if fewer than three valid culture results are obtained; if it has not been tested with the ElephantTB STAT-PAK® Assay performed by a USDA-employed veterinarian trained and certified to perform the test; or a reactive Stat-Pak test that has not been followed by a MAPIA ™

Appendix 2A: Summary of trunk wash cultures* of *currently alive* elephants at PAWS sanctuary (Pen=Pending; Neg=Negativ

AFF1		AFF2		AFF3		ASF1		ASF3		ASF2		
Date of TW Collection	Result	Date of TW Collection	Result	Date of TW Collection	Result	Date of TW Collection	Result	Date of TW Collection	Result	Date of TW Collection	Result	Dat Col
2012-02-09	Pen	2012-02-08	Pen	2012-02-08	Pen	2012-04-21	Pen	2012-04-22	Pen	2012-04-21	Pen	2012
2012-02-08	Pen	2012-02-07	Pen	2012-02-07	Pen	2012-04-17	Pen	2012-04-17	Pos	2012-04-17	Pen	2012
2012-02-07	Pen	2012-02-06	Pen	2012-02-06	Pen	2012-04-16	Pen	2012-04-16	Pen	2012-04-16	Pen	2012
										2012-02-08	Neg	
										2012-02-07	Neg	
										2012-02-06	Neg	
2011-02-20	Neg	2011-02-20	Neg	2011-02-20	Neg	2011-12-04	Neg	2011-12-04	Neg	2011-12-04	Neg	2011
2011-02-18	Neg	2011-02-16	Neg	2011-02-18	Neg	2011-12-03	Neg	2011-12-03	Neg	2011-12-03	Neg	2011
2011-02-16	Neg	2011-02-13	Neg	2011-02-16	Neg	2011-12-02	Neg	2011-12-02	Neg	2011-12-02	Neg	2011
						2011-09-11	Neg	2011-09-11	Neg	2011-09-11	Neg	2011
						2011-09-10	Neg	2011-09-10	Neg	2011-09-10	Neg	2011
						2011-09-05	Neg	2011-09-05	Neg	2011-09-05	Neg	2011
						2011-07-04	Neg	2011-07-04	Neg	2011-07-04	Neg	2011
						2011-06-30	Neg	2011-06-30	Neg	2011-06-30	Neg	2011
						2011-06-29	Neg	2011-06-29	Neg	2011-06-29	Neg	2011
										2011-06-21	Neg	
										2011-06-20	Neg	
						2011-05-01	Neg	2011-05-01	Neg	2011-05-01	Neg	
						2011-04-27	Neg	2011-04-27	Neg	2011-04-27	Neg	
						2011-04-26	Neg	2011-04-26	Neg	2011-04-26	Neg	
						2011-02-23	Neg	2011-02-23	Neg	2011-02-23	Neg	
						2011-02-22	Neg	2011-02-22	Neg	2011-02-22	Neg	
						2011-02-21	Neg	2011-02-21	Neg	2011-02-21	Neg	
2010-05-27	Neg	2010-05-27	Neg	2010-05-27	Neg	2010-05-27	Neg	2010-05-27	Neg	2010-05-27	Neg	
2010-05-24	Neg	2010-05-20	Neg	2010-05-20	Neg	2010-05-20	Neg	2010-05-20	Neg	2010-05-20	Neg	
2010-05-19	Neg	2010-05-19	Neg	2010-05-19	Neg	2010-05-19	Neg	2010-05-19	Neg	2010-05-19	Neg	
2009	Neg	2009	Neg	2009-01-30	Neg	2009	Neg	2009-02-12	Neg	2009-08-05	Neg	
2009	Neg	2009	Neg	2009-01-29	Neg	2009	Neg	2009-02-11	Neg	2009-08-04	Neg	
2009	Neg	2009	Neg	2009-01-27	Neg	2009	Neg	2009-02-10	Neg	2009-08-01	Neg	
2008-07-31	Neg	2008-06-10	Neg	2008	Neg	2008-06-07	Neg	2008	Neg	2008-08-05	Neg	
2008-07-30	Neg	2008-06-07	Neg	2008	Neg	2008-06-10	Neg	2008	Neg	2008-08-04	Neg	
2008-07-25	Neg	2008-06-06	Neg	2008	Neg	2008-06-11	Neg	2008	Neg	2008-08-01	Neg	
										2008-03-19	Neg	
										2008-03-15	Neg	
										2008-03-15	Neg	
										2008-05-04	Neg	

												2008-05-03	Neg
												2008-05-01	Neg
2007-05-14	Neg		2007-05-14	Neg	2007-05-14	Neg	2007-05-14	Neg				2007-11-16	Neg
2007-05-13	Neg		2007-05-13	Neg	2007-05-13	Neg	2007-05-13	Neg				2007-11-15	Neg
2007-05-10	Neg		2007-05-10	Neg	2007-05-10	Neg	2007-05-10	Neg				2007-11-14	Neg
												2007-09-14	Neg
												2007-09-13	Neg
												2007-09-12	Neg
												2007-07-27	Neg
												2007-07-26	Neg
												2007-07-25	Neg
												2007-05-01	Neg
												2007-04-30	Neg
												2007-04-29	Neg
												2007-04-20	Neg
2006-03-13	Neg		2006-03-13	Neg	2006-03-13	Neg	2006-03-13	Neg					
2006-03-09	Neg		2006-03-09	Neg	2006-03-09	Neg	2006-03-09	Neg					
2006-03-08	Neg		2006-03-08	Neg	2006-03-08	Neg	2006-03-08	Neg					

* Trunk wash culture results mentioned as positive/negative for any *Mycobacterium tuberculosis* complex pathogen only; Not for atypical mycobacteria

Appendix 2B: Summary of trunk wash cultures* of *previously dead* elephants at PAWS sanctuary

ASFX1		ASMX2		AsFX3		AFFX4		ASFX5		ASFX6		A
Date of TW Collection	Result	Date of TW Collection	Result	Date of TW Collection	Result	Date of TW Collection	Result	Date of TW Collection	Result	Date of TW Collection	Result	Date of Collect
2002	Pos. Swab	Prior to death, all TW cultures were negative		Prior to death, all TW cultures were negative		Prior to death, all TW cultures were negative		Prior to death, all TW cultures were negative		Prior to death, all TW cultures were negative		Prior t TW cul ne

* Trunk wash culture results mentioned as positive/negative for any *Mycobacterium tuberculosis* complex pathogen only; Not for atypical mycobacteria

Appendix 3A: Summary of Elephant TB STAT-PAK®(S) and MAPIA®(M) testing of *currently alive* elephants at PAWS sanctuary

AFF1			AFF2			AFF3			ASF1			ASF3			ASF2			
Date	S	M	Date	S	M	Date	S	M	Date	S	M	Date	S	M	Date	S	M	D
2012-06-20	NR	--	2012-06-20	NR	--	2012-06-20	NR	--	2012-06-20	NR	--	2012-06-20	R	R	2012-06-20	R	R	20 06
2011-06-27	NR	--	2011-06-27	NR	--	2011-06-28	NR	--	2011-06-27	NR	--	2011-06-27	R	R	2011-06-27	R	R	
2010-05-20	NR	--	2010-05-20	NR	--	2010-05-20	NR	--	2010-05-20	NR	--	Not Done			2010-05-20	R	NR	20 12

Appendix 3B: Summary of Elephant TB STAT-PAK®(S) and MAPIA®(M) testing of *previously dead* elephants at PAWS sanctua

ASFX1			ASMX2			AsFX3			AFFX4			ASFX5			ASFX6			
Date	*S*	*M*	*Date*	*S*	*M*	*Date*	*S*	*M*	*Date*	*S*	*M*	*Date*	*S*	*M*	*Date*	*S*	*M*	*D*
2010-12-10	R	R	2011-06-27	R	R	Death before the 2010 USDA testing requirements			2010-05-19	NR	--	Death before the 2010 USDA testing requirements			Death before the 2010 USDA testing requirements			2(

Appendix 4: Details of post-mortem examination of elephants conducted at the PAWS sanctuary

Elephant ID	Year of Death	Gross Nodules in Any Organ	*M. tuberculosis* Isolation from Nodule Culture
ASFX1	2010	Yes, in lungs	Yes
ASMX2	2012	Yes, in lungs	Yes
AsFX3	2008	No	--
AFFX4	2011	No	--
ASFX5	2009	No	--
ASFX6	2008	No	--
ASFX7	2005	No	--

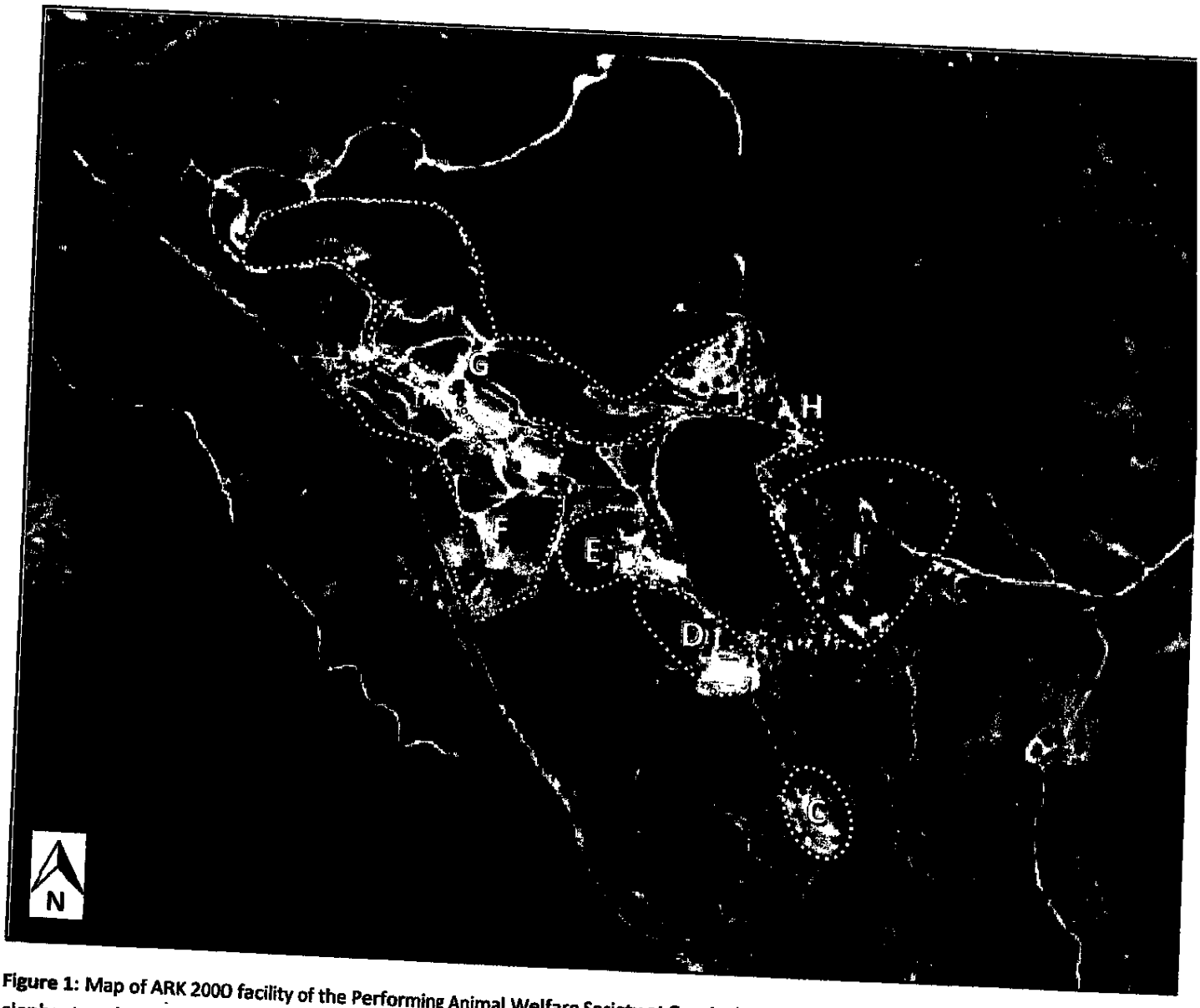

Figure 1: Map of ARK 2000 facility of the Performing Animal Welfare Society at San Andreas, California [A-Main entrance; B-Main office; C-V elephant enclosures and barns; F-Asian female elephant enclosures and barn; G-African female elephant enclosure and barn; H-Veterinariar enclosures] *Courtesy: Google Maps*