

Guidelines for TB management in captive elephants in the European Elephant TAG

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Introduction

Tuberculosis (TB) is caused by bacteria in the genus *Mycobacterium*. Mycobacteria infect a broad range of species (mammals, birds, reptiles, amphibians and fish). Species susceptibility to specific Mycobacteria varies. In mammals, the term “tuberculosis” is used to define disease caused by *Mycobacterium tuberculosis*-complex organisms (*M. tb-complex*), which include *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. canetti*, *M. caprae*, *M. pinnipedii*, *M. orygis* and *M. mungi*. *M. tuberculosis*-complex has a zoonotic potential to elephant keepers, other zoo staff in contact with elephants and zoo visitors.

Mycobacterium tuberculosis is the predominant infection-causing agent in elephants although cases caused by *M. bovis* have occurred. *Mycobacterium szulgai*, an uncommon non-tuberculous Mycobacterium species, was associated with fatal disease in two African elephants and *Mycobacterium elephantis*, a rapidly growing Mycobacterium, was isolated from a lung abscess of an elephant that died of chronic respiratory disease. *Mycobacterium avium* is commonly isolated from elephants and is not generally associated with disease although a single fatal case has been reported (e.g. *Mycobacterium avium* ssp. *hominissuis* in an African elephant).

TB testing in elephants is a concern for all elephant keeping institutions. In Europe, no validated immunological tests for elephants, approved by EU-authorities are available at this time. However, veterinary authorities may request elephants to be tested on TB when they are moved to other countries. Zoos that receive elephants must be well aware of the risk of the import of TB in their collection. This makes it very important to build up a well documented history of elephant herds, including regular testing of elephants and monitoring other animals and personnel.

In the recommendations of the European Association of Zoo and Wildlife Veterinarians (EAZWV) for the approval of zoos according to the EU Directive 92/65, the trunk wash procedure for regular testing on tuberculosis (TB) has been discussed. In the final recommendations, a more general text was chosen (see point C.5.h.): “Specific guidelines for the systematic testing of specific animal species may be developed and recommended by the Infectious Diseases Working Group of EAZWV”. This means, that at this time, no official guidelines for TB-testing of elephants are described under the BALAI directive.

However, this does not mean that zoos keeping elephants should refrain from establishing a routine testing protocol. The risk of introducing TB in a zoo through elephants is quite realistic as we can see from cases in the past decades both in the USA as well as in Europe. This document does not touch the issue of introduction of TB through other animal species and human contact, but should leave no doubts that a complete TB-surveillance is the only way to reduce the risks of TB in a zoo.

Every national or regional government may have its own interpretation about how to deal with suspected TB-cases. Much depends on the relationship between the zoo veterinarian and the official veterinary authorities. Generally, it helps if the zoo has a clear policy regarding its health surveillance system. Zoos that make all efforts to be certified according to the EU Directive 92/65 should have the intrinsic desire to stay free of infectious diseases like TB.

This document describes methods that can be used to collect information about the TB-status of individual elephants and an elephant herd. This document can only help to convince institutions that keep elephants to use all means available to minimize the risk of contracting and spreading tuberculosis in their elephants and personnel. Finally, it may help decision making in case of a

planned elephant transfer; the reliability of a “TB-history report” depends a priori on the amount of data collected over the years, not only of a single test taken “on the day of transport”.

Tools for TB-diagnosis in *live* elephants

A. Bacteriological tests (culture and PCR of *M. tb* complex):

A positive culture and/or PCR confirms the presence of TB. Any positive culture should be confirmed by DNA-sequencing to trace the origin of the infection and exclude sample contamination at the lab. A negative result does not exclude infection and can implicate either: a truly negative animal, a TB-positive animal, but not yet shedding (closed TB, latency), or a TB-positive animal, but shedding low numbers of bacteria (open TB).

Samples to be taken from elephants for culture and PCR can be obtained by one of the following methods:

Trunk wash (TW):

The trunk wash procedure is an active manipulation at the elephant trunk, which can be performed in free and protected contact systems in non-immobilized elephants after they are conditioned for this procedure. The principle is that a sterile 0,9% saline solution (approx. 100 ml) is injected in each nostril of the trunk. The trunk has to be lifted actively by the elephant or passively by the keeper so that the solution is running up to the base of the trunk. The mixture of the solution and trunk mucus is collected in sterile plastic bags by active blowing of the elephant through its trunk (training required). The staff should protect themselves against spilling trunk content into their face. A full trunk wash procedure requires 3 different trunk washes performed within a period of 7 days. Each sample must be sealed and stored at 4°C. Depending on the quality of the samples, the diagnostic lab can decide to pool the samples for culture/PCR. Samples must be shipped to the TB-diagnostic lab immediately after the 3-rd sample has been taken. The maximum storage period at 4°C is 7 days. NB: follow the EU guideline for shipment of potentially hazardous biomaterials. Trunk wash in a non-contact situation requires a full anesthesia of the elephant and a portable fluid pump and sucking system, which allows the operation under sterile condition. The external pump and sucking system will be connected to a sterile PVC tube (1 cm diameter, with distance markers) with a length of approx. 2 meter. The amount of sterile solution and the collection bag are like described before. In non-contact situations, a bronchoalveolar lavage (BAL) under standing sedation is the preferred procedure (see below).

Bronchoalveolar lavage:

The BAL can be performed under standing sedation. Two methods for approaching the deeper bronchi are being practiced currently.

- (1) The BAL can be performed through the trunk. In addition to the sedation, a local block anesthesia in the trunk base is required in order to get relaxation of the cartilage “valves” present in the trunk base. This procedure requires a 5 m flexible endoscope.
- (2) Another approach for the BAL is through the mouth. A mouth gag is required to protect the arm of the veterinarian who carries out the procedure. A 3.5 m long flexible endoscope can be advanced deep into the trachea, guided by the finger tips of the operator.

BAL allows visualization of the major bronchi. Different samples are taken by injecting 100-150 ml sterile 0,9% saline solution in different bronchi through a disposable catheter. Fluid is recovered by using a suction pump or 60 ml syringes. A typical sample contains watery fluid with some mucous material and air bubbles. The oral approach can also be used for

obtaining a gastric lavage sample by advancing a flexible tube into the esophagus without using an endoscope). Such an additional sample increases the chance to detect mycobacteria originating from swallowed sputum and can also be used for culture and PCR.

Culture of suspected material should be performed at the National Veterinary Laboratory of the EU-member state. A final test result for culture may take up to 4 months. PCR-results are usually obtained within a few days. PCR should always be combined with culture.

B. Immunological tests

A positive immunological test confirms a prior contact of the animal's immune system with mycobacterial antigens. This may indicate either a TB-positive animal with active infection, a prior contact with TB, which lead to sterile immunity (no TB infection present), or a false-positive reaction due to contact with closely related non-pathogenic mycobacteria.

A negative result does not exclude infection and can implicate either: a truly negative animal, a TB-positive animal in which an immune response has not (yet) developed (closed TB, latency), or a TB-positive animal with advanced stage anergy (immunological non-responsiveness) (late stage clinical TB).

Immunological tests highly depend on the quality of the antigens used to read out the immunological reaction.

Tests to measure cell-mediated immune responses to *M. tb* complex:

- **Interferon-gamma (IFN- γ) test:** developed at the Veterinary Faculty in Utrecht. Like other diagnostic immunological TB-tests for elephants this test cannot be validated properly, because it is not possible to get enough samples from proven TB-positive and it is impossible to identify live proven TB-negative elephants. After stimulation of leucocytes with positive and negative controls, PPD-B, PPD-A and MTB-Complex specific recombinant antigens, the in vitro production of IFN- γ is measured using an elephant IFN- γ specific ELISA. Heparinized blood has to be delivered at the lab within 8 hours after collection. Cross reaction with antigens from related Mycobacterium spp. may cause a "false" positive test result. The interpretation of this promising test is still very difficult, but it is considered to contribute to the understanding of the immunological reaction of the elephant. At this moment the test can only be performed after making arrangements with the Div of Immunology, Dept of Inf Dis and Immunology, Fac of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands (Please contact Prof. Victor Rutten, v.rutten@uu.nl). If the time for transporting the sample to Utrecht (NL) exceeds 8 hours, in consultation with Victor Rutten a regional TB-diagnostic lab can be approached to perform the first step in preparing the blood sample for conservation during prolonged transport.
- **Comparative skin test:** this "classical" test uses PPD-derived M.bovis-tuberculin and PPD-derived M.avium-tuberculin that is obtained from the National Veterinary Institute in each EU-country. Due to the special skin properties of elephants the use of the comparative skin test in this species has no diagnostic value. There is some evidence, that repetitive skin tests can booster the immune response in TB-positive animals. To measure this booster-effect a heparin blood sample must be taken 2-3 weeks after tuberculation (cells to be used for the IFN- γ , plasma for antibodies). However, more data are needed to confirm this phenomenon.

Tests to measure humoral immunity against *M.tb* complex:

In the past years several tests have been recommended and used, but the diagnostic value of measuring antibodies for tuberculosis in elephants using those tests has proven to be questionable. Several **non-validated ELISA's** have been routinely available at the Central Veterinary Institute Lelystad (Netherlands). Antigens that were used: M.bovis crude antigens, M.avium crude antigens, and recombinant MPB70. However, in spring 2018 (after a validation test of the ELISA's performed in

284 elephant samples) the CVI-Lelystad announced that the routine testing has been discontinued, as the sensitivity of the test is too low to use it as a predictive test for individual elephants. For specific situations (i.g. for screening a TB-confirmed herd), CVI-Lelystad is developing new ELISA's. (Address: Wageningen Bioveterinary Research, Houtribweg 39, 8221 RA Lelystad, the Netherlands).

The formerly used Elephant TB STAT-PAK Assay and MAPIA (Chembio, USA) are no longer available for Europe. TB STAT-PAK has been replaced by the **Elephant DPP-VetTB test**; this test has been used in the past years (USA and Europe) and has been subject of many debates because of the high number of (probably) false-positive results in assumed negative elephants and negative results in confirmed positive elephants. The availability of this test in Europe has been and still is unpredictable. At this moment the vet advisors of the European Elephant TAG do not recommend its use on a routine basis as interpretation of the high number of (possibly) false-positive results caused a lot of confusion and conflict with veterinarian authorities and participants of the breeding programs formerly.

Monitoring the TB status of elephants in zoos

The recommended way to monitor the TB status in individual elephants and in the elephant herd is based on:

1. History of TB in that particular zoo, including other mammalian species.
2. Annual trunk washes or tracheal washes performed in all elephants of 4 years and older followed by culture and PCR. This procedure includes 3 trunk washes performed in one week (non-sedated animal) or 1 bronchoalveolar lavage (standing sedation). Each trunk wash sample must be submitted as an individual sample for culture and PCR.
3. Full necropsy of every dead elephant.

The EEP-coordinator sends out an annual survey in which culture results should be provided.

Pre-transport testing

When an elephant will move to another institution, the EEP-coordinator shall contact the exporting institution in order to collect relevant data about the history of the animal(s) and to make arrangements for the required diagnostic procedures.

1. **A report about the history** of the animal(s) that will be moved must be provided, including the following data:
 - Species / ID / date of birth / location of birth
 - Locations where the animal has been kept during its entire life, including dates of entry, known history of TB cases at these locations (endemic areas e.g.)
 - Has the animal previously been suspected of tuberculosis or treated for this disease?
 - Has there been any known direct or indirect contact with confirmed or suspected TB cases in herd mates or other mammalian species, including humans?
 - List of data when blood was sampled, tested and stored at below -20°C
 - All results of TB-tests performed in the animal and in the herd in the past 5 years
 - Does the animal show clinical signs that are suggestive for tuberculosis?
2. **TB testing:** Each elephant that will be moved to another institution must be subjected to 3 trunk washes performed in 1 week or 1 bronchoalveolar lavage (BAL), 4 months* prior to transport (culture and PCR). At the same time, an Interferon-gamma test (IGRA) must be performed. Subsequently, the culture and PCR procedure must be repeated 2 months* prior

to transport. Each trunk wash sample must be submitted as an individual sample for culture and PCR.

NB: BAL should always replace the trunk wash in case of:

- a. Unexplained chronic weight loss
- b. Any positive result from a serological test (IGRA and/or DPP)

** The standard culture period for Mycobacterium sp. may differ per country. If the reference lab in the country of the sender requires a culture period longer than 2 months, the culture and PCR-procedures should be performed with a 2 months interval, scheduled in such a way that the results of the last procedure are received not more than 2 weeks prior to the expected transport.*

The animal history and the TB-test results are communicated to the EEP-coordinator, who in turn sends the results to the veterinary advisors of the European Elephant TAG. In case of doubt, the veterinary advisors will consult specialists in the field and provide a final decision about the transport.

Mtb-complex exposure Risk Categories for Elephants

Elephants are placed into one of four groups depending on their risk of exposing the environment with Mtb-complex. Testing requirements for elephants vary according to what risk group they belong to. Note that these groups are determined by two factors: herd history and a positive trunk wash/BAL culture, but not primarily by immunological test results. The veterinary advisor for the TAG can recommend increasing the amount and type of testing for any elephant based on the concerns and history of the animals involved.

Category A elephants:

Risk of infection with Mtb-complex: Low

Known exposure to an Mtb-complex culture/PCR-positive animal: None within the past five years.

Test history: Negative for past five years (or shorter in animals under the age of 8 years) on annual triple TW testing/BAL by culture/PCR.

Recommended testing: Routine. Triple TW culture technique or single BAL done minimally annually.

Travel restrictions: None.

Category B elephants:

Risk of infection with Mtb complex: doubtful

Known exposure to an Mtb-complex culture/PCR-positive animal: Exposure to a Mtb culture-positive elephant, keeper or another mammal has occurred within the past five years.

Test history: negative test results of the previous 5 years have no consequences for the actual status.

Recommended testing: single BAL and IGRA should be performed immediately after confirmed TB (Mtb-complex) in a herd member, a keeper or another mammal. This is followed by quarterly triple TW for one year. After 12 month a single BAL and IGRA should be performed. If all culture/PCR tests remain negative, these elephants return to Category A status. If culture/PCR are positive (including sequencing of the Mycobacterium sp.), these elephants go to Category C. All test results should be communicated with the vet advisor-group.

According to the guidelines of the national authorities regarding confirmed TB-infection: proper hygiene measures must be implemented, including medical control of staff members with access to the elephant facility, proper disposal of animal excretions, species-specific TB-testing of all animals in close contact with the elephants (mixed exhibits), disinfection of the exhibit (inside and outside).

If a Category D elephant (see below) is present at the institution, the B-status will apply to all other herd members as long as this Category D elephant is present at the institution plus the required period for the testing protocol as described above.

Travel restrictions: no travelling neither receiving elephants from other institution.

Category C elephants:

Risk of infection with Mtb-complex: high.

These elephants are positive for Mtb-complex on PCR or culture, including DNA-sequencing. An action plan following a positive test result should be discussed immediately with the vet advisor-group.

Depending on the national legislation and the Mycobacterium subspecies, the options for category C animals are:

- Treatment: based on the antibiogram of the Mycobacterium. These elephants go to Category D.
- Euthanasia in case:
 - o treatment is not allowed by the national authorities;
 - o Mycobacterium strain(s) is (are) not sensitive for any of the suitable antibiotic compounds;
 - o treatment protocol cannot be fulfilled for the recommended treatment time;
 - o this is the zoo's decision.

Travel restrictions: no travelling neither receiving elephants from other institution.

Category D elephants:

Risk of infection with Mtb-complex: unknown to high.

These elephants are treated elephants that belonged to Category C.

Recommendations: annual monitoring the treatment efficacy by BAL and IGRA for the rest of its life for staff safety reasons. Whatever the success of the treatment and the test results are, these elephants can never be considered as TB-free animals.

Travel restrictions: only to zoos with Category D elephants

Notifications of an Mtb-complex culture positive elephant

After an elephant tests positive for Mtb-complex via culture and DNA-sequencing derived from TW/BAL or any other body fluids (NB uterine discharge and faeces!), the diagnostic testing laboratory will contact the official veterinarian and the attending veterinarian. Once a positive culture is received, the elephant is considered infected with Mtb-complex, and the following notifications and steps are necessary:

1. Regulatory personnel such as:
 - State Veterinarian
 - State/local Public Health officials
 - TAG vet advisor-group
 - Species coordinator
2. Facility personnel including:
 - All staff working with the elephant including barn staff, veterinary staff and volunteers
 - Upper management & legal teams
 - Public relations, marketing, and communications teams
 - Human resources (for TB-testing the staff)
3. Notification of other facilities where the Mtb-complex culture-positive elephant has been in the past.

4. Discussions regarding safe elephant handling, use of personal protective equipment (PPE) such as N-95 respirators, and barn cleaning protocols to prevent zoonotic transmission or spread to other elephants in the herd should occur in collaboration with regulatory and facility personnel.
5. Mixed exhibit: animals sharing the exhibit with a TB-positive elephant should be considered as potential TB-carriers and tested and handled accordingly.

Summary: What to do when tuberculosis has been confirmed in an elephant?

In the unfortunate event that tuberculosis has been confirmed either during necropsy or from culture or PCR of samples taken from a living elephant, the official authorities should be informed. Although not explicitly mentioned under BALAI, *Mycobacterium bovis* is a notifiable disease when confirmed in *Mammalia*, in particular *Antilocapridae*, *Bovidae*, *Camelidae*, *Cervidae*, *Giraffidae*, and *Tragulidae* (Annex A to Directive 92/65/EEC).

Though elephants are not specified as such, potentially close contact with the above mentioned species and the close contact with humans justify the close cooperation with official veterinary and human medical authorities. The zoo veterinarian should form a small group of experts, including a representative of these authorities, in order to make a surveillance and monitoring program. This plan should include (1) screening of zoo staff and individuals that have been in close contact with the affected elephant, (2) screening of contact elephants as well as other species sharing the same enclosure, (3) informing the zoo where the elephant has come from and (4) informing any zoo that has received contact animals during the period that shedding may have taken place. Treatment of TB-confirmed or suspected elephants is an option to be discussed with the zoo staff as well as with the official authorities. One should be aware of the fact that there is evidence that even after intensive treatment of some elephants, shedding has reoccurred in these animals after several years (see category D). Some countries may not allow treatment of confirmed or suspected cases. Screening of contact elephants follows the methods described in this document (see category B).

Necropsy protocol in relation to TB

In addition to the TAG elephant necropsy protocol, the following recommendations should be taken into account when TB is suspected in an elephant.

Wear a mouth and nose protector when necropsy is performed in case of a TB-suspect case and work with a minimal staff at the site of necropsy.

All elephants undergoing necropsies should have a careful examination of:

- The tonsillar regions and submandibular lymph nodes for tuberculous appearing lesions. Take any nodes that appear caseous or granulomatous for culture (samples should be shipped for culture instantly at 4°C according to EU-legislation for shipment of hazardous biomaterials), and a separate part for histology (fixation in buffered 10% formalin). Back-up samples should be stored preferably at -80°C.
- Split the trunk from the tip to its insertion and take samples of any plaques, nodules or suspicious areas for TB diagnosis as above. Look for and collect possible extra-thoracic TB lesions, particularly if there is evidence of advanced pulmonary TB.
- Open the trachea and look for nodules or plaques and process as above. Regional tracheal lymph nodes should also be examined and processed accordingly. Take samples of any suspicious lesions. The thoracic organs carefully for early stages of TB as follows: after removal of the lungs and trachea, locate the bronchial nodes at the junction of the bronchi from the trachea. Use clean or sterile instruments to section the nodes. Freeze half of the lymph node and submit for TB culture (even if no lesions are evident). Submit sections in formalin for histopathology. Carefully palpate the lobes of both lungs from the apices to the caudal borders to detect any firm (nodular size) lesions. As the pleura in elephants are fused, the lungs are firmly attached to the thoracic wall. This makes handling of the elephant lungs more difficult than in other species. Regional thoracic lymph nodes should also be examined and processed accordingly. Take samples of any suspicious lesions.

In case of any suspicion of TB in an elephant the Veterinary advisor-group of the elephant TAG must be informed immediately:

Willem Schaftenaar, DVM
Jasmijnhof 10
2665 GS Bleiswijk – The Netherlands
Tel: +31 6 22789442
E-mail: W.Schaftenaar@rotterdamzoo.nl

Michael Flügger, DVM
Tierpark Hagenbeck
Research
Lokstedter Grenzstrasse 2
22527 Hamburg - Germany
Tel: +49 40 53 00 33-350
E-mail: michael.fluegger@hagenbeck.de

Endre Sós, DVM
Budapest Zoo, Állatkerti krt. 6-12
1146 Budapest - Hungary
Tel: +36 205593150
E-mail: drsos@zoobudapest.com

Prof. Thomas B. Hildebrandt, DVM
Leibniz Institute for Zoo & Wildlife
Alfred Kowalke Strasse 17
10315 Berlin – Germany
Tel: +49 30 5168-440
E-mail: Hildebrand@izw-berlin.de