Short Communication

Mycobacterium tuberculosis in a Primagam negative wild caught captive olive baboon (*Papio anubis*) in Ethiopia

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Abstract

A free-roaming wild olive baboon (*Papio anubis*) was caught in the compound of a hospital and kept in captivity pending reintroduction to the wild. The animal had a sporadic dry cough but was TB negative on the blood-based assay PRIMAGAM (IFN-γ test). Six years later, the animal was found dead without any prior clinical signs. The lungs were severely affected. Laboratory analysis included Ziehl-Neelsen staining, GenExpert, culture, deletion typing and spoligotyping. *M. tuberculosis* was isolated. The spoligotype was SIT 53 (lineage 4) and no Rifampicin resistance was detected. This case report raised challenges on accurate diagnosis of TB in Non-Human Primates in Ethiopia, the question of latency in baboon and the lack of spread of a highly virulent TB strain in the Non-Human Primate colony. It also highlighted the potential role of TB transmission between Non-Human Primates and people in Ethiopia with impacts as well on public health as on primate conservation.

Key words: Baboon; diagnostics; Ethiopia; Mycobacterium tuberculosis; tuberculosis

Introduction

TB remains worldwide a leading cause for morbidity and mortality. Ethiopia has a high TB burden, ranking third in Africa and eighth globally among the 22 countries with highest TB prevalence (WHO 2018). Moreover, Multi-drug

resistant TB (MDR-TB) has been increasingly observed in Ethiopia, with prevalence ranging from 3.3 and 46.3%, providing additional challenges to the national TB control program (Biadglegne *et al*., 2014).

Non-human primates (NHP), like their human counterparts, can get infected with TB and show identical disease epidemiology (e.g. transmission, immunology, type and course of disease) hence they are often used as model for human TB (Gormus *et al*., 2004; Scanga and Flyn 2014). Old world monkeys are considered to be the most susceptible species for TB among all NHP species (Montali *et al*., 2001). NHP are primarily affected by *M. tuberculosis* but *M. bovis* infections have also been observed. The disease does not exist naturally in free-ranging NHP (Montali *et al*., 2001). However, TB becomes a major health risk for NHP when they are exposed to humans with TB (Fourie and Odendaal 2003) or when they consume BTB infected meat (Tarara *et al.,* 1985; Thorel *et al*., 1998; Keet *et al*., 2000). TB in captive NHP colonies has major health and economic implications (human exposure, animal losses, and cost for disease control and staff therapy) but also in terms of conservation (risk of TB to wild populations during reintroduction programs). Animals with active TB may be symptomless for weeks and months while they transmit the disease (Gibson 1998). Latent TB, although not infectious can be reactivated at any time of the animal's life. Latent TB is not detected by traditional screening methods such as the skin test (Lerche *et al*., 2008). Therefore, early TB detection in captive NHP is crucial. Unfortunately, there is currently no diagnostic gold standard test for TB. Traditionally, NHP are tested by skin test (eye-lid or abdominal skin PPD skin injections). This can be stressful for the animal, and requires two anesthesia within 72 hours. In addition, the skin test lacks specificity (84-87%) and sensitivity (84%), does not always account for high environmental TB and does not detect latency (Garcia *et al*., 2004; Lerche *et al*., 2008; Lin *et al*., 2009). Rapid blood based *in-vitro* assay such as the PRIMAGAM test offer an alternative, with relatively good sensitivity (68%) and excellent specificity (97%) and able to detect TB at a very early stage (Garcia *et al*., 2004). PPD antigens are presented to lymphocytes in whole blood culture and the resulting production of interferon-γ (IFN-γ) by the TB-exposed lymphocytes is detected using a monoclonal antibody-based sandwich enzyme immunoassay (EIA). Lymphocytes of non-infected animals does not produce any IFN-γ. We present here a case of *M. tuberculosis* detected in a captive wild caught olive baboon, who tested negative with the blood-assay TB-PRIMAGAM test and discuss the TB diagnostics challenges in NHP in Ethiopia.

Material and methods

History

A juvenile female olive baboon (*Papio Anubis*) was roaming freely on the premises of a large hospital in Addis Ababa, was then captured by the Ethiopian Wildlife and Conservation Authority and kept in captivity in a large enclosure close to Addis Ababa, Ethiopia before considering a potential release into the wild. Upon arrival, the animal underwent quarantine. She looked healthy with normal body condition. No visible clinical signs were observed with the exception of a very sporadic dry cough that would persist over the following years. The animal was housed with other rescued olive baboons. The large enclosure was on natural ground (shrubs, dirt ground, grass and rocks) surrounded by a meshed cage. Diet consisted on scattered mix of various fresh fruits and vegetables as well as supplemental grass and acacia tree branches with pods or flowers. All animals were screened shortly after arrival for tuberculosis (TB) using the commercially available primate interferon-γ test (Primagam® blood essay; Prionics AG). All tested animals were negative to the TB test. Six years later, the baboon was found dead in the enclosure without showing any prior signs of disease except a very sporadic dry cough that she kept throughout the years.

Post-mortem examination

A standard post-mortem examination was carried out within 2 hrs. of death. Specimens of lung tissues were collected into sterile containers (one third without transport media, which were frozen at -80°C, one third contained 10% formalin solution and one third contained PBS (phosphate buffered saline) and brought to the laboratory at the Armauer Hansen Research Institute (AHRI), Addis Ababa within 15 minutes of collection.

Laboratory diagnosis

Suspicion of TB lead to follow all diagnostic procedures according to the institute and national TB SOP's (TB program quality assurance) (e.g. use of P3-TB lab for all diagnostic procedures; wearing of face masks and gloves). Smears from lung tissue samples were prepared followed by Ziehl- Neelsen (ZN) staining and observation for acid fast bacilli under the light microscope. *Mycobacterium* culture was done on Lowenstein-Jensen (LJ) medium following the procedure described in Mycobacteriology Laboratory Manual (Global Labo-

ratory Initiative, 2014). Tissue sample was homogenized with 3 ml phosphate buffered saline (PBS) using sterile mortar and pestle. *Mycobacterium* culture was done on Lowenstein-Jensen (LJ) medium. Digestion-decontamination of tissue sample was performed by N-acetyl L-cysteine /Sodium Hydroxide method (NALC/NAOH) with a final NaOH concentration of 1%. An equal volume of standard NALC/NaOH solution was added to tissue sample and incubated for 15 minutes. After neutralization by PBS and centrifugation (15 minutes at 3000g), the sediment was re-suspended in 1ml sterile PBS. Finally, 200µl of sediment was used to inoculate on two LJ slants. The remaining sediment was used for smear preparation followed by Ziehl- Neelsen (ZN) staining, and examined for acid-fast bacilli (AFB) using regular light microscopy (Federal Democratic Republic Ethiopia Ministry of Health, 2014). The AFB grading was done according to WHO smear grading scale for ZN staining (WHO, 1998). Culture was checked daily during the first week, then weekly thereafter. Colonies from positive culture were removed from the surface of LJ medium and suspended in 300 µl of Molecular grade water and the mixture heated at 80°C for 1 hr. in water bath. After centrifugation, the supernatant was collected and used for RD9 deletion typing and spoligotyping.

RD 9 Deletion Typing

Mycobacterial species was identified using polymerase chain reaction (PCR) based region of difference (RD9) typing as described by Brosch *et al*. (2002) using previously published RD9_FlankF, IntR and FlankR primer sequence (Berg *et al*., 2009). PCR was performed on heat-killed cells. *M. tuberculosis* H37Rv, *M. bovis*, and Qiagen water was used respectively as positive and negative controls.

Spoligotyping

Spoligotyping was performed following the method described by Kamerbeek *et al*. (1997).The spoligotype pattern was entered in an Excel spreadsheet, compared with those in the International Spoligotyping Database (SITVITWEB) of the Pasteur Institute of Guadaloupe (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/), and a spoligotype international type (SIT) was assigned.

In addition, for the sake of diagnostic speed, 0.5 ml of homogenized tissue sample was subjected for GeneExpert analysis by mixing with the supplied sample reagent in a 1:3 ratio, vortexed and incubated for 15 minutes. Two ml of the reagent-sample mix was then transferred to an Xpert cartridge using a

pasteur pipette and the cartridge loaded onto Expert machine. The GeneXpert automates all following steps, including sample work-up, nucleic acid amplification, detection of the target sequence and result interpretation (Geleta *et al*., 2015).

Further investigation of personnel

Four primate animal personnel, who had close physical contact to the TB positive animal over the years but showed no clinical signs of TB, wished to be tested for TB as well following the result of the animal. The gamma interferon test (QuantiFERON-TB Gold Plus) was used. Four ml of venous blood was drawn into lithium-heparin tubes and processed within 2 hours at AHRI. One ml of blood was dispensed into each QFT plus tubes (Nil, TB1, TB2, mitogen) followed by mixing by inversion and incubation at 37°C for 20 hrs. After incubation, the tubes were centrifuged at 3000 RPM for 10 minutes, the supernatants removed and the amount of IFN gamma measured by ELISA. IFN-γ ELISA was performed according to the QFT-Plus protocol and the result was interpreted using the software supplied by the manufacturer.

Results

The animal showed a normal to thin body condition based on the body scoring system by Clingerman and Summers (2005). No external changes were observed with the exception of hyperaemic conjunctivae. Superficial lymph nodes were normal. Intra-abdominal examination showed that all organs were hyperaemic without other visible pathological abnormalities. Lungs were severely enlarged and abnormally shaped, having entirely lost their normal lung shape, with hard bumpy sections and ballooning. The lungs filled the entire thorax cavity with fibrous adhesions to the pleura that made extraction from the thorax cavity difficult. The lungs were removed en-toto using sharp and blunt dissection. The lung texture was hard fleshy for most part of the organ with hardly any normal looking lung tissue left. Granulomas, soft in texture could be felt throughout the lungs. Upon dissection, the lungs were entirely filled with various sized cavities (from 1 to 5 cm in diameters) sometimes coalescent/confluent filled with little liquid creamy looking pus and ill-defined walls (Figure 1). When pressure was applied to the lungs, pus would ooze out from everywhere, including bronchi. No calcifications were observed. Lung lymph nodes could not be identified. The heart was enlarged with pericardial fluid.

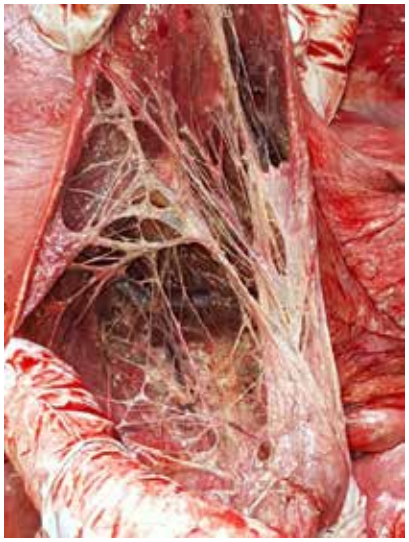

Figure 1. Macroscopic close-up of a dissected lung lesion in an olive baboon (*P. anubis*)

Laboratory results

Baboon sample

Ziehl-Neelsen staining of three slides (direct lung smear) and one slide with colonies from culture were graded as +++, implying the presence of more than 10 Acid Fast Bacilli per field. The result of the GenExpert was positive for *M. tuberculosis* and no rifampicin resistance was detected. Results from culture showed colony growth within two weeks of inoculation that were creamy white dry, rough, raised, irregular with wrinkled surface and confluent growth was observed. Figure 2 shows the results of RD9 deletion typing. The isolate had an intact RD 9 region identifying it as *M. tuberculosis*. The spoligo pattern was *M. tuberculosis* SIT 53, belonging to Lineage 4.

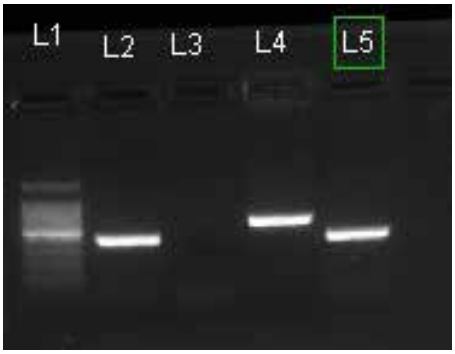

Figure 2. Electrophoretic pattern of the amplified element of PCR products, Lane=100bp ladder; Lane 2= H37Rv (*M. tuberculosis* positive control), Lane 3= Qiagen H2O (negative control); Lane 4 = *M. bovis* (positive control); Lane 5= DNA from heat killed culture

Human samples

One out of 4 people were positive to the quantiferon test, hence making *M. tuberculosis* infection likely. However, due to the limitation of the test, definitive diagnosis of whether the infection is active or most probably latent since the person did not show any signs of active TB, requires further combination of epidemiological, historical, medical and complementary diagnostic findings. Furthermore, the study could not link with certainty the infection in the animal and the person due to the lack of *M. tuberculosis* isolation in the sampled people.

Discussion

This case-study highlighted two major issues regarding TB in NPH, namely: 1) poor detection of TB by the blood-based assay PRIMAGAM and 2) the unusual very long disease course and lack of further spread. In hindsight, we assume that the animal had likely already an active TB, when he was tested 6 years ago considering the chronic cough she had. Animals with active TB can be symptomless for weeks to months (Gibson, 1998). However, although TB can be a chronic debilitating disease, having an active TB for so many years has, to our knowledge not been observed. Furthermore, the animal kept his good body condition to the end and no disease outbreak occurred in the colony. TB is usually described as highly contagious within a NHP colony. All primates

underwent monthly health checks during these 6 years (assessment of body condition, cough, appetite etc). Furthermore, autopsies were performed as a standard procedure on several NHP during these 6 years that died from any cause. No visible lesions suggestive for TB were ever observed, and smears of samples collected routinely from autopsies were ZN negative. In addition we know that the isolated strain belonged to a virulent most commonly found lineage in Ethiopia (modern lineage), which makes it more surprising for not having spread fast. It is well possible that the animal was already in an advanced state of anergy during the testing. However, it would be unlikely she would have survived another 6 years. TB latency is a growing concern in the NHP TB epidemiology. Studies have shown that a latent TB can be reactivated for example by a pregnancy and develop quickly into a fatal active TB (Martino *et al*., 2006). Hence, the importance to diagnose TB at the latent stage. The last possibility is that the animal was negative at the time of the testing and it acquired the disease while in captivity. However, this scenario is the least plausible since no other baboons sharing the enclosure became clinically sick with TB, and later performed autopsies did not reveal any visible TB-like lesions and were ZN negative. A TB latency could not to be ruled out in the remaining animals of the group, However, latency over several years in a captive primate colony has never been previously described.

The standard testing procedure in NHP remains the Tuberculin Skin testing (TST), which detects delayed type hypersensitivity (DTH) to tuberculin antigens. Classically, the SIDT test is performed in the eyelid and swellings observed after 72 hours. The procedure requests two anesthesia and is subject to the observer's interpretation leading to subjectivity. The required antigen concentration to detect reactivity for TB is higher than in humans but the exact quantity of tuberculin units (TU) is often not provided in PPD for NHP and same testing is often used as in humans. The TST is known for its poor sensitivity and specificity and leads to false negative animals (Lerche *et al*., 2008). Moreover, the TST does not detect TB latency. Various blood tests have been used to screen NHP with diverse results (Parsons *et al*., 2004; Vervenne *et al*., 2004). More recently, non-invasive TB screening was done among chimpanzees and baboons in Tanzania, using fecal IS6110 PCR (Wolf *et al*., 2016). Blood tests, such as the PRIMAGAM, reduces the stress in animals since only one anesthesia is required. Unlike the TST, it is a quantifiable test. It detects TB reactivity by measuring the IFN-γ production by lymphocytes that had contact to Mycobacteria. It is highly specific (97%) and can detect TB at a very early stage (Garcia *et al*., 2004). However, studies have clearly shown that no

single test has the high enough sensitivity and specificity to detect correctly all positive animals in a group and a combination of tests might be necessary, highlighting the still existing challenge to diagnose accurately TB.

TB in NHP is a concern in Ethiopia in light of the high numbers of illegally kept primates with close contact to humans, rehabilitated/rescued primates that are set to be released into the wild and the many free-ranging NHP in close vicinity to human dwellings, particularly urban wild NHP. The baboon of this study was a wild baboon initially roaming freely in a hospital compound. In light of the high TB burden in Ethiopia and the emergence of MDR-TB, these wild roaming NHPs can become infected by TB and can then also potentially act as vehicle for further spread to humans and other NHP. Awareness campaigns are warranted. They are a crucial step in controlling the spread of TB between humans and NHP but also for overall conservation purposes, by promoting the stop of illegal ownership of NHP as pets and banning illegal wildlife trade.

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Conflict of interest

The authors declare that there is no conflict of interest

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