Short communication

Important role of Non Tuberculous Mycobacteria in Non-Human primate TB testing in Ethiopia

Rea Tschopp [1,2,3] and Liya Wassie[1]*

[1] Armauer Hansen Research Institute (AHRI), PO Box 1005, Jimma Road, Addis Ababa, Ethiopia

[2] Swiss TPH, Socinstr 57, 4002 Basel, Switzerland

[3] University of Basel, Basel, Switzerland

** Corresponding author: Rea Tschopp, rea.tschopp@swisstph.ch*

Abstract

Tuberculosis is an important disease in captive non-human primates (NHP) but remains challenging to diagnose accurately. The tuberculin skin test (TST) remains the standard test used worldwide, whereby the single intradermal tuberculin test (SIDT) in the eye-lid is the most frequently used method to diagnose TB in NHP. In this study, 9 NHP (5 baboon *spp*.) and 4 geladas (*T. gelada*) were tested for TB using the Primagam (whole blood immunoassay) in order to assess the feasibility of relocation into the wild. Although the results showed that all species reacted to non-tuberculous mycobacteria (NTM), the geladas showed a consistent distinct reaction pattern with very high reactivity to NTM. The results suggest that using the SIDT in Ethiopian primates, particularly the endemic geladas, would lead to a high number of false positive animals, highlighting the current challenge to accurately diagnose NHP for TB, which could help in the conservation efforts in the country.

Key words: Ethiopia; TB; Non-human primate; Gelada; non-tuberculous mycobacteria

Introduction

Tuberculosis (TB), although not occurring naturally in wild non-human primate (NHP) populations, is a major health risk for animals that have close contact to people and/or that consume meat from TB infected animals (Keet *et al*., 2000, Montali *et al*., 2001, Martino *et al* 2006). *Mycobacterium tuberculosis* (*M. tuberculosis*) and to a much lesser extent *M. bovis* are the two main patho-

gens causing TB in NHP (Kaufman and Anderson 1978). TB, besides being a public health threat, is a highly contagious and costly disease in captive NHP in terms of cost of animal losses, cost of diagnostics, and cost of treatment for exposed staff. It requires regular and accurate screening to avoid outbreaks in these populations. Reactors are usually eliminated in order to prevent further disease spread. The choice of diagnostic test is hence of importance, in order to avoid false negative animals that can continue to spread the disease and false positive animals that would be sacrificed unnecessarily. The test should also ideally be able to detect the disease at an early stage as well as detect latent cases. Like in humans, TB diagnostic however, remains a challenge in NHP. There is currently not a single test that meets all requirements on sensitivity, specificity, user friendliness, low cost and available logistics.

The tuberculin skin testing is a standard diagnostic tool to screen NHP for TB in captive animals (Bushmitz *et al*., 2009). The eyelid test is usually preferred as compared to abdominal testing, where purified protein derivative (PPD) is injected intradermally into the eyelid under general anesthesia and reaction (grade of swelling/drooping) observed and interpreted after 72 hours, under a second anesthesia. The test is based on a delayed hyper sensitivity response against a mycobacterial antigen (delayed-type hypersensitivity) (Garcia *et al.,* 2004; Lerche *et al*., 2008; Lin *et al*., 2008). Blood tests such as *in-vitro* immune stimulation assay are also used to a lesser extent. They are quantifiable, require general anesthesia only once, have good sensitivity and specificity and can detect TB at an early stage (Garcia *et al*., 2004).

Material and Methods

In this small study conducted in 2016 in Ethiopia, 9 captive NHP from 2 different facilities in Addis Ababa were screened for TB. The main objective for screening was to assess the possibility of releasing individuals into the wild. Four olive baboons (*P. Anubis*), 1 Hamadryas baboon (*P. hamadryas*) and 4 Geladas (*T. gelada*) were included in the study. Two were juveniles, 3 were sub-adults and 4 were adults. Six out of 9 were males (67%). Due to a shortage of PPD and anesthetics, a blood test was solely performed on all NHP, hence requiring only one anesthesia. All procedures were performed by a trained professional wildlife veterinarian, assuring that welfare standard for animals were met. Volume of drawn venous blood was dependent on the size of the animal (maximum 2 ml), drawn into heparinized tubes and transported to the TB lab at the Armauer Hansen Research Institute (AHRI), Addis Ababa within 2

hours. The commercial PRIMAGAM-primate IFG test (Prionics AG, Schlieren, Switzerland) test was used. a The test procedure followed as indicated by the manufacturer. The PRIMAGAM is derived from the classical BOVIGAM test used in cattle. The test is a rapid *in-vitro* lymphocyte stimulation assay, where lymphocytes are presented to antigens in whole blood culture. It detects reactivity to tuberculin antigens by measuring the IFN-γ produced by lymphocytes that had previous contact with the pathogens using a monoclonal antibody-based sandwich enzyme immunoassay (EIA). It has relatively good sensitivity (68%) and excellent specificity (97%) as compared to the TST (Garcia *et al*., 2004). The tests were run in duplicates. Optical density (OD) was measured for bovine and avium PPD. The test results were interpreted as positive for TB if the OD of PPD-B – PPD-A > 0.05 OD units.

Results

The tests results are shown in Fig. 1. Following the definition cut-off of the manufacturer, none of the 9 tested animals were positive for TB. Five out of nine (55%) animals had positive reactions to bovine PPD. These animals would have most likely reacted positively in the eye lid test. However, taking into account reactions to avium PPD, the final diagnosis was negative for TB in all cases. Gelada monkeys (individuals 6, 7, 8 and 9) showed a consistent distinct pattern of *M. avium* PPD reactions by being more elevated than the reaction with bovine PPD, which were consistently high as well.

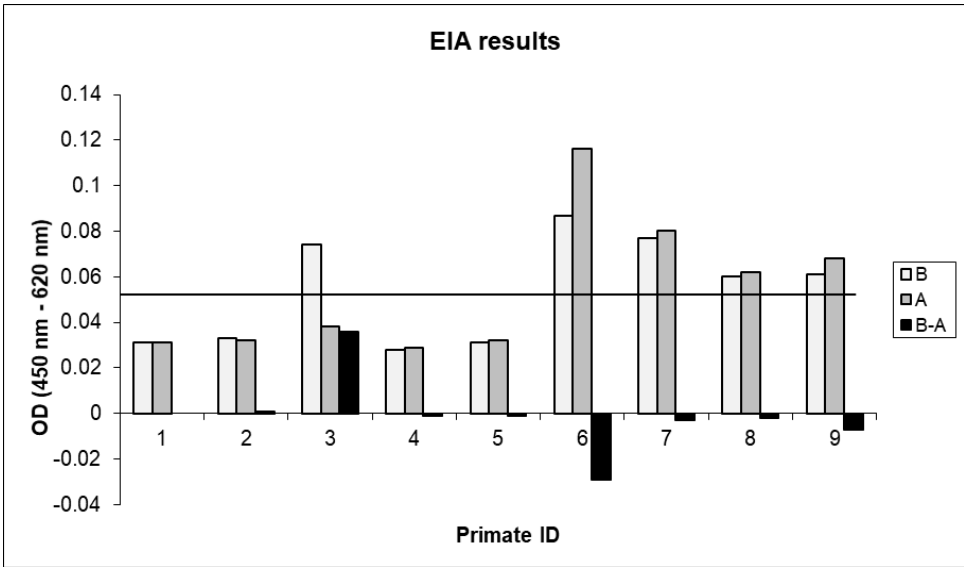

Fig 1. PRIMAGAM results of 9 captive non-human primates. B= OD PPD bovine; A= OD PPD avium; B-A = difference OD PPD bovine and OD PPD avium. Cut-off for TB positivity set at B-A > OD=0.05 (shown by a horizontal line).

Discussion

To our knowledge this is the first published information on TB testing in wild caught gelada monkeys. Geladas are a species of old-world monkeys, endemic to the Ethiopian Highlands. They are terrestrial, gregarious and the only graminivorous primates (true grazers). They spend their days foraging on grass and herbs, hence being constantly close to the ground. This feeding behavior is very likely an explanation for the high exposure to environmental mycobacteria as compared to the baboon species.

In Ethiopia, the non-tuberculosis mycobacterial burden has been shown to be high in humans (Mathewos *et al.,* 2010, Workalemahu *et al.,* 2011, Gumi *et al*., 2012), livestock (Berg *et al.,* 2009) and wildlife species (Tschopp *et al.,* 2010). The single intradermal skin test (eye-lid test), as used per recommendation of the European Primate Veterinary Association Working Group is in the Ethiopian context is thus not advisable. Over half of the NHP would have been false positive reactors in this study. Comparative skin testing is hence mandatory.

Also, a high number of anergic cases have been observed in old world monkeys, while using the TST, particularly in baboons (Montali *et al*., 2001). Although highly specific, and able to detect early stages and latency of TB, the IFN-γ test is known to have some pitfalls as well leading to false negative reaction (Garcia *et al.,* 2004). Hence, for both the skin test and blood test, results are affected by the primate species tested. For instance, *Cynomolgus macaques* have been described as having a lot of false negative results with the TST as with the primagam (Garcia *et al.,* 2014). Since both tests fail to 100% identify TB positive animals, a combination of different tests would increase the likelihood of detecting these positive reactors. In addition, species specific diagnostic approaches should be taken in account, particularly with baboon spp and geladas, particularly in the context of the conservation of these species. However, in a poor resource country like Ethiopia, combination diagnostics could prove challenging (e.g. lack of wildlife veterinary expertise, reagent and lab availability, costs of diagnostics).

Acknowledgements

The authors are thankful to the staff of AHRI for their logistic and financial support, to EWCA and to the Born Free Foundation for purchasing the reagents.

References

Berg, S., Firdessa, R., Habtamu, M., Gadisa, E., Mengistu, A., Yamuah, L., et al. 2009. The Burden of Mycobacterial Disease in Ethiopian Cattle: Implications for Public Health. *PLoS ONE* 4(4): e5068. https://doi.org/10.1371/journal.pone.0005068

Bushmitz, M., Lecu, A., Verreck, F., Preussing, E., Rensing, S., et al. 2008. Guidelines for the prevention and control of tuberculosis in non-human primates: recommendations of the European Primate Veterinary Association Working Group on tuberculosis. *J. Med. Primatol*., 38(1), 59-69. https://doi.org/10.1111/j.1600-0684.2008.00303.x.

Garcia, M.A., Yee, J.A.L., Bouley, D.M., Moorhead, R. and Lerche, N.W. 2004. Diagnosis of Tuberculosis in Macaques, Using Whole-Blood In Vitro Interferon-Gamma (PRIMAGAM) Testing. *Comp. Med*., 54(1), 86-92.

Gumi, B., Schelling, E., Berg, S. et al. 2012. Zoonotic Transmission of Tuberculosis Between Pastoralists and Their Livestock in South-East Ethiopia. *EcoHealth*, 9 (2), 139-149. Doi:10.1007/s10393-012-0754-x.

Kaufmann, A.F., and Anderson, D.C. 1978. Tuberculosis control in nonhuman primate colonies, p227-234. In R.J, Montali (ed) Mycobacterial infections in zoo animals. Smithsonian Institution Press, Washington DC.

Keet, D.F., Kriek, N.P.J., Bengis, R.G., Grobler, D.G., and Michel, A. 2000. The rise and fall of tuberculosis in a free-ranging chacma baboon troop. *Onderstepoort J. Vet. Res.*, 67(2),115-122.

Lerche, N.W., Yee, J.A.L., Capuano, S.V., and Flynn, J.L. 2008. New Approaches to Tuberculosis Surveillance in Nonhuman Primates. *ILAR* J., 49(2), 170-178. https://doi.org/10.1093/ilar.49.2.170.

Lin, P.L., Rodgers, M., Smith, L., Bigbee, M., Myers, A., Bigbee, C., Chiosea, I., Capuano, S.V., Fuhrman, C., Klein, E., and Flynn, J.A.L. 2009. Quantitative Comparison of Active and Latent Tuberculosis in the Cynomolgus Macaque Model. *Infect. Immun.*, 77(10), 4631-4642.

Mathewos, B., Kebede, N., Kassa, T., Mihret, A., and Getahun, M. 2015. Characterization of mycobacterium isolates from pulmonary tuberculosis suspected cases visiting

Tuberculosis Reference Laboratory at Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia: a cross-sectional study. *Asian Pac. J. Trop. Med.*, 8(1), 35-40. doi: 10.1016/S1995-7645(14)60184-X.

Montali, R.J., Mikota, S.K., and Cheng, L.I. 2001. *Mycobacterium tuberculosis* in zoo and wildlife species. *Rev Sci Tech.*, 20(1), 291-303. http://dx.doi.org/10.20506/rst.20.1.1268.

Martino, M., Hubbard, G.B. and Schlabritz-Loutsevitch, N. 2006. Tuberculosis (*Mycobacterium tuberculosis*) in a pregnant baboon (*Papio cynocephalus*). *J. Med. Primatol.*, 36 (2).108–112. doi:10.1111/j.1600-0684.2006.00166.x

Tschopp, R., Berg, S., Argaw, K., Gadissa, E., Habtamu, M., Schelling. E., Young, D., Aseffa, A., and Zinsstag, J. (2010). Bovine tuberculosis in Ethiopian wildlife. *J. Wildl. Dis.*, 46(3). 753-762. https://doi.org/10.7589/0090-3558-46.3.753

Workalemahu, B., Berg, S., Tsegaye, W., Abdissa, A., et al. 2013. Genotype diversity of mycobacterium isolates from children in Jimma, Ethiopia. *BMC Res. Notes*, 6, 352.