

African giant rats for tuberculosis detection: a novel diagnostic technology



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Tuberculosis (TB) continues to be a major public health problem especially in developing countries. More than 2 billion people (i.e. one third of the world's total population) are infected with TB bacilli (*Mycobacterium tuberculosis*, the bacteria causing TB). One in every 10 of those people will become sick with active TB in their lifetime. People with HIV/AIDS have more risks of TB infections. Globally, there were 1.77 million deaths from TB in 2007, including 456 000 people infected with HIV, which is nearly 4800 deaths per day¹.

HIV has caused TB incidence to triple in sub-Saharan Africa, where in some countries 80% of TB patients are co-infected with HIV. Thirteen out of the 15 countries with the highest TB burden are in Africa¹. Despite TB killing more people with HIV than any other disease, in 2008 only 1% of people with HIV had a TB screening. This is a drawback and major challenge to the global efforts towards achieving the TB control targets as well as the UN Millennium Development Goals for 2015². Although there are more sophisticated methods in the developed world, the diagnosis of TB in affected countries is mainly based on microscopic examination of TB bacilli (Acid-fast bacilli-AFB) in sputum smears stained by the Ziehl Neelsen (ZN) method. Other rarely used methods include fluorescent microscopy (FM) and culture (gold standard) that requires laboratory facilities that are not readily available in resource-limited areas. For example, Tanzania has only three TB culture laboratories serving nearly 40 million people². This makes the ZN method (microscopy) a vital tool for TB control in developing countries. The case detection rate in these countries (e.g. Tanzania) is around 47%, far below the global target of 70%³. This is attributed to the poor sensitivity of the method; the huge workload, which causes fatigue to the microscopist and may result in significant number of false-negatives (misdiagnosis); and to difficulties in diagnosing TB in patients co-infected with HIV because most of them produce negative smear results which delays the diagnosis and treatment in the absence of culture facilities⁴. Therefore, a fast, simple and more efficient diagnostic system is highly demanded.

To alleviate this diagnostic problem in sub-Saharan Africa, Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling (APOPO) came up with a novel technology: training and utilizing African giant pouched rats (*Cricetomys gambianus*) for TB diagnosis. This paper reports the successes achieved so far with this novel technology, the ongoing research activities, the way forward and the challenges to be overcome to finally make the technology fully operational. To this effect, a case study conducted on samples collected from selected urban health centres in Tanzania that resulted in a significant increase in TB case detection, as well as demonstrating detection of difficult sputum smear negative specimens, is presented.

APOPO is an award-winning social enterprise⁵ that researches, develops and deploys detection rat technology for humanitarian purposes. It utilizes a local resource, the African rats, for its detection technology. Using these rats as detectors is advantageous due to their efficiency in this role, and their acute sense of smell. Other benefits include their low cost for husbandry and maintenance, ease of transport and their wide availability. It has also been shown that once trained, they can reliably carry out repetitive tasks. Moreover, it is an appropriate tool since it does not depend on high tech equipment or highly skilled labour, increasing its accessibility in the countries of operation⁶. Initially, APOPO proved the concept of its innovative technology for the detection of landmines and this result is currently operational in some countries seriously affected by landmines^{6,7}. As an extension of its knowledge and as a separate wing of its research activities, APOPO envisaged a completely new idea of using its technology for the benefit of the larger poor: rats detecting TB! This idea is based on the fact that the mycobacteria emit specific volatile organic compounds^{8,9}, that are likely to be detected by the rats through olfactory perception and the proof of this concept was recently published¹⁰.

To explain the training and evaluation procedure briefly, beginning at the age of four weeks rats learn to associate the pop sound of a metal clicker, made by the trainer, with a reward of food (peanuts or banana pulp). They learn to



Figure 1: Cassettes containing sputum samples for TB detection (a), a sniffer rat is being rewarded after positive indication of a tuberculosis sample in the evaluation line cage (b).

indicate positive samples by keeping their nose in the “sniffing holes” of a specially designed cage, under which the target samples are placed (see video at www.herorat.org or www.apopo.org). The rat ignores sniffing holes with negative samples after sniffing for less than a second, and proceeds to sniff the next hole in a row until it “hits” a positive hole where it keeps the nose fixed for five seconds. The training and evaluation set-up for the analysis of sputum samples consists of a configuration (cage), in which samples are lined up to be sniffed by the trained rats, to indicate positive targets in return for a food reinforcer (Figure 1a and b). The samples are inserted into cassette-carriers containing 10 samples each, which are placed under corresponding sniffing holes in the cage. The sniffing holes are covered with metal plates in between testing to keep headspace vapour in the air above the sputum and to allow systematic sniffing of the samples by the rats. The trainer uncovers the sniffing holes, such that the rats have to sufficiently sniff a sample first before the lid of the next sample is opened. This set-up allows processing (analysis) of 140 sputum samples in one session of 15–20 minutes.

A recent case study conducted on sputum samples collected from four selected Direct Observation Treatment Short-Course (DOTS) centres in Dar es Salaam, Tanzania (namely: Amana, Mwananyamala, Magomeni and Tandale) from January 2008 to May 2009 demonstrates the advantage of APOPO's technology over the conventional ZN microscopy. The rats screened samples systematically, indicating positive specimens by keeping the nose in the sniffer hole for five seconds while scratching paws on the cage floor. Positive indications were confirmed by microscopy (ZN) and the positive findings for patients undetected/missed by microscopy in DOTS centres were communicated to DOTS centres for tracing and further confirmation by the DOTS centres. Out of 15 041 patients the DOTS centres (ZN microscopy) detected a total of 1838 (12.2%) patients whereas sniffer rats detected 2415 patients (16.1%) with increased case detection in all four DOTS centres (Figure 2). The cases detected by rats but missed by DOTS centres ($n = 577$) increased TB detection by 31.4% which is found to be statistically significant ($p < 0.001$). Statistical tests also confirmed the agreement between results of the positive

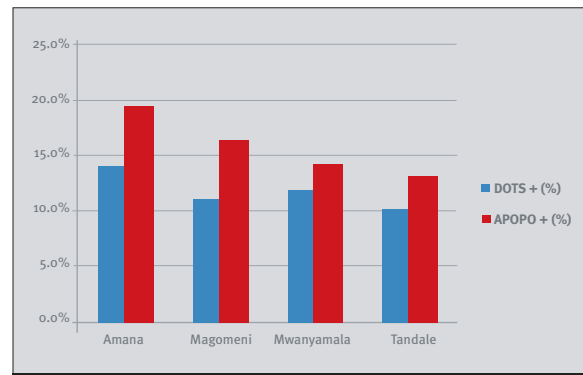


Figure 2: Tuberculosis case detection in the four selected DOTS centres in Dar es Salaam (microscopy) and APOPO trained African giant pouched rats (*Cricetomys gambianus*) ($n = 15\ 041$ patients).

smear microscopy from the DOTS centres and APOPO's microscopy as well as rats' detection.

APOPO's research outputs so far asserted that sniffer rats can analyse sputum samples faster and reliably with high sensitivity and specificity¹⁰. Furthermore, it demonstrated that rats can analyse an average of 70 sputum specimens in 20 minutes whereas an average of 20 specimens can be analysed by a microscopist in a day of eight working hours. Hence, using rats for detection reduces workload significantly in the TB diagnosis centres and improves the quality of results, which diminishes with increasing workload. The use of rats can also reduce transmission/spread of TB bacilli from infected patients who are undetected or misdiagnosed due to various reasons including workloads in laboratories and HIV status¹¹. For example, the new 577 patients who were detected by rats but missed/undetected in the DOTS centres would have been able to transmit TB to between 5770 and 8655 people per year if they had remained undetected (one untreated person can transmit TB to between 10 and 15 people per year^{2,12}). This shows the potential use of sniffer rats as a first-line screening tool in active case finding and referring suspected patients to DOTS centres for confirmation and early treatment. Similarly, culture laboratories could benefit from dealing with screened suspect positive samples only, which may improve work in these laboratories as well as the drug susceptibility testing facilities (DST). These may contribute to achieving the Global Plan to Stop TB and the target to save 14 million lives and achieve the proper treatment of 50 million people.

Current research activities are geared towards further improvement of the sensitivity and specificity of rats' detection; refining sample collection, transport and pre-treatment procedures so as to avoid any cue the process might leave for the sniffer rats; automation of the evaluation cage so that any human error (bias from animal trainers) could be minimized; and installing a quality assurance and internal accreditation scheme for individuals or groups of trained rats. We are also planning to launch a clinical trial in Cape Town, South Africa where TB as well as TB-HIV co-infection is rampant but the expertise and the technology to run the confirmatory tests are present. Due to the relative technological advances in South Africa, the accreditation

procedure is in place and we anticipate swift accreditation once we successfully finish the clinical trial. Once we gain experience there, we may transfer this knowledge to Tanzania where the accreditation procedure is non-existent, assist our Tanzanian colleagues to establish one, and seek accreditation to implement the technology. The last steps will pave the way for seeking international accreditation to be officially part of the tool to curb the TB burden worldwide.

Access to funding remains the main challenge for APOPO's innovative technology to be embraced in the global fight against TB. So far a number of organizations and foundations (such as The World Bank Development Marketplace, UBS Optimus Foundation and the US National Institutes of Health) have provided funding for this project. But as explained in the preceding paragraph we have a long way to go and numerous research activities to conduct to reach our ultimate goal. APOPO looks forward to continued partnering with interested parties as we work towards containing the spread of TB. □

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Bart Weetjens is a product development engineer with a focus on appropriate technologies for developing countries. In 1998, Bart initiated the use of HeroRATS: trained giant African pouched rats as an alternative and sustainable landmine detector, in response to the global landmine challenge. Since 2000 APOPO has addressed humanitarian detection challenges in Africa: the detection of landmines and screening for TB. HeroRATS have received much international recognition. Bart is an ASHOKA Fellow and a SCHWAB Fellow, and a permanent member to the Global Agenda Councils. In 2009, he won the SKOLL Awards for social entrepreneurship. Bart is a Zen Buddhist monk and lives with his wife and two daughters in Tanzania.

Georgies Mgode is pursuing his PhD study at the Max Planck Institute for Infection Biology, Berlin, Germany. He has a Bachelors degree in zoology/botany from the Open University of Tanzania, and a Masters degree in zoology from the University of Pretoria, South Africa. At Sokoine University of Agriculture, Tanzania, Georgies has been involved in research on rodent-transmitted diseases of public health and veterinary importance (such as plague and leptospirosis), as well as rodent taxonomical studies. He is currently investigating the target compounds from TB organisms detected by the trained African giant pouched rats.

B Witkind (Wit) Davis is a volunteer for APOPO as part of her Masters of Public Health coursework in epidemiology and

Key messages

- ✦ Using African giant pouched rats for TB detection significantly increases case detection including detection of sputum smear negatives mostly missed by microscopy.
- ✦ Utilizing sniffer rats reduces the time required to screen for TB (less than 6 minutes are needed to analyse an average of 20 specimens which may require 8 hours for examination by microscopy) in turn reducing the workload in DOTS centres, which is directly related to the quality of diagnostic results.
- ✦ There are a number of steps to pass through to reach to the ultimate goal of using APOPO's new technology for the global fight against TB and the main challenge for the accomplishment of this task is funding.

biostatistics at the Tufts University School of Medicine in Boston, USA. Prior to APOPO, Wit worked for the International Fund for Animal Welfare responding to international disasters and overseeing scientific documentation and policy development. Wit holds an MSc from the Tufts University School of Veterinary Medicine and a BSc from Bates College. She maintains a long-standing commitment to identifying ways where animals and people can improve each other's health and welfare.

Christophe Cox is a Belgian national who has an MSc in product development and an MSc in development cooperation. He obtained three years' grassroots experience working in Kenya as a volunteer in a rural community development project between 1994 and 1997. He then joined APOPO at the start of the project. Having been involved in all aspects of the development of APOPO, Christophe gained knowledge in animal behaviour, chemistry and olfaction, as well in financial and administrative management. He was appointed CEO of APOPO in 2008.

Negussie Beyene, PhD, is APOPO's analytical chemist seconded by GICHD to manage APOPO's analytical explosives chemistry lab at SUA, in support of the REST research programme. He was an assistant professor at Addis Ababa University, Ethiopia before resuming his current post. Negussie has conducted research in laboratories in Austria, South Africa and Spain. His prior experience as a clinical chemist and head of the Regional Public Health laboratory in Ethiopia, lectureship of clinical chemistry and research experience in the development of sensors and biosensors for compounds of biomedical importance has led to a keen interest in biomedical sciences and to his active contribution in APOPO's TB project.

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