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ACTIVE TUBERCULOSIS DETECTION BY POUCHED RATS IN 2014: MORE THAN 2,000 NEW PATIENTS FOUND IN TWO COUNTRIES

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Tuberculosis (TB) is a major problem in poor countries because sensitive diagnostic tools are unavailable. In 2014, our pouched rats evaluated sputum from 21,600 Tanzanians and 9,048 Mozambicans whose sputum had previously been evaluated by microscopy, the standard diagnostic for TB. Evaluation by the rats revealed 1,412 new patients with active TB in Tanzania and 645 new patients in Mozambique, increases of 39% and 53%, respectively, when compared to detections by microscopy alone. These results provide further support for the applied use of scent-detecting rats.

Key words: applied behavior analysis, discrimination training, tuberculosis, pouched rats

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Many people contributed to the work we report. Notable among them are Bart Weetjens, APOPO's founder, and the trainers and technicians employed by APOPO. We appreciate all that they have done and are doing and offer heartfelt thanks for their good efforts.

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Worldwide, roughly one in three people have been exposed to tuberculosis (TB), which kills about 1.5 million people each year (World Health Organization [WHO], October 2015). Tuberculosis can be treated effectively with antibiotics if it is detected early, but the absence of an inexpensive and accurate diagnostic technology is a major problem in economically disadvantaged areas, where sputum-smear microscopy is widely used to detect the disease (Perkins, Roscigno, & Zumla, 2006). This is problematic because sputum-smear microscopy often misses around half of the cases of active TB (Reid & Shah, 2009).

Personnel at Anti-Persoonsmijen Ontmijnende Product Ontwikkeling (APOPO) have shown that pouched rats can detect human TB by sniffing sputum (i.e., mucus secreted from the lungs, bronchi, and trachea and ejected through the mouth) and that using the rats in second-line screening, in which they analyze samples initially screened by sputum-smear microscopy, increased the new case detection rate by 44% in 2009 (Poling et al., 2010) and by 43% in 2010 (Mahoney et al., 2011). Presumptive TB patients in these studies were Tanzanians who came to clinics (i.e., directly observed treatment, short-course [DOTS] centers) in Dar es Salaam seeking help with respiratory problems. Because of the clinical value of the rats as second-line TB screeners for this population, a second clinic was established in Maputo, Mozambique in 2013. We herein report the results obtained there and in Tanzania during 2014. When considered with prior findings, these data allow the performance of the rats to be compared across time and location, which is important in determining whether APOPO's TB-detection technology is exportable and generally useful. If second-line screening by the rats substantially increases TB detection at different points in time in two different countries, then that finding suggests that the TB-detection technology is likely to be of value in other low-income countries where TB is a problem.

METHOD

Procedure

In Tanzania, sputum samples were collected at 19 DOTS centers in Dar es Salaam and 3 DOTS centers in Morogoro. In Maputo, samples were collected from 13 TB clinics. Each presumptive TB patient provided two samples. Following preparation of a slide for smear microscopy at the clinic, each sample was frozen and transported to one of APOPO's labs. Lab technicians heated the samples to kill infectious microorganisms, then placed them in small pots for presentation to the rats.

The manner in which pouched rats are used to detect human TB is detailed elsewhere (Poling et al., 2011) and videos depicting the process are available online (apopo.org). In brief, the rats were trained to perform an operant discrimination task, where they paused above sputum samples known to contain the *mycobacteria* that cause TB and not to pause above other sputum samples. They worked in a chamber in which 10 holes were located an equal distance apart along the long axis of the floor. Pots containing sputum samples were placed immediately below these holes, which could be open or closed by moving sliding covers. A feeding hole in one end wall allowed reinforcers (mouthfuls of mashed banana mixed with crushed rat chow) to be delivered via a syringe inserted into the chamber.

When used operationally, a rat was placed in the chamber, the leftmost hole was opened, and the rat's response to that hole was observed by two trainers. One trainer was not aware of the clinical status of the sample contained below the hole, that is, whether a microscopist had evaluated it as TB-positive or TB-negative, but the second trainer had this information. If the rat paused above the hole for 3 s, the first trainer verbally stated that an indicator response had occurred and the second trainer verbally indicated whether or not a reinforcer should be delivered. Note that a single observer determined whether a rat paused for 3 s; no measure

of interobserver agreement was taken during operational TB detection.

Holes were opened and closed in sequence as the rat moved from left to right and sniffed at them. If the rat paused for 3 s above a sample that was TB-positive according to the clinic, a click was sounded and a trainer inserted a food-filled syringe into the feeding hole. Once the rat ate from the syringe, it was withdrawn, the cover over the hole with the positive sample was closed, the cover over the next hole was opened, and operations proceeded as just described. If the rat sniffed the hole above a sample but did not pause for 3 s, the cover was closed and the cover over the next hole was opened, regardless of whether the sample was clinic-positive or clinic-negative. The same procedure was employed if a rat made an indicator response above a clinic-negative sputum sample. This process was repeated until all 10 of the holes were sniffed. The presence or absence of the rat's indicator response (i.e., whether or not it paused for 3 s) at each hole was recorded and entered into a database. Once all holes were sniffed, new sputum samples were placed below the holes and the process recurred.

The total number of rats involved in operational TB detection differed somewhat across time, depending on whether the rats were needed for other research studies, but each sample was almost always evaluated by 10 different rats in Tanzania and by 9 different rats in Maputo (only 10 trained rats were available there, and one died in 2014). If one or more of those rats emitted an indicator response to a clinically-negative sample, then that sample was identified as a rat-positive sample. Laboratory technicians used fluorescent microscopy (FM) of concentrated sputum (Uddin et al., 2013) to determine whether or not such samples actually were TB-positive. All patients who provided one or two sputum samples that were determined to be TB-positive by this procedure were assumed to have active TB and the relevant clinics were informed of such patients and charged with finding and treating them. Ethical clearance to conduct the reported research was obtained from the Tanzanian National Institute for Medical Research and the Mozambican National Bioethics Committee. Because there was no direct contact with patients, the need for informed consent was waived.

RESULTS AND DISCUSSION

In Tanzania, sputum samples from 21,600 presumptive TB patients were evaluated, first by microscopists at DOTS centers, then by pouched rats at APOPO. The DOTS centers found 3,604 smear-positive TB patients and the rats detected an additional 1,412 patients with active TB, increasing the new case detection rate by 39%. Sputum samples from 9,048 patients in Mozambique were evaluated. The TB clinics found 1,217 smear-positive TB patients and the rats detected an additional 645 TB patients, increasing the new case detection rate by 53%. The upper and lower frames of Figure 1 show, by month, the cumulative number of patients with active TB detected in Tanzania and Mozambique, respectively, by first-line screening via microscopy alone and by microscopy followed by second-line screening via pouched rats with confirmation by FM of concentrated sputum. The two lines would overlap if the rats conferred no benefit. If the rats did confer benefit, the data paths would diverge, with the degree of divergence indicating the relative effectiveness of the intervention (i.e., second-line screening by the rats). There is substantial divergence in the data paths.

The magnitudes of the increases in new-case detections observed in Tanzania (39%) and in Mozambique (53%) in 2014 are similar to the 44% and 43% increases observed in 2009 and 2010, respectively, in Tanzania (Mahoney et al., 2011; Poling et al., 2010). Such sizeable and repeatable increases indicate that our second-line TB screening technique is exportable and can be upscaled; therefore, we are planning to open

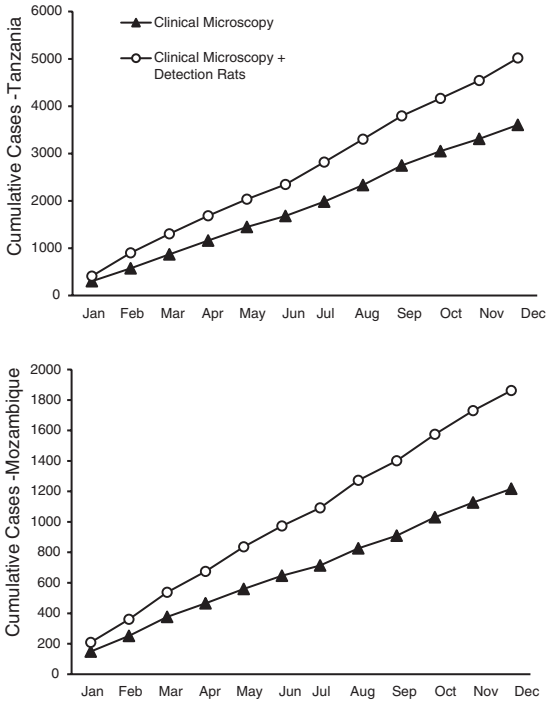

Figure 1. The cumulative number of cases of active TB detected across months in 2014 by microscopists at clinics alone and by microscopists at clinics and APOPO's pouched rats combined.

additional facilities in other metropolitan areas with high TB prevalence rates and inadequate TB-detection programs (e.g., Addis Ababa, Ethiopia). Of course, further research independent of APOPO is needed to determine the range of conditions under which this second-line screening is practical and useful. So far, only two labs have evaluated the technology and both operated under the auspices of APOPO.

This limitation notwithstanding, the applied significance of the present data is clear. In 2014, APOPO's rats detected more than 2,000 patients with active TB, which if undetected and hence untreated is severely debilitating—it is no accident that the disease was once called "consumption"—and now ranks alongside HIV as the leading cause of death worldwide (WHO, 2015). Moreover, every person with active TB is likely to infect others; a conservative estimate is that a person with active TB infects, on average, 10 other people per year (WHO, October 2015).

The criterion we used in designating a clinic-negative sample as rat-positive (one or more of the group of rats pausing for 3 s above it) is a liberal one that maximizes the likelihood that a sample that is actually positive for TB will be identified as TB-positive by the rats. On the down side, this criterion also maximizes the likelihood of false positives. Therefore, it is unsurprising that 91% of clinic-negative, rat-positive samples evaluated in Tanzania, and 89% of those evaluated in Mozambique, were not confirmed as TB-positive by FM of concentrated sputum, which was used to determine actual new-case detections. These new-case detections were reported to the appropriate clinics for treatment and it is for this reason that they, rather than all rat-positive patients, are reported in Figure 1.

Of course, this confirmatory technique is imperfect, and many of the samples not confirmed as TB-positive with it would likely have been so confirmed if a more sensitive technique, such as the "gold standard" for TB assessment, culturing followed by polymerase chain reaction analysis of cultures showing growth, was used. We did not use this technique because it is too slow, expensive, and difficult to be feasible under our working conditions. Although using fluorescent microscopy typically increases the number of cases of active TB detected relative to using light microscopy, and analyzing concentrated sputum does the same (Getahun, Harrington, O'Brien, & Nunn, 2007), we do not know how many of what appear to be false positives would be false positives relative to the highest quality reference standard. Be that as it may, the rats identified 52% and 49% of the clinic-negative samples as TB-positive in Tanzania and Mozambique, respectively. We recently completed a study which found that most of

the cases of active TB that can be detected by analyzing all of a set of clinic-negative samples with FM of concentrated sputum can be detected by analyzing only those samples identified as TB-positive by pouched rats (Edwards, Valverde, Mulder, Cox, & Poling, 2016). If this holds true with the samples used in the present study, then despite the use of the most liberal detection criterion possible, the rats were of considerable practical value.

Moreover, using a more conservative criterion for defining a sputum sample as rat-positive can substantially reduce the relative level of false positives without substantially reducing new case detections (e.g., Mahoney et al., 2012). A liberal criterion was used in 2014 because both APOPO labs had sufficient capacity to conduct a relatively large number of FM evaluations each day, which allowed us to prioritize maximization of case detections over efficiency. This will not be the case in all situations, and we are currently conducting cost-effectiveness analyses that will be helpful in determining how, and in some cases if, pouched rats should be used for TB detection.

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