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Short Report: Using Giant African Pouched Rats to Detect Tuberculosis in Human Sputum Samples: 2009 Findings

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Abstract. In 2009, giant African pouched rats trained to detect tuberculosis (TB) evaluated sputum samples from 10,523 patients whose sputum had previously been evaluated by smear microscopy. Microscopists found 13.3% of the patients to be TB-positive. Simulated second-line screening by the rats revealed 620 new TB-positive patients, increasing the case detection rate by 44%. These data suggest that the rats may be useful for TB detection in developing countries, although further research is needed.

Tuberculosis is a major public health problem in developing countries,[1] where sputum smear microscopy is the most common technique for diagnosing the disease. This method has limitations, the most serious being inaccuracy, when large numbers of sputum samples are analyzed.[2] Hence, alternative diagnostic tools are badly needed. In recent years, researchers have explored the possibility of using trained giant African pouched rats (*Cricetomys gambianus*), to screen sputum samples rapidly and accurately. A recent study[3] reported that 18 of 20 pouched rats could detect the presence of *Mycobacterium tuberculosis* in sputum samples at least as well as, and more quickly than, trained microscopists using the standard Ziehl Neelsen (ZN) method.[3]

In 2009, the rats analyzed sputum samples collected from five Direct Observation Treatment Short-Course (DOTS) centers in Dar es Salaam, Tanzania.[4] Sputum samples were used to prepare microscope slides that were stained by the ZN method and evaluated under light microscopes by technicians at the DOTS centers. Sputum remaining after smears were prepared was frozen and the samples were sent to Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling (APOPO) for subsequent analysis by the rats. Thus, the rats were used in a context that approximated second-line tuberculosis (TB) screening. This work describes their performance in that capacity.

Ethical clearance to conduct the reported research was obtained from the Tanzanian National Institute for Medical Research. The manner in which rats were maintained, trained, and used in evaluating sputum samples is detailed elsewhere.[3,5] In brief, they were rewarded with a mouthful of banana for pausing at sputum samples known to contain *M. tuberculosis* but not for pausing at other sputum samples. Through such training, they learned to pause reliably only at samples that were positive for TB. In simulated second-line screening the rats worked in a 10-hole stainless steel cage 205 cm long, 55 cm wide, and 55 cm high, shown elsewhere.[3] Sputum samples obtained from the DOTS centers and autoclaved to kill infectious microorganisms were presented in small pots located immediately below the holes. The status of samples with respect to microscopy was known and the rats were rewarded with food when they kept their nose in a hole above a TB-positive sample for at least 5 seconds, which defines an indicator response. Indicator responses to samples deemed by microscopists to be TB-negative were of particular interest, because such responses may indicate detection of TB-positive cases initially missed by the DOTS centers.

Ten rats evaluated every sample. Any sample reported as TB-negative by a DOTS center but TB-positive by two or more rats was evaluated by a second ZN microscopic analysis performed by a technician in our laboratory. Those cases found positive in this analysis were designated as new case detections. They were reported to the appropriate DOTS Centers for follow-up testing and, if appropriate, treatment.

In 2009, 23,101 sputum samples from 10,523 patients were screened by DOTS center microscopists, and then by the rats. The DOTS center microscopists found 2,487 positive sputum samples, taken from 1,403 different patients (13.3% of all patients tested). The rats as a group identified 2,274 of these samples and 1,335 of these patients as TB-positive. The rats also identified as TB-positive 3,012 DOTS-negative samples and 1,418 DOTS-negative patients. Analysis of smears by APOPO's microscopists confirmed the presence of *M. tuberculosis* in 927 of these smears, which came from 620 different patients. Thus, the use of rats in simulated second-line screening increased the new-case detection rate by 44%. Overall, there were 2,085 rat-positive samples and 898 rat-positive patients in which the TB bacillus was not evident to microscopists at the DOTS centers or at APOPO. Thus, when multiple rats evaluated the same sample, the overall sample-wise and patient-wise specificities were 89% and 90%, respectively. Table 1 shows the performance of 10 individual rats. Note that in all determinations sensitivity (and specificity) was determined by comparing rats' evaluations of samples to single evaluations of samples by DOTS-center microscopists. The sample-wise and patient-wise sensitivities of individual rats ranged from 82% to 90% and 85% to 91%, respectively, and their sample-wise specificities ranged from 91% to 95%. Our data did not allow us to calculate meaningful patient-wise specificities for individual rats. For samples and patients found negative at the DOTS center but positive by an individual rat, 28–47% and 24–45%, respectively, were confirmed positive by APOPO's microscopists. Respective mean values were 38% and 33%.

The present findings suggest that sniffer rats can be of value in second-line TB screening. The rats evaluated many samples quickly, offering the possibility of an inexpensive diagnostic,

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TABLE 1
Performance of individual rats

Sensitivity (%)*		Specificity (%)*		Confirmed (%)†	
Rat	Sample-wise	Patient-wise	Sample-wise	Sample-wise	Patient-wise
1	89	90	91	28	24
2	86	87	95	46	41
3	85	85	93	41	34
4	85	86	93	43	36
5	82	85	94	38	34
6	88	90	92	30	26
7	87	80	92	35	30
8	85	86	92	34	31
9	85	89	93	47	45
10	85	86	92	35	31

*Relative to the results of Direct Observation Treatment Short-Course (DOTS) center microscopists viewing each side on one occasion.
†DOTS-negative, rat-positive samples, and patients confirmed by Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling (APOPO) microscopists as tuberculosis (TB)-positive.

and they substantially increased the case detection rate relative to ZN microscopy alone. However, microscopy often fails to detect a substantial proportion of patients who have active TB, especially when done in poorly resourced laboratories in the developing world. For example, a recent study conducted in Nigeria revealed an initial sensitivity of only 23%.[6] Moreover, a second examination of sputum smears can increase TB detection.[7] No DOTS-negative and rats-negative samples were examined by APOPO's microscopists and it is unclear how many new cases would have been detected if they had examined the same number of slides as in the present study (3,012), but selected those slides at random from the 20,614 DOTS-negative slides. Because 30% of the DOTS-negative, rats-positive samples and patients were confirmed to be TB-positive, a level substantially higher than the DOTS centers' sample-wise detection rate of 10.7%, it certainly appears that the rats facilitated detection. Nonetheless, it is crucial to determine their sensitivity and specificity as TB detectors relative to culturing, which is the established gold standard for TB detection. Only in that way can their true accuracy be ascertained.

In the only comparison to culturing published to date,[3] two rats were tested with samples that were confirmed as positive or negative by culturing. Over 7 days, they evaluated 817 samples, of which 67 were TB-positive. Sensitivities, which reflect the ability to detect the presence of TB, were 73.1%, 73.1%, and 86.6% for the two rats as individuals and for the two together, respectively. Respective specificities, which reflect the ability to detect the absence of TB, were 93%, 93.8%, and 89.1%. These data suggest the rats are at least as sensitive and accurate as smear microscopy as usually conducted in developing countries, but further data are needed to confirm this suggestion.

Also needed are evaluations of the actual status of samples identified as TB-positive by the rats but negative in two evaluations by microscopists, one (before rat evaluation) in the DOTS centers and one (after rat evaluation) in our laboratory. Smear microscopy is notoriously poor at detecting low concentrations of *M. tuberculosis*,[2,8] and it is possible, even probable, that some of the apparent false indications by the rats were in fact actual, but unrecognized, case detections. Of course, they may simply be false indications. If the latter, it would be interesting to determine whether they contained other members of the genus *Mycobacterium*, which might share odor cues with *M. tuberculosis*.

Although not normally pathogenic in humans, these bacteria might produce opportunistic infections in people with human immunodeficiency virus (HIV), hence compromised immunity. Previous research indicates that *M. tuberculosis*, which is a major cause of death in people with HIV in the developing world,[9] is especially difficult to detect in sputum smears provided by members of this population. The HIV status of patients was not determined in this study, and another area for further research is determining the value of *Cricetomys* in detecting TB in people with HIV. Finally, research is needed to determine how to maximize accuracy while screening a large number of samples. For example, a sample was deemed positive in this study if at least 2 of 10 rats indicated it to be so. This criterion was based on pilot data, but full analysis of the receiver operator characteristics of rats as a detection system is needed if they are to be used optimally. In sum, *Cricetomys* hold promise as a tool for detecting TB in the developing world, but further research is needed to document their worth and to ascertain their appropriate applications. We have planned further studies, which are expected to be conducted in automated cages to facilitate data recording and reduce the likelihood of experimenter error, and intend to begin them in the near future.

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