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Pediatric tuberculosis detection using trained African giant pouched rats

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BACKGROUND: Tuberculosis (TB) diagnosis in children is a challenge with up to 94% of children with TB treated empirically in TB high-burden countries. Therefore, new diagnostic tests are needed for TB diagnosis. We determined the performance of trained rats in the diagnosis of pediatric TB and whether they can improve detection rate compared to the standard of care.

METHODS: Presumptive TB patients in 24 TB clinics in Tanzania were tested. Samples indicated as TB-positive by rats underwent confirmation by concentrated smear microscopy. TB yield of bacteriologically confirmed pediatric TB patients (≤5 years) was compared with yield of standard of care.

RESULTS: Sputum samples from 55,148 presumptive TB patients were tested. Nine hundred eighty-two (1.8%) were the children between 1 and 5 years. Clinics detected 34 bacteriologically positive children, whereas rats detected additional 23 children yielding 57 bacteriologically TB-positive children. Rats increased pediatric TB detection by 67.6%. Among 1–14-year-old children, clinics detected 331 bacteriologically positive TB whereas rats found the additional 208 children with TB that were missed by clinics. Relative increase in TB case detection by rats decreased with the increase in age ($P < 0.0001$).

CONCLUSION: Trained rats increase pediatric TB detection significantly and could help address the pediatric TB diagnosis challenges. Further determination of accuracy of rats involving other sample types is still needed.

Tuberculosis (TB) is a deadly disease, which killed 1.3 million people worldwide in 2016, of whom 130,000 were children (1). Sub Saharan Africa and South East Asia are among the regions with the highest burden of TB (2). A large proportion of TB patients remain undetected in most high-burden areas due to the poor sensitivity of smear microscopy widely used in these areas. In many low-income countries, the diagnosis of pediatric TB is solely based on clinical evidence and smear microscopy (3,4). Pediatric TB is difficult to diagnose due to paucibacillary that complicates mycobacteriological confirmation by smear microscopy and culture which yields <15% and between 30 and 40% sensitivities, respectively. The sensitivity of adult sputum culture is 80% (4). In addition, for children it is challenging to produce a sufficient volume of good-quality sputum needed for smear microscopy. As a result, many children with TB are not bacteriologically confirmed or not even diagnosed, which has major implications for their treatment success rate (5). In northern Tanzania it was found that pediatric TB contributed to 13% of the total TB burden and 75% of these had pulmonary TB, of which only 5.8% of all TB patients were bacteriologically confirmed (5). Nearly all of the children with TB were thus treated empirically (5).

There is a need for new diagnostic tests to enhance TB detection in children especially in low and middle-income countries. Trained TB detection rats have been in use in Tanzania and Mozambique as an enhanced case-finding tool under research, after smear microscopy, whereby they have increased TB case detection by over 40% (6,7). In recent years the percent increase in TB detection due to the trained rats has slightly dropped from 40 to 35–37% annually, probably reflecting the improved quality of smear microscopy in the collaborating DOTS centers. The current study on pediatric TB diagnosis by trained rats is unique as is specifically focusing on TB in children, whereas previous reports (6,7) focused on adult TB.

Detection rats target a specific combination of volatile organic compounds produced by *Mycobacterium tuberculosis* (8). They distinguish *M. tuberculosis* cultures from cultures of other microorganisms, and can also distinguish clinical sputum containing *M. tuberculosis* from the sputum containing other pathogens (9–11). The objectives of this study were to determine the ability of trained rats to detect pediatric TB, and whether rats could enhance the detection of pediatric TB over standard of care.

Methods

Setting

The study was conducted in Dar es Salaam, Coast region, and Morogoro, Tanzania from January 2011 to June 2015. Over 90% of the participants were from Dar es Salaam city with a population of nearly 5 million people (12).

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Received 18 July 2017; accepted 12 January 2018; advance online publication 4 April 2018. doi:10.1038/pr.2018.40

Study Population and Sampling

Presumptive TB patients who presented themselves in 24 directly observed treatments, short-course (DOTS) clinics in Dar es Salaam, Coast, and Morogoro region, Tanzania, were prospectively sampled for this study between 2011 and 2015. Sample collectors reaching collaborating DOTS centers across the city by using motorbikes collected all sputum samples daily and securely transported to the central TB detection rats' laboratory for evaluation. The rationale for this sampling was to determine the ability of rats to detect pediatric TB within a programmatic setting. During this period the non-profit organization partnered with Sokoine University of Agriculture known as Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling or "Anti-Personnel Landmines Detection Product Development" in English (APOPO) started working with a civil society organization in tracing back the additional patients found by rats to start the treatment. Presumptive TB patients were informed that their sputum samples would also be tested for TB by rats following the testing by microscopy in DOTS centers, whereby sputum smears are stained by Ziehl Neelsen (ZN) or auramine stain and are examined under bright field and fluorescent microscopy (FM), respectively. From January 2011 to June 2015, a total of 4,793 TB patients missed by DOTS centers were detected by rats and confirmed by concentrated smear microscopy (approved ZN or FM), whereby 2,289 were called back and started the treatment under the National tuberculosis and Leprosy Program. Patient tracking improved from 0.3% (1/386) found in 2011 to 56% (1020/1807) in 2013 and to 70% (444/633) found from January to June 2015, a period for current study. A steady increase in patients starting treatment after being diagnosed by TB detection rats and confirmation by microscopy continued being realized from 2015 onward and is attributed to the strengthened patient tracking and increasing acceptance of the research in use intervention. A nucleic acid amplification test for Mycobacterium tuberculosis (MTB) DNA and resistance to rifampicin (RIF) known as Xpert MTB/RIF was also available at some clinics for testing single specimens hence samples from those participants were not available for rat evaluation except when such children provided another sample. The bacteriological confirmation was thus through smear microscopy that according to the World Health Organization (13) defines a bacteriologically confirmed TB case as an individual from whom a biological specimen is positive by smear microscopy, culture or Xpert MTB/RIF (13).

TB Testing by Rats

Sputum samples already examined for TB through smear microscopy in the clinics were securely transported to APOPO's TB laboratory at Sokoine University of Agriculture, Morogoro, for rat evaluation. Detailed rat training and rat evaluation procedures have been previously described (14). In brief, samples were first heat inactivated at 100 °C for 30 min to kill microorganisms and render them safe for subsequent handling and evaluations. Samples were randomly arranged in containment bars and presented to trained rats. Animals were rewarded upon correct identification of known TB-positive control sputum samples. All other rat-indicated samples were pre-concentrated by adding 4% sodium hydroxide and centrifuging to obtain sediment for concentrated smear microscopy that is more sensitive than direct smear (15). Results of TB patients found by rats and confirmed by light-emitting diode fluorescence microscopy (LED-FM) (16) were submitted to the respective DOTS center and also to the healthcare workers for tracking the respective patients to start TB treatment. Contact details including telephone numbers of presumptive TB patients were collected by community healthcare workers working with a civil society organization called "Mapambano ya Ukimwi na Kifua Kikuu Temeke" (MUKIKUTE) for subsequent tracking of the additional TB patients detected by rats missed in hospitals.

Use of Animals

The use of the African giant pouched rats (*Cricetomys ansorgei*, formally *C. gambianus*) was approved by the Sokoine University of Agriculture, and we obtained Animal Welfare Assurance from the Department of Health and Human Service, National Institute of Health (NIH). Animals were treated humanely and received good care, including shelter, food, water, veterinary care, wooden toys, and a running wheel for physical exercise.

Data Analysis

We compared the TB yield by rats vs. smear microscopy (standard care) in children, adolescents, and adults. To compare the TB detection rate in children vs. the adolescents and adults, data were stratified by age group: children from 1 up to 5 years old; children 6–10 years old; adolescents 11–18 years old; and adults above 18 years of age. The detection rate was also evaluated in the total pediatric group consisting of children of 1–14 years old. The differences in proportions of case detection between the age groups were assessed by Pearson χ^2. Associations were considered statistically significant when *P*-values were ≤ 0.05. Statistical analyses were conducted using MedCalc (17,18).

Ethical Considerations

The Medical Research Coordinating Committee of the National Institute for Medical Research (NIMR) granted permit for TB screening in humans using trained rats (Permit no. NIMR/HQ/R.8c/Vol.II/495). Up to 70% of the additional TB patients detected by rats and confirmed by concentrated smear microscopy were traced back and received TB treatment in the respective health centers under the National Tuberculosis and Leprosy Program. The ethical committee waived the need for informed consent for this operational research study undertaking second-line TB testing after DOTS centers routine TB diagnostic care.

RESULTS

A total of 55,148 presumptive TB patients were tested, out of which 982 (1.8%) were children aged 1–5 years with sex ratio (male:female 1:1). There were 2,121 (3.8%) 6–10-year-old children (sex ratio 1:0.9), whereas 4,960 (9.0%) participants were 11–18-year old (sex ratio 1:1). Participants older than 18 years were 47,085 (85.4%, sex ratio 1:0.7) (**Table 1**). DOTS centers detected 34 bacteriologically confirmed patients aged 1–5 years, whereas detection rats detected additional 23 bacteriologically confirmed TB-positive children (13), yielding

Table 1. TB patients detected in different age groups by DOTS smear microscopy and by trained rats confirmed by LED-FM

Age group, years	No. of presumptive TB patients (%)	DOTS smear positive TB	Detection rats and LED-FM confirmed TB	Increase in case detection through rats and LED-FM % ($P<0.0001$)
1–5	982 (1.8)	34	57	67.6
1–14	4,629 (8.3)	331	539	62.8
6–10	2,121 (3.8)	94	129	37.2
11–18	4,960 (9.0)	775	952	22.8
>18	47,085 (85.4)	7,448	9,958	33.7

a total of 57 bacteriologically confirmed children in this age group. Rats therefore increased pediatric TB case detection by 67.6% (23/34 children with TB). DOTS centers detected 94 children aged 6–10 years, and rats detected an additional 35 patients, yielding 129 bacteriologically confirmed patients and increasing case detection in this age group by 37.2%. In adolescents, DOTS centers detected 775 patients, and rats detected additional 177 patients, yielding a total of 952 bacteriologically confirmed patients. Rats increased case detection of adolescents by 22.8% (177/775 patients). For a typical pediatric group (1–14 years) with a sex ratio of 1:0.997 (male:female), DOTS centers detected 331, and rats found additional 208 TB-positive children increasing case detection by 62.8%. In adults, DOTS centers detected 7,448, and rats found additional 2,510 patients increasing case detection by 33.7% (**Table 1**). The increase in pediatric TB case detection rate (67.6%) was statistically significantly higher than the increase found in age groups older than 5 years ($P<0.0001$, 95% CI 26.1–33.4, $\chi^2=239.44$, and DF = 1).

The TB yield by detection rats was higher in children and decreased with increase in age, whereby it reached a plateau in adulthood. The highest increase in case detection through detection rats was found in children under the age of 5 (**Figure 1**).

Bacterial Levels and Detection Rates

The quantity of TB bacilli in sputum (smear grade) was low (scanty) at a younger age where rats found more additional TB patients than in adults with a large number of bacteria in sputum samples (1+, 2+, and 3+), which were highly detected by TB clinics (**Table 2**).

The number of bacteria in sputum samples determined by smear grading was lower in children and increased with the increase in age (**Table 2**). The additional TB yield by rats was higher in sputum with scanty bacilli than in sputum with more bacilli (1+, 2+, and 3+) (**Figure 2**).

Over the 5-year period, the detection rats detected more TB patients in children aged 1–5 years, yielding a prevalence of 5.8% compared with 3.5% by TB clinics (**Figure 3**). This difference in TB yield by rats and TB clinics was statistically significant ($P=0.0155$, $\chi^2=5.855$, and DF = 1).

DISCUSSION

This study showed that in Tanzania, detection rats combined with concentrated LED-FM increased the detection of bacteriologically confirmed pediatric TB by 68% over standard of care. Children up to 5 years old are known to produce poor-quality sputum, compromising the quality of smear-microscopy results in TB clinics (5). The proportion of smear-positive TB patients missed by DOTS microscopy who were detected by rats was statistically significantly higher in

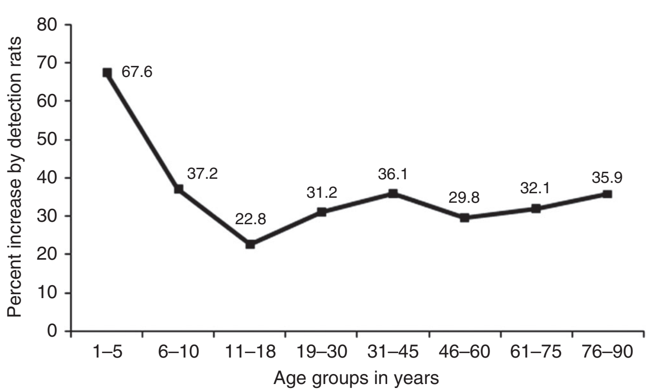

Figure 1. Tuberculosis detection rate by age group.

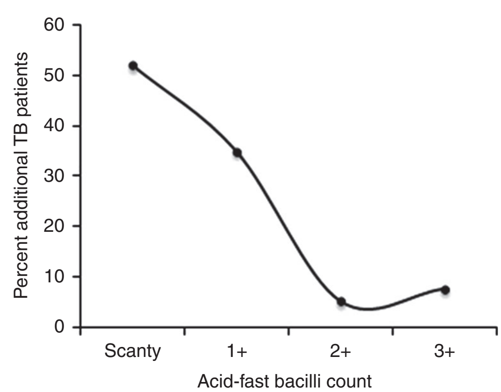

Figure 2. Proportion (%) of additional children detected by rats by smear microscopy grading.

Table 2. Tuberculosis yield in different smear grades by TB clinic microscopy and detection rats

	1–5 years		1–14 years		>18 years	
	DOTS smear	Additional by rats	DOTS smear	Additional by rats	DOTS smear	Additional by rats
Patients (*n*)	34	23	331	208	7,448	2,510
Scanty	6 (17.6%)	10 (43.5%)	62 (18.7%)	108 (52%)	1,149 (15.4%)	1,060 (42.2%)
1+	11 (32.4%)	10 (43.5%)	128 (38.7%)	73 (35%)	2,471 (33.2%)	765 (30.5%)
2+	7 (20.6%)	0 (0%)	70 (21.14%)	11 (5.3%)	1,503 (20.2%)	360 (14.3%)
3+	10 (29.4%)	3 (13%)	71 (21.5%)	16 (7.7.%)	2,325 (31.2%)	325 (13%)
Total yield	57		539		9,958	

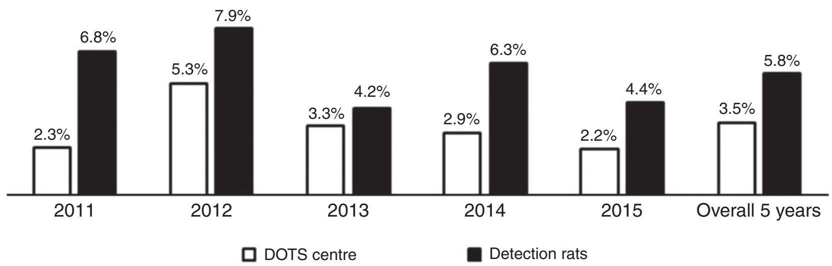

Figure 3. Tuberculosis yield by DOTS smear microscopy and detection rats for children (1–5 year) in 5-year period.

children aged 1–14 years than in adults, indicating that trained rats can help address the difficulties of detecting pediatric TB associated with sputum quality and bacilli load that can be below the detection limits of conventional smear microscopy. The observation that rats produced higher additional yield in samples with scanty bacilli than in samples with more bacilli suggests that despite quality and quantity, sputum from children with TB contains an adequate amount of *Mycobacterium tuberculosis*-specific volatile-odor compounds (8–10), enabling rat detection.

Although the proportion of smear-positive TB was higher among adults than in children, the percentage of additional TB case detection by rats dropped from 67.6 to 33.7% in adults. This suggests improved sample quality and quantity in adults and higher bacterial load, which improved smear-microscopy results. Nevertheless, still more than a quarter of the adults with smear-positive TB were missed by the DOTS clinics. Previous studies have shown that the TB case detection rate increases by over 40% in the general population when trained rats are used as an enhanced case-finding tool (6,7). In the present study, rats increased case detection by 33.7% in adults that is slightly lower than 40% reported previously. The higher proportion of case detection (67%) in children suggests that TB yield by rats is a function of the study-population age group in which conventional microscopy do not perform well.

This research intervention involving TB testing by trained rats and community-based patient tracking of new TB patients missed by hospitals enables treatment initiation up to 70% that is a significant proportion, given that these additional patients were considered TB negative in hospitals, and hence majority were initially left untreated.

The limitation of this study includes the absence of Xpert MTB/RIF or culture as a reference standard for comparing the yield of DOTS microscopy and detection rats technology. These need to be considered in further investigation on the potential of detection rats in enhancing TB diagnosis in children.

In conclusion, the detection rats show high potential for enhanced case finding among pediatric presumptive TB patients. This could be a major step forward in TB control. Further investigation is needed with more sensitive diagnostic tools to determine the actual ability and limitations of detection rats in the diagnosis of pediatric TB. Such studies should also consider investigating the ability of trained rats to detect TB in other samples than sputum, such as urine, gastric lavage, and nasopharyngeal aspiration. Urine could provide noninvasive sampling, whereas gastric and nasopharyngeal aspiration is widely used when children cannot produce sputum samples.

ACKNOWLEDGMENTS

We thank medical staff in collaborating DOTS centers in Dar es Salaam, Coast, and Morogoro for their cooperation. Patient tracking by MUKIKUTE and PASADA healthcare workers is much appreciated.

STATEMENT OF FINANCIAL SUPPORT:

This work was funded by UBS Optimus Foundation; Carraresi Foundation; and the Belgium Development Cooperation.

DISCLOSURE

G.F.M., C.L.C., S.M., and C.M. are fully working with APOPO project.

Disclosure: The authors declare no conflict of interest.

REFERENCES

1. World Health Organization. Global tuberculosis report 2017. 2017.
2. World Health Organization Global Tuberculosis Report 2015, 20th edn. WHO/HTM/TB/2015.22 http://www.who.int/tb/publications/global_report/en/ Accessed 4 December 2015.
3. Cruz AT, Starke JR.. Clinical manifestations of TB in children. Pediatr Respir Rev 2007;8:107–17.
4. Eamranond P, Jaramillo E.. Tuberculosis in children: reassessing the need for improved diagnosis in global control strategies. Intl J Tuberc Lung Dis 2001;5:594–603.
5. Mtabho CM, Irongo CF, Boeree MJ, Aarnoutse RE, Kibiki GS.. Childhood tuberculosis in the Kilimanjaro region: lessons from and for the TB programme. Trop Med Intl Health 2010;15:496–501.
6. Poling A, Weetjens BJ, Cox C, et al. Using giant African pouched rats to detect tuberculosis in human sputum samples: 2009 findings. Amer J Trop Med Hyg 2010;83:1308–10.
7. Mahoney AM, Weetjens BJ, Cox C, et al. Using giant African pouched rats to detect tuberculosis in human sputum samples: 2010 findings. Pan Afr Med J 2011;9:28.
8. Mgode GF, Weetjens BJ, Nawrath T, et al. *Mycobacterium tuberculosis* volatiles for diagnosis of tuberculosis by Cricetomys rats. Tuberculosis 2012;92:535–42.
9. Mgode GF, Weetjens BJ, Nawrath T, et al. Diagnosis of tuberculosis by trained African giant pouched rats and confounding impact of pathogens and microflora of the respiratory tract. J Clin Microbiol 2012;50:274–80.
10. Mgode GF, Weetjens BJ, Cox C, et al. Ability of Cricetomys rats to detect *Mycobacterium tuberculosis* and discriminate it from other microorganisms. Tuberculosis 2012;92:182–6.
11. Reither K, Jugheli L, Glass TR, et al. Evaluation of Giant African pouched rats for detection of pulmonary tuberculosis in patients from a high-endemic setting. PLoS ONE 2015;10:e0135877.
12. Ministry of Finance. Population and Housing Census: Population Distribution by Administrative Areas. Dar es Salaam: Tanzania National Bureau of Statistics, 2012.
13. World Health Organization Definitions and reporting framework for tuberculosis – 2013 revision. Updated December 2014.

14. Weetjens BJ, Mgode GF, Machang'u RS, et al. African pouched rats for the detection of pulmonary tuberculosis in sputum samples. Int J Tuberc Lung Dis 2009;13:1–7.
15. Uddin MKM, Chowdhury MR, Ahmed S, et al. Comparison of direct versus concentrated smear microscopy in detection of pulmonary tuberculosis. BMC Res Notes 2013;6:291.
16. Anthony RM, Kolk AH, Kuijper S, Klatser PR.. Light emitting diodes for auramine O fluorescence microscopic screening of *Mycobacterium tuberculosis*. Intl J Tuberc Lung Dis 2006;10:1060–2.
17. Campbell I. Chi-squared and Fisher-Irwin tests of two-by-two tables with small sample recommendations. Stat Med 2007;26: 3661–75.
18. Richardson JTE. The analysis of 2 x 2 contingency tables - yet again. Stat Med 2011;30:890.