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ORIGINAL ARTICLE

Laboratory development and field testing of sentinel toys to assess environmental faecal exposure of young children in rural India

Belen Torondel[a,*], Yaw Gyekye-Aboagye[a], Parimita Routray[a], Sophie Boisson[a], Wolf Schimdt[a] and Thomas Clasen[a,b]

[a]*Faculty of Infectious Diseases, London School of Hygiene and Tropical Medicine, Keppel St, London, WC1E 7HT, UK;* [b]*Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA*

*Corresponding author: Tel: +44 (0) 20 7636 2934; E-mail: Belen.torondel@lshtm.ac.uk

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Background: Sentinel toys are increasingly used as a method of assessing young children's exposure to faecal pathogens in households in low-income settings. However, there is no consensus on the suitability of different approaches.

Methods: We evaluated three types of toy balls with different surfaces (plastic, rubber, urethane) in the laboratory to compare the uptake of faecal indicator bacteria (*Escherichia coli)* on their surface. We performed bacteria survival analysis under different environmental conditions and tested laboratory methods for bacteria removal and recovery. In a field study we distributed sterile urethane balls to children <5 from 360 households in rural India. After 24 hours, we collected and rinsed the toys in sterile water, assayed for thermotolerant coliforms (TTC) and explored associations between the level of contamination and household characteristics.

Results: In the laboratory, urethane foam balls took up more indicator bacteria than the other balls. Bacteria recovery did not differ based on mechanic vs no agitation. Higher temperatures and moisture levels increased bacterial yield. In the field, the only factor associated with a decreased recovery of TTC from the balls was having a soil (unpaved) floor.

Conclusions: Sentinel toys may be an effective tool for assessing young children's exposure to faecal pathogens. However, even using methods designed to increase bacterial recovery, limited sensitivity may require larger sample sizes.

Keywords: Environmental contamination, Hygiene, Indicators, Microbiology, Sanitation, Water

Introduction

In the study of environmental health interventions such as improving water access or sanitation, researchers, implementers and programme evaluators often seek to estimate the level of environmental exposure of a population to faecal pathogens. Non-laboratory methods include household surveys, community transect walks and sanitary surveys.[1–3] These however, are only proxy markers for actual pathogen exposure. Complex microbiological sampling techniques, such as microbial source tracking, are increasingly used to measure pathogen exposure directly.[4–7] While efforts are being made to develop comprehensive approaches,[8] most of the current approaches involve sampling for faecal contamination in drinking water, on hands, household surfaces (including latrines) and flies (mechanical vectors). To date, however, these methods have shown limited sensitivity to detect differences in exposures that may be associated with disease.

A further approach that focuses specifically on the young child is the use of sentinel toys. A microbial survey of faecal contamination and selected diarrheal pathogens in households of peri-urban Bagamoyo, Tanzania, reported high levels of *Escherichia coli* and *Enterococci* on formites (including toys) and on surfaces.[9] Vujcic et al.[10] used sentinel toys as a standard fomite to investigate whether faecal contamination of children's toy was a marker of household contamination, by evaluating the association between household cleanliness and faecal contamination of toys in rural Bangladesh. They also tested whether the levels of contamination of toys provided by the study were different to the toys already owned by the households. Vuic et al. showed that contamination of households toys was correlated with contamination of study-provided balls, and also that toys from

cleaner households (defined as a household with a toilet and with no visible human faeces in the living or adjacent place) had fewer faecal coliforms than less clean households. They did not observe differences in faecal streptococci levels. They also found good correlation between faecal coliform contamination in household toys and study-provided toys.[10] A study in Honduras[11] examined the effect of four water and sanitation conditions (access to latrines, improved sanitation, improved water and the plastic biosand filter) on the levels of total coliforms in existing and introduced toys. They found higher levels of *E. coli* on toys in households without latrines and/or improved sanitation.[11]

Although both studies suggested that using sentinel toys could be a good method to estimate the impact of wash intervention in pathogens in the environment, the amount of bacteria measured in the ball may depend on many environmental and methodological factors that need to be investigated to enable standardisation of methods.

This study consisted of both a laboratory component and a field component. In the laboratory, we aimed to determine: whether there is a difference in bacteria retention on different types of toy balls; which technique of bacteria recovery achieves the highest yields; and whether bacteria survival is affected by soil type, time and temperature. In the field, we used the lessons from the laboratory to explore whether the level of contamination on toys distributed among children aged under five in rural India correlates with household characteristics.

Material and methods

Laboratory tests

Ball selection

We chose playing balls as they have been used previously in other studies as standardised fomites to which young children are exposed serving as an indicator of faecal contamination level in the domestic and peri-domestic environment. Three types of balls, all of a similar size (approximately 7 cm diameter) and a non-porous surface were tested: a coated urethane foam, rubber, and high density polyethylene plastic.

Recovery of bacteria from different balls

Twenty units of each type of ball were sterilized by washing with ethanol alcohol, allowed to dry and placed in a sterile bag. Tryptone Soya Broth (TSB) (Oxoid CMO129, Basingstoke, Hampshire, UK) was spiked with *E. coli (ACTC 25922)* to a concentration of 1.2×10^2 CFU/ml. All balls were fully immersed in the contaminated fluid for 30 min. The contaminated toy balls were then removed from the solution and left at room temperature (approximately 22°C) for 15 min. Thereafter, each individual contaminated ball was placed in a sterile bag (Whirl-Pak bags, Nasco, Fort Atkinson, WI, USA) with 300 ml of distilled water. The bacteria from half of each type of ball were recovered using a laboratory shaking device (Thermo Scientific MaxQ HP, Waltham, MA, USA) at 50 rpm and the other half was left in the bag without shaking (static) for 1 min. Using the membrane filtration method,[12] 100 ml of each rinse was then filtrated through a 0.45 micron sterile membrane (Oxoid). The membrane was placed on Brilliance *E.coli*/coliform agar CM1046 (Oxoid) and incubated at 37°C for 24 h. After 24 h the number of colony forming unit (CFU) on the membrane was counted and then divided by the surface area of each type of ball.

Survival of bacteria on different balls

In the second experiment, we compared the number of CFU of bacteria recovered from each type of artificially contaminated ball at different time points. Balls were contaminated using the same protocol as above and then left at room temperature (approximately 22°C) for different times: 15 m, 3 h, 6 h and 24 h. We used 10 balls of each type for each different time point. We choose the non-mechanical agitation method for further laboratory and field experiments. We then selected the ball type that retained most bacteria on the surface (coated urethane foam balls) and measured the amount of bacteria remaining after 24 h under two different temperature conditions: 22°C and 37°C (16 units for each type of ball), again using the same contamination and assay protocol described above.

Soil contamination test

In order to explore how soil moisture content (10 and 40%) influences survival and uptake of bacteria on balls at two different time points (15 min and 24 h), we used commercially available soil (Top Soil, Homebase, Milton Keynes, Buckinghamshire, UK) that was dried and sterilized in an oven (Fisher Isotemp 100 Series Model 116G Lab Oven, Loughborough, Leicestershire, UK) at 100°C for 1 hour. Then, 40 samples of 50 g of sterile soil each were prepared; 20 samples each were mixed with phosphate buffer saline (PBS) to achieve 10% vs 40% moisture content. Two millilitres of *E. coli* suspension containing 1.2×10^4 CFU/ml was added to each sample and then mixed thoroughly by agitation. The content was emptied into a bigger Petri dish where each individual ball was placed and then left on a shaker for 30 min. Each ball was then removed from the soil and placed in a sterile beaker at room temperature (approximately 22°C) and left for 15 min vs 24 h. Each individual ball was placed in a sterile bag washed with 300 ml of sterile PBS by using a laboratory shaker; 100 ml of this rinse was assayed for *E. coli* as described above.

Field experiments

Study setting

We conducted this study in rural India, Puri District (Odisha) in the context of a randomised trial to evaluate the health impact of a sanitation intervention conducted between July 2010 and October 2013. The setting and characteristics of the study population have already been described.[13]

Field work description: distribution, collection and other data collected

Coated urethane foam balls purchased in the local market were sterilized and distributed to children <5 from a sample of 360 households. Participants were selected using convenience sampling, as we visited the same households where other environmental samples were collected. We gave one ball to the youngest child present in the selected household on the day of the visit and encouraged the child's caretaker to have the child

play with the ball in the household setting. The following day we came back at the same time. The ball was placed in a Whirl-Pak sterile bag with 300 ml of distilled water. Upon collection the enumerator shook the bag for 15 s, rubbed the ball from the outside of the bag on all sides for 1 min and then removed the toy from the bag. The toy rinse was placed on ice and processed within 6 h of collection to assess levels of thermotolerant coliforms (TTC). Microbiological assessment was performed using the membrane filtration technique[14] on membrane lauryl sulphate medium (Oxoid) using a DelAgua field kit (DelAgua Water Testing Limited, Marlborough, Wiltshire, UK).

During the household visit, we also collected data on household characteristics using a questionnaire survey covering presence of a hand washing facility, animals at home, the use of cow dung for plastering the floor and soil type. We also collected information on the household's water source, presence of dirt on the child's fingers, whether any member of the household practiced open defecation, child faeces disposal method and the reported frequency of child having played with the ball. We used demographic data and data on functional latrine coverage collected in the main study at the mid-point of follow up.

Data and statistical analysis

Bacteria counts were log transformed (log10). A value of 1 was added to the counts prior to log transformation to remove zero counts. As the data were not normally distributed, we used the Kruskal-Wallis non-parametric test in order to compare the logarithmic mean concentration of bacteria recovered between more than two groups, and the Wilcoxon rank sum test for the comparison of two groups. For the field experiments, we dichotomized the data in the presence/absence of TTC since bacteria counts of TTC assays presented strong zero-inflation (46% of values) and right-truncation (too numerous to count—17% of values). Univariate analysis of associations between household characteristics and bacteria counts from toys was conducted using log-binomial regression (log-link function, binomial distribution), which calculates the risk ratio of contamination (counts>0) between each level of exposure. All statistical analysis was performed with the statistical package STATA version 11.0 (StataCorp LP, College Station, TX, USA).

Results

Laboratory results

Foam balls retained more test bacteria after 15 min of contamination, than rubber balls and plastic balls (4.37, 4.18 and 4.03 mean log CFU/cm^2 respectively) but the differences were small. For all three types of balls, recovery of bacteria after contamination was similar after mechanical shaking compared with no shaking (static). For the remaining experiments, bacterial recovery was done without mechanical automatic shaking (Table 1).

In the second set of experiments we observed that after 3 h of contamination the amount of bacteria decreased by around 80% regardless of ball type. After 24 h the concentration of bacteria was reduced by 99.9%. After 3 and 6 hours foam balls retained more bacteria than plastic and rubber balls (p=0.03 and 0.05 respectively). Therefore we chose to continue all the experiments with foam balls (Table 2).

Table 1. Laboratory results comparing bacteria retention after 15 min of contamination in different types of ball materials, and comparing recovery using mechanical shaking vs no shaking

	Mean (log CFU/cm^2) (SD)		
Type of ball	Mechanical shaking	No shaking	p-value[a]
Foam	4.34 (0.70)	3.91 (0.93)	NS
Plastic	4.03 (0.57)	4.10 (0.78)	NS
Rubber	4.18 (1.09)	3.96 (0.69)	NS

NS: not significant.
[a] Wilcoxon rank sum test.

Table 2. Laboratory results showing bacteria survival on the surface of the balls after artificial contamination at different times

	Type of ball, mean (log CFU/cm^2) (SD)			
Time	Foam	Plastic	Rubber	p value[b]
15 min	3.91 (0.93)	4.10 (0.78)	3.96 (0.69)	NS
3 h	0.71 (0.21)[a]	0.42 (0.16)[a]	0.61 (0.33)[a]	0.036
6 h	0.57 (0.15)[a]	0.40 (0.13)[a]	0.49 (0.14)[a]	0.05
24 h	0.02 (0.02)	0.01 (0.01)	0.02 (0.05)	NS

NS: not significant.
[a] Recovery of bacteria at 3 or 6 h was greater than after 24 h, (p<0.001); Wilcoxon rank sum test.
[b] Kruskal-Wallis test.

We further tested how temperature influences the survival of bacteria on foam balls. We observed that, after 24 h, more bacteria survived at higher temperatures (mean (log CFU/cm^2)=0.28 at 37°C vs 0.09 at 22°C, p=0.003).

In the last set of experiments we simulated bacteria uptake by toys from floors and surfaces. We observed that balls contaminated in soils with higher moisture (40%) retained more bacteria than when contaminated in less humid soils (10%) (mean log CFU/cm^2=4.10 vs 3.19 respectively, p=0.04 n=20) (Table 3). After 24 hours of contamination bacteria retention declined 99.6% in less humid soils and 99.3% in soils with higher moisture.

On the whole the results suggested the use of foam balls for field testing.

Field results

A total 326 households were sampled from 60 villages. The average number of persons per household was 3.63 (SD=2.33) (Table 4). The majority of the households 277/326 (84.6%) had or claimed to have a government issued below-poverty-line (BPL) card. From 326 households 108 (33.1%) were 'pucca'

Table 3. Laboratory results of bacteria retained in foam toys after soil contamination

	Mean (log CFU/cm^2) (SD)		
Humidity soil levels	10%	40%	p value
15 min	3.19 (1.01)[a]	4.10 (0.53)[a]	0.04
24 h	0.01 (0.01)	0.03 (0.03)	NS

NS: not significant.
[a] Recovery of bacteria at 15 min and 24 h (p=0.001) Wilcoxon rank sum test.

Table 4. Socio-economic characteristics of study households at baseline survey (n=326)

Characteristics	Total, n=326
Average persons per household (SD)	3.63 (2.3)
Education level of household head	
None	45 (13.8%)
Literate without formal schooling	48 (14.7%)
Primary school not completed	58 (17.8%)
Primary school completed	143 (43.9%)
Some secondary school or more	32 (9.8%)
Education level of caregiver	
None	35 (10.7%)
Literate without formal schooling	18 (5.5%)
Primary school not completed	37 (11.4%)
Primary school completed	199 (61.0%)
Some secondary school	37 (11.3%)
Has BPL card	277 (84.6%)
House structure	
Cement wall and roof (pucca)	108 (33.1%)
Cement wall (semi pucca)	75 (23.0%)
No cement (kucha)	143 (43.9%)
Electricity	253 (77.7%)
Owns agricultural land	245 (75.2%)
Owns poultry/livestock	179 (54.9%)
Water source	
Piped	6 (1.8%)
Deep tube well	133 (40.8%)
Shallow tube well	140 (42.9%)
Open well	9 (2.8%)
River/lake/pond/canal	25 (7.7%)
Other	13 (4.0%)
Location of water source	
In own dwelling	55 (16.9%)
In own compound	43 (13.2%)
Outside compound	228 (69.9%)

BPL: below poverty line.

(concrete), 143 (44%) were 'kucha' (mud and dung) and the rest were semi-pucca. Most of the participants owned agriculture land 245/326 (75.2%) and 179/326 (54.9%) owned poultry or livestock. Few households 6/326 (1.8%) had tap water as main water source, while the majority obtained water from deep or shallow tube wells 274/326 (84.1%). Most of the households 228/326 (69.9%) had the water source located outside the compound and only 55/326 (16.9%) had their water source in their own dwelling.

Fewer toys were contaminated in households that were situated in a village with more than 50% latrine coverage (post-intervention), reported no open defecation, had no animals, used plaster floors with cow dung, had hand-washing facilities with soap, practised safe disposal of child faeces, had protected source water in the dwelling, but statistical support for these differences was low (Table 5). There was statistical evidence that households with floors of soil or mud surfaces in the entrance (p=0.02) and living area (p=0.05) had a lower amount of bacteria on toys compared with cement floors. Having a latrine or a functional latrine was not associated with fewer bacteria on toys. Nearly all mothers (310/326, 95.1%) reported that the child played many times with the toy in the 24 h period versus 16/326 (4.9%) who reported that the child did not play with the toy at all. No difference in contamination was found (data not shown). Socioeconomic factors were not associated with different bacteria levels in toys.

Discussion

Our laboratory experiments suggest that the type of balls used and ambient temperature and humidity can influence the bacteria recovered after artificial contamination. After optimising the methodology based on the laboratory studies, our field study failed to identify strong predictors for bacterial contamination, except floor type with soil surfaces decreasing toy contamination.

Laboratory results showed that after 3 h of contamination, *E. coli* recovered decreased almost 80% in the three types of ball, foam balls being the ones that retained more bacteria. This may be explained by factors affecting the degree of microbial adhesion and survival on a surface, including material geometry, porosity, roughness, composition and hydrophobicity.[15] We further tested two different methods of bacterial recovery and concluded that a mechanical shaker did not improve yield. Higher temperatures and moisture levels in the soil increased *E. coli* survival in artificially contaminated balls. These results are consistent with other studies that showed that *E. coli* could survive longer in environments that present higher moisture levels.[15,16] Moisture not only influences survival but also transfer efficiency. Gerardo et al. examined the effect of different humidity on fomite-to-finger transfer efficiency of five model organism from different inanimate surfaces (fomites)[17] and showed that transfer efficiency was greater under high relative humidity for most organisms tested. Non-porous surfaces had greater transfer efficiency than porous surfaces, especially under higher relative humidity levels. This can explain why we recovered fewer bacteria on balls from households that had soil floor surfaces compared to smooth concrete surfaces. Another possible explanation for our finding is that houses with soil are more prone to be plastered

Table 5. Univariate analysis assessing association between household characteristics and the risk ratio of contamination on the ball (counts >0)

Household characteristics	Denominator (individuals)	Number of toys contaminated	Effect size (RR)[a]	95% CI	p value
Having a latrine (n=285)					
No	159	82			
Yes	126	75	1.15	0.93–1.42	NS
Having a functional latrine (n=285)					
No	200	105			
Yes	85	52	1.16	0.94–1.44	NS
Villages latrine coverage (n=314)					
<50%	274	151			
>50%	30	14	0.84	0.56–1.25	NS
Household practising open defecation (n=314)					
Yes	274	151			
No	40	21	0.95	0.69–1.30	NS
Animals (n=314)					
Yes	215	119			
No	99	53	0.96	0.77–1.20	NS
Plaster with cow dung (n=314)					
Yes	238	130			
No	76	42	1.01	0.80–1.27	NS
Exterior entrance surface (n=322)					
Cement	197	116			
Soil	128	58	0.77	0.62–0.97	0.03
Interior Floor surface (n=322)					
Cement	193	113			
Soil	129	61	0.8	0.63–1.00	0.05
Cooking area floor surface (n=322)					
Cement	42	23			
Soil	280	151	0.98	0.73–1.32	NS
Child defecates in the compound (n=314)					
Yes	176	91			
No	138	81	1.13	0.92–1.38	NS
Child's fingers visibly dirty (n=279)					
Yes	144	81			
No	135	76	1	0.81–1.23	NS
Water source (n=260)					
Protected	223	125	1		
Unprotected	37	18	0.86	0.60–1.22	NS
Water source located (n=260)					
In own dwelling	45	29	1		
Outside dwelling	215	114	0.82	0.64–1.06	NS
Washing facility with soap (n=314)					
Yes	30	16			
No	284	156	1.02	0.72–1.46	NS
Having below poverty line card (n=325)					
Yes	205	113	1		
No	55	30	0.98	0.75–1.29	NS
Education of head of household (n=260)					
None or primary not completed	234	126	1		
Primary completed or more	26	17	1.2	0.89–1.63	NS

NS: not significant.
The first row of the dichotomous variables are the reference category.
[a] Risk ratio from binomial regression (categories: 0, 1–300 colony forming units).

with cow dung, which may have antiseptic properties[18–20]; however our study did not show any difference in contamination levels of toys between households plastered and not plastered with cow dung. Further research should explore this counter-intuitive result regarding concrete floors.

Differences in moisture, temperature, type of sentinel toy material or time of contamination may well explain the disparity of findings between our results and previous studies. Further, the amount of environmental contamination and the transfer efficiency of this contamination from surfaces or hands to the fomite is likely to influence bacterial yield.

To enable comparison we measured TTC in toys distributed in the field as main indicator for faecal contamination via other transmission routes (hands, water and flies) in the main sanitation trial. Faecal bacteria have been used as indicator of the potential presence of pathogens in different environments, such as soils, water bodies, or hands. Because of the difficulties of detecting clinically relevant pathogens such as *Shigella* sp, *Salmonella* sp, diarreaogenic *E. coli*, *Giardia lamblia*, *Cryptosporidium parvum*, enteric viruses concentrations of faecal bacteria including TTC, enterococci and *E. coli*, are used as proxy indicators. However, the limitations of these indicators must be acknowledged.[21]

In our field experiments we left the ball with children for 1 day before collection and bacteria recovery analysis. In previous studies different times of collection of toys were used varying from 3 or 4 days[10] to 2 weeks.[11] A comparison of results in the field with laboratory findings suggests that the *E. coli* bacteria measured on the objects by these studies probably are due to contamination within the 3 h immediately prior to collection. This would imply that leaving the ball for one or more days with a child may not influence the amount of *E. coli* recovered. As the comparability of laboratory and field conditions cannot be assumed, however, further research is required to determine the optimal period for leaving the object in the field. This research must also address the variability of contamination, both in terms of concentrations and time.

We did not observe any difference in levels of toy contamination in households with and without latrines. Divita et al.[22] measured *E.coli* in sentinel toys and found that *E. coli* counts were low and not different between households with improved and unimproved latrines. These results are in contrast with Vuic et al.[10] and a study from Honduras[11] both of which found differences in levels of faecal coliforms in sentinel toys between households with a latrine or without a latrine. Vuic and colleagues were measuring faecal streptococci and they did not observe any difference in the same toys. The Honduras' study also found lower levels of *E. coli* in houses that have access to latrines or improved sanitation.

In our study, houses that belong to a village where sanitation coverage was higher than 50% had fewer bacteria in the toys than households in villages with lower sanitation coverage. Less contamination was also seen in households where no one in the household was practicing open defecation. These findings may suggest that sanitation coverage at communal level could affect toy contamination, as could the sanitation practices of all members of the same household. However, statistical support for both findings was poor. Our findings suggest that either contamination rates between households were broadly similar, or that our method of detection and the use of TTC as indicator bacteria is not sensitive enough to reflect differences in actual exposure to pathogens (which are likely to exist). The sanitation intervention in which context this study was undertaken did not achieve any measurable reduction in sentinel toy contamination.[23] This may again have been due to the insensitivity of our method, the unsuitability of TTC as indicator bacteria or because the intervention was ineffective in reducing exposure. Considering the other outcomes from the trial, we found strong evidence for the latter.[23]

Conclusions

Hand-to-mouth transmission is an important source of exposure for young children.[24] Sentinel toys offer the potential for measuring faecal contamination in a domestic environment. While this study advances our understanding of the method, further research is required in order to demonstrate the reliability and sensitivity of sentinel toys and to optimize the procedures for using them.

Authors' contributions: BT and TC conceived and designed the experiments. YG performed the laboratory experiments. BT, PR and SB were responsible for data collection and field management. BT and WS did the data analysis. BT, TC and WS wrote the paper. All the authors read and approved the final version. BT is guarantor of the study.

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Competing interests: None declared.

Ethical approval: The study was approved by the ethics committees of the London School of Hygiene & Tropical Medicine and Xavier University (Bhubaneswar).

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