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'Unfit for human consumption': a study of the contamination of formula milk fed to young children in East Java, Indonesia

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Abstract

Objectives: To examine levels of bacterial contamination in formula feeding bottles in Sidoarjo, East Java, and to assess the preparation practices that may have been responsible.

Methods: Cross-sectional study. We randomly selected 92 households with children under the age of two who were bottle-fed formula. In each household we carried out video observation of mothers/caregivers preparing bottles, and examined samples of formula for coliform bacteria and Escherichia coli (*E. coli*). In-depth interviews were conducted with a sub-sample of 20 mothers.

Results: 88% of the formula feeds were contaminated with total coliforms at a level >10 MPN/ml, and 45% contained *E. coli*. These feeds were defined as 'unfit for human consumption'. In the video observations, none of the mothers complied with all five WHO-recommended measures of hygienic formula feed preparation. Only two mothers washed their hands with soap prior to formula preparation. Most mothers also failed to clean or sterilise the bottle and clean the preparation area. In-depth interviews confirmed that such suboptimal hygiene practices were common.

Conclusion: The high levels of contamination found highlight that bottles are an important faecal-oral exposure pathway resulting from poor hygiene practices during bottle preparation.

Keywords: nutrition; hygiene; diarrhoea; infant formula; Coliform Bacilli; *Escherichia coli*

Introduction

Globally, diarrhoea kills around 760,000 children under five every year (1). Ingestion of food and water contaminated with faecal material can expose infants to the pathogenic bacteria that cause a substantial proportion of diarrhoeal diseases and associated malnutrition in young children (2)(3)(4). The introduction of complementary food prepared in unhygienic conditions has been identified as the riskiest period for the ingestion of enteric pathogens (4)(5)(6). Before the introduction of complementary foods, exclusive breastfeeding in the first six months plays a protective role, and mitigates many of the risks of exposure to pathogens, as milk can be consumed directly from the breast (7)(8). Bottle-fed infants are more susceptible to diarrhoeal disease (7)(9) and data from low- and middle-income countries (LMICs) suggest that a non-breastfed child is 14 times more likely to die in the first six months of life than a breastfed child (10). This statistic may in part be explained by a lack of appropriate hygiene during bottle preparation.

The repeated cycle of faecal-oral infection of the intestinal tract from the transmission of pathogenic bacteria predisposes infants to malnutrition (11)(12). Stunting, or low height for age, is the most prevalent form of malnutrition, which affects almost a quarter of children worldwide under five years of age, the vast majority of whom live in sub-Saharan Africa and South Asia (13). As the causes of childhood growth restriction are multifactorial, dietary interventions alone have proved insufficient to promote optimal growth in low-income settings, only reducing the burden of stunting by around a third (14). While only pathogenic bacteria cause diarrhoea, recent evidence suggests that repeated exposure to other microbes of faecal origin may contribute to environmental enteric dysfunction (EED), a subclinical inflammatory condition of the gut without overt diarrhoea (15). EED has been hypothesised to be an important causal factor in childhood stunting (15)(16)(17).

Formula is a major source of nourishment for many infants and young children, particularly in the Asia Pacific region (18). For bottle-fed infants, WHO preparation guidelines state that the caregiver must properly clean and disinfect hands, bottles, teats and surfaces and throw away any feed that has not been consumed within two hours (19). WHO recommends the use of cups instead of bottles in areas where sanitation and clean water may be a problem, as bottles are particularly difficult to clean (20). Milk products make an ideal breeding ground for harmful bacteria (21), and consumption of contaminated products is particularly dangerous for infants younger than two years as their immune systems are not yet fully developed (22). In LMIC settings, caregivers may face additional challenges due to unpredictable fuel and electricity supply to boil water and refrigerate formula, having surfaces that are difficult to clean, and inadequate sanitation facilities leading to faecal

contamination of the environment (23). Additionally, ambient temperatures in LMICs often favour the replication of pathogens in foodstuffs (24)(25)(26). The bottle feeding of formula milk, when not strictly necessary, is therefore a major concern, especially in countries with a high burden of infectious disease, such as Indonesia, where less than half of children under six months (41.5%) are exclusively breastfed and one in three (36.4%) children are stunted (27). Mixed feeding is the most common practice for infants under six months in Indonesia, and by two months of age, more than one third of breastfed infants also bottle-feed with infant formula (28). Consequently, this study explored formula feeding bottles as a potential source of exposure to bacterial contamination for infants and young children, and assessed the bottle preparation practices of mothers and caregivers that may have resulted in contamination.

Methods

Study Setting

This mixed-methods cross-sectional study was conducted in Sidoarjo, East Java. Sidoarjo is one of 38 districts in East Java, situated approximately 20 km from Indonesia's second largest city, Surabaya. Sidoarjo has a population of around 2.12 million, with an estimated population density of 2,964 per km^2, and a mean household size of three (29).

Household food expenditure accounts for 42% of total expenditure and 83% of households have their own toilet (30). In 2015, there were 1,779 *Posyandu* (integrated health posts) in Sidoarjo (29).

Participant Selection

Households were selected using multi-stage sampling. Using probability proportional to size, 12 villages were randomly selected from two subdistricts in Sidoarjo, namely Krembeung and Sidoarjo. Once a village was selected, the local *Posyandu* was informed and a list of all households with formula-fed children was generated by the *kaders* (volunteer nutrition workers) from a larger list of all households within the *Posyandu* catchment area. *Kaders* meet with children and mothers in the community on a weekly or monthly basis, so they are aware of who is formula-fed. Households were randomly selected from the list and a *kader* accompanied the researchers around each village to assist in identifying the households. Eligible households were those with children under 24 months who were bottle-fed, either exclusively with formula, or mixed fed. If there were multiple children under two in the household, the first born was selected.

Data Collection

Data collection began after a one-day training period followed by a pre-test. First interviews were carried out with mothers using a questionnaire to collect demographic and household characteristics and infant and young child feeding practices. The interviews were conducted by local enumerators in Bahasa Indonesia. The caregiver was then asked to prepare a feed for the researchers to capture on video, and a 100ml sample of the formula was collected. Small hand-held video cameras (Panasonic SDR-50) were used. Videos were reviewed on laptops and preparation practices were scored on the five-point hygiene checklist of WHO guidelines on 'How to Prepare Formula for Bottle-Feeding at Home' as follows:

1) Wash hands with water and soap before commencing.
2) Clean and disinfect a surface on which to prepare the feed.
3) Wash equipment (bottle and teat) thoroughly with soapy water.
4) Use a brush to wash equipment.
5) Sterilise the equipment (with boiling water or sterilisation equipment).

For the qualitative element of the study, a sub-sample of 20 mothers were purposively selected to ensure there was maximum variation based on socio-economic status, age, number of children, age of children and education level. The mothers were interviewed regarding their perceptions of and practices with regard to bottle-feeding. All interviews were recorded using a digital voice recorder.

Microbiological Analysis

Milk samples of 100 ml were collected by carefully pouring the prepared formula feeds into sterilised containers which were then placed in a cold box at a temperature below 10 °C. The samples were transported within six hours to a nearby private laboratory in Surabaya and processed with a hydrophobic grid membrane filter using the most probable number method (MPN). Microbial analysis included total *E. coli* count and total coliform count. *E. coli* is a good proxy indicator for faecal contamination as approximately 90% of faecal coliforms are *E. coli* (31). Total coliforms are widely used to reflect overall hygiene during food preparation and are commonly used as an indicator of the potential for contamination with disease-causing pathogens (31). The growth medium used was ES Colimark agar which contains two chromogenic substrates.

Data Analysis

Data on microbial counts were double-entered and compared for accuracy and then analysed using STATA 14. There is no standardised cut-off for when contamination levels of total coliforms in milk may become dangerous, but many governments, including the National Agency of Food and Drug

Control in Indonesia, and WHO, limit the number of coliforms permitted in milk and milk products to no greater than 10 MPN per millilitre (31)(32). Therefore, in this study, samples with over 10 MPN/ml were classified as unfit for human consumption.

Analysis of the video footage involved creating descriptive codes, such as hand washing and bottle cleaning method, and summarising them in relation to critical actions that may have resulted in contamination. Additionally, each video observation was scored out of five for adherence to the WHO hygiene checklist.

Qualitative data were analysed thematically, through a 'top down' analysis based on the study objectives (33). The qualitative interviews were transcribed verbatim by a trained transcriber. Full transcripts were uploaded onto Dedoose software and coded using *a priori* codes that included hygiene perceptions, hygiene practices and causes of diarrhoea.

Ethics and Consent

Ethical approval for this research was granted by the London School of Hygiene and Tropical Medicine and the Faculty of Medicine, University of Indonesia. Additional approval was received from the Ministry of Home Affairs, Republic of Indonesia and the subsequent provincial, district and subdistrict government offices in Surabaya and Sidoarjo. Households were informed about the purpose of the study and written informed consent was obtained from each mother or caregiver. Families were comfortable being video recorded (probably due to the ubiquity of camera phones). Two families refused to participate and one of the 92 participants refused to be recorded on video.

Results

Demographic and Household Characteristics

A total of 92 households with formula-fed children under age two were surveyed in August 2016. Boys comprised 57% of the sample, and the mean age of the children was 12 months (SD = 6.01; range= 1-23 months). Households were categorised into wealth quintiles: lowest 16%, second 12%, middle 26%, fourth 20% and highest 26% based on distribution of household asset indices. The mean age of the mothers was 29.4 (SD= 5.01; range 21-43 years), and the majority of mothers (57%) had completed senior high school/vocational training. Over half of the households (55%) reported that they purchased drums of purified water as their primary water source. These required boiling before use. Of the sample, 45% of the households had private water taps inside the house, 87% had a working refrigerator, and 86% had their own toilet.

Health and Nutritional Characteristics

Among the sample under six months of age, 69% were mixed-fed breast milk and formula, 31% were exclusively formula-fed, and 13% were also receiving complementary food. From six to 11 months of age, 36% were mixed-fed breast milk and formula and 64% were exclusively fed formula milk. For infants 12-23 months old, 27% were mixed-fed breast milk and formula and 73% were exclusively fed formula milk. All children aged 6 to 23 months were also receiving complementary food. Nineteen (21%) of the children had diarrhoea in the two weeks preceding the survey based on mother's reports.

Microbiological Results

The levels and distribution of total coliforms and *E. coli* contamination of the formula feeds are shown in Table 2. Of the 92 samples, 88% contained more than 10 MPN/ml of total coliforms. Total coliform counts ranged from 0 to 5,127 organisms with a median of 1,631 MPN/ml (IQR: 144.5-5,127). *E. coli* was isolated from 45% of the samples and counts ranged from 0 to 5,127 with a median of 0 MPN/ml (interquartile range IQR: 0-13.6). It was not possible to see the entire distributions for *E. coli* and total coliform count as the maximum recorded value by the laboratory was 5,127 MPN/ml. As all the bottles contaminated with *E. coli* also contained more than 10 MPN/ml of total coliforms, a total of 88% (n=81) of the feeds were classified as unfit for human consumption. There was little sign on any major differences in levels of feed contamination according to wealth, educational level, water source or sanitation status.

Video Observation

Of the 91 mothers and caregivers who agreed to be video-recorded preparing a formula feeding bottle, none complied with all five points on the WHO hygiene checklist. Only one completed four points: disinfecting a surface, washing the equipment with a brush and soap, and sterilising the equipment, but did not wash her hands with soap first. Women who completed each hygiene action are shown in Table 2.

Rates of hand washing were very low with only two women in total washing their hands with soap. The microbial findings show that the bottles prepared by these two women contained no *E. coli*. Another five (5%) women rinsed their hands in water without soap. Only two women washed the equipment thoroughly with soap, one using a brush to do so, and the other using a cloth. The most common practice for cleaning the bottles was to rinse the bottle out with tap water or warm (previously boiled and stored in a thermos flask) water without soap. A total of 31 (34%) women performed this action. Three other women rinsed the equipment with freshly boiled water without

soap. The rest of the women (60%) did not clean the equipment before preparation, and three of those women prepared a bottle with leftover milk inside from a previous feed. Four women sterilised the equipment, three with boiling water and one with an electric steam steriliser. However, none washed their hands with soap first, allowing for re-contamination immediately after sterilisation. Sterilisation equipment was seen, but not employed in two other household videos.

A significant potential sources of contamination was the practice of placing an unwashed finger or hand over the teat, once attached to the bottle, to shake and mix the formula. Thirty mothers and caregivers (33%) performed this action. This could either contaminate the milk directly through the hole in the teat, or contaminate the teat itself which comes into direct contact with the child's mouth. Some women would also use their unwashed hand to finger the inside of the teat when attaching it to the ring, or wipe the inside edges of the bottle after scooping the powder into the bottle, further potential contamination routes. Nine (10%) women used a spoon that was not cleaned or sterilised to mix the formula. The microbial findings show that 10 of the 11 women who prepared bottles that were fit for human consumption did not place an unwashed finger or hand over the teat or inside the bottle at any point during bottle preparation.

Formula feeds were prepared in kitchens mostly in the same location where family foods were prepared, and it was notable that mothers rarely followed packet instructions concerning using measuring quantities or special preparation techniques.

Qualitative Findings

Hygiene Perceptions and Practices in the Community

When the 20 in-depth interviewees were asked how they prepare formula bottles, three said they wash their hands first, six said that they sterilize the bottle before use, and three said that they wash the bottle with soap and a brush. Some described suboptimal behaviours: '*I wash the bottles, though it sometimes has some milk left inside'* (25 years old; high SES).

Participants were more readily able to describe good food hygiene practices, and over half (n=12) mentioned the importance of washing food and cooking utensils, and eight mentioned the importance of hand washing before preparing food for their child. When questioned about other mothers in the community, low standards were often reported, in particular feeding in the street which was believed to be unhygienic (n=6).

'*I observe mothers feeding their children in the dusty streets without covering the food'* (37 years old; high SES).

'Judging from how the mothers feed their children, I can say that it is not really … well not hygienic at all. So much dust and pollution, I am afraid the child will eat that. So it is better to feed the child inside, not in the road' (25 years old; high SES).

Causes of Diarrhoea

The participants suggested a range of factors that might cause diarrhoea in children under two. The most commonly mentioned cause of diarrhoea was through 'dirty' food (n=8), especially flies coming into contact with the food and contaminating it: '*The flies land on the food and the child directly eats that in. That's why they get diarrhoea*' (36 years old; middle SES). Six women believed formula milk causes diarrhoea. Other suggested causes included spicy food, sour food, playing in water and lack of hand washing.

'*The food is causing diarrhoea ... like the spicy one, some unhealthy snack. If children have diarrhoea, it might be that their parents did not control their foods*' (30 years old; middle SES).

Discussion

This study showed that bacterial contamination of formula feeds is a major problem in this setting. The microbiological results of this study are consistent with other studies in LMIC settings that show high levels of total coliform contamination >10 MPN/ml ranging from 59% in Ethiopia to 86% in northern India (34)(35)(36)(37). Previous research shows *E. coli* contamination of bottles ranging from 25% in Brazil to 62% in South Africa (36)(38)(39)(40). Studies have noted positive correlation between the length of time from preparation and coliform contamination of milk (41). Thus, it is likely that contamination levels would have been higher if samples had been collected at the point of consumption, rather than of preparation, since feeds are often kept for long periods in this population.

The multiple failures of good hygiene practices noted in the videos during bottle preparation are probably causes of the high levels of contamination found in the freshly prepared feeds. Evidence indicates that suboptimal caregiver hygiene practices relative to food preparation and infant feeding can increase diarrhoeal risk, although findings are inconclusive (42)(43).

These findings are particularly arresting given that mothers and caregivers were likely to demonstrate what they thought would be the ideal way to prepare a bottle due to the fact they were being observed by researchers (44). Day-to-day bottle preparation is thus likely even less hygienic than what was observed. These findings are consistent with previous studies in Indonesia, Thailand and South Africa documenting poor hygiene practices when preparing formula feeding bottles (45)(39)(43)(46).

The presence of *E. coli* in almost half of the bottles implies that mothers may not be washing their hands after risk of faecal contact (24). Hand washing is considered as a primary infection control practice and one of the most effective ways to reduce diarrhoea (47)(48). Results from our study support findings from other observational studies in LMIC settings including Indonesia that show low rates of hand washing before preparing formula feeds of between 1% and 11% (43)(49)(50)**.** Other studies that involved self-reporting of hand washing before infant feeding consistently record much higher rates of between 42% and 68% likely due to social desirability bias (38)(39)(51)(52).

The predominant practice of cleaning the bottles by rinsing with tap or warm (previously boiled) water was inadequate given that teats and screw tops can be particularly difficult to clean (20). A qualitative study in Tangerang, Indonesia, demonstrated that mothers did not like to use soap when cleaning because they believed it left a bad smell in the bottle (53). This emphasises the importance of promoting the use of cups instead of bottles in areas where cleaning habits may be difficult to change. Additionally, a clearer understanding of infection prevention along with more aggressive promotion of hand washing is needed to reduce contamination of bottles.

The qualitative interviews supported the idea that suboptimal hygiene practices are common in the community. Self-reported bottle hygiene practices were poor but altogether more hygienic than the video observations. However, few mothers seemed to recognise bottle hygiene as a serious issue for child health or treat the preparation of formula feeds with especial care as compared to the preparation of any family food. Mothers predominantly attributed diarrhoea to contaminated foods and unhygienic environments. This outcome is comparable to findings in other studies in Indonesia, Pakistan and the Gambia (53)(54)(55). Mothers reported safer food preparation practices than bottle preparation practices indicating that health promotion interventions in this area may have placed more focus on complementary food hygiene than bottle hygiene (56).

Limitations

This study only included households who were registered at a *Posyandu* which may limit the generalisibility of the study, although data suggests around 80% of the population use *Posyandu* services across Indonesia (57). The sample was designed to investigate levels of contamination and not its determinants. Future larger studies than can quantitatively pinpoint risk factors for poor hygiene behaviour, bottle contamination and infant diarrhea and stunting respectively would be worthwhile, as would more detailed theory-based formative research that could pinpoint interventions capable of changing such practices.

Conclusion

This study suggests that almost all of the milk fed to infants and young children from feeding bottles in this setting is so contaminated that it is unfit for human consumption. The study documents a pattern of poor hygiene practices during bottle preparation, particularly a lack of hand washing, sterilisation and bottle-cleaning, which are likely to be the primary causes of this contamination.

These findings suggest that formula feeding is an inappropriate practice in countries such as Indonesia. Formula has become normalised as ordinary food in many low-income settings, and many mothers see it as an essential component of their child's diet. As bottle feeding is discouaraged by health agents, programmes have tended to ignore it rather than point out its risks and explain how it should be prepared. In our view, formula should be presented as 'medicine' to be used only in special cases. As *kaders* meet with mothers and children regularly, they could serve as important agents of change to promote and support safe infant formula practices. The dangers of poor bottle hygiene need to be explained in WASH and nutrition programmes, and should at least be given equal status to complementary food hygiene in intervention efforts. This may act as a deterrent to bottle-feeding, once women are made aware of how difficult it is to avoid contamination during its preparation.

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Table 1. Legend?

	Whole Population
Population Characteristics (n=92)	n (%)
Age of Child (months)	
0-5	16 (17)
6-11	25 (27)
12-17	34 (37)
18- 23	17 (19)
Sex of Child	
Female	40 (44)
Male	52 (57)
Subdistrict	
Krembeung	43 (47)
Sidoarjo	49 (54)
Maternal Age	
<26	29 (32)
26-32	39 (42)
>32	24 (26)
SES	
Lowest	15 (16)
Second	11 (12)
Middle	24 (26)
Fourth	18 (20)
Highest	24 (26)
Maternal Education	
Primary school	3 (3)
Junior high school	15 (16)
Senior high school	52 (57)
University	22 (24)
Water Supply	
Tap water	10 (11)
Protected well	3 (3)
Unprotected well	1 (1)
Protected spring water	4 (4)
Water seller (drum)	51 (55)
Bottled water	23 (25)
Refrigerator	
Yes	80 (87)
No	12 (13)
Toilet (not shared)	
Yes	79 (86)
No	13 (14)
Water Tap (inside)	
Yes	41 (45)
No	51 (55)

Table 2. Legend??

	Whole Population
Health Data (n=92)	n (%)
Mixed Feeding	
Yes	34 (37)
No	58 (63)
Receiving Complementary Foods	
Yes	78 (85)
No	14 (15)
Diarrhoea	
Yes	19 (21)
No	73 (79)
Coliform Counts per Millitre of Milk	
Total coliform count	
0	6 (7)
>0-10	5 (5)
11-100	9 (10)
101-1000	17 (18)
1001-5000	27 (29)
>50000	28 (30)
E. coli count	
0	51 (55)
>0-10	17 (19)
11-100	10 (11)
101-1000	5 (5)
1001-5000	6 (7)
>50000	3 (3)
Observed Hygiene Behaviours	
Wash hands with soap and water before commencing	2 (2)
Clean and disinfect a surface on which to prepare feed	1 (1)
wash equipment thoroughly with soapy water	2 (2)
use a brush to wash equipment	1 (1)
sterilise equipment	4 (4)