

TOWARD A HYGIENIC ENVIRONMENT FOR INFANTS AND YOUNG CHILDREN

A Review of the Literature



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FEBRUARY 2018

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ACRONYMS AND ABBREVIATIONS

BCC Behavior Change Communication

CF Complementary Food CFU Colony-Forming Unit

CHV Community Health Volunteer
CLTS Community-Led Total Sanitation
DHS Demographic Health Survey
ECD Early Childhood Development
EED Environmental Enteric Dysfunction

ENGINE Empowering the New Generation to Improve Nutrition and Economic Opportunities

FIB Fecal Indicator Bacteria FSM Fecal Sludge Management

GEMS Global Enteric Multicenter Study
HACCP Hazard Analysis Critical Control Point

HAZ Height-for-Age Z-Score

IE Impact Evaluation

IYC Infants and Young Children
IYCF Infant and Young Child Feeding
LMIC Low and Middle-Income Country

MST Microbial Source Tracking

OD Open Defecation

OR Odds Ratio

RCT Randomized Controlled Trial

RR Relative Risk

SBCC Social and Behavior Change Communication

SD Standard Deviation SDW Stored Drinking Water

SHINE Sanitation Hygiene Infant Nutrition Efficacy

STH Soil-Transmitted Helminth
TSC Total Sanitation Campaign

UN United Nations

UNICEF United Nations International Children's Emergency Fund

US United States

USAID United States Agency for International Development

WASH Water, Sanitation, and Hygiene

WASHPaLS Water, Sanitation, and Hygiene Partnerships and Learning for Sustainability

WAZ Weight-for-Age Z-Score
WHO World Health Organization
WHZ Weight-for-Height Z-Score

WSP Water and Sanitation Program of the World Bank

EXECUTIVE SUMMARY

INTRODUCTION

As of 2014, 24 percent of children under age five globally were stunted, with the vast majority living in low- and middle-income countries (LMICs) (UNICEF, WHO, & World Bank 2015). Undernutrition and stunting are related to poor overall health, increased susceptibility to infections, lower economic productivity, cognitive deficits and learning disabilities, and increased mortality. Concurrent exposures of inadequate diet and poor water, sanitation, and hygiene (WASH) conditions predispose infants and young children (IYC) to a debilitating cycle of infections and undernutrition in early life.

Additionally, over the past decade, environmental enteric dysfunction (EED), a condition characterized by inflammation of the small intestinal lining that inhibits permeability and nutrient absorption, has been identified as a potential major mediating pathway linking poor WASH conditions and chronic undernutrition. Though the etiology of EED is not fully understood, it has been linked with ingestion by IYC of high loads of human and animal fecal microbes. The EED hypothesis proposes chronic exposure to fecal microbes, independent of the effects of diarrhea, as a significant underlying cause of child growth faltering. EED is thought to explain why even the most rigorous dietary interventions have only a modest effect on reducing child stunting.

For nearly six decades, routes of pathogen transmission have been identified and summarized in a seminal "F-diagram" via fluids, fingers, flies, fields (floors, earth, dirt), fomites (surfaces), and food (Wagner & Lanoix, 1958). The traditional F-diagram focuses exclusively on human excreta, tracing the transmission of pathogens through different exposure routes into water and food and onto hands that are then ingested by a host. Based on existing evidence, we suggest that two refinements be made to the F-diagram: (1) including domestic animal excreta as a distinct and potentially important source of risk, and (2) in the case of IYC, framing ingestion of pathogens via eating dirt (geophagy) and/or human and animal excreta and through mouthing behaviors as an additional *direct* transmission pathway not disrupted by the traditional suite of WASH measures targeted at adults and older children.

WASH interventions to disrupt transmission pathways in the F-diagram have traditionally focused on increasing access to an improved water supply, improving drinking water quality, and refining hand hygiene and sanitation measures (through the reduction of open defecation and the adoption of improved toilets). These strategies have paid less attention to behaviors and technologies separating IYC from human and animal feces through interventions such as clean play spaces, IYC mouthing behaviors, and safe disposal of IYC feces.

The USAID Water, Sanitation, and Hygiene Partnerships and Learning for Sustainability (WASHPaLS) project conducted a review of the scientific and gray literature, complemented by dozens of key informant interviews with researchers and field implementers, to synthesize the latest understanding of key pathways of fecal microbe ingestion by IYC and their links to diarrhea, EED, and poor nutrition and development outcomes. Specifically, the review sought to:

- Consider both human and animal sources of fecal contamination and the pathways presenting major
 exposure risks to IYC, including sources and pathways previously underemphasized (and to the
 extent possible, seek to understand the relative potential importance of the various pathways in
 terms of magnitude of pathogen transmission);
- Examine technological and behavioral interventions to disrupt the transmission pathways (including
 established WASH measures) as well as interventions that address the underemphasized pathways,
 such as clean play spaces, animal husbandry, IYC feeding practices, and IYC-focused WASH
 behaviors; and

• Identify areas of priority for implementation research to address the issue of clean play spaces for infants and young children.

The findings of the literature review will inform the design of research to measure the additive effects of specific measures to reduce IYC exposure to fecal pathogens and other fecal microbes in their home environments when coupled with independent or integrated WASH and nutrition interventions.

PRINCIPAL FINDINGS

The review began by documenting the traditional transmission pathways outlined in the F-diagram and the effectiveness of traditional rural WASH interventions to disrupt them. Key findings are as follows:

- The evidence of WASH interventions reducing the risk of diarrhea among under-five-year old children is mixed and is stronger for particular categories of WASH interventions such as water supply and point-of-use water treatment than for others.
- An emerging body of evidence from high-quality (experimental) studies also shows mixed effects of
 improved sanitation on health outcomes such as diarrhea, EED, soil-transmitted helminths (STHs),
 and child growth outcomes. However, a recent community-led total sanitation (CLTS) trial provides
 evidence suggesting that promoting intensive behavior changes over a 12-month period can improve
 child linear growth (Pickering et al. 2015).
- Evidence also suggests that unless threshold levels of community sanitation coverage are reached to achieve herd effects, household sanitation improvements may be insufficient to mitigate fecal-oral pathogen exposure and achieve desired health benefits.
- Substantial evidence has been found on the effectiveness of handwashing with soap in preventing diarrhea and STH infections. However, the magnitude of the effect differs between studies and across contexts.
- The additive or synergistic effects of different WASH interventions on child health are complex and not well understood, and evidence on the effect of focused and combined WASH interventions on child health outcomes, such as diarrhea and growth, is far from definitive.
- Food hygiene is largely a neglected aspect of WASH, and the few existing intervention studies are weak in quality and not easily generalized.

While evidence of the impact of traditional WASH interventions on improving health outcomes is mixed, the review suggests other underemphasized sources and pathways for fecal pathogens that may force some re-evaluation of the types of interventions needed to limit pathogen exposure in IYC.

A NEGLECTED CONTAMINANT SOURCE: ANIMAL EXCRETA IN THE DOMESTIC ENVIRONMENT

Risks from human excreta have been well documented, but uncontained animal feces also are abundant in rural areas in developing countries, constituting an important reservoir of zoonotic pathogens and non-commensal fecal microbes to the domestic environment and water supply sources. As an example, at a study site in rural India, over 50 percent of household-stored water samples, 90 percent of mothers' and children's hands, and 74 percent of public ponds were contaminated with animal feces (mainly ruminants) across 24 villages (Schriewer et al. 2015). Several other studies documented significant sources of animal fecal contamination in the domestic environment, including ruminants in urban Bangladesh and both ruminants and avian species in rural Bangladesh (Harris et al. 2016), poultry and livestock in rural Ethiopia (Headey & Hirvonen 2016) and poultry in Zimbabwe (Ngure et al. 2013),

Zambia (Reid et al. 2018) and peri-urban Peru (Marquis et al. 1990). Overnight corralling of poultry and/or livestock within the same room as IYC also is associated with elevated markers of EED (George et al. 2015) and stunting (George et al. 2015; Headey & Hirvonen 2016). While the relative magnitudes of pathogen exposure from these sources remain largely unknown, substantial data exist to demonstrate that animal feces contribute significant contamination to the home environment.

AN UNDEREMPHASIZED PATHWAY: DIRECT INGESTION OF FECES AND SOIL

Direct ingestion of fecally contaminated soil and/or animal feces is a critical pathway for exposure of fecal pathogens by IYC. IYC ingest dirt and feces through mouthing soiled fingers, play objects, and household items (Marquis et al. 1990; Ngure et al. 2013; Reid et al. 2018) as well as through exploratory ingestion of contaminated soil and/or poultry feces. Contamination by ingestion is common in low-income environments (Marquis et al. 1990; Ngure et al. 2013; Reid et al. 2018; Shivoga & Moturi 2009; George et al. 2015a). Soil ingestion among IYC is associated with increased risk of diarrhea (Shivoga & Moturi 2009), elevated markers of EED, and stunting (George et al. 2015a).

PROTECTING IYC: NEW INTERVENTIONS TO ADDRESS UN-DISRUPTED PATHWAYS

Barriers: In addition to the interventions intended to disrupt the pathways of the traditional F-diagram (e.g., increasing use of improved latrines, improving access to safe water, handwashing with soap at critical junctures, and to a lesser extent emphasizing food hygiene), efforts have been made to construct barriers that prevent fecal contamination of the domestic environment and associated ingestion of soil and animal feces by IYC. These barriers include finished flooring; improved animal husbandry practices, such as corralling and other means to separate IYC and animals; playmats for immobile infants; and a playmat/playpen combination for crawling and mobile infants.

While the introduction of barriers is gaining attention in IYC and nutrition communities of practice, to date there have been no published studies finding that protective play spaces prevent fecal ingestion or improve child health.¹ Likewise, a few implementing organizations have conducted formative research and executed interventions to address poultry feces through modified animal husbandry practices; however, data are not generally available on the effectiveness of these approaches. Moreover, poultry-corralling interventions have not largely been successful due to individual household preference for free-range poultry and eggs, and various cultural, structural, and economic barriers (Harvey et al. 2003). Similarly, there is some initial evidence of the potential protective effect of improved flooring: an observational study of a Mexican government program that replaced dirt floors with cement floors found that child diarrhea and parasitic infestations were reduced significantly in households with the new floors (Cattaneo et al. 2009).

Animal Feces Management: Reducing or eliminating animal feces in household compounds is challenging. Both ruminant and fowl feces are used productively for fuel, farm fertilizer, and building materials. Because of its utility, households are reluctant to get rid of what they consider to be a valuable resource. Storage and handling of fresh animal feces for such purposes expose household members including children to contamination, suggesting that a focus on safer management of animal feces and handwashing after handling may be more successful than focusing on eliminating animal feces from household compounds altogether.

Management of Child Excreta: Researchers have measured the impact of uncontained feces on the health and growth of the IYC cohort, but until recently, little attention has been focused on feces

A protective play space refers to the combination of a playmat and an enclosure or playpen for use by IYC to prevent direct contact with soil and feces (Humphrey et al., 2015).

management of the IYC cohort. This review highlights findings from recent analyses indicating that IYC feces disposal is sub-optimal in many LMICs. Field practitioners have responded to these findings with interventions that promote the safe disposal of IYC feces out of the belief that promoting age cohort-specific behaviors (supported by enabling technologies such as potties, diaper cloths, and hoes) facilitate improved IYC feces disposal practices. Data supporting this recommendation, however, have come from program evaluations with low rigor. In some deployments, CLTS has begun to include a focus on safe disposal of IYC feces as part of triggering, follow-up, and verification of open defecation-free status. Nutrition programs also have begun to include safe disposal of IYC feces and handwashing after handling child feces (often not regarded as "dirty" by their caregivers) as part of comprehensive IYC growth initiatives.

DISCUSSION

The relative magnitudes of transmission pathways of enteric microbes in IYC are not well defined and may be highly context specific, making it difficult to conclude which pathways represent the highest risk. A number of ongoing studies (among them WASH Benefits, the Global Enteric Multicenter Study [GEMS], Sanitation Hygiene Infant Nutrition Efficacy [SHINE], and NOURISH [see Annex B]) may offer important insights into pathways when findings are published. Still, the growing attention to risks from direct and indirect consumption of animal waste and direct consumption of contaminated soil point to the potential benefits of separating IYC from fecal pathogen sources in the home environment.

Playmat and play space interventions may be promising, but assessing their true potential requires a better understanding of the biological plausibility of their protective effects against fecal contaminant risk. Extended periods of protection on a mat or within a play yard may not be sufficient to prevent risk posed by even short periods of time spent in areas of increased hazard (e.g., on bare soil with excreta present or eating contaminated food from contaminated hands). A number of studies of water treatment interventions argue that there is an exposure threshold above which infection becomes probable (Engell & Lim 2013; Brown & Clasen 2012; Hunter et al. 2009); however, the exposure-response relationship for ingestion of animal excreta or contaminated soil remains a source of considerable uncertainty. Due to the heterogeneous nature of the formative and observational studies of fecal microbe transmission pathways, developing findings on the relative magnitudes of fecal microbe exposures across different pathways was not possible in this review; further research is needed to make better and more informed programmatic decisions.

Analysis of the available literature, as well as feedback from key informant interviews, underscores how little is understood about the etiology of EED. Given that EED may facilitate linear growth failure independently of diarrhea, the historic research focus on diarrhea as the primary outcome variable may have missed key relationships among various WASH interventions, EED, and child growth. Measuring EED, however, is far more complex and expensive than measuring diarrhea (including specific diarrhea-causing pathogens). There is a need to develop less costly proxy measures predictive of EED and child stunting.

This review underscores a significant evidence gap for various technologies and social behavior change interventions promoted through current programmatic efforts. Additional research is needed to investigate the effectiveness, adoption, constraints, and scale potential of measures to reduce IYC exposure to fecal pathogens, including safe play spaces, improved animal husbandry practices, and flooring, among others. This research should explore the additive benefits of these interventions when coupled with traditional WASH measures such as expanded water supply, improved water quality, utilization of improved toilets, handwashing with soap, and promotion of other hygienic behaviors.

I.0 BACKGROUND

I.I WASH and Child Undernutrition

Infants and young children (IYC) in low- and middle-income countries (LMICs) are exposed to the combined risks of inadequate diet and recurrent infections during the critical first 1,000 days of life (from conception to 24 months of age), when gut health, immune competence, growth, and development trajectories are established. Poor water, sanitation, and hygiene (WASH) conditions; inadequate caregiving²; and food insecurity underlie these immediate causes of child growth faltering. The Global Burden of Disease study estimates diarrheal disease to be the ninth leading cause of death globally, at about 1.3 million deaths per year, but the fourth leading cause of death in children under five years of age, with roughly 499,000 deaths annually (GBD 2015 Mortality and Causes of Death Collaborators 2016). Using earlier data, it was estimated that inadequate WASH was responsible for over half of the global diarrheal disease burden, amounting to 842,000 deaths in 2012 (and over 5 percent of the global mortality of infants and children under the age of five) (Prüss-Ustün et al. 2014). In 2014 it was estimated that globally, 22.9 percent of children under five years old were stunted (UNICEF et al. 2015), with more than one-half living in Asia and more than one-third in Africa (Black et al. 2013). In 2011, 43 percent of 6- to 59-month-old children were anemic (WHO 2015). Africa and Asia account for the highest prevalence of childhood anemia, at 60 percent and 42 percent, respectively. Stunting and anemia can negatively affect child development, adult intellectual capacity, and future economic productivity (Black et al. 2013; Walker et al. 2011). Together with other social and environmental factors, the effects of poor diet and infectious diseases occur both concurrently and cumulatively during early life, preventing millions of children from achieving their full development potential (Walker et al. 2007; Black et al. 2013).

Poor water, sanitation, and hygiene conditions contribute to undernutrition and, in turn, growth stunting and impaired development in children. Of the approximately 660 million people lacking access to improved drinking water sources in 2015, nearly half live in sub-Saharan Africa, and another fifth live in South Asia (WHO & UNICEF 2015). Meanwhile, about 950 million people practice open defecation (OD). Southern Asia accounts for two-thirds of the world's OD, occurring nearly three times as much as in sub-Saharan Africa. At the same time, the number of people practicing OD in Southern Asia is reported to have decreased by roughly one-fifth between 1990 and 2015 (from roughly 770 million [65 percent] to roughly 610 million [34 percent]), while the absolute number increased in sub-Saharan Africa, as the region's population nearly doubled over this period (WHO & UNICEF 2015). The increase in access to sanitation has not kept pace with population growth in sub-Saharan Africa, with only 36 percent of the additional population gaining access (WHO & UNICEF 2015).

Moreover, global efforts to expand non-sewered sanitation, combined with population growth, make attention to the full sanitation value chain all the more vital. Proper sanitation entails not only the elimination of OD and the delivery of an improved toilet, it also requires timely emptying of pits and storing of sub-surface excreta, as well as safe handling, transport, treatment, and disposal or reuse. In an analysis relying on Demographic Health Survey (DHS) data, it was reported that 63 percent of the populations in 58 LMICs (constituting approximately 1.8 billion people) use facilities that lack proper fecal sludge management (FSM) (Berendes et al. 2017).

Inadequate WASH represents the dominant cause of diarrheal diseases globally (Prüss-Ustün et al. 2014), and diarrhea, in turn, increases susceptibility to acute respiratory infections (Ashraf et al. 2013;

I

[&]quot;Caregiving" refers to adequate child-care practices such as stimulation, protection, infant feeding, hygiene, and health seeking. Caregiving behaviors include care for pregnant/lactating women, feeding/breastfeeding, psychosocial and cognitive stimulation, hygiene behaviors, health seeking, food preparation, and storage (Engle, Menon & Haddad, 1999).

Schlaudecker et al. 2011; Schmidt et al. 2009). The vicious cycle between recurrent childhood infections and undernutrition is well recognized (Petri et al. 2008). Evidence on the magnitude of the relative contribution of diarrhea to chronic child undernutrition, as reflected by stunting, is mixed. In one study, evidence was found consistent with the hypothesis that a higher cumulative burden of diarrhea increases the stunting risk (Checkley et al. 2004), whereas Briend (1990) referred to earlier studies that questioned whether diarrhea-induced growth faltering was short term or sustained. Reduction of diarrheal morbidity and employment of rigorous dietary interventions have proven to be only modestly successful at reducing the child height deficit among the poor in sub-Saharan Africa and South Asia (Bhutta et al. 2008; Dewey & Adu-Afarwuah 2008; Humphrey 2009).

In light of these uncertainties, a growing body of evidence has hypothesized that environmental enteric dysfunction (EED), an energy-intensive subclinical condition characterized by chronic inflammation of the gut and subsequent immune stimulation, as the primary pathway linking inadequate WASH to child stunting and anemia (Humphrey 2009; Humphrey et al. 2015; Prendergast et al. 2014; Prendergast et al. 2015). EED appears to be linked to chronic enteric pathogen and non-commensal fecal bacteria overload of the small intestine,³ and is common in resource-poor contexts with inadequate sanitation (Humphrey 2009).

1.2 IYC Exposure to Excreta in Home Environments

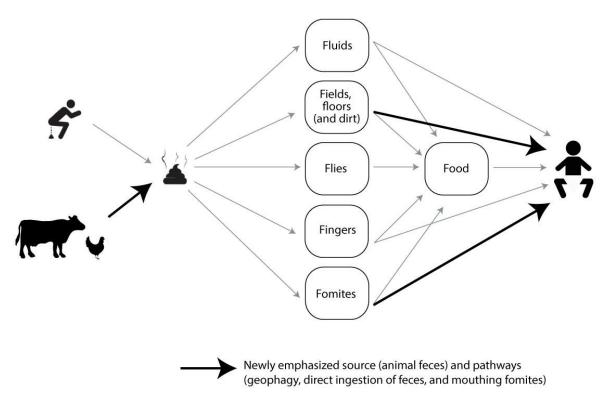
The major transmission pathways for infectious disease (as well as their barriers) were originally summarized in the iconic F-diagram (Wagner & Lanoix 1958). Introducing barriers to transmissions are the basis for such WASH interventions as drinking water treatment, expanded water supply, improved sanitation, handwashing, and food hygiene. However, the current suite of evidence-based WASH measures do not appear to be alone sufficient to protect IYC in low-income countries from ingesting large quantities of disease-causing fecal microbes (Ngure et al. 2013; Ngure et al. 2014). IYC are frequently at a high risk of infection by fecal pathogens and soil-transmitted helminths (STHs) as a result of crawling on contaminated areas and mouthing behaviors that are common in early child development (Ngure et al. 2014). Even when children have no detectable infections, the microbe-laden environment may provide low-level chronic immune stimulation plausibly linked to anemia, inflammation, and catabolic processes that result in impaired growth (Humphrey 2009).

As noted in Mills & Cumming (2016), "a greater understanding is needed of the impact of non-traditional/new WASH interventions, such as safe disposal of child feces, complementary feeding hygiene, hygienic play areas, and others." Increased focus is needed on how best to interrupt underemphasized fecal-oral transmission pathways among under two-year-old IYC by paying increased attention to animal feces as a pathogen reservoir and direct ingestion of feces and contaminated dirt as transmission pathways Ngure et al. (2014). The additional reservoir and pathways are reflected in a modified F-diagram (Figure 1). We highlight the pathways of greatest interest to this review: direct exposure to infants and young children through direct ingestion of animal feces, dirt (geophagy), and mouthing play objects and household items.

To address these pathways, a combination of behavioral and technological inputs have been suggested to support a more hygienic environment in which IYC can thrive and play (Ngure et al. 2014). Proponents argue that exposures, possibly leading to EED, can be minimized by frequent baby handwashing at critical times, as well as by creating a hygienic and protective play environment to complement hygienic infant

We note that the etiology of EED is not currently well understood. In this review we focus on the transmission of fecal/enteric pathogens to represent pathways for other general, non-commensal fecal bacteria that might exacerbate diet-induced changes in gut microbiota (Brown et al. 2015) and induce inflammation of the small intestine mucosal lining (Humphrey 2009).

Figure I: Modified F-diagram that highlights geophagy and direct feces ingestion by IYC



This diagram is a derivative of Figures 1 and 3 in Penakalapati et al., 2017, under a Creative Commons CC-BY 4.0 Usage Agreement with the American Chemical Society.

feeding,⁴ providing safe drinking water, promoting handwashing by caregivers before food preparation and feeding, and implementing improved sanitation measures.

Undeniably, poor WASH conditions are concentrated in the most disadvantaged households (Mason & Mosello 2017). Households with low socioeconomic status experience conditions of inadequate water supply, poor water quality, inferior sanitation infrastructure, and lack of access to sanitation services and goods. Poor households also are more likely to be crowded, have dirt floors, cohabit with animals, and lack refrigeration for foods (Keusch et al. 2006). Within these households, women, who are principally responsible for procuring water and maintaining household hygiene, experience significant time poverty due to their involvement in market-based work and non-market work (Kes & Swaminathan 2006). Time poverty also constrains the ability of women, the primary caregivers, to provide comprehensive child care including practices that prevent diarrheal disease. It is thus unsurprising that the highest prevalence of diarrheal disease is among poor populations. Further, poor children who experience diarrhea are also less likely to receive treatment than children from more well-off households (Barros et al. 2010). Lack of resources leading to late diagnosis and treatment of diarrheal disease as well as limited access to and affordability of health services are key causes of the higher mortality associated with diarrheal disease among poor children (Keusch et al. 2006). In turn, the greater burden of diarrheal disease further compounds the disadvantage experienced by poor individuals by leading to conditions such as undernutrition and poor child development with long-run sequelae on health and well-being.

^{4 &}quot;Play" refers to any time the infant or child is not in a protected position (sleeping or on the caretaker's back, lap. or hands) or left unattended (sitting on the ground, crawling, using toys, or exploring its environment).

1.3 Objectives and Organization of the Review

This review synthesizes the latest understanding of key pathways of fecal microbes, in particular, enteric pathogens and STHs, ingestion by IYC, and the link to diarrhea, EED, and poor nutrition and development outcomes. The review is based on a thorough search of the scientific and gray literature on WASH, child undernutrition, and health, including snowball sampling (chain sampling) from citations within articles. The electronic searches and review of the literature were complemented by key informant interviews with researchers and field implementers to examine sources and pathways of fecal microbe transmission as well as the interventions to disrupt them.

This report seeks to:

- Consider both human and animal sources of fecal contamination and the pathways presenting major
 exposure risks to IYC, including sources and pathways previously underemphasized (and to the
 extent possible, seek to understand the relative potential importance of the different pathways in
 terms of magnitude of pathogen transmission);
- Examine the exposure pathways to which children in the first two years of life may be most susceptible to enteric pathogens and their potential importance; and
- Identify interventions to break these pathways, including established WASH measures and underemphasized risk factors such as animal husbandry, IYC feeding practices, and IYC-focused WASH behaviors.

Findings of the literature review will be used to design field research activities to study the additive effects of specific measures to reduce IYC exposure to fecal pathogens and other fecal microbes in their home environments when coupled with independent or integrated WASH and nutrition interventions.

Following this background section, Section 2.0 reviews key evidence on sources of fecal contamination and Section 3.0 provides the transmission pathways of fecal pathogens and intestinal parasites. This is followed in Section 4.0 by a presentation and discussion of the evidence on the effectiveness of various interventions to disrupt the transmission pathways. Section 5.0 provides a discussion of major findings and recommendations for further areas of research.

2.0 SOURCES OF HOUSEHOLD FECAL CONTAMINATION AND HEALTH RISKS

Human and animal fecal contamination of household environments is pervasive in LMICs where OD, poor environmental hygiene, and close animal husbandry practices are common. Inadequate human and animal excreta disposal, poor wastewater conveyance, and use of animal feces for fuel and housing material are each sources of fecal pathogens and STHs. Inadequate IYC feces disposal and OD by older children are common in many LMIC contexts. Where fecal contamination is abundant, both humans and animals may spread it throughout the living environment (Curtis et al. 2000).

2.1 Adult Feces

Environmental contamination by human feces due to OD and poor FSM represents a key risk factor for diarrhea, acting as a reservoir for specific bacterial pathogens (*Campylobacter jejuni*, enteropathogenic strains of *Escherichia coli*, and *Salmonella* species) (Byers et al. 2001; Curtis et al. 2000; Marquis et al. 1990) as well as protozoan parasites, viruses, and other infectious disease agents (Curtis et al. 2000; Pickering & Davis 2012). It has been asserted that human feces is the definitive source of diarrheal disease, because even feces from healthy people likely contain more human pathogens than that of animals, and should therefore be targeted as the first priority unless there is evidence to the contrary (Curtis et al. 2000). Potential pathogens were identified in 83 percent of children with diarrhea and 72 percent of controls in the large prospective multicenter case-control study, the Global Enteric Multicenter Study (GEMS) (Kotloff et al. 2013). Similarly, one or more pathogens were commonly detected in children without diarrhea from early infancy (64.9 percent versus 76.9 percent of non-diarrhea compared to diarrhea specimens) in a multi-site birth cohort study, MAL-ED⁵ (Platts-Mills et al. 2015). These data highlight the frequency of enteric pathogen carriage even in asymptomatic individuals.

OD presents negative effects within communities and could be especially harmful where population density is much higher (Spears 2013), although the evidence on population density effects is mixed (Jarquin et al. 2016). It has been asserted that OD predicts stunting in India, with between 35 percent and 55 percent of the average difference in stunting between Indian districts attributable to differences in OD (Spear, 2013). In the context of the MAL-ED study, OD was associated with elevated risks of enteric infection among all age groups (younger than 5, 5–12 years, and 12 and older) in Vellore, India; similarly, the presence of a private toilet was associated with decreased risks of enteric infection (Berendes et al. 2016).

The same study points to inadequate FSM representing a growing challenge as access to on-site dry and pour-flush latrines accelerates in both rural and urban households. Direct discharges of lower-water content fecal biosolids into the immediate environment (particularly through flood events) elevate the risks of enteric infection among children in a drain-flooding area in Vellore; meanwhile, even as toilets within the household were associated with reduced enteric infection, toilets emptying to drains were *not* effective at reducing risks of enteric disease during the rainiest season in that area (Berendes et al. 2016).

2.2 Child Feces

Unsafe management of child excreta is common in many countries (Rand et al. 2015; Freeman et al. 2016; Majorin et al. 2014; Miller-Petrie et al. 2016; Mara et al. 2010; George et al. 2016; Gil et al. 2004). Unsafe disposal is defined as feces that are not deposited into any kind of improved or unimproved

⁵ Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health Study

toilet or latrine. Safe disposal of child feces includes helping a child use a toilet or latrine or, for very young children, placing or rinsing their feces into an improved or unimproved toilet or latrine (Rand et al. 2015). In an analysis of Multiple Indicator Cluster Surveys and DHS data, more than 50 percent of households in 15 out of 25 LMICs practiced unsafe disposal of feces of children under the age of three (Rand et al. 2015). The highest levels of unsafe child feces disposal were reported among poor, rural households; among the youngest children; and where other household members were practicing OD. Children younger than three years old were more likely to practice OD across all 25 countries, independent of household defecation practices. All countries reported some unsafe child feces disposal practices even in households with improved sanitation. In India, for example, 56 percent of households with improved sanitation practiced unsafe child feces disposal, with 23 percent of these households leaving child feces in the open (Rand et al. 2015).

Older children are increasingly more likely to use toilets/latrines by themselves, unlike younger ones for whom primary responsibility for feces disposal and training is with the caregiver. In Haiti, for example, 59 percent of households with children less than 12 months old reported safe disposal, as compared to 72 percent of those with children older than four (Rand et al. 2015). The transition toward safe disposal of child feces increasing with child age is happening in many countries, although OD of the older child cohorts remains high. Income is also a predicting factor, with safe disposal of child feces for under-three-year-olds more likely among the 40 percent of the wealthiest households than among the least wealthy 60 percent (Rand et al. 2015).

Inadequate infant feces disposal was associated with a 23 percent increase in the risk of diarrhea among children in Peru (Lanata et al. 1998) and an 11 percent increase among children in India (Bawankule et al. 2017). In a prospective cohort study of children less than 30 months old in rural Bangladesh (George et al. 2016), unsafe disposal of infant feces was associated with elevated markers of EED, lower weight-for-height z-score (WHZ,) and lower weight-for-age z-score (WAZ). Unsafe child feces disposal also was associated with five times greater odds of detecting diarrheagenic *E. coli* in areas where children were observed playing (George et al. 2016). In households where soil samples were collected in children's outdoor play areas, 97 percent had at least one sample with detectable *E. coli*, and 14 percent had at least one sample with detectable to human and/or animal feces.

2.3 Animal Feces

Increasingly, animal feces are being investigated as an additional source of disease risk from zoonotic pathogens. Penakalapati et al. (2017) provide a systematic review of literature addressing: (a) human exposure to poorly managed animal feces; (b) negative human health outcomes from exposure to animal feces (e.g., diarrhea, gastroenteritis, EED, trachoma, STH infections, child growth [anthropometric] outcomes, and infection by zoonotic pathogens); and (c) animal feces contamination of the environment (e.g., water or fields), identifying 62 articles meeting the inclusion criteria. Their findings of the consequences of animal feces exposure include the following:

- Observed effects of feces on human diarrheal illness are heterogeneous.
- Child growth may be impaired, although these effects do not consistently appear in the literature.
- Trachoma (*Chlamydia trachomatis* infection) risk may increase by potentially providing breeding sites for flies that propagate the infection.
- Evidence suggests that cat and dog excreta in particular can lead to an increased risk of STH infections.

Animal feces are important sources of zoonotic bacteria (enteropathogenic E. coli, Campylobacter, and Salmonella) and protozoa (Cryptosporidium and Giardia) (Kotloff et al. 2013). Animal feces are more

widespread in contexts where free-range animal husbandry is practiced and animals are corralled within environments where children sleep and play. For instance, validated microbial source tracking (MST) markers of animal fecal contamination were widely detected in households (as well as water sources) in rural India, indicating widespread risks of exposure to animal feces and zoonotic pathogens; nearly every fecal-oral pathway explored was contaminated with animal feces in both the public and household domains (Schriewer et al. 2015). Over 50 percent of household-stored water samples, 90 percent of mothers' and children's hands, and 74 percent of public ponds were contaminated with animal feces (Schriewer et al. 2015). Meanwhile, in a dense, urban low-income setting in Dhaka, Bangladesh, ruminant fecal contamination of surfaces and hands was also common (Harris et al. 2016). Widespread ruminant and avian fecal contamination was also found in rural settings in Bangladesh, where animal fecal contamination increased with number of animals owned (Boehm et al. 2016). In a multi-country study, presence of animal feces in the domestic environment was negatively associated with hand cleanliness in mothers and children (Headey & Hirvonen 2016).

Free-scavenging poultry are especially common in rural and peri-urban settings (Marquis et al. 1990; Ngure et al. 2013; Harvey et al. 2003). Several studies have isolated pathogenic bacteria (Simango 2006; Marquis et al. 1990) and other fecal bacteria from soil and chicken feces sampled from the domestic environment (Pickering & Davis 2012; Ngure et al. 2013). In rural India, levels of both human and animal (mainly ruminant) fecal contamination in households showed significant associations with subsequent child diarrhea, suggesting that exposure to animal (in addition to human) fecal contamination may increase the risk of child diarrhea in this setting (Odagiri et al. 2016). *Cryptosporidium* and *Giardia* were detected in 12 percent of clinic-admitted diarrhea patients in areas where animal loading estimates suggested cattle were the greatest contributor of *Cryptosporidium* oocysts and *Giardia* cysts to the environment in the region (Daniels et al. 2015). In a recent systematic review, 69 percent of the studies reported an association between exposure to domestic food-producing animals (such as poultry and livestock) and diarrheal illness (Zambrano et al. 2014). Exposure to domestic poultry more than doubled the risk of human campylobacteriosis, and failure to identify the microbial cause of disease might have actually underestimated the effect (Zambrano et al. 2014). Elevated prevalence of *Giardia duodenalis* among Ethiopian children was associated with cattle and manure contact (Wegayehu et al. 2013).

There is evidence of the direct child health hazards of household animal husbandry. In a prospective cohort study in Western Kenya, children from households with animals with digestive disorders experienced slower growth than children in households with healthy animals (Mosites et al. 2015). Explanations offered for this association include the possibility that sick animals were an indicator of household poverty, food shortage, or overall poor household health (Thumbi et al. 2015; Zambrano et al. 2014). Another hypothesis is that sick animals are a source of zoonotic pathogens leading to infectious diarrhea and subsequent growth failure (Checkley et al. 2008; Schlaudecker et al. 2011). Within the same cohort, livestock disease reports were associated with simultaneous human disease reports (Thumbi et al. 2015). While controlling for household size, the odds of human gastrointestinal, febrile, and respiratory illness increased by 31 percent for every 10 cases of animal illness or death observed within the household. Similarly, a 6 and 10 percent increase in the probability of human gastrointestinal and respiratory illness, respectively, was associated with each additional case of the respective illness observed in animals in the same household during the same visit.

In Bangladesh, having an animal corral within a child's sleeping room was associated with elevated EED scores and doubled the odds of stunting (George et al. 2015). The most common animals corralled in children's sleeping rooms were chickens and cows, both important sources of common zoonotic diseases *Campylobacter*, diarrheagenic *E. coli* and *Cryptosporidium* (Bender & Shulman 2004). Presence of

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⁶ Molecular studies also provide compelling evidence that identical protozoal strains of *Cryptosporidium* and *Giardia* exist in domestic animals and humans (Zambrano et al., 2014).

animal feces in the household environment was negatively associated with child height-for-age z-score (HAZ) among 6- to 24-month-olds in Ethiopia and Bangladesh, but not in Vietnam (Headey & Hirvonen 2016). Poultry ownership was strongly associated with observed animal feces in the three countries.

It is important to note, however, that these costs may co-occur with potential nutritional benefits offered by increased food access from domestic animal husbandry (Kaur et al. 2017). Mosites et al. (2015) report a modest child growth benefit of owning household livestock, with a ten-fold increase of livestock ownership associated with lower stunting prevalence in Ethiopia and Uganda (though not in Kenya). The authors adjusted their analysis for such confounding factors as child age at baseline, child sex, number of household members, household baseline wealth, and baseline household livestock ownership count, concluding that "ownership of healthy livestock may improve child baseline health and decrease the impact of infectious diseases that would otherwise lead to stunting" but that the "pathway has not been well evaluated and further research is needed."

A recent cross-sectional analysis of a large agricultural survey in rural Ethiopia suggests that while poultry ownership may be beneficial to child growth, overnight corralling of poultry within the household dwelling may present a concurrent risk for undernutrition via increased risk of infectious diseases (Headey & Hirvonen 2016). With their distinctly mixed findings within a cross-sectional meta-analysis of DHS data from 30 sub-Saharan African countries, Kaur et al. (2017) reinforce the hypothesis that domestic animal husbandry is, at the same time, "protective against stunting, an indicator of chronic malnutrition, and a risk factor for all-cause mortality in children." They identify 13 countries for which domestic livestock ownership is a risk factor for elevated child diarrhea, but in another 10, found a protective association (Kaur et al. 2017). The authors acknowledge that their results reveal considerable heterogeneity among countries for which further data would be needed to infer the relative costs and benefits of the practices within a particular geography.

3.0 PATHWAYS OF FECAL PATHOGEN TRANSMISSION

The transmission pathways for human- and animal-sourced zoonotic pathogens are multiple; however, the relative contribution of these various pathways is not well documented. Similarly, the context-specific environmental, behavioral, technological, and infrastructure factors that affect these pathways and their relative predominance across time and space also are not well understood. What is clear is that contamination of the household environment permeates all other pathways of fecal pathogen transmission including soil, hand cleanliness, food, and water at source and during storage.

The general household physical environment in low-income settings is frequently contaminated with child, adult, and animal feces. The sources of contamination provide a microbial-laden environment where IYC frequently eat, crawl, and play. Maintenance of personal and household hygiene, as well as inculcating healthy hygiene habits in young children, falls primarily to women who are already constrained by many other demands on their time. The same social and cultural norms that hold women responsible for household hygiene consequently stigmatize caregivers for household contamination and sick children.

Poor hygiene of the domestic environment has been linked to EED and growth faltering. In an observational follow-up study of 10- to 48-month-old children in Bangladesh, young children living in a more hygienic household environment had lower levels of parasitic infection, improved gut function, and improved growth compared to their peers living in a contaminated environment (Lin et al. 2013). Children living in cleaner household environments had 0.54 higher HAZ, 22 percent lower stunting, and 0.32 standard deviation (SD) lower lactulose:mannitol ratio than children living in dirtier households.⁷ A clean environment was defined as a household with good water quality (median E. coli <10 CFU/100mL in quarterly drinking water samples collected over 24 months), improved sanitation (flush/septic/piped sewerage or a pit latrine with a slab and a water seal), and hygienic handwashing conditions (a dedicated handwashing location stocked with soap and water). In contrast, a contaminated environment was defined as a household with poor water quality (median E. coli ≥10 CFU/100mL), inadequate sanitation (OD, open pit latrines, slabs with broken water seals, toilets that flush to somewhere else, or hanging toilets), and unhygienic handwashing conditions (a dedicated handwashing location that lacked either water or soap, or the absence of such a dedicated location). In a cross-sectional study in Ethiopia, a hygiene index representing personal and household cleanliness was associated with child growth or stunting among children aged two to five years, independent of water quality, the presence of sanitation facilities, infant feeding practices, recent morbidity, household food security, and socio-economic status (Ngure, 2012). The hygiene index was an additive score of eight attributes, including mother's hand cleanliness, child's hand cleanliness, compound appearance relative to hygiene, general appearance inside the house, whether the area around the house needed to be swept, presence of garbage around the house/compound, whether the floor inside the house needed to be swept, and piles of dirty clothes.

3.1 IYC Soil and Feces Ingestion

Exploratory mouthing of hands and objects occurs often and is theoretically desired in early stages of child development. However, IYC frequently explore, crawl, sleep, and feed in contaminated environments, exposing themselves to risk as well as opportunity (Teunis et al. 2016). Ingestion of dirt and feces by IYC through mouthing soiled fingers, play objects, and household items, as well as exploratory ingestion of contaminated soil and/or poultry feces, is common in low-income environments (Marquis et al. 1990; Ngure et al. 2013; Reid et al. 2018) . Hand and object mouthing is frequent and

⁷ The lactulose:mannitol ratio is a measure of intestinal permeability, which is an indicator of EED.

common among 3- to 18-month-old children in rural Bangladesh (Kwong et al. 2016). An in-depth observation study of 23 households in rural Zimbabwe, lasting over six hours, found infants ingested nearly a dozen handfuls of soil and two direct ingestions of chicken feces (Ngure et al. 2013). Although based on a small non-random sample (with wide variance around the centers of spread), exploratory soil and chicken feces ingestion were identified as potentially significant pathways for fecal matter among 3to 18-month-old children due both to a high indicator bacterial load in chicken feces and the frequency of ingestion. On average, 69 CFU and 10 million CFU of E. coli were detected per gram of soil from laundry areas and chicken feces respectively. In a similar study in rural Zambia, 93 percent of mothers surveyed reported observing their children eating soil and 17 percent reported observing their child eating chicken feces (Reid et al. 2018). Ingestion of soil was also reported in 18 percent of 7- to 30month-old children in rural Bangladesh (George et al. 2015a), a context in which 97 percent and 14 percent of the households exhibited detectable E. coli and pathogenic E. coli in the soil, respectively. In a longitudinal study of 175 households in two rural locations in Njoro, Kenya, 37 percent of the children between one and four years old were observed occasionally ingesting less than a handful of soil, while 12 percent ingested a handful or more (Shivoga & Moturi 2009). Ingestion of chicken feces by young children also was reported in an in-depth observation study in a peri-urban setting in Lima, Peru (Marquis et al. 1990). In the Peruvian study, Campylobacter jejuni, a pathogenic bacterium that causes dysenteric diarrhea, was isolated in chicken feces up to 48 hours after deposition.

A Monte Carlo Simulation using fecal indicator bacteria (FIB) data from hand rinses and stored drinking water estimated that Tanzanian children ingested more feces each day from hand-to-mouth contact than from drinking water (Mattioli et al. 2015). Hand-to-mouth contact also far outweighed contaminated water as the dominant source of FIB for children less than six months old. (Many infants rely on breastfeeding and thus drink the least amount of water.) A recent cross-sectional study in urban slums in Nairobi, Kenya, found diarrhea to be associated with caregiver-reported soil ingestion by IYC between three months and five years of age (Bauza et al. 2017). *E. coli* and human-associated *Bacteroides* fecal marker (HF183), enumerated with locally validated methods, were detected in 100 percent and 93 percent of soil samples, respectively, collected near each of the 54 households in the study sample. Diarrhea in children was also associated with soil ingestion in rural Kenya (Shivoga & Moturi 2009).

3.2 Contaminated Water

Fecal contamination of drinking water sources, including piped water sources, is widespread and affects an estimated 1.8 billion people globally (Bain et al. 2014). In general, drinking water in rural areas is more likely to be contaminated with feces than water in urban areas, although the relative safety of rural water versus urban water is dependent upon the mix of local sources and the operational success of individual water service providers.

An inverse relationship between the distance of a water supply from a latrine and the level of fecal contamination of such a water supply has been reported (Sclar et al. 2016). Although groundwater contamination often occurs downgradient from latrines, pollutant transport distances, recommendations based on empirical studies, and latrine siting guidelines are not well aligned (Graham & Polizzotto 2013).

By using more traditional fecal indicator tests as well as novel MST markers, a compelling picture was presented of the extent and mix of human and animal fecal contamination of water (among other fecal-oral pathogen transmission pathways) (Odagiri et al. 2016). In the cross-sectional study of 60 villages in Odisha, India, 74 percent of household stored drinking water (SDW) tested positive for thermotolerant coliforms, while 53 percent of SDW samples tested positive for *Bacteroidales* (Odagiri et al. 2016). The authors highlight significant animal fecal contamination (33 percent of samples) and human fecal contamination (19 percent of samples) of SDW. These findings further support the notion that the domestic environment is important for transmission of animal-sourced zoonotic pathogens in addition to human pathogens (Schriewer et al. 2015).

More than 50 percent of tubewells sampled contained detectable fecal contamination (measured by the total *Bacteroidales* fecal marker), with such specific pathogens as rotavirus, *Cryptosporidium*, and *Giardia* detected in between 8 and 15 percent of protected groundwater sources (Odagiri et al. 2016). Tubewells have long been considered among the safest of the groundwater sources because they are less susceptible to contamination than open wells, protected springs, or such unimproved surface water sources as streams, rivers, and ponds. It was also noted that increased village prevalence of child diarrhea in the six weeks following sampling conducted at each additional tubewell was detected with a tested diarrheal pathogen (Odagiri et al. 2016). More than half of the ponds in the study area exhibited domestic animal feces contamination, with this pattern persisting throughout the monsoon season (Daniels et al. 2015). The study population was characterized by low sanitation coverage (10 percent of households) and high domestic animal ownership (59 percent), mostly of cattle. Critically, the use of open ponds for non-drinking purposes such as anal cleansing after defecation, brushing teeth, and domestic hygiene activities was common in more than 50 percent of the households (Odagiri et al. 2016).

3.3 Hand Hygiene

Unhygienic hands are important vectors of fecal pathogen transmission. In Bagamoyo, Tanzania, enteric viruses were frequently detected on hands, suggesting that these are vectors of viral illness transmission (Mattioli et al. 2015). *E. coli* virulence genes were detected with roughly equal frequency in stored water and on hands, suggesting that both hands and water were important pathways for bacterial pathogen transmission. Similarly, enteric pathogen markers in SDW were more likely to be found in households where these markers were detected on hands as well as those with unimproved water supply and sanitation infrastructure (Mattioli et al. 2015). These findings are consistent with the idea that contaminated hands can affect the quality of SDW, as suggested by studies testing FIB (Pickering et al. 2010) and those using human and animal MST fecal markers in rural India (Schriewer et al. 2015). In places where hand feeding is common, IYC may directly ingest high dosages of fecal pathogens through caregivers' hands.

Visible soiling of caregiver hands was associated with elevated levels of fecal calprotectin (a fecal marker of EED) in a cross-sectional study of 216 children under the age of 30 months in rural Bangladesh (George et al. 2015). In a low-income urban setting in Dar es Salaam, Tanzania, an increase in FIB contamination of caregivers' hands was found after toilet use, cleaning up child feces, sweeping, cleaning dishes, preparing food, and bathing (Pickering et al. 2011). Fecal contamination of mothers' and children's hands appeared to drive post-collection contamination of household SDW (Mattioli et al. 2015; Schriewer et al. 2015). Separately, the use of lower-than-recommended volumes of water for daily handwashing and hand-water or hand-food contacts was associated with elevated enteric pathogen markers on mothers' hands in Bagamoyo, Tanzania (Mattioli et al. 2013). These findings underscore the role of hands as an important vector in contamination of the home and in disease transmission, and emphasize the difficulty of maintaining good personal hygiene in resource-poor settings that lack access to high-quality water supplies and improved sanitation.

3.4 Complementary Food Hygiene

Consumption of contaminated food, compounded by its preparation with contaminated water, is a significant vehicle for bacteria, virus, and parasite transmission to IYC, particularly when foods and liquids are added to the diet of previously exclusively breastfed children (M. A. Islam et al. 2012; Saha et al. 2009). Food is one of the most important factors in transmitting pathogens that cause diarrheal illness (Motarjemi et al. 2012). While a wide range of pathogens can cause foodborne diseases, viruses, bacteria, and parasites pose the greatest share of preventable foodborne threats (Lee et al. 2014; Fischer-Walker et al. 2013). Food serves as a vehicle for virus and parasite transmission to a new host;

however, for many bacteria, food offers an opportunity to grow exponentially to infectious levels. Some bacteria, such as *Staphylococcus aureus* and *Bacillus cereus*, will produce toxins while growing in food, resulting in foodborne intoxications or food poisoning. More than 120 important viral, bacterial, and parasitic agents transmitted by food have been identified. Thirty-two of these are significant public health problems, and more than half cause diarrhea, either alone or in combination with other adverse symptoms (Motarjemi et al. 2013)

Water is also a critical component of food hygiene, playing a significant role at multiple points in food preparation, including washing ingredients, rinsing utensils, cooking, and handwashing (or not). At the point of consumption, it is challenging to attribute the extent to which food in isolation (exclusive of water) contributes to disease transmission. Therefore, many research studies that evaluate household food hygiene include water as a component of food (Woldt & Moy 2015). Up to an estimated 70 percent of diarrheal episodes among young children could be due to pathogens transmitted through food (Motarjemi et al. 1993; Esrey & Feachem 1989).

Fecal contamination of complementary foods (CF) is important in the transmission of bacteria and pathogens to IYC,⁸ although the burden of diarrhea attributable to CF contamination is still not yet well documented. CF contamination is determined by several factors, including poor water quality, water scarcity, contaminated hands of food preparers and child-feeders, contaminated food preparation surfaces (fomites), contaminated cooking utensils, cross-contamination by raw food and flies, improper cooking and storage, and exceeded food storage time. Child feeding practices, such as bottle-feeding, use of unclean hands by caregivers, and self-feeding, may also increase the risk of fecal pathogen ingestion.

In rural and urban Bangladesh, 18 percent of tested complementary food samples for children were contaminated with high levels of fecal coliforms (≥ 100 CFU/g of food) and 29 percent were positive for *E. coli* (M. A. Islam et al. 2012). Freshly prepared foods had lower FIB counts compared to leftover foods meant for multiple feeding, and microbial counts increased with the duration of storage at room temperature for the leftover food. As in many other LMICs, most of the decline in HAZ among rural Bangladeshi IYC occurs during the complementary feeding age (Saha et al. 2009). In a Tanzania study, FIB counts increased significantly for complementary foods stored longer than four hours, compared to freshly prepared food (Kung'u et al. 2009). In the Tanzania study, no drinking water source samples met the World Health Organization's guidelines for drinking water safety, and thus may have served as a source of contamination in the preparation of commercially fortified and instant porridge flours.

A number of pathogenic *E. coli* strains, including enterotoxigenic, enteropathogenic, and Shiga-toxin *E. coli*, also were detected in complementary food samples in Bangladesh (M. A. Islam et al. 2012). The report also indicated that elevated aerobic plate counts were associated with the presence of *E. coli* in CF as well as with the frequency of feeding the same food for multiple meals over time.

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Complementary foods are foods introduced to complement breastfeeding, ideally only after the first six months of exclusive breastfeeding. In many contexts, complementary liquids are given in early infancy and complementary feeding can start as early as three months of age.

4.0 INTERVENTIONS TO BREAK PATHWAYS FOR FECAL PATHOGEN INGESTION

The interventions discussed below are approaches to disrupt major pathogen transmission pathways discussed in the previous section. Diarrheal disease reduction strategies have generally focused on increasing water supply, improving drinking water quality, facilitating better hand hygiene, and improving sanitation, both through the reduction of OD and the installation and use of latrines. However, interventions to disrupt pathogen transmission pathways typically have not been designed to address animal feces as a source of pathogens nor the underemphasized pathways. Thus, interventions have not typically incorporated animal husbandry practices, proper disposal of animal wastes, or direct ingestion of feces by IYC.

As discussed in some detail below, there is considerable variance in observed effects across interventions, which is not surprising given the differences in baseline conditions of water and sanitation access and hygiene behaviors, as well as in geography and context. Most of the high-quality intervention studies reviewed in this section or included in previous systematic reviews are randomized controlled trials (RCTs) conducted under near-ideal and rigorous experimental conditions and have limited scaling potential because of the intensity to replicate at scale. There is a dearth of implementation and process evaluation data (such as fidelity of intervention delivery and household uptake of promoted behaviors and practices) from RCTs or observational, programmatic, and gray literature to inform future programming and scaling efforts. The few studies available are included in this review. Additional such data are expected from the Sanitation Hygiene Infant Nutrition Efficacy (SHINE) research trial (Humphrey et al., 2015).

4.1 WASH Interventions

4.1.1 WATER

Published studies of water and diarrhea have considered a wide variety of interventions (e.g., water supply improvements, community-level water quality improvements, and household-level water quality improvements) and baseline conditions (e.g., reliance on unimproved sources, on improved supply community sources, or on basic piped delivery to premises with continuous supply). There is substantial evidence on the effects of improved water supply and point-of-use water treatment in reducing the risk of diarrhea among children under five years old. The magnitude of risk reduction varies with the type of intervention and study context. Such evidence is mixed for water supply interventions alone. There is limited and suggestive evidence on the effects of: (1) access to improved water supply on child stunting and wasting and (2) water treatment on weight-for-age body measurement for underweight children.

In a systematic review updating reviews conducted by Fewtrell et al. (2005), Waddington et al. (2009), and Cairncross et al. 2010), Wolf et al. (2014) provided a comprehensive schematic depiction of the myriad combinations of baseline conditions and interventions addressed in the literature, concluding that large *potential* reductions in diarrheal disease risk could result from water interventions. At the same time, the authors caution that results must be considered carefully because of inconsistencies among baseline conditions/intervention combinations. Combining random-effect meta-analysis and Bayesian meta-regression (as well as statistical tools to adjust for both bias and discrepancies between blinded and non-blinded studies), it was estimated that the largest water-related diarrhea risk reductions occurred when households lacking improved water supply were provided with continuous, high-quality piped water (Wolf et al. 2014).

Evidence of the effects of improved water supply on diarrhea (without accompanying water treatment interventions) is mixed. The seminal meta-analysis yielded a pooled estimate of a 27 percent diarrhea reduction attributable to water supply interventions (Esrey et al. 1991). By contrast, it was reported that the water supply interventions included in their meta-analysis average "a negligible and insignificant impact on diarrhea morbidity compared to controls" (Waddington et al. 2009).

It was estimated that water quality interventions (point-of-use water treatment using chlorine) reduced diarrhea morbidity by 17 percent compared to contaminated water (Esrey et al. 1991). Such interventions had an average effect, reducing diarrhea morbidity by 42 percent relative to controls (Waddington et al. 2009). Household filters generally outperformed household chlorination, and in each case, safe storage provided significant additional protection (on the order of an additional 12 to 15 percent risk reduction) (Wolf et al. 2014).

With respect to water-related interventions specifically and child growth, we are aware of three published attempts to disaggregate child growth impacts of water from those attributable to more general WASH interventions:

- 1. An analysis of the Young Lives Younger Cohort data from Ethiopia, India, Peru, and Vietnam (Dearden et al. 2017), where access to improved water was associated with a reduction in stunting and wasting/thinness at one and five years of age among children in Ethiopia.
- 2. An analysis of DHS data that yielded an 8 percent reduction in odds of mild to severe stunting from improved water supply access (Fink et al. 2011).
- 3. A study of the benefits of household solar disinfection, which detected significant increases in WAZ scores among children under five years old attributable to the intervention (du Preez et al. 2011).

Articles forthcoming from the WASH Benefits Study trial in Kenya and Bangladesh should provide additional insights on water-related interventions and child growth,⁹ although the study is limited to water treatment (as opposed to water supply) interventions (Arnold et al. 2013).

4.1.2 SANITATION

Based on evidence drawn largely from observation (non-experimental) studies, improved sanitation is associated with a lower risk of diarrhea and STH infections. An emerging body of evidence from high-quality experimental studies shows mixed effects of improved sanitation on health outcomes such as diarrhea, EED, STH, and child growth.

Improved sanitation is one of the primary barriers to fecal-oral transmission of pathogens. Sanitation improvements can take a variety of forms (from pit latrines with slabs to pour-flush latrines with septic tanks to piped conveyance of sewerage, among others). However, "improvements" do not necessarily equate to "safety." It was estimated that diarrhea was reduced about 16 percent after a transition from unimproved to improved (but *non*-sewered) sanitation, but much larger benefits were found with progression up the sanitation ladder, with risk reductions in the order of 60 to 70 percent from the introduction of piped sewerage (the number of studies considered was quite small) (Wolf et al. 2014). More generally, current literature emphasizes diarrhea reductions ranging from 22 percent to 36 percent attributable to improved sanitation (Fewtrell et al. 2005; Waddington et al. 2009; Cairncross et al. 2010; Engell & Lim 2013; Wolf et al. 2014). A Cochrane review of interventions to improve disposal of human feces found the interventions to be protective against diarrhea in 11 out of 13 studies (Clasen

The WASH Benefits Study will provide rigorous evidence on the health and developmental benefits of water quality, sanitation, handwashing, and nutritional interventions during the first years of life. The study includes two cluster-randomized controlled trials to measure the impact of intervention among newborn infants in rural Bangladesh and Kenya. The studies are large in scope (> 5,000 newborns per country) and will measure primary outcomes after two years of intervention.

et al. 2010), but the poor quality and heterogeneous nature of the studies limited an estimation of effect size. Evidence from a recent systematic review shows no effect of sanitation interventions on water quality (source or household), hands or sentinel toys, food, household and latrine surfaces, or soil fecal contamination (Sclar et al. 2016). Household toilets were associated with reduced risk for bacteria and parasites (although statistically insignificant for parasites), but no association for viruses was observed in a low-income urban setting in Vellore, India. Meanwhile, toilets emptying to conveyances from the households were protective against enteric infection in the dry season but were ineffective during the rainiest season in India (Berendes et al. 2016).

The combined effects of increased availability and use of sanitation facilities appear to reduce the odds of STH infection for Ascaris lumbricoides, Trichuris trichiura, and hookworm combined (Ziegelbauer et al. 2012) and for any STHs (Strunz et al. 2014). However, evidence from more recent higher-quality studies on the impact of sanitation interventions on diarrhea, STH infections, and child growth is mixed. Two randomized controlled evaluations of the Indian government's Total Sanitation Campaign (TSC) showed no impact on diarrhea, gastrointestinal illness, helminth infection, growth, and anemia in rural Madhya Pradesh (Patil et al. 2014) or on fecal contamination of the household, diarrhea, STHs, and child HAZ in Odisha (Clasen et al. 2014). The absence of an observable health effect in both Indian cases was likely due to low utilization of sanitation facilities. Qualitative research in India determined that toilet use did not increase in sites where toilet access rapidly increased because many of the government latrines were poorly constructed and, in many cases, lacked a nearby water supply source for anal cleansing (Routray et al. 2015). People are more likely to use latrines when they are functional, well maintained, accessible, clean, private, and provide amenities for practicing hygienic behaviors such as anal cleansing and menstrual management (Garn et al. 2017).

In addition to latrine coverage as a response parameter in a systematic review of sanitation interventions, an estimated 13 percent increase was observed in latrine *usage* likely resulting from CLTS combined with other programming (such as sanitation marketing), education, and total sanitation campaigns (Garn et al. 2016). It must be noted, however, that only 10 studies are included in this analysis (as opposed to other analyses on latrine coverage drawing from 27 countries), and the overall pooled estimate is driven largely by three outliers: a 34 percent increase in latrine utilization observed in Mali (Pickering et al. 2015), a 39 percent increase resulting from the introduction of community health clubs in Zimbabwe (Waterkeyn & Cairncross 2005), and a 27 percent increase in households with any observable latrine use under India's TSC in Odisha (Clasen et al. 2014). Studies also determined latrine usage to be highly sub-optimal in Odisha, which could explain why the apparent high usage did not lead to health gains (Jenkins et al. 2014). (Latrine use was frequently non-exclusive and limited to one or two household members, and OD persisted at high rates even with latrine ownership (as is common in rural India). In addition, threshold levels of coverage to achieve herd effects may not have been reached, and household sanitation improvements may be simply insufficient to mitigate fecal-oral pathogen exposure (Clasen et al. 2014).

Two recent randomized controlled evaluations of CLTS interventions are also worth noting. The first, a national-scale CLTS roll-out in Indonesia achieved a significant (44 percent) reduction in roundworm infestation, but discerned no other impact on diarrhea (perhaps unsurprisingly, since it resulted in only a 3.3 percent increase in toilet construction) (Cameron & Shah 2017). The second evaluation took place in the Koulikoro district of Mali, which also found no impact on diarrhea despite a 33 percent reduction in OD between treatment and control arms (Pickering et al. 2015). Diarrhea was only measured at one time during the dry season.

There are several possible explanations for the lack of impact on diarrhea in these studies. Some were underpowered to detect meaningful reduction in diarrhea and other health outcomes (Arnold et al. 2010; Clasen et al. 2014); others achieved insufficient latrine coverage and/or use to bring about measurable impact (Hammer & Spears 2013; Patil et al. 2014).

It is important to note that the CLTS intervention tested in Mali involved intensive behavior change interventions over a 12-month period, which resulted in greater adoption of toilets and of handwashing with soap than observed in other trials (Pickering et al. 2015). Children in the CLTS communities were significantly taller (0.18 increase in HAZ) and less likely to be stunted (6 percent reduction in prevalence) at the end line compared to those from the control communities, with effects largely driven by gains in children under two years of age at enrollment (0.24 increase in HAZ) (Pickering et al. 2015). This study suggests that CLTS may have prevented growth faltering through pathways other than diarrhea, and possibly through reduction in EED.

A sub-group analysis within a recent systematic review of 29 studies, most of which were observational studies and low in quality, suggested an 80 percent coverage threshold beyond which sanitation interventions are more effective at reducing levels of observed feces (Sclar et al. 2016). Studies from India, Indonesia, Mali, and Tanzania identified a fairly linear relationship between OD and a measure of child growth, in which conversion of a community from full OD to ODF status corresponded to a 0.44 standard deviation increase in child height (Gertler et al. 2015). (The slope of the curve suggests that small to modest OD reductions are unlikely to result in measurable child height changes.

4.1.3 INFANT AND YOUNG CHILD FECES MANAGEMENT

Most of the previously discussed sanitation interventions do not emphasize or are not explicit on strategies to address child feces disposal. TSC and CLTS interventions do not explicitly focus on adequate child feces disposal (Freeman et al. 2016). More recently, some CLTS programs have begun to incorporate a focus on IYC feces disposal (Rand et al. 2015).

Evidence from observation studies suggest that the use of enabling technology, such as child potties, sani-scoop, and diapers, facilitate adequate disposal of child feces. In a survey of 130 households in 21 villages across two provinces in Cambodia, hygienic disposal of children's feces was associated with child age, years of latrine ownership, caregiver's age, consistency of adult latrine use, and presence of child feces management tools in the latrine (Miller-Petrie et al. 2016). Notably, the youngest caretakers with the newest latrines and the youngest children were the least likely to manage IYC excreta hygienically, and thus represent a key target for behavior change (Miller-Petrie et al. 2016). Child potties and diapers were helpful in facilitating hygienic disposal of child feces in this context, but locally available potties were designed for generic household use and not specifically for ease, safety, and convenience of use by IYC, making them less convenient for caregivers. Reusable diapers with easy-to-use covers, child-friendly potties, and child-safe latrine seats were attractive "enabling products" for caretakers, offering a time and cost savings, easy disposal, and easy cleaning. Disposable diapers were less likely than reusable diapers to be disposed of hygienically and presented a solid waste disposal challenge, and therefore may not be an ideal product (Miller-Petrie et al. 2016). The use of potties or chamber pots have been culturally accepted and used in improving hygienic disposal of child feces in Nigeria and Peru (Jinadu et al. 2007; Yeager et al. 1999).

In the context of the formative research preceding the WASH Benefits Study trial in Kenya and Bangladesh, an observation study of 20 households in rural Bangladesh suggested that a light and easy-to-use mini-hoe, redesigned from a heavy-duty farming hoe, was well accepted for removal of child feces from the household courtyards into a proper latrine (Sultana et al. 2013). In this context, most children under the age of three defecated in the open within the household courtyard. Caretaker preference for a particular method of feces disposal was driven by disgust and convenience, availability, and affordability of materials/tools. This mini-hoe was later adapted for use as an enabling product (a locally developed sani-scoop) for child and animal feces management in the full trial (Arnold et al. 2013). While providing enabling technologies for disposal of child feces, the WASH Benefits Study trial did not factor willingness-to-pay into the design, a limitation to adoption and scaling up of technologies that is common with such trials, including SHINE. In a study in Cambodia, the adoption of scoops and shovels for

transporting feces to latrines was rare due to consumer fears about filling pits with dirt, even as these same tools were used widely for burying feces (Miller-Petrie et al. 2016). Although scoops were useful for burying child feces, this practice was deemed "unhygienic" by a consultation group of sanitation experts because natural disturbances may uncover the feces (Bain & Luyendijk 2015).

A USAID project in Bangladesh conducted qualitative research to develop and program age cohort-specific IYC feces disposal options. Outcomes included documentation of age cohorts recognized by mothers and families and age-specific options for effective feces disposal that were considered feasible by mothers and households. Age cohorts included the lap child, crawling baby, toddler, and young child, with feces disposal recommendations for each. In Bangladesh, the WASHplus activity saw a 13 percent increase in safe disposal of child feces (for all age cohorts) through technological and behavioral inputs, and a 14 percent drop in feces being disposed of indiscriminately (USAID / WASHPlus Project 2016a). The study methodology, however, was conducted pre-program and post-program, at random project focal areas without a control group.

Also under WASHplus, an integrated WASH-nutrition project in Mali included a focus on child handwashing and safe disposal of IYC feces, and documented a 33 percent increase in the practice of safe disposal of infant feces. A similar intervention in Kenya documented a 25 percent increase, through both intensive interpersonal communication and promotion of enabling technologies such as potties (no products were actually distributed to households) (USAID / WASHPlus Project 2016b; USAID / WASHPlus Project 2016c).

Access to water within households was associated with hygienic disposal of child feces. In Burkina Faso, mothers with access to tap water in the yard reported hygienic disposal of infant feces three times more often than those who relied on wells outside the compound, and twice as often as mothers who used public standpipes or wells within the yard (Curtis et al. 1995). The authors concluded that improved water service provisions encouraged safe disposal of child feces by reducing the time spent collecting water and allowing more consistent hygienic excreta disposal.

Identifying age cohort-specific actions and technologies for the improved disposal of IYC feces was critical to improving practices, with interventions specifying safer disposal options for each developmental stage. Low perception of risk of IYC feces influences proper disposal, as does lack of enabling technologies, feasible behaviors, and water supply to facilitate proper disposal. Modifications of household latrines to make them more "child friendly" may lead to increased use by young, mobile children, as in Bangladesh, Mali, and Kenya, where households were encouraged to make latrines less "scary" and more stable, and were associated with increases in safe feces disposal (USAID, 2016a, 2016b, and 2016c).

4.1.4 HANDWASHING

Substantial evidence exists on the effectiveness of handwashing in preventing diarrhea and STH infections. This ranges from weak evidence from observational studies to high-quality evidence from RCTs. However, the magnitude of effect size ranges between studies and contexts.

A recent Cochrane review concluded that handwashing promotion in LMIC communities (as opposed to school settings specifically) can prevent about 28 percent of diarrhea episodes, based on eight trials with a total of roughly 14,000 participants (Ejemot-Nwadiaro et al. 2015). Notably, however, six of the eight trials were from Asia, with one study each for South America and Africa evidence categorized as being of "moderate quality" (meaning that further research is likely to have an important impact on confidence in the estimate of effect and may change the estimate according to the GRADE Working Group). Among the best known studies is a cluster randomized trial in Karachi, Pakistan, in which intervention households receiving both soap and a program of household-level handwashing promotion reported 53

percent less diarrhea than did those in control households (Luby et al. 2006). Handwashing with soap is associated with reducing diarrhea risks by up to 48 percent in several systematic reviews (Cairncross et al. 2010; Curtis & Cairncross 2003; Fewtrell et al. 2005; Waddington et al. 2009), some of which included studies of much lower quality that were likely to be subject to reporting bias. Handwashing before food preparation and/or child feeding is a frequently missed opportunity for habituating hygienic behaviors (Halder et al. 2010; Winch et al. 2013) and reducing childhood diarrhea. Handwashing with water before food preparation was associated with reduced risk of diarrhea among Bangladeshi children (Luby et al. 2011). A much higher risk reduction was associated with handwashing with soap. A large-scale IYC feeding program in Bangladesh, Ethiopia, and Vietnam conducted by Alive & Thrive demonstrated large and rapid increases in handwashing behaviors before food preparation and child feeding. The initiative's comprehensive program consisted of advocacy, interpersonal communication, community mobilization, mass communication, and strategic use of data (Sanghvi et al. 2013).

Evidence from a meta-analysis of cross-sectional studies and five RCTs suggested a protective effect of handwashing on STH infection (Strunz et al. 2014). Handwashing before eating and after defecating was associated with lower risks of A. lumbricoides infection. Availability and use of soap as well as handwashing after defecation were also associated with lower infection with any STHs. Two school-based studies also found an additive effect of hygiene education when coupled with the administration of deworming medication, significantly reduced rates of reinfection (Bieri et al. 2013; Gyorkos et al. 2013).

4.1.5 IMPROVED COMPLEMENTARY FOOD HYGIENE

Evidence drawn from a few recent studies suggests that interventions promoting key complementary food hygiene behaviors are effective in reducing FIB. However, this evidence comes from observation studies of short duration, and it is unclear if these behaviors are sustained in the long term.

Most food hygiene interventions promote improved food hygiene practices by focusing on health and safety benefits. A food hygiene intervention that followed hazard analysis critical control points (HACCP) quality control and quality assurance¹⁰ conducted to identify CF contamination points in Bangladesh significantly reduced fecal coliforms by 1.74 and fecal streptococci by 1.83 log10 CFU/g of CF (M. S. S. Islam et al. 2012). The food hygiene behaviors encouraged in the study were sustained three months after the intervention. After training and observation, recommendations were made for: (1) washing hands with safe water (defined as "water free from harmful microorganisms and substances") and soap before starting meal preparation or feeding a child, after cleaning a child's bottom, and after using the toilet; (2) using safe water to wash utensils and prepare food; (3) cooking and reheating foods until boiling; and (4) covering food with a lid during storage. A similar study in peri-urban Mali demonstrated substantial reduction in the number of fresh, stored, and reheated CF samples contaminated with fecal coliforms (at >10 CFU/g) after a food hygiene intervention based on a modest resource HACCP approach (Touré et al. 2013), with better results three months after the intervention. A complementary food hygiene intervention promoting five key behaviors, following the HACCP approach, in rural Nepal reduced the mean coliform count by 100 and E. coli CFU/g tenfold after adjusting for baseline counts (Gautam 2015). In the Nepalese context, contamination of water at baseline was low compared to food, and did not improve after the intervention.

4.1.6 COMBINED WASH INTERVENTIONS

The evidence of the effect of combined WASH interventions on child health outcomes, such as diarrhea and growth, is far from definitive. Some evidence obtained from observation studies suggests improved

For more information on HACCP, see http://www.fsis.usda.gov/Oa/background/keyhaccp.htm. The seven principles of HACCP in the food industry address: I) hazard analysis, 2) critical control point identification, 3) establishment of critical limits, 4) monitoring procedures, 5) corrective actions, 6) record keeping, and 7) verification procedures (U.S. Department of Agriculture, 1998).

water and sanitation conditions are associated with improved linear growth and weight gain in under five-year-old children. Similarly, evidence from a few experimental studies of low quality suggests a modest effect of WASH interventions on HAZ scores in children under five years old.

The additive or synergistic effects of different WASH interventions on child health are complex and not well understood. A synergistic effect of water and sanitation on child growth among infants in Lesotho was documented in a prospective study (Esrey et al. 1992) but not in Sudan (Merchant et al. 2003); however, the lack of an effect in Sudan was potentially due to modification of hygienic conditions of the external environment or hygiene practices. Using a non-randomized but robust matched cohort design, no health impacts were found from a combined sanitation promotion, water supply, and hygiene intervention on diarrhea and HAZ among infants and children under the age of five in rural southern India. The absence of a detected benefit in that study may be explained by the low prevalence of endemic diarrhea in the area (and consequent under-powering of the study) (Ejemot-Nwadiaro et al. 2015). In a simulation that modeled different fecal-oral transmission pathways for enteric pathogens, the benefits of a household water quality intervention depended on existing sanitation and hygiene conditions, with water quality improvements having minimal effects in contexts with poor sanitation conditions (Eisenberg et al. 2007). This study supported an integrated WASH approach to address the interdependent and complex pathways of fecal pathogen transmission (Eisenberg et al. 2007). Evidence from observation studies (VanDerslice & Briscoe 1995), analysis of DHS data (Esrey 1996) and water quality intervention trials (Gundry et al. 2004) supported the assertion that the interaction of different levels of water and sanitation service improvements may impact child health.

Other evidence from published RCTs questioned the synergy among WASH interventions specifically with respect to diarrhea. Clasen et al. (2015) noted that "the effectiveness of [household water treatment] interventions in settings without improved sanitation contradicts earlier findings that interventions to improve water quality are effective only where sanitation has already been addressed." It was also found that, while household water treatment and handwashing with soap reduced child diarrhea by 51 percent and 64 percent, respectively, there was no additional reduction in diarrhea among children in Pakistan who were exposed to both interventions (55 percent) (Luby et al. 2006). An earlier meta-analysis of 46 studies on interventions to improve WASH found that combined interventions were no more effective in reducing diarrhea than those with a single focus (Fewtrell et al. 2005). The need for high-quality evidence on the independent and combined effects of WASH motivated the design of the WASH Benefits Study trials in Kenya and Bangladesh (Arnold et al. 2013), results from which are forthcoming.

Evidence from a sub-study of a multi-armed randomized trial (from the WASH Benefits Study) found limited reduction in overall fecal contamination in the household environment from a sanitation intervention in rural Bangladesh (Boehm et al. 2016). The limited effect was attributed to widespread ruminant and avian fecal contamination. The sanitation intervention included provision of a hygienic dual-pit latrine, child potty, metal scoop for removal of child and animal feces from the environment, and behavior change promotion on the use of latrines and accompanying hardware inputs. Despite limited overall reductions in fecal contamination in the household, the interventions did, however, reduce ruminant fecal contamination in drinking water and general fecal contamination of the soil, likely due to the use of the sanitary scoop to reduce the amount of ruminant feces in the environment and ultimately in water sources.

Integrated WASH measures related to child growth are exceedingly complex. A Cochrane review of evidence on WASH interventions and child nutritional status identified no evidence of an effect of WASH interventions on WAZ or WHZ, but did identify a borderline statistically significant effect on HAZ, with findings supported by data from more than 5,300 children from five cluster-randomized controlled trials (Dangour et al., 2013). However, the reported modest effect on HAZ was specifically for solar water disinfection, provision of soap, and improvement of water quality for intervention studies

of relatively short duration, none of which was of high methodological quality. Other studies that detected beneficial effects on caregiver-reported diarrhea among IYC did not detect improvements in linear growth (Bowen et al. 2012; Arnold et al. 2010; Langford et al. 2011; Luby et al. 2006; Stanton et al. 1988).

At the same time, a number of studies observed improved linear growth resulting from sanitation and water supply improvements (Esrey et al. 1992; Esrey 1996; Cousens et al. 1990). A prospective cohort pilot study from rural Bangladesh enrolled in a pilot for the WASH Benefits Study trial found lower parasite infection, improved biomarkers for EED, and better growth among children raised in "cleaner" households (those with improved sanitation, hygiene, and water quality conditions) than those in "dirtier" ones (Lin et al. 2013). A similar study from Peru found children in the cleanest households to be, on average, one centimeter taller at 24 months of age than those from the most contaminated households (Checkley et al. 2004).

4.2 Other Interventions to Improve Environmental Hygiene

While the WASH interventions discussed in Section 4.1 directly improved environmental hygiene by addressing containment of human feces, such interventions are not designed to address contamination from animal feces. Several non-WASH interventions do exist that focus on technologies and behavior change to encourage corralling animals, improving hygiene of animal corrals and animal feeding practices, improving household flooring, and creating clean protective play spaces. Below are highlighted some of the available evidence and ongoing and recently concluded research projects focusing on these interventions. The summary of ongoing programmatic efforts includes age-focused WASH and environmental hygiene improvement interventions (herein referred to as "BabyWASH" through management of animals and animal feces, playpens, mats and protective spaces, and flooring.

A growing number of development projects, as well as private-sector organizations, are addressing the neglected pathways through programs focusing on the BabyWASH suite of interventions to prevent fecal contamination of the domestic environment and associated ingestion of soil and feces by IYC. Many of these interventions draw on the available evidence base, and conduct qualitative research to develop feasible practices with the promise of improving environmental hygiene and creating barriers between IYC and both human and animal feces. Some have best practice pre- and post- evaluation designs to document changes in household practice. However, none are rigorous research designs with random assignment or controls to establish causality, and therefore primarily document association.

These initiatives are included in this literature review because they elucidate the panorama of interventions that come closest to meeting our literature review objectives:

- Examine interventions to break these pathways, including established WASH measures as well as
 underemphasized risk factors such as animal husbandry, IYC feeding practices, and IYC-focused
 WASH behaviors; and
- Help inform a WASHPaLS field research design to study the additive effects of specific measures to reduce IYC exposure to fecal pathogens and other fecal microbes in their home environments.

Annex A provides a summary of several recent or current interventions, their BabyWASH focus, intervention modality, the use of formative research (or not) to design the intervention, and notes on any rigorous research and learning agenda associated with the intervention. As illustrated in the matrix, interventions cover a range of BabyWASH focal areas, including caregiver handwashing; baby hygiene

The term "BabyWASH" was first coined by and thoroughly defined in Ngure et al. (2014). It is worth noting that the use of the term in this report does *not* refer to the BabyWASH Coalition that integrates WASH, nutrition, early childhood development, and health programming for improved nutrition, health, and development outcomes.

(hands, mouth, face, body); safe disposal of baby feces; food hygiene, particularly focused on complementary feeding during the first 6 to 24 months; clean/safe water; feeding practices; use of playmat/playpen; and animal husbandry practices, including corralling during day and/or night, modified husbandry practices, safe management of productive feces, and regularly cleaning yard of stray feces.

4.2.1 ANIMAL HUSBANDRY PRACTICES

Promoting improved housing, feeding, and health care of poultry and livestock has great potential to reduce the transmission of zoonotic diseases via animal feces (Gelli et al. 2017). However, there is scarce evidence on the effectiveness of interventions to reduce human health risks from poor animal husbandry practices. Over the years, programs have focused on promoting frequent sweeping of feces from courtyards, suggesting disposal in latrines and/or garbage pits. Other interventions have generally promoted separating children's sleeping areas from domestic animals and corralling animals in the daytime. However, no rigorous evaluation of these programs is available in the literature.

Low-income households face various barriers and constraints in corralling and improving livestock housing and hygiene. Some of the knowledge barriers include a low perception of the risks to child health from close proximity to livestock and poultry. There is also a widespread belief that free-range chicken eggs and meat taste better. Other constraints include the fear of theft or loss of animals to predators when animals are kept outside; the cost of animal feed, vaccinations, and materials for constructing corrals; and time required to feed corralled animals.

An additional challenge to reducing or eliminating animal feces in household compounds is its productive use as fuel, farm manure, and building material. Residents are reluctant to rid the households of what they consider to be a valuable resource. Storage and handling of fresh animal feces for such purposes exposes household members and children to contamination, suggesting that a focus on safer management of feces and handwashing after handling may be more successful than a focus on eliminating animal feces from household compounds altogether.

The Women's Poultry Program to Improve Income and Nutrition (Soutenir l'Exploitation Familiale pour Lancer l'Élevage des Volailles et Valoriser l'Économie Rurale, or SE LEVER) in Burkina Faso is using an integrated approach that combines revenue generation, women's empowerment, and nutritional behavior change interventions to improve child nutrition and health (details in Annex B). The program's interventions include improving access to poultry value chain services (such as vaccinations), financing, and training on flock management (including housing). A formative research phase of the project is underway to: (I) assess WASH and animal husbandry risks related to diarrhea, chest infections, EED, and child stunting; and (2) inform the design of an impact evaluation (IE) of the project. The proposed IE will employ an RCT design to measure whether incorporating comprehensive WASH and improved hygiene of poultry housing to a package of agricultural and nutrition interventions results in additive benefits on children's nutrition status and morbidity.

Separately, USAID's Empowering the New Generation to Improve Nutrition and Economic Opportunities (ENGINE) Project in Ethiopia (2011–2016) integrated agricultural systems to strengthen livelihood interventions focused on improving agricultural productivity through provision of sheep, goats, and dual-purpose breeds of poultry for meat and eggs. Farmers were first required to construct poultry corrals before receiving chicks and feed. A total of 41 percent of the households did not follow ENGINE's guidelines on preparing enough food for the flocks, even when provided with a start-up supply of poultry feed by the project, indicating the persistence of free scavenging and only intermittent poultry corralling throughout the intervention (Save the Children 2017). No health outcomes associated with husbandry practices were measured.

In a two-month trial performed within a larger observation study in peri-urban Peru on the use of corrals in 12 households, the majority of participants were willing to corral poultry after extensive

orientation and technical assistance (Harvey et al. 2003). However, birds were corralled intermittently due to time and financial costs associated with corralling. Corralling poultry requires household members to provide adequate feed, as well as water, in addition to the time costs of bird care and maintaining corral hygiene. In rural Zimbabwe, households were resistant to corralling poultry during the day due to feed constraints (Mbuya et al. 2015; Ngure et al. 2013). Such challenges are common in other rural contexts where small-scale production is characterized by low-input scavenging systems with minimal investment in housing, feeding, and health care, and consequently weak biosecurity, high disease, and high mortality rates (Dessie et al. 2013). Fear of theft and predation also serve as impediments to building poultry corrals further away from sleeping and food preparation areas (Headey & Hirvonen 2016; Save-the-Children/Ethiopia and The-Manoff-Group-Inc. 2014)

Although most participants in the Peruvian study viewed bird feces in the house and yard as dirty, few saw its connection with illness (Harvey et al. 2003). In this context, free-range birds were perceived as healthier, happier, and able to produce better quality meat and eggs. Similar observations were made in formative research in Ethiopian villages in the aforementioned ENGINE Project (Save-the-Children/Ethiopia and The-Manoff-Group-Inc. 2014).

These findings suggest that while behavior change strategies may address key knowledge and attitudinal barriers on the importance and practical approaches to hygienic livestock management, more comprehensive behavior change approaches are required to address the myriad financial, logistical, and structural constraints to adoption of a broader set of improved livestock practices, including management of feed and water, cleaning, and construction of improved poultry housing.

4.2.2 CLEAN PLAY SPACE INTERVENTIONS

In contexts where domestic poultry and livestock farming is common and where households do not corral animals, protecting IYC from soil and animal feces ingestion is critical. There are numerous programmatic and research efforts promoting the use of playmats and/or playpens to protect IYC from soil and feces ingestion, among other BabyWASH interventions. However, there is limited evidence on the uptake, uses, and effectiveness of these interventions in reducing soil and feces exposure and ingestion by IYC, and ultimately on health outcomes such as reducing diarrhea, EED, STHs, and improved child growth outcomes. The SHINE project trials hypothesized that a prescribed and hygienic space would protect IYC from key pathways of enteric pathogens and bacterial ingestion (Humphrey et al. 2015), especially direct ingestion of soil and feces (Ngure et al. 2013; Ngure et al. 2014). The concept of a safe play space is designed to protect, stimulate, and promote learning for IYC. The SHINE protective space is a playmat for immobile infants and a combination of both a playmat and playpen for older and mobile IYC (Mbuya & Humphrey 2016). The SHINE trial used commercially available sturdy and brightly colored plastic playpens. In the context of a research activity, it was important to use a product that had been tested for strength and physical stability to support a child while practicing how to stand up. The behavior change elements of the SHINE BabyWASH framework (Mbuya & Humphrey 2016) were designed to elicit motivating emotions for hygiene (e.g., disgust or nurture) and to encourage a full range of BabyWASH practices.

In the SHINE trial, soil and feces ingestion by IYC was reduced by about 50 percent between the WASH intervention and non-WASH households in an in-depth observation of 180 households.¹² These initial

A WASH household received a ventilated pit latrine, tippy taps, monthly liquid soap, a water treatment solution, and a protective play space, and accompanying interpersonal communication interventions promoting feces disposal in a latrine, handwashing with soap, drinking water treatment, hygienic weaning, food preparation, and preventing babies from putting dirt and animal feces in their mouths. Non-WASH households were either drawn from the control arm (receiving only standard of care) or the nutrition arm (receiving nutrition interventions, including 20 g/d nutri-butter from 6–18 months, interpersonal communication interventions promoting optimal use of locally

data suggest that the behavior change messages and use of a playpen were effective in raising perception of risk and changing exploratory behavior to reduce soil and animal feces ingestion. Even with minimal use of playpens for children above 12 months of age, as children became more mobile, the SHINE researchers speculate that the children who previously used playpens during the crawling stage would be less likely to ingest soil as they grew older (Dadirai Fundira, personal communication); however, this has not been confirmed through tests.

In Zambia, an innovative play yard intervention was developed and designed locally, with local materials, intended for caregivers of IYC. Under 24-hour recall, caregivers reported placing their children in the play yard for extended periods of time (more than 1.5 hours) in eight of the ten households observed (Reid et al. 2018). Although drawn from a small pilot study and based on self-reporting, these data suggest that such an intervention may have potential to limit time IYC spend on bare contaminated household soil.

Through a community-based approach, the ENGINE project provided WASH products through microenterprises and local women's saving groups in 46 kebeles (neighborhoods) within 10 woredas (subdistricts) in Ethiopia. The products offered included concrete latrine slabs, subsidized playmats made out of PVC pipe, subsidized water filters, and tippy taps. The goal of this approach was to improve WASH, nutrition, and health in households with children under the age of two years. Ninety-six percent (n=4,286) of targeted households bought the PVC playmats at 42 percent of the market price based on a previous willingness-to-pay study by the same group and in the same context. According to a small follow-up consumer satisfaction survey, playmats were well received and reported to be regularly used for intended purposes (77 percent reported using them always and another 9 percent occasionally); all 22 households that participated in the survey were satisfied with their purchase and cited cleanliness and health as the major reasons for buying the playmats (Save the Children 2017). Four out of the five saving groups sold out of the mats in the fourth year of the ENGINE project, and two groups planned to purchase more in the near future; however, in the end, none of the groups purchased more mats to sell in the fifth year. This was attributed to the subsidized pricing that could not be sustained after the project ended. Lack of established supply chains to provide the playmats at market value made these efforts unsustainable (Daniel Abbott personal communication).

4.2.3 IMPROVED HOUSEHOLD FLOORING

Children in rural and low-income peri-urban settings are exposed to fecal microbes and STHs through bare and loose courtyard soil. Indoor dirt floors also provide primary breeding grounds for microbial pathogens due to pockets of high moisture content from spilled water and food. Though such floors are commonly swept, they are hard to clean and sanitize. Improving indoor and courtyard flooring by providing a hard and impermeable surface will potentially reduce microbial exposure to IYC. Such surfaces are easier to clean with soap and water and dry off in case of spills. Importantly, these interventions assume a household has access to sufficient quantities of water to clean the floors; otherwise, the increase in the household's water collection duties (usually, on the part of women and girls) can reduce the positive benefits of the intervention.

In an impact evaluation of a large-scale Mexican program called Piso Firme, replacement of dirt floors with cement flooring was associated with a nearly 20 percent reduction in incidence of parasitic infestations, an approximately 13 percent reduction in prevalence of diarrhea, and a 20 percent reduction in prevalence of anemia compared to the control group. The program also was associated with improvements in child cognitive development (Cattaneo et al. 2009). No significant differences

available foods for complementary feeding after 6 months old, continued breastfeeding, and feeding during illness). All of the households in the trial received standard care interventions that included promotion of exclusive breastfeeding for all infants from birth to 6 months, strengthened PMTCT (prevention of mother-to-child transmission of HIV) services and village health worker system.

were observed in HAZ and WAZ, but the program was associated with significant improvements in adult welfare, as indicated by increased satisfaction with housing and quality of life and lower depression and perceived stress scores. These benefits were realized at an average cost of \$150 per household. The authors do caution, however, that these results are not necessarily able to be generalized in rural contexts, where the share of total area under cement floor might be significantly less than in the Piso Firme urban context. Households in this urban context also had water and sanitation facilities, which might not generally be the case in rural contexts.

Given the high cost of cement floors, market-based efforts are developing and promoting inexpensive floors that can be washed, cleaned, and used to support a healthier home environment for poor consumers. The EarthEnable commercial enterprise in Rwanda is marketing improved floors made of a multi-layer surface that allows for drainage yet seals the surface to make it waterproof, washable, durable, and appealing to customers. As of January 2017, EarthEnable had installed earthen-finished flooring to replace dirt in roughly 1,400 households, at costs significantly lower than that of cement (Gayatri Datar, Personal Communication). No health data are yet available; however, the enterprise will be examining reduced IYC exposure of contaminated soil and feces as well as positive health outcomes such as reducing the risk of diarrhea, EED, and STHs, and improved child growth.

4.2.4 INTERVENTIONS TO ADDRESS SOCIAL DETERMINANTS

Other types of interventions, such as policies and programs that address the underlying social determinants of diarrheal disease, can serve as critical, complementary strategies to focus on barriers to contamination pathways. In Brazil, economic growth combined with reduced income disparities between the rich and the poor, increased educational attainment for women, water and sanitation infrastructure improvements, and increased provision of oral rehydration salts led to decreased infant mortality (92 percent from 1990 to 2007) due to diarrheal disease (Victora et al. 2011). In a dated study from Bangladesh, higher maternal education and household wealth were associated with lower risk of diarrheal disease among children; seven more years of maternal education were associated with a more than 50 percent decrease in diarrheal disease prevalence, and those in the wealthiest quintile had a 41 percent lower risk of diarrhea compared to those in the lowest quintile (Mahalanabis et al. 1996). In Ghana, children of illiterate mothers were found to experience greater risk of diarrhea in conditions of poor water supply and lack of toilets (Gyimah 2003). These studies lend evidence that social determinants have important implications for lowering diarrheal disease prevalence. There are opportunities for integrating these interventions with those focusing on the proximal determinants, with the potential for synergistic effects (Casanovas et al. 2013).

5.0 DISCUSSION AND NEXT STEPS

This review of the recent literature on key pathways of fecal microbe ingestion by IYC and their links to diarrhea, EED, and poor nutrition and development outcomes identified several important findings and gaps in the evidence with implications for future research and programming. The findings highlight a clear path for WASHPaLS research toward a hygienic environment for IYC.

Practitioners and researchers have underestimated potentially key pathways of disease transmission in Wagner & Lanoix's 1958 "F-Diagram."

The growing attention to risks from direct and indirect consumption of animal and human waste and direct consumption of contaminated soil point to the need for separating IYC from fecal pathogen reservoirs in the home environment.

Animal feces contamination may be extensive and could be more abundant than human feces in low-income rural, peri-urban, and urban contexts where free-scavenging poultry and livestock husbandry practices are common. Animal feces are a primary source of zoonotic organisms and contamination of public and private water sources. Presence of animal feces in the immediate household environment increases the risk of contamination of hands and, consequently, of water and food. Overnight corralling of poultry and/or livestock within the same room as IYC has been linked to EED (George et al. 2015) and stunting (George et al. 2015; Headey & Hirvonen 2016). However, the role of animal density with respect to diarrhea, EED, and child stunting is not well understood (Headey & Hirvonen 2016).

Exploratory mouthing behaviors of children using contaminated household objects, as well as direct consumption of fecally contaminated soil, serve as an additional pathway of transmission for IYC. Additionally, many of the current WASH interventions until recently overlooked infant and child feces disposal, which is sub-optimal in many LMICs and contributes to diarrhea, EED, and other health consequences. In general, CLTS and other sanitation approaches have not addressed child feces disposal, or at least not with any emphasis. However, some CLTS programs have begun recently to incorporate a focus on IYC feces disposal (Rand et al. 2015).

The relative importance of different disease transmission pathways is not well documented.

The relative magnitude of transmission pathways of enteric microbes faced by IYC is not well defined and may be highly context specific, making it difficult to conclude which pathways may represent the highest risks. The disease transmission pathways may vary greatly as infants progress through development levels with increasing levels of mobility. We expect that a number of research studies (e.g., WASH Benefits, GEMS, SHINE, and NOURISH) will offer important insights when findings are published.

EED as a mediator of child growth faltering is not yet well understood.

Analyzing the available literature and key informant interviews underscore how little is understood about the etiology of EED, specifically the issues of intermittent exposure to contagion, thresholds, and dose-response relationships that are critical to developing effective interventions.

Despite some important gains in interventions reducing diarrheal morbidity, achieving widespread reductions in child stunting in LMICs remains elusive. This has been increasingly attributed to pervasive EED (Humphrey 2009), hypothesized to be caused primarily by fecal microbes and pathogens ingested from contaminated soil and feces. Given that EED may facilitate linear growth failure *independent of diarrhea*, research focusing on diarrhea as the primary outcome variable may have missed key relationships between various WASH interventions, EED, and child growth in both positive and negative directions. Measuring EED, however, is far more complicated and expensive in terms of research methods and biomarkers than measuring diarrhea outcomes, particularly studies relying on *reported*

rates of diarrhea. Development of inexpensive proxy measures that are predictive of EED and child stunting is needed.

Implementers are increasing their focus on preventing IYC from exposure to fecal pathogens through previously neglected pathways, but there is little evidence to support the effectiveness of such interventions.

At least 17 distinct medium-to-large-scale service delivery programs employ measures that fall within the scope of BabyWASH, including the distribution of mats and playpens, and promotion of behavior change interventions directed at keeping play spaces clean and corralling animals away from IYC.

Despite recent interest in rolling out the playmat/play space interventions through various programmatic efforts, there is no empirical evidence on the efficacy or effectiveness of these interventions in reducing the risk of soil, human, and animal feces ingestion and improving child growth and health.

Playmat and play space interventions are interesting, but assessing their true potential requires a better understanding of the biological plausibility of their protective effects against fecal contaminant risk. More specifically, intermittent exposures to microbial pathogens may still result in risk; extended periods of protection on a mat or within a play yard may not be sufficient to prevent risk posed by even short periods of IYC time spent in areas of increased hazard (on bare soil with excreta present) or eating contaminated food from contaminated hands. A number of studies of water treatment interventions argue that there is an exposure threshold above which infection becomes probable (Enger et al. 2013; Brown & Clasen 2012; Hunter et al. 2009), and that the exposure-response relationship for ingestion of animal excreta or contaminated soil remains a source of considerable uncertainty.

Data from the sub-study of the SHINE trial and other less rigorous play space studies discussed above raise additional research questions about the relative importance of reducing soil and chicken feces ingestion for children between 3 and 12 months and the persistence of changes in caretaker and IYC behavior after 12 months. Is the risk perception by caregivers sustained post-playmat use, and how does the risk perception translate into changes in caregiver behavior over time? Do changes in infant practices at earlier ages continue as they age and increase mobility, even in the absence of restraints?

Evidence on the impact of improved flooring on child nutrition and health is limited to a single study in urban Mexico, where most of the households had cement floors (Cattaneo et al. 2009). Notably, open courtyards with bare loose soil are common in rural areas, where households are less likely to have cement floors. Human and animal density and flow of livestock in such contexts may influence the effectiveness of such improved floors on child health outcomes. The positive impact of cement floors on adult psycho-social well-being prompts further research questions on whether finished flooring is likely to improve WASH behaviors like cleaning the floor and keeping poultry and animals away, and more generally impact child health and growth in rural areas as well as the Mexican urban context.

This review underscores a significant evidence gap for various technologies and social behavior change interventions promoted through current programmatic efforts. Additional research is needed to investigate the effectiveness, adoption, constraints, and scale potential of measures to reduce IYC exposure to fecal pathogens, including safe play spaces, improved animal husbandry practices, and flooring. This research should allow exploration of the additive benefits of these interventions when coupled with traditional WASH measures such as expanded water supply, improved water quality, delivery and utilization of improved toilets, handwashing with soap, and promotion of other hygienic behaviors.

ANNEX A. RECENT OR CURRENT PROJECTS WITH BABYWASH FOCUS

ORGANIZATION SPECIFIC BABYWASH FOCUS AND **FORMATIVE RESEARCH TO DEVELOP RIGOROUS DESIGN** (FUNDER) PROJECT **TYPE OF INTERVENTION INTERVENTION OR TEST CONCEPTS -**TO DOCUMENT IMPACT DATES **FORMAL OR INFORMAL** OF BABYWASH **COUNTRIES** ON BEHAVIORS, EED, OR GROWTH ISI lead with HKI, others Interventions vary across 8 countries, focusing Trial of improved practices (TIPS)¹³ in Sierra No rigorous learning agenda. on various combination of: Project pre- and post- evaluations to be Leone, with other formative research in Guinea. (USAID) conducted, but not particularly focused on Ghana, and Bangladesh. **S**trengthening • Safe disposal of child feces BabyWASH elements. Partnerships, Results, • Playpens and playmats Highlights of findings include: Monitoring data showed Bangladesh and Innovations in • Managing animals and feces (sweeping and participants much more likely to wash hands **Nutrition Globally** Sierra Leone: Main objections to playpens modified chicken coops) after defecation (75%) than non-participants (SPRING) Project seemed to be motivated by lack of familiarity • Handwashing of child and caretaker (6%). with the practice and low perception of risk of • Treated water for 6- to-24-month-olds 2011-2016 with children's contact with feces. Parents generally extension year through preferred sweeping to playpens. While interventions vary across countries, most 2017 include community infant and young child Ghana: Most households had a chicken coop feeding (IYCF) counselling package, including Bangladesh, Burkina Faso, that opens into an enclosed courtyard. Research training of outreach cadre (volunteer and paid) Ghana, Guinea, India, identified families willing to create a clean play and counselling cards to support consistent and Niger, Nigeria, Sierra space by turning the chicken coops to open behavior-focused messaging; community videos; Leone outside of the courtyard, reducing the time enhanced CLTS (WASH 1,000 focusing on the chickens are in a courtyard and thus reducing first 1.000 days); improved chicken husbandry: child contact with chicken feces. encouraging caging/corralling of chickens when feasible; and playmats. https://www.spring-nutrition.org/aboutus/news/springghana-presents-wash-1000-39thannual-water-engineering-and-development-

centre

TIPS is a formative research methodology that involves target audiences ascommunity consultants to develop and test feasible and effective solutions for a range of health and development areas. Various behavior and/or behavior-technology dyads are developed, verified for efficacy or biological plausibility, and then tested over time for effectiveness, feasibility, and popularity. Most often, the outcome is a menu of feasible and effective options to integrate into programs. TIPS can be more or less rigorous in their application. Unlike other methods, subjects are actively engaged as consultants, and modifications are often made throughout the course of the study.

ORGANIZATION (FUNDER) PROJECT DATES COUNTRIES	SPECIFIC BABYWASH FOCUS AND TYPE OF INTERVENTION	FORMATIVE RESEARCH TO DEVELOP INTERVENTION OR TEST CONCEPTS – FORMAL OR INFORMAL	RIGOROUS DESIGN TO DOCUMENT IMPACT OF BABYWASH ON BEHAVIORS, EED, OR GROWTH	
	https://www.spring-nutrition.org/stories/spring-promotes-nutrition-sensitive-agriculture https://www.spring- nutrition.org/publications/posters/developing- effective-messages-better-nutrition-and-hygiene- tonkolili-tips			
Food for the Hungry (USAID/Title II Food for Peace) Tuendelee Pamoja II Development Food Assistance Project October 2017 — September 2022 Democratic Republic of the Congo	Geophagy Playmats/play pens	Study with Johns Hopkins University (Christine Marie George) investigating EED and stunting, and effective methods for decreasing EED and reducing stunting. Currently undertaking extensive formative research activities including repeated household observations and measures of pathogenic contamination of sentinel objects. Building on formative findings, researchers will develop a playmat/ play space randomized trial measuring health outcomes.	A prospective cohort study will investigate the relationship between exposure to enteric pathogens through geophagy, EED, and stunting. The study will include an RCT to determine which activities (e.g., playmats or playpens) are the most effective strategy in preventing geophagy and decreasing EED. The findings will be incorporated into a BabyWASH lesson plan in the "Essential Hygiene Actions" Care Group module in FH programming in DRC.	
Food for the Hungry (Private funds) Part of on-going programming, not a project Ongoing Ethiopia	 Hygienic play spaces Handwashing, including for children, emphasizing BabyWASH times Managing animal feces Latrines, including child-friendly ones to increase use Promotes essential hygiene actions through care groups (refer to column 4) to encourage improved behaviors through support, increased perception of risk of contact with feces and geophagy, skills, and changing norms. 	Conducting a formative "barrier analysis" to assess determinants of practicing essential hygiene actions, including BabyWASH-related practices.	No rigorous evaluation design following evidence-based program development.	

ORGANIZATION SPECIFIC BABYWASH FOCUS AND FORMATIVE RESEARCH TO DEVELOP **RIGOROUS DESIGN** (FUNDER) PROJECT TYPE OF INTERVENTION **INTERVENTION OR TEST CONCEPTS -**TO DOCUMENT IMPACT **DATES FORMAL OR INFORMAL** OF BABYWASH **COUNTRIES** ON BEHAVIORS, EED, OR GROWTH Save the Children. The Standard best-practice program monitoring and **Playmats** Conducted two studies to inform BCC and evaluation design used (not high rigor). ENGINE Manoff Group and others • Poultry corrals social marketing of WASH products: monitoring showed about 5,000 households (USAID) Empowering Subsidized WASH products (latrine concrete bought PVC playmats, and qualitative follow-up Water, Sanitation and Hygiene (WASH) in Rural slabs. PVC playmats, and water filters) were the New Generation showed most used them as intended A Households in Amhara, Oromia, SNNP and Tigray: to Improve Nutrition marketed through micro-enterprises and local significant majority of the households (77%) Findings and Recommendations for Social and and Economic women's saving groups. The added products reported always using the playmat for the Behavior Change Communications. (2014) **Opportunities** were a late addition in 10 districts (out of 116) intended purpose, and another 9% reported (ENGINE) to reduce fecal bacteria and parasite ingestion. Draft report on household's willingness to pay for using the mat occasionally for intended purpose Sensitization was done by the women's saving household water filters, inmproved child floor mats, with IYC. 2011-2016 groups, with mothers in the groups as the first construction of separate livestock room, at ten Poultry corrals were mandatory (with subsidized districts of Oromia, SNNPR, Tigray and Amahara target group. Ethiopia materials and feed provided) in order to receive Regions, Ethiopia (2014) https://ethiopia.savethechildren.net/ENGINE ruminants and poultry as part of agricultural systems strengthening efforts. Most program participants complied to some extent; however, intermittent poultry corralling and freescavenging local poultry were still practiced. No data explain why, but anecdotal information suggests householders still supplemented "costly" food by letting animals graze. There was no measure of the WASH intervention's impact on child health outcomes. WASH was introduced as a relatively small-scale/ pilot study. Qualitative case studies and further conversation with program managers suggest that lack of messages or sensitization at the community level on the use of playmats contributed to low demand at population level. In addition, lack of established supply chains to provide the playmats at market value and weak linkages between suppliers and the saving groups made these efforts unsustainable.

ORGANIZATION (FUNDER) PROJECT DATES COUNTRIES	DER) PROJECT TYPE OF INTERVENTION INTERVENTION OR TEST CONCEPTS DATES FORMAL OR INFORMAL		RIGOROUS DESIGN - TO DOCUMENT IMPACT OF BABYWASH ON BEHAVIORS, EED, OR GROWTH	
Save the Children lead with World Vision, Tufts, PSI and The Manoff Group (USAID) Growth through Nutrition (GtN) 2016–2021 Ethiopia	 Separation of animals from IYC Safe Play Space(possibly) Private sector marketing of infant potties GtN works through the government Health Extension and Agriculture Outreach Programs, as well as through four implementing NGOs to deliver evidence-based behavior change programming through household visits, community conversations and agricultural outreach to promote separation of children from animals and their feces. Through a private sector strengthening component, they will establish 30 sanitation markets that will include a locally-designed child potty. 	A follow-on to the ENGINE Project, the GTN project had a rigorous health outcome study on their learning agenda; however, they cancelled due to logistical challenges related to country regulations on the research methods	No, standard project evaluation.	
CARE Nutrition at the Center Project 2012–2017 Bangladesh, Benin, Ethiopia, Zambia	Playpens, playmats Animal corralling to reduce exposure to poultry feces Aimed at decreasing child stunting and anemia in women and children; promoting animal corralling, especially in men's groups and Village Savings and Loan Associations (VSLA). Explicit EED module focused on building caretaker awareness/perception of risk of EED-related behaviors. Project collaborates with SHINE Project in Zambia. https://nutritionatthecenter.wordpress.com/	Formative research in Zambia regarding women's perceptions of geophagy (more positive than negative). Formative research conducted on community-built (mud-brick) playpens and plastic playpens (following SHINE trial play yard intervention). Semi-structured interviews suggest that both are equally acceptable (~30 HH).	Observation results under review at the American Journal of Tropical Medicine and Hygiene (AJTMH) intervention results currently being written up. Presently testing the impact of 24 small-scale, community-operated egg production facilities on the egg consumption and growth of children in the Luangwa Valley, Zambia. These facilities and roosting structures help restrict poultry feces to a contained area.	
Feed the Children (Private funds) Part of programming, not a project Ongoing (beginning in 2015)	 Playmats Toy sanitization Using pens or cages to separate livestock from IYC 	Formative research conducted to develop intervention, identify behavioral determinants, and address barriers through solutions generated or tested by householders to assure feasibility. (See detail in column 2).	No data to be collected from activity.	

ORGANIZATION (FUNDER) PROJECT DATES COUNTRIES	SPECIFIC BABYWASH FOCUS AND TYPE OF INTERVENTION	FORMATIVE RESEARCH TO DEVELOP INTERVENTION OR TEST CONCEPTS – FORMAL OR INFORMAL	RIGOROUS DESIGN TO DOCUMENT IMPACT OF BABYWASH ON BEHAVIORS, EED, OR GROWTH
Kibera, Kenya	Focused care group module for groups of 10-12 women, every two weeks for approximately three months.		
	Caregivers encouraged to provide a "clean" playmat; sanitize the playmat once a week and whenever soiled with food or dirt; clean and sanitize toys and other items that babies frequently place in their mouths at least two or three times per week, when the baby is recovering from an illness, and when other children have put the items in their mouths. Requested that parents ask caretakers at day care center to use clean mats and clean and sanitize mats and toys regularly.		
Project Concern International [PCI] (USDA) Food for Education Project III 2016–2021 Tanzania	 Playmats and clean toys Handwashing of child and before food preparation Food hygiene Integrates early childhood development (ECD/education with health/nutrition/WASH (handwashing, food hygiene), playmats, and cleaning toys. Uses education cascade groups, weekly sessions led by a trained outreach worker, and home follow-up. Trains grandparents or primary caretakers in a range of skills. Each household receives a kit (\$6) that includes easy DIY stim toys and a playmat. PCI pilot of the approach in 2017, scaling-up to 5,376 HHs (10%) in 2018, then to 54,000 HHs by 2020. 	Modeled after Hawaii ECD initiative with extensive formative research. Concepts tested and modified for Tanzania.	Longitudinal study starting in 2018.
World Vision (WV) with The Manoff Group	Clean play spaces Interventions not yet developed; aims to determine culturally acceptable and practical play	TIPs research on BabyWASH including "clean play spaces" underway.	No rigorous design yet.

ORGANIZATION (FUNDER) PROJECT DATES COUNTRIES	SPECIFIC BABYWASH FOCUS AND TYPE OF INTERVENTION	FORMATIVE RESEARCH TO DEVELOP INTERVENTION OR TEST CONCEPTS – FORMAL OR INFORMAL	RIGOROUS DESIGN TO DOCUMENT IMPACT OF BABYWASH ON BEHAVIORS, EED, OR GROWTH
Part of WV on-going programs, not a project 2017-2018 Kenya, Somalia, Uganda	space options in both development and emergency contexts.	Findings will guide a menu of viable options for clean play spaces to incorporate into WV BabyWASH programming, "test" in-country, and share with the global community. For Somalia, will look at an emergency context to determine if similar behaviors may be utilized. Research carried out in Summer 2017, report not yet released.	
World Vision Osiligi Integrated BabyWASH Program Part of WV on-going programs, not a project June 2017–2019 Kenya	Full BabyWASH Program including play space, in pilot stage. The program will focus on one community, bringing together health, WASH, and ECD in both community and health facility settings. The facility will be rehabilitated. Communities will be encouraged through a modified CLTS to become open defecation free (including focus on children under 2) and will have parenting programs to keep children from harmful environments.	Operational research under way to define culturally acceptable "clean play spaces."	The WV Kenya team is working with Jomo Kenyatta University to determine if a coordinated BabyWASH approach is more effective than individual interventions carried out without coordination. Research questions and methods are still being developed.
Johns Hopkins Center for Communications Programs, Health Communication Capacity Collaborative (USAID) Western Highland Integrated Program (WHIP) 2014—September 2017 Guatemala	 Caregiver handwashing IYC hygiene (hands, mouth, face, body) Safe disposal of baby feces Food hygiene Active feeding/engaging child for better eating and ECD Use of playmats Corralling domestic animals Comprehensive program to reduce chronic malnutrition and address maternal health, family planning, crops diversification, home gardens, and gender equity. https://healthcommcapacity.org/where-we-work/guatemala/ 	Literature review (generalized findings similar to Nepal-specific findings below) and original formative research, "Opening our Minds," on aspirations and family dynamics in relation to hygiene and nutrition. Findings included an openness to protecting children from being in contact with feces, and a more comprehensive understanding of mother/baby/child well-being (health, nutrition, hygiene, caring and love, etc.). Comparison of household observations of positive deviants and non-positive deviants (in progress). Study completed and the publication will be ready in July 2017.	No evaluation data forthcoming.

ORGANIZATION (FUNDER) PROJECT DATES COUNTRIES	SPECIFIC BABYWASH FOCUS AND TYPE OF INTERVENTION	FORMATIVE RESEARCH TO DEVELOP INTERVENTION OR TEST CONCEPTS – FORMAL OR INFORMAL	RIGOROUS DESIGN TO DOCUMENT IMPACT OF BABYWASH ON BEHAVIORS, EED, OR GROWTH
Save the Children with HKI, Johns Hopkins Center for Communication Programs, JHPIEGO, and a host of other cooperating agencies and national partners (USAID) Suaahara 2011–2016 Nepal	 Washing hands with soap and water before feeding the baby. Creating physical barriers between children and floor, particularly separate from animals (including chickens), dirt, and all feces. Integrated nutrition, WASH, and agriculture program. Modeled improved BabyWASH behaviors through all communication activities, mass media (including radio entertainment education, and radio spots), community activities, and one-on-one interactions, including home visits. Developed a video to demonstrate how to manage improved chicken coops, especially when a family has a small child: https://www.youtube.com/watch?v=HINVLiul0m A Open defecation free community mobilization element: https://www.usaid.gov/nepal/fact-sheets/suaahara-project-good-nutrition 	 No regular handwashing by adults, and baby handwashing not practiced. Inconsistent use of latrines. Baby feces considered "normal" and to not contain germs. Domestic animals around children considered normal with little perceived risk associated. Putting animals in pens perceived to increase costs and amount of work for parents. 	No rigorous research design, but using Lot Quality Assessment Sampling (LQAS) monitoring and a process evaluation to document campaign effects. People exposed to the campaign were significantly more likely to have a handwashing station with soap or ash and know that they should wash their hands before feeding children, have a clean toilet, use water treatment, or use covered water. 121 villages were declared open defecation free. The 2015 earthquake interrupted plan for endline survey.
Save the Children (USAID) Nurture Project 2016–2018 Laos	 Child feces disposal IYC handwashing No intervention yet, research underway. https://www.usaid.gov/asia-regional/fact-sheets/usaid-nurture 	Informal research ("mini-TIPS") conducted to identify feasible actions to dispose of child feces safely, and to learn from families how IYCs' hands can be washed with soap before feeding/eating.	Forthcoming
CARE (USAID Food for Peace) Shouhardo III September 2015–2020	Separating children from animal feces through cleaning the area where children play Incorporate preventive measures to reduce risk of EED in community health volunteers (CHV) Health, Hygiene, and Nutrition module, the	Intervention based on extensive formative analysis findings and development of a comprehensive SBCC strategy.	Expect project evaluation data at close of project in 2020.

ORGANIZATION (FUNDER) PROJECT DATES COUNTRIES	SPECIFIC BABYWASH FOCUS AND TYPE OF INTERVENTION	FORMATIVE RESEARCH TO DEVELOP INTERVENTION OR TEST CONCEPTS – FORMAL OR INFORMAL	RIGOROUS DESIGN TO DOCUMENT IMPACT OF BABYWASH ON BEHAVIORS, EED, OR GROWTH	
Bangladesh	Farmer Field and Business School module, and School-Based Teen Brigade module. By adding to these modules, BabyWASH is now part of courtyard sessions and CHV household visits. http://carebangladesh.org/shouhardolll/	Formative research guided focus on cleaning/ sweeping the compound and safely disposing of animal feces in compost pits.		
WaterSHED Child Potties: Sanitation for the Most at Risk April 2017–2018 Cambodia	Safe disposal of IYC feces Initiative to test if IYC sanitation products (i.e., potties) can be marketed sustainably and used consistently in rural areas to improve safe disposal of IYC feces, reducing exposure to diarrheal disease pathogens.	Formative research documented evidence of the efficacy of potties in hygienically managing child feces, and information on consumer demand and consumer preferences through product testing. Link to formative research paper: http://www.watershedasia.org/cambodia-iycs-study/	No rigorous design to document behaviors or impact. Results from sales records and HH surveys anticipated in 2018.	
 (Conrad N. Hilton Foundation) THRIVE Project ECD initiative providing 15,000 children <5 affected by HIV with a "sustainable culture of care and support." 2016–2018 (Phase 2) Includes direct provision of services (training to 		Working with Emory University using TIPS for social and behavior change communication activities focusing on three thematic packages. I. Food preparation and storage 2. Mealtime 3. Clean compound, clean family TIPS planned to run through March 2018 with report available by June 2018.	Quasi-experimental design looking at development milestones, uptake of IYCF and WASH behaviors, health-seeking behaviors, and maternal well-being.	

ANNEX B. OVERVIEW OF RECENT AND CURRENT RESEARCH TRIALS AND TOOLS

- WASH Benefits Study (2012–2016): This study was motivated by the need to generate quality evidence on the effects of individual (water quality, sanitation, handwashing, and nutrition) and combined interventions on diarrhea and growth when delivered to IYC. WASH Benefits included two cluster-randomized trials to assess improvements in water quality, sanitation, handwashing, and child nutrition (alone and in combination) to rural households with pregnant women in Kenya and Bangladesh. The study randomized geographically matched clusters (groups of household compounds) to one of six intervention arms or control. The studies aimed to enroll newborn children (5,760 in Bangladesh and 8,000 in Kenya) and measure outcomes at 12 and 24 months after intervention delivery. Primary outcomes included child length-for-age Z-scores and caregiver-reported diarrhea. Secondary outcomes included stunting prevalence, markers of environmental enteropathy, and child development scores (verbal, motor, and personal/ social) (Arnold et al., 2013).
- Sanitation Hygiene Infant Nutrition Efficacy (SHINE) Trial (2012–2017): The SHINE trial was motivated by the premises that EED is a major underlying cause of both stunting and anemia, chronic inflammation is the central characteristic of EED mediating these adverse effects, and EED is primarily caused by high fecal ingestion from living in poor WASH conditions. SHINE was a proof-ofconcept, 2x2 factorial, cluster-randomized, community-based trial in two rural districts of Zimbabwe that tested the independent and combined effects of protecting babies from fecal ingestion (Factor I, operationalized through a WASH intervention) and optimizing nutritional adequacy of infant diet (Factor 2, operationalized through an IYCF intervention) on length and hemoglobin at 18 months of age. Within SHINE, two causal pathways will be measured. The program impact pathway will evaluate processes and behaviors linking implementation of the interventions with the two-child health primary outcomes. This pathway will be modeled using measures of fidelity of intervention delivery and household uptake of promoted behaviors and practices. This pathway will also measure a range of household and individual characteristics, social interactions, and maternal capabilities for childcare to explain heterogeneity along these pathways. The biomedical pathway comprises the infant biologic responses to the WASH and IYCF interventions that ultimately result in attained stature and hemoglobin concentration at 18 months of age. This pathway will be elucidated by: (1) measuring biomarkers of intestinal structure and function (inflammation, regeneration, absorption, and permeability); (2) microbial translocation; (3) systemic inflammation; and (4) hormonal determinants of growth and anemia among a subgroup of infants enrolled in an EED sub-study (Humphrey et al., 2015).
- Cambodia Integrated Nutrition, Hygiene and Sanitation (NOURISH) Project Impact Evaluation (2014–2019): The IE for the USAID-funded NOURISH project will use a cluster randomized control trial design with four arms: (1) nutrition only; (2) WASH only, (3) nutrition and WASH; and (4) control (MSI, 2016). Project interventions are focused on four key strategies: (1) improving community delivery platforms to support improved nutrition through the Baby-Friendly Community Initiative (BFCI); (2) creating demand for health and WASH practices, services, and products through the use of conditional cash transfers, CLTS, vouchers, and SBCC; (3) using the private sector to advance supply of WASH and nutrition products; and (4) building the capacity of government and civil society in nutrition. The IE will evaluate the effectiveness of co-delivery of WASH and nutrition interventions and the separate effect of each in a field setting (scalable conditions), a step further than the current efficacy studies (WASH Benefits and SHINE trial) integrating WASH and nutrition. Primary outcomes will be HAZ, WAZ, and WHZ; secondary

- outcomes will be prevalence of enteric infections and measures of EED; and tertiary outcomes will be self-reported diarrheal disease and all-cause infant mortality.
- Global Enteric Multicenter Study (GEMS) (2007-2011): GEMS was motivated by the need for a well-designed study to obtain information on the etiology and burden of more severe forms of diarrheal disease to guide global investment and implementation decisions. The study was designed to overcome limitations from earlier studies, and determine the etiology and population-based burden of pediatric diarrheal disease. GEMS included one of the largest case/control studies of an infectious disease syndrome ever undertaken (target approximately 12,600 analyzable cases and 12,600 controls). The study was rolled out in four sites in sub-Saharan Africa (Gambia, Kenya, Mali, and Mozambique) and in three sites in South Asia (Bangladesh, India, and Pakistan). Each site is linked to a population under demographic surveillance (total approximately 467,000 child years of observation among children <five years of age). GEMS data will guide investment and help prioritize strategies to mitigate the morbidity and mortality of pediatric diarrheal disease (Levine et al. 2012).
- Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health (MAL-ED) Study (2009-2014): The MAL-ED Study, led by the Fogarty International Center of the National Institutes of Health and the Foundation for the National Institutes of Health, has been established at sites in eight countries with historically high incidences of diarrheal disease and undernutrition. Central to the study is the hypothesis that enteropathogen infection contributes to undernutrition by causing intestinal inflammation and/or by altering intestinal barrier and absorptive function. It is further postulated that this leads to growth faltering and deficits in cognitive development. The effects of repeated enteric infection and undernutrition on the immune response to childhood vaccines are also being examined in the study. MAL-ED uses a prospective longitudinal design that offers a unique opportunity to address a complex system of exposures and health outcomes in the community directly-rather than rarer circumstances that lead to hospitalization—during the critical period of development of the first two years of life. Among the factors being evaluated are enteric infections (with or without diarrhea) and other illness indicators, micronutrient levels, diet, socioeconomic status, gut function, and the environment. MAL-ED aims to describe these factors, their interrelationships, and their overall impact on health outcomes in unprecedented detail, and to make individual, site-specific, and generalized recommendations regarding the nature and timing of possible interventions aimed at improving child health and development in these resource-poor settings.
- Sanitation Trial in Maputo, Mozambique (MapSan (2015–2018): MapSan is control beforeand-after trial to estimate the health impacts of an urban sanitation intervention in informal
 settlements in Maputo, Mozambique, with assessment of localized population density as an effect
 modifier (Brown et al. 2015). The intervention consists of private pour-flush latrines (to a septic
 tank) shared by multiple households in a compound or household cluster. Outcomes will be
 measured in approximately 1,000 children (500 children accessing interventions and 500 controls
 using existing shared private latrines in poor sanitary conditions) at two points: immediately before
 the interventions and at follow-up after 12 months. The primary outcome will be combined
 prevalence of enteric infections among under-five-year-old children. Secondary outcome measures
 include STH re-infection in children following baseline deworming and prevalence of reported
 gastrointestinal illness. The study will make use of exposure assessment, fecal source tracking, and
 microbial transmission modeling to examine whether and how routes of exposure for diarrhea
 pathogens and STHs vary and the impact of effective sanitation on transmission of pathogens.
- Childhood Hygiene Intervention on Enteric Infections and Growth Faltering in Lowincome Settlements of Kisumu, Kenya, SAFE START, (2016–2018): SAFE START is a cluster randomized control trial to evaluate the impact of child hygiene interventions (targeting food preparation and infant feeding practices) on prevalence of enteric infections (primary outcome),

height and weight adjusted for age (secondary outcomes), and infant mortality (tertiary outcome) (Mumma & Esteves-Mills, 2016). Other intermediate objectives include the effect of the interventions on observed and reported household behaviors, fecal contamination of children's environments (food, water, and fomites), and presence of flies. The study will be conducted in two informal settlements in the city of Kisumu, Kenya, a context found to have high fecal contamination even in households with improved water and sanitation in a previous cross-sectional study by the same partners (http://www.shareresearch.org/project/safe-start). Children will be enrolled at 3 months and will be followed until 18 months.

- SaniPath Exposure Assessment Tool: The SaniPath Tool aims to increase the evidence base available to sanitation policy makers and implementers in low-income urban communities (http://sanipath.org). It is designed to assess risk related to poor sanitation and to help prioritize sanitation investments based on the exposures that have the greatest public health impact. Data from simple environmental microbiology methods and behavioral surveys are combined to assess the relative exposure to fecal contamination. Relevant exposure pathways include open drains, drinking water, surface waters, public latrines, soils, wastewater-irrigated produce, and floodwater. The tool automatically generates risk profiles for each pathway using data entered by the user. The risk profiles show users which pathways contribute the greatest risk and where interventions may have the greatest impact on reducing exposure to fecal contamination.
- Women's Poultry Program to Improve Income and Nutrition (Soutenir l'Exploitation Familiale pour Lancer l'Élevage des Volailles et Valoriser l'Économie Rurale, or SE LEVER) Impact Evaluation (2015-2020): SE LEVER is a five-year project implemented by Agribusiness Systems International (ASI) in Burkina Faso in partnership with local NGOs, private institutions, and governmental services. This poultry value chain program leverages agriculture development strategies and nutrition to increase poultry production and improve the nutritional status of women and children in the Centre Ouest and Boucle de Mouhoun regions. The project uses an integrated approach combining revenue generation, women's empowerment, and nutritional behavior change interventions, including:
 - Improved access to value chain services. This component includes vaccinations, financing, and training on poultry flock management (including housing);
 - Behavior change communication (BCC) on nutrition and health provided through women's
 groups, poultry producer groups, and local community leaders. The BCC content includes the
 promotion of improved diets at key stages of the lifecycle, and additional content focusing on
 nutrition during the first 1,000 days, including IYC feeding practices and basic hygiene practices;
 and
 - Community-level sensitization on women's economic empowerment and gender equity, including strengthening of women's groups.

In addition to these activities, SE LEVER is undertaking formative research on the need for a more intensive WASH/health component to improve health and hygiene in the context of poultry flock management that could be integrated into an enhanced SE LEVER package.

An IE, using a cluster randomized control trial design, will be overlaid on the SE LEVER project. The IE will include two groups: I) the SE LEVER intervention group; and 2) the control group with no intervention. An additional intensive WASH/health component designed to improve child health and nutrition in the context of poultry flock management will be added if the results of the formative research suggest the need for such an intervention. A sub-study would be undertaken within the SE LEVER intervention, and the SE LEVER clusters would be randomly allocated to either: I) SE LEVER only or 2) SE LEVER + with an intensive WASH/health component. The primary outcomes of the

study include the mean probability of adequacy of diets for women and children (two to five years), IYCF practices for caregivers of children aged zero to two years, and household poultry output and sales.

ANNEX C. REFERENCES

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