THE IMPACT OF LATRINE CONSTRUCTION ON DENSITIES AND PATHOGEN INFECTION RATES OF SYNANTHROPIC FLIES AND *CULEX QUINQUEFASCIATUS* MOSQUITOES IN ODISHA, INDIA

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“We hope that, when the insects take over the world, they will remember with gratitude how we took them along on all our picnics.”

- Bill Vaughan

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Abstract

Diarrhoeal disease is one of the leading causes of mortality and morbidity globally. Non-biting synanthropic flies are of public health importance due to their habit of flying between faecal matter and households, potentially transmitting pathogenic bacteria by regurgitation, defecation or mechanical transmission via legs or wings. Lymphatic filariasis (LF), caused by *Wuchereria bancofti* and transmitted by *Culex quinquefasciatus*, affects approximately 120 million people in Asia. Controlling the mosquito vector can have a significant impact on LF incidence rates.

It is assumed that increasing the coverage of latrines will reduce the amount of open defecation and environmental faecal contamination resulting in a healthier population. However, few studies have measured the impact of building latrines on human health and even fewer have demonstrated its impact on synanthropic fly populations or their bacterial carriage and associated diarrhoeal disease. Similarly, although *Cx. quinquefasciatus* has long been associated with pit latrines, the resulting impact through the construction of improved pour-flush latrines on population densities has yet to be explored. Initial experiments were conducted to determine the best methodology for trapping both synanthropic flies and *Cx. quinquefasciatus,* to inform the design of the entomological component of a cluster randomised control trial (cRCT). Thereafter, the focus was on determining the impact of latrine construction on the exposure of households to populations of flies and the bacteria that they carry, and on *Cx. quinquefasciatus* densities and *W. bancofti* prevalence.

Results indicate that latrine construction had no impact on the density of flies within households or on the carriage of bacteria. There was no statistical difference between control and intervention arms in the population density of synanthropic flies (IRR=0.89; 95%CI=[0.76-1.03]; p=0.131). There was a significant correlation between fly numbers and rainfall. Data were analysed by season for between arm differences; in the monsoon season 40% fewer flies were caught in the intervention arm compared to the control arm (p=<0.001). Most flies caught belonged to the Muscidae family:

Musca domestica or *M. sorbens*. Of the flies tested for bacteria, 60.3% were positive for at least one of either *Escherichia coli* 0157:H7, *Vibrio cholerae*, *Salmonella* spp. or *Shigella* spp.. *Escherichia coli* was detected most frequently with 46% testing positive. Numbers of *Cx. quinquefasciatus* caught (IRR=0.88; 95%CI=[0.74-1.06]; p=0.178). between the control and intervention villages. Less than 1% of *Cx. quinquefasciatus* tested positive for *W. bancrofti*, equivalent to an LF prevalence rate of 0.0034% within the vector population.

Latrine construction was not associated with a reduction in the densities of vectors and their pathogens, compared to villages without latrines. However, it should not be concluded that latrines have little value in improving public health. Reducing open defecation is one step towards limiting fly population densities and bacterial contamination in the environment, by reducing available larval habitats. However, latrine coverage by itself is not enough to prevent open defecation; construction needs to be supported by behaviour change. These findings reflect that there are multiple pathways for the spread of bacteria in the environment, one of which is fly-borne. In environments with high bacterial transmission reducing fly numbers alone is not enough to impact the transmission of diarrhoeal pathogens.

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List of Abbreviations

Ab	Antibody
Ag	Antigen
CDC	Center for Disease Control and Prevention
CFU	Colony Forming Unit
CRT	Cluster Randomised Trial
DEC	Diethylcarbamazine
ELISA	Enzyme Linked Immunosorbent Assay
EPS	Expanded polystyrene beads
GPELF	Global Programme to Eliminate Lymphatic filariasis
ICT	Immunochromatographic Card Test
IFAT	Indirect Immunofluorescent Test
IQR	Interquartile Range
IRS	Indoor Residual Spraying
L3	Third stage larvae of *Wuchereria bancrofti*
LF	Lymphatic filariasis
MDA	Mass Drug Administration
Mf	Microfilariae
MX	Molecular Xenomonitoring
ORS	Oral Rehydration Solution
PCR	Polymerase Chain Reaction
C-PCR	Conventional Polymerase Chain Reaction
qPCR	Real Time Polymerase Chain Reaction
RCT	Randomised Control Trial
RDT	Rapid Diagnostic Test

SAFE	Surgery, Antibiotics, Facial cleanliness, Environmental improvement
SD	Standard Deviation
SDI	Socio Demographic Index
TAS	Transmission Assessment Survey
TCS	Total Sanitation Campaign
TNTC	Too Numerous to Count
ULV	Ultra Low Volume spray
VIP	Ventilated Improved Pit
WASH	Water, Sanitation and Hygiene
WHO	World Health Organisation

Chapter 1: Introduction

1.1 Diarrhoeal diseases

Recent estimates show that 1.3 million people die from diarrhoea each year (GBD, 2016). Approximately 500,000 are children under 5 years die from diarrhoea each year, accounting for 8.6% of all deaths in this age group. In 2015, diarrhoeal diseases were ranked as the fifth leading cause of mortality, an increase from 2005 when they were ranked sixth. However, mortality rates decreased by 37.4% across all ages, and 35.8% specifically in children under five years old, from 2005 to 2015. Rotavirus is the leading cause of death for diarrhoeal disease in children under five years (29.3%) and all age categories (15.2%) (GBD, 2017).

Diarrhoea is defined as the 'passage of loose watery stools, usually at least three times in a 24-hour period' (WHO, 2005). There are four clinical types of diarrhoea; 1) acute and watery, 2) acute and bloody, 3) persistent, and finally 4) diarrhoea with severe malnutrition (WHO, 2005). Diarrhoeal episodes last several days. The leading cause of death from diarrhoea has most frequently been caused by dehydration, lost though sweat, vomit and liquid stools. However, co-infections and septicaemia are becoming more frequent as the use of oral rehydration salts increases (Nabukeera-Barungi et al., 2018). This is especially true in children who are malnourished or people who have impaired immunity, for example, with HIV.

Treatment with zinc tablets can help to shorten the episode of diarrhoea and in all cases, treatment with oral rehydration solution (ORS) is recommended (WHO, 2005). For severely malnourished children, giving nutrient rich foods and longer term, a nutrient rich diet, can prevent the cycle of diarrhoea contributing to malnourishment. Rotavirus immunisation is a key measure to prevent diarrhoea, whereas water, sanitation and hygiene practices prevent the bacterial and parasitic infections that cannot be vaccinated against (UNICEF, 2017).

The highest burden of diarrhoeal disease is found in countries where there are few established health systems. The most common aetiological causes of diarrhoea are Rotavirus and *Escherichia coli* (GBD, 2017). Many types of bacteria, protozoa, viruses and the fungus, *Candida* spp., can cause diarrhoea (Table 1.1). The leading factor contributing to diarrhoeal disease is pathogen contaminated water and a lack of access to sanitation facilities, as well as poor personal hygiene spreading infection from person to person (Pengpid and Peltzer, 2012). Diarrhoeal disease incidence was reduced by 13.4% due to the provision of access to safe water and sufficient access to sanitation and hygiene facilities (GBD, 2017). Malnourished children are more likely to suffer from diarrhoea. Each episode of diarrhoea contributes to increasing malnourishment as the body is deprived of nutrients necessary for growth during this time.

Table 1.1 Deaths and population percentage for diarrhoeal causing diseases in 2015 (GBD, 2016).

2015	**Children under 5 years**		**All ages**	
Diarrhoea	Deaths (thousands)	Population (%)	Deaths (thousands)	Population (%)
Cholera	28.8	5.8	68.4	5.2
***Salmonella* infections**	38.5	7.7	90.3	6.9
Shigellosis	54.9	11.0	164.3	12.5
Enteropathogenic *E. coli*	11.3	2.3	12.0	0.9
Enterotoxigenic *E. coli*	23.6	4.7	74.1	5.6
***Campylobacter* enteritis**	30.9	6.2	37.5	2.9
Amoebiasis	15.5	3.1	67.9	5.2
Cryptosporidiosis	60.4	12.1	64.8	4.9
Rotaviral enteritis	146.5	29.3	199.2	15.2
Aeromonas	7.3	1.4	56.8	4.3
Clostridium difficile	0.8	0.2	9.4	0.7
Norovirus	14.8	3.0	29.7	2.3
Adenovirus	46.0	9.2	7.02	5.4

1.2 Environmental Interventions

Open defecation and a lack of good sanitation facilities contributes to the transmission of diarrhoeal diseases in four important ways: 1) direct contamination of food with human faeces, the result of

food being grown in fields close to or in open defecation areas, which is later ingested; 2) lack of sufficient personal hygiene, for instance after anal cleansing, leading to transference of bacteria to surfaces that can be ingested; 3) contamination of water with human faeces that can later be ingested; and 4) transference of bacteria, from flies that have developed in or landed on faeces, to food being prepared (WHO and UNICEF, 2009) (Figure 1.1).

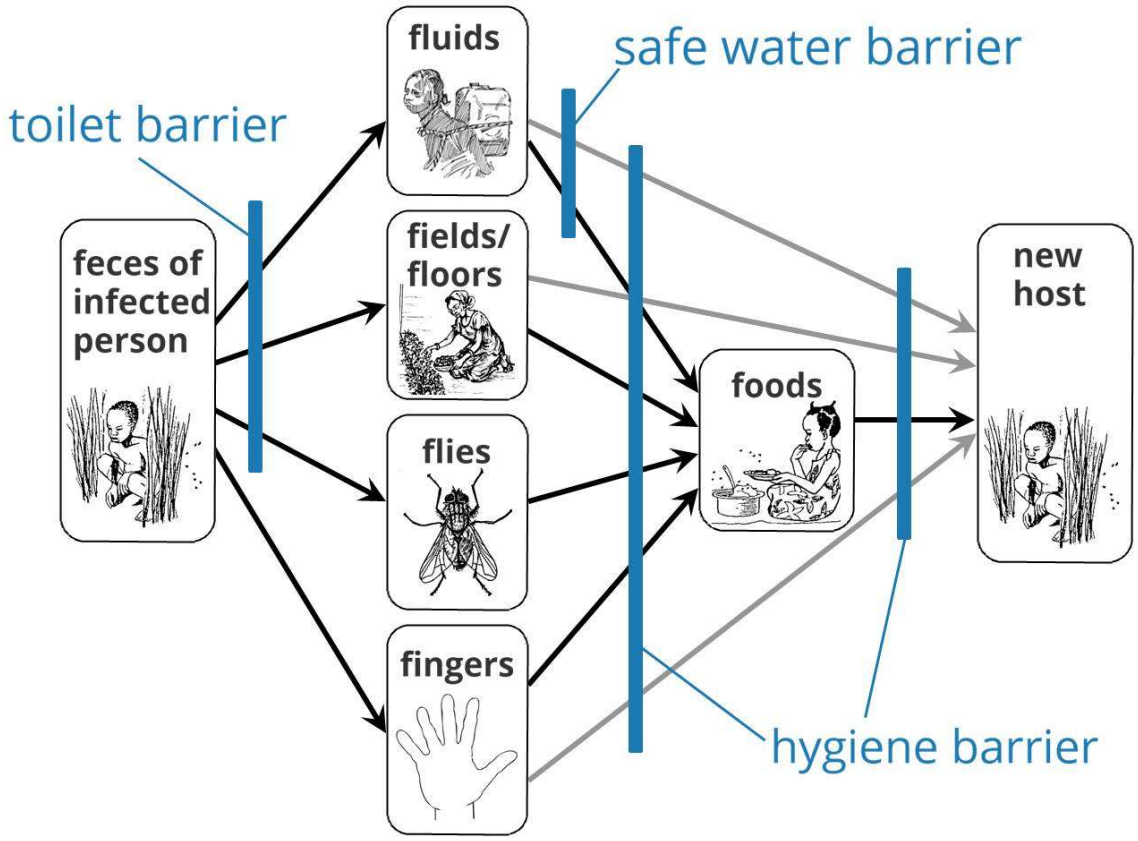

Figure 1.1. The F diagram showing the four main pathways to contamination with faecal matter that can lead to diarrhoeal disease. https://water1st.org/problem/f-diagram/

Control usually consists of targeting one or two of these pathways: improving water quality and/or improving hygiene and sanitation practices. One diarrhoea transmission pathway is dependent on another; improved water quality is frequently very dependent on improved sanitation practices (Eisenberg et al., 2007). A report published as a joint collaboration between the World Health Organisation (WHO) and United Nations Children's Fund (UNICEF) (2009) outlined the main elements required to prevent diarrhoeal diseases. Prevention was based on the main methods of bacterial

transmission as part of Water, Sanitation and Hygiene (WASH) interventions; including hand washing with soap and water, preventing contamination of drinking water, decreased open defecation and safer disposal of faeces (WHO and UNICEF, 2009). Several successful schemes have achieved reduction in diarrhoeal disease incidence, including those focusing on hand washing and personal hygiene (Beau De Rochars et al., 2011). Strategies, like the one outlined for the prevention of trachoma: SAFE (surgery, antibiotics, face washing and environmental change) is successfully implemented for a range of diseases, including diarrhoeal diseases (Roba et al., 2010, WHO, 2010b). However, few interventions have come close to the reduction in diarrhoeal incidence as those that have provided latrines to individual households or village communities (Semba et al., 2011, Markovic Baluchova and Mamova, 2017).

1.2.1 Latrines

Provision of latrines and encouraging people to move away from open defecation practices improves sanitation in the local environment and removes the primary source of faecal contamination; this in turn can lead to reduction in diarrhoeal diseases (Cairncross, 1993, Cha et al., 2017). Increasing the coverage and use of latrines is a worldwide challenge that works towards part of millennium development goal 7: halving the population without access to basic sanitation or safe drinking water and since 2015, sustainable development goal 6: providing access to water and sanitation for all (UNICEF, 2017).

As of 2015, 12% of the world's population was still considered to be practicing open defecation (892 million people), a further 20% still using unimproved or limited facilities that are improved but shared with multiple households and 29% using basic facilities that are improved and not shared with other households (UNICEF, 2017). Altogether, 2.3 billion people do not have access to even a basic sanitation facility.

Latrines are an important part of human waste disposal. There are several varieties of latrines that can be built depending on the available resources; the most common of which is the pit latrine, a type of unimproved sanitation facility. A typical pit latrine comprises a circular opening in the ground that drops down into a specially dug pit beneath to contain the faeces (Cairncross, 1993). It must be built away from water sources and the home but is the easiest and cheapest type of latrine to construct. The disadvantages of this type of latrine are unpleasant smells and access of insects to the latrine. A ventilated improved pit (VIP) latrine is an improved pit latrine where a vent pipe reduces problems with flies and smell (Ryan and Mara, 1983). This also needs to be built away from homes and water supplies. The fly screen on the vent pipe needs to be well maintained to ensure lack of availability to faeces for flies to breed. The primary disadvantage of pit and improved pit latrines is the need to relocate the pit once full. Although most materials used to construct pit latrines can be reused, there is an investment in time and effort required each time (Bhagwan et al., 2008).

More advanced (improved) latrines include the pour flush latrine, found frequently throughout India (Cairncross, 1993). This enables a water seal to be formed between the pit and the latrine itself in a toilet pan, providing better protection against smells from escaping but also, and more importantly, against flies and other insects from accessing the faeces contained within the pit (WHO, 1996). Pour flush latrines can be built much closer to homes and are much more easily cleaned but do require good access to water and are more expensive to construct. They can also be built as part of an offset, two part pit system that allows for switching to another pit when the first is full, allowing the full pit to be left for sufficient time that it can be used for fertiliser, usually after a period of two years (Hussain et al., 2017).

Other types of sanitation, although used infrequently, include composting toilets: a dry pit where kitchen waster and excreta are stored that is broken down and used for fertiliser. They do not need to be moved but are more expensive and difficult to maintain. Septic tanks are chambers where all

faeces and urine is emptied, built below ground. Tanks need to be mechanically emptied and are expensive to construct as well as requiring large supplies of water. Sewerage connections, which require large amounts of water and specialists to maintain, are generally unfeasible in most developing countries due to the prohibitive costs involved (WHO, 2002b).

1.3 Fly borne diarrhoeal transmission

Transmission of bacteria by flies was shown to be a major contributor in maintaining diarrhoeal disease in a military camp in Israel (Cohen, 1991) and in other countries (Levine, 1991, Emerson et al., 1999a). Synanthropic flies, or filth flies, are commonly found around human dwellings and have the potential for pathogen transmission due to their habit of landing and feeding on household waste, faeces and food (Greenberg, 1973). They are ideal carriers of bacterial fauna and enteric pathogens including those that cause cholera, dysentery salmonellosis and shigellosis (Table 1.2). In addition to trachoma and other serious pathogens, flies can infect wounds and cause myiasis (Greenberg, 1965). Synanthropic flies generally belong to three families: Muscidae, Calliphoridae and Sarcophagidae, of which, the most widespread are the common housefly *Musca domestica*, the bazaar fly *M. sorbens*, the oriental latrine fly *Chrysomya megacephala* and the African latrine fly *Chrysomya putoria* (Crosskey, 1993). Outbreaks of diarrhoeal disease are often closely associated with increases in fly numbers, usually during the wet season, and at times when sanitary conditions and hygiene are absent or reduced, especially during periods of flooding (Graczyk et al., 2005).

The method of pathogen transmission associated with filth flies is mechanical and can occur through a number of different ways. The tarsi of the fly are covered in a sticky substance allowing greater adhesion to vertical surfaces but also adhesion of bacteria, viruses and cysts to the fly. These can then be displaced onto the next surface visited by the fly (Graczyk et al., 2005). Electrostatic charging of housefly exoskeletons also facilitates the uptake of particles and transference to another surface (McGonigle and Jackson, 2002). Pathogens can also be conveyed between surfaces through the

ingestion and then regurgitation habits of flies. Bacteria are ingested to the alimentary tract and are then regurgitated and deposited via vomit drops and or deposited through faecal drops that occur during feeding or resting (Graczyk et al., 2005). It was shown that bacteria are readily transmitted between flies and sterile hospital surfaces (Fotedar et al., 1992, Rahuma et al., 2005) as well as food (De Jesús et al., 2004).

Table 1.2. Diarrhoea causing enteric pathogens and method of detection from synanthropic flies.

Bacteria detected	Location	Method of detection	Flies sampled	Reference
***Shigella* sp, *Salmonella* sp**	Various, Beirut, Lebanon	Agar culture	Muscids and Calliphorids	(Bidawid et al., 1978)
***Cryptosporidium* spp., *Giardia* spp.**	Livestock, USA	Flourescent *in situ* hybridisation and immunofluorescent antibody analysis	Muscids, Calliphorids and Sarcophagids	(Conn et al., 2007)
E. coli*, *Shigella* sp, *Vibrio cholerae* and *V. fluvalis	Thailand	Agar culture	Muscids	(Echeverria et al., 1983)
Intestinal Helminths and protozoan parasites [1]	Ethiopia	Fomol-ether, ziehl neelsen staining	Muscids and Calliphorids	(Fetene and Worku, 2009)
***E. coli*, *Klebsiella* spp. *Staphylococcus* spp. and *Campylobacter* spp**	Germany	Agar culture	*M. domestica* and *Stomoxys calcitrans*	(Forster et al., 2009a, Forster et al., 2009b)
Staphylococcus* spp., *Klebsiella* spp., *E. coli, V. cholerae	India	Agar culture	*M. domestica*	(Fotedar, 1992, Fotedar, 2001)
Cryptosporidium parvum	Lab conditions and USA	Immunoflourescence microscopy	*M. domestica*	(Graczyk et al., 1999, Graczyk et al., 2000)
Intestinal parasites and protozoan parasites[1]	Ethiopia	Fomol-ether, ziehl neelsen staining	Calliphorids and Muscids	(Getachew et al., 2007)
Helicobacter pylori	Lab conditions	Agar culture	*M. domestica*	(Grubel et al., 1997)
Shigella* sp, *Salmonella* sp *E.coli	Pakistan	Agar culture	*M. domestica*	(Khalil et al., 1994)
***V. cholera, E. coli*, *Shigella* sp. and *Salmonella* sp.**	Myanmar	Agar culture	*M. domestica*	(Khin Nwe et al., 1989)

***acinetobacter* sp., *bacillus* sp., *Escherichia* sp., *enterobacter* sp., *klebsiella* sp. and *proteus* sp.**	Malaysia	Agar culture	*M. domestica*	(Nazni et al., 2005)
V. cholerae	Lab conditions	Agar culture	*M. domestica*	(El-Bassiony et al., 2016)
***Bacillus* sp., *E. coli (o157)*, *salmonella* sp., *shigella* sp. and 11 other bacteria**[2]	Thailand	Agar culture	*C. megacephala* and *M. domestica*	(Chaiwong et al., 2014)
Clostridium difficile	Lab conditions	Agar culture	*M. domestica*	(Davies et al., 2016)
E. coli	Bangladesh	Agar culture	*C. megacephala* and *M. domestica*	(Lindeberg et al., 2018)

[1] *Ascaris lumbricoides, Trichus trichiura,* hookworm, *Hymenolepsis nana, Taenia* spp., *Strongyloides stercoralis, Entamoeba histolytica/dispar, Entamoeba coli, Cryptosporidium* spp., *Giardia lamblia.*
[2] *Citrobacter* sp., Coagulase negative staphylococci, *Enterobacter* sp., *Enterococcus* sp., *Klebsiella* sp., *Morganella* sp., *Proteus* sp., *Providencia* sp., *Pseudomonas aeruginoisa*, *Staphylococcus aureus*, *Streptococcus* group D non-enterococci

1.4 Synanthropic flies

1.4.1 Muscidae

Most species of Muscidae are not synanthropic, although the most common, *M. domestica,* is ubiquitous throughout the world and is the fly most likely to be found within houses as it has adapted to life in human settlements (Omar et al., 2003). The flies are 7-9 mm in length, grey, with darker stripes on the thorax. Houseflies breed in different types of decaying material, although preferentially in human faeces (Greenberg, 1973). If the preferred breeding sites are not available, *M. domestica* will breed in animal faeces, carrion and decomposing food (Ruiu et al., 2008, Shah et al., 2016). Flies can lay up to 120 eggs at one time and hatch within a few hours. Larvae burrow into and feed off the organic material present. Depending on the moisture content of the breeding material, they may burrow up to several centimetres. In moist content, burrowing is restricted as they require oxygen from the environment. Larvae develop through 3 instars and can take as little as 6 days at the optimum temperature of 35°C and an abundance of food (Wang et al., 2018).

Development from the pupa to adult can take from 2-10 days. Adult mating and oviposition happens after a few days and a female will usually lay no more than 5 times in a life time (Butler et al., 2013). The average life span is from 2-4 weeks, dependent on temperature (Keiding, 1986). Temperature can also effect fly density and the optimal temperature for development is between 20 and 25°C. Below 10°C and above 45°C fly numbers reduce significantly. In very low temperatures, adult flies and pupae can become dormant. Mating and ovipositon stop below 15°C (Wang et al., 2018).

Muscidae adults, both male and female, can feed on a range of substrates such as human food, garbage and faeces (Greenberg, 1959). A few species, such as *Stomoxys calcitrans,* bite animals or humans to draw blood for oviposition (Salem et al., 2012). All Muscids can survive on sugar water but the females require protein to reproduce (Greenberg 1959; Yamamoto and Jensen 1967). Food needs to be semi liquid or soluble for ingestion by salivary secretions. Muscidae need to feed at least 2 or 3 times a day to survive and will not survive more than 48 hours without access to water (Fisher et al., 2017). Musicdae can also obtain liquid from milk, blood, sugar among other common food items found in human habitation.

Unlike *M. domestica*, *M. sorbens* preferentially lays eggs in exposed human faeces and will not use latrines as larval development sites (Emerson et al., 2005). *Musa sorbens* feeds on proteinaceous substrates, specifically human secretions; tears, ocular and nasal discharges, saliva and pus exudates (Crosskey, 1993).

Musca domestica and *M. sorbens* are diurnal. They feed and mate during the day then rest at night. Resting places during the night includes ceilings and overhead beams indoors (Hertz et al., 2011). Alternative resting sites outside include bushes and other vegetation as well as fences or washing lines. Resting places are usually near sources of food and breeding sites, usually sheltered from wind and are not normally more than five metres high (Baumgartner, 1988).

1.4.2 Calliphoridae

Calliphoridae are commonly known as blowflies, or carrion flies, and are scavengers which lay eggs in faeces, carrion, open sores, and other decaying organic animal matter. There are approximately 1000 species within the family, of which not all are synanthropic (Wolff and Kosmann, 2016). Many are asynanthropic, i.e. avoid human habitation and some are hemisynanthropic; live on the periphery of human habitation and in zones of transition from rural to urban areas. Calliphoridae have a worldwide distribution but their habitat preferences differ depending on the local geography and availability of resources (Wolff and Kosmann, 2016). The flies range in size from 8-10mm, with some specimens larger and are often metallic green or blue in colour. Similar to species belonging to the family Muscidae; Calliphoridae are mainly outdoor flies, more common in farm areas, and congregate around outdoor marketplaces, garbage, damaged fruit, carrion, open wounds, human and animal excreta (Chaiwong et al., 2014).

Chrysomya are one of the most important synanthropic fly genera, which breeds extensively in pit latrines and areas where liquid faeces can be found (Irish et al., 2013, Lindsay et al., 2013). They will preferentially breed in human faeces but eggs can also be found in cattle dung and corpses (Badenhorst and Villet, 2018). Calliphoridae adults can lay between 120-200 eggs at a time and the life cycle progresses in the same way as for Muscidae; from egg to larva in a few hours, through 3 larval instars, which can take between 6 to 11 days, and finally a pupal stage that will emerge as an adult between seven to fourteen days later (Anderson, 2000). Development rates are dependent on temperature; as low as 16°C, maturation can take 32 days from eggs to adults. Optimal development occurs around temperatures of 25-30°C where the cycle from egg to adult can take as little as 14 days (Kamal, 1958, Gruner et al., 2017). The average development time from egg to adult takes approximately twenty days. Adults live up to 54 days at 25-29°C and 75% relative humidity with longer life spans at lower humidity (Ngoen-klan et al., 2011). Females can lay up to 2000 eggs during their life (Greenberg, 1973).

Chrysomya megacephala is frequently found around garbage containers and feed on carrion and other waste or excreta but can also be found visiting plants with strong smells closely resembling rotting meat (Chaiwong et al., 2014). In contrast, *Calliphora vicina* primarily breeds in carrion and other meat but can be found breeding in human faeces but more solid mass than the liquid preferring *Chrysomya megacephala* (Greenberg, 1973).

Calliphoridae are insects of economic importance as well as public health concern due to the problem of myiasis in livestock, attacking live, healthy tissue, causing in excess of several billion dollars worth of damage worldwide each year (Vargas-Terán et al., 2005). Mammals like humans, dogs, deer, sheep and cattle can become infested with maggots (Francesconi and Lupi, 2012). Eggs are laid in or near wounds or ulcers, open sores or mucus membranes. The larvae then burrow into the tissue and begin to feed. Damage caused during this process produces an odour that attracts more flies to the wound for more eggs to be laid. The primary myiasis causing flies are the new world and old world screwworm (Francesconi and Lupi, 2012). The new world screwworm, *Cochliomyia hominivorax,* was found primarily throughout Central and South America. After a successful program releasing sterile males in southern USA, the screwworm was eradicated (Baumhover, 1966). Although an outbreak has since been reported in Florida (Skoda et al., 2018), the elimination of the screwworm from the Americas, has reportedly saved the livestock industry 1.3 billion dollars (Vargas-Terán et al., 2005). The old world screwworm, *Chrysomya bezziana,* can be found throughout sub-Saharan Africa, South Asia, Indonesia, Philippines and many Pacific Islands. It was reported that it is also susceptible to control via the sterile insect technique but application is more difficult as rural areas are harder to reach, and maintain the necessary level of surveillance required to prevent re-infestation from other areas (Hall et al., 2009).

1.4.3 Sarcophagidae

Commonly known as flesh flies these flies oviposit in carrion, faeces, and decaying matter, with some species ovipositing in open wounds (Pape, 1996). The size of sarcophagids varies from 6-

23mm. Sarcophagids are commonly viviparous, laying larvae directly onto the chosen site, although there are a small number of species that do lay eggs (Sukontason et al., 2014). They usually have black and grey longitudinal stripes on the thorax and a chequered pattern on the abdomen. Adults feed predominantly on carrion but will also feed on decaying vegetable, organic matter and excrement. Their lifecycle lasts between 10-17 days, depending on temperature. There are approximately 3000 species within the family of which only a few are associated with faeces and humans (Verves et al., 2017). A few species have also been implicated in causing myiasis (Colwell and O'Connor, 2000).

1.5 Monitoring Fly densities

There are many methods that have been used for the capture and assessment of synanthropic flies but there is little uniformity in techniques used in various locations around the world (Table 1.3). Trap types include baited traps, or sticky traps. Sweep nets can be used to catch individual or groups of flies. Scudder grills can be used to assess density but do not capture flies.

Each design has its own advantage and disadvantage. Jug baited traps allow capture of large amounts of flies and be classed as a control measure due to the high quantity captured. From the purposes of population density monitoring, these traps allow the identification of species, and monitoring of numbers to measure the effect of control methods on a targeted population. The disadvantage lies in the inability to catch flies individually and prevent flies from contaminating one another for the purpose of testing for bacteria transmission. Sweep nets can be used to catch individual flies or groups of flies. Individual capture of flies does allow for bacteria isolation. However, catching flies individually and in sufficient quantity to determine the effect of control measures would be a very labour intensive way of monitoring population densities in a given area. Scudder grills allow for the determination population density but there is no option for catching or identifying the species surveyed. Sticky traps allow the capture of flies in large quantities, but separately, to minimise bacterial contamination between flies. Very few traps used in commercial

situations use a sticky element as the primary motivation usually is to reduce populations by catching as large a number of flies as possible rather than analysing the bacterial load being carried (Geden, 2005, Geden, 2006). Fly ribbons or spiral sticky traps are generally used in domestic as opposed to commercial situations. These spiral sticky traps have limited surface areas for catching large quantities of flies but can be used to assess species and bacteria carriage (Rady et al., 1992).

Baited traps using meat or viscera have been shown to be very effective at attracting flies from a range of different families. However, the use of baits is very specific to the type of fly (Pickens, 1994, Pickens, 1995, Boonchu et al., 2003). To be a universal fly trap the bait used would have to attract a range of different flies rather than specific species. Synthetic lures have also been tested and tried in field conditions with proven efficacy for catching flies in preference to fresh baits (Nurita et al., 2008, Aak et al., 2010). These are specific for particular species and would not attract a range of flies. Problems with baited traps arise not just from the selection of bait to use but also the design of the trap. There are many different structural designs that have been recommended and used (Pickens, 1995, AFPMB, 2006, Geden, 2006, Carlson and Hogsette, 2007, Geden et al., 2009) and there have been few studies performed that have shown significant efficacy of one design over another.

Few recent studies have explored the use of traps to assess species and bacterial carriage as well as population densities but these studies were not conducted on a large scale within houses (Talley et al., 2009, Lindsay et al., 2012). Most experiments have either focused on the species and bacterial load or the population density and species (Table 1.3).

Table 1.3. A sample of published articles showing different trapping methodologies for assessment of synanthropic fly population densities, species and bacterial load.

Trap type used	Country	Location	Population density monitored?	Species identified?	Bacteria detected?	Reference
Baited jug traps	Florida, USA	Rural - commercial cattle farms	Yes	*Musca domestica*	No	(Geden, 2005)
Sweep net	California, USA	Rural - commercial cattle farms	No	*Musca domestica*	Yes	(Mian et al., 2002)
Sweep net	Addis Ababa, Ethiopia	Urban – various locations	No	*Chrysomya rufifacies, Musca domestica, Musca sorbens*	Yes	(Getachew et al., 2007)
Baited jug trap	Northern Thailand	Rural – various locations	Yes	*Chrysomya megacephala, Musca domestica*	No	(Ngoen-klan et al., 2011)
Sweep net and sticky traps	California, USA	Rural - organic farms	Yes	*Musca domestica, Calliphoridae*	Yes	(Talley et al., 2009)
Baited glass slide and sweep net	Malaysia	Rural – various locations	No	*Musca domestica*	Yes	(Nazni et al., 2005)
Sweep net	Northern Thailand	Urban - food markets	No	*Chrysomya megacephala, Musca domestica*	Yes	(Sukontason et al., 2007)
Scudder grill and sticky trap	Muheza, Tanzania	Rural - houses	Yes	*Musca domestica*	No	(Raybould, 1966a, Raybould, 1966b)
Emergence and odour	Upper River Region,	Rural - Pit latrines	Yes	*Chrysomya putoria*	Yes	(Lindsay et al., 2012)

baited traps	The Gambia

1.6 Control of synanthropic flies

Until recently, an often overlooked method of diarrhoeal disease prevention was the control of flies as vectors. In the early 1990s fly control was classified as ineffective; partly due to the uncertainty surrounding the role of flies in the transmission of diseases stemming from a lack of studies successfully showing the correlation between seasonal fly densities and prevalence peaks in disease, as well as little evidence of the sustainability of fly control (Esrey, 1991). Since then, there have been a few key studies that not only have shown conclusively that flies can be mechanical vectors of numerous pathogenic diseases but also the effectiveness of fly control in the reduction in diarrhoeal cases (Cohen, 1991, Emerson et al., 1999a, Emerson et al., 2004b) resulting in some countries adopting specific regulation targeting bacterial transmission via flies (Olsen, 1998).

1.6.1 Physical and mechanical control

Human and animal faeces are common breeding media for synanthropic flies. Significant reductions in fly populations cannot occur if the number of breeding sites remains high. Interventions involved in providing communities with sanitation facilities must also ensure that health education is made a priority (Pattanayak et al., 2009) and removal of livestock from within villages is facilitated, reducing the risk of the flies breeding in animal faeces in the vicinity of human habitation (Emerson et al., 2005). Their might be resistance to moving livestock from within the confines of houses where there is more security and control of access to livestock.

Removing sources that would attract flies to domestic or residential areas can also help to reduce diarrhoeal disease incidence. Removing faeces from around the house, specifically child faeces, and disposing in latrines removes a primary source of attraction (Majorin et al., 2014). Garbage piles close to houses, could also act as an attractant to flies into the domestic environment (Chaiwong et

al., 2014). Waste, especially organic, can be a potential breeding site and removal or preventing access through a shelter or other structure can reduce populations close to houses.
Finally preventing flies coming into contact with surfaces and food where they could transmit bacteria can help to reduce diarrhoeal disease incidence (Lindeberg et al., 2018). Baited traps can have a lower overall impact on fly numbers but they are a relatively inexpensive method of control and, in a relatively contained area like a kitchen, could be effective in preventing fly/food contact (Boonchu et al., 2003). However, in the Pakistan study, fly baited traps were not found to be effective (Chavasse et al., 1999). Specific environmental factors, such as the amount of sunlight, proximity to a fire source and wind strength, have all been shown to have an impact on the efficacy of fly traps (Raybould, 1966a).

It is possible that synanthropic flies could be prevented from entering properties through the use of screens on windows, air vents or other opening that allow flies access. Mesh size of 3-5mm will exclude larger flies from accessing properties and could potentially deter smaller flies, as well as mosquitoes, although air flow can be reduced (Lindsay et al., 2003). Curtains placed across doorways can have a similar affect and are cheaper to install and maintain than screens.

As a method of control, screening houses is far from ideal. The design and construction of many Kutcha houses (large gaps in the eaves, walls constructed from mud but in some places only bamboo screens) would prevent effective screening of the entire house. Flies would still be able to access the house even with all the main entryways covered. Preventing access of flies to kitchen areas outside where food might be prepared or cooked would not be possible with screens. Screening does not control the source of the problem at larval development sites so fly densities will remain high within a village.

A commonly seen method for killing flies is through the use of an ultraviolet (UV) light trap. These are frequently mounted on walls in restaurants (Lillie and Goddard, 1987). Attracted flies are killed by electrocution after contact with an electric grid (Pickens and Thimijan, 1986). UV light traps

require a consistent electrical supply and can be expensive to maintain. It is possible that they could be effective over much smaller space, such as placement within areas where flies are known to congregate, like kitchens. Pickens and Mills (1993) showed that solar-powered traps could be effective in killing large numbers of flies (Pickens, 1991, Pickens and Mills, 1993). A solar powered trap would be much more practical for use in villages. Despite the efficacy of these traps for killing flies, it has been shown that electrocuting insect traps have the potential to aid in the spread of bacteria and viruses by releasing bacteria when the fly is electrocuted (Urban and Broce, 2000).

1.6.2 Biological control

Musca virus (MdSGHV) or Salivary gland hypertrophy virus of *M. domestica* is a virus that replicates in housefly salivary glands, causing them to become enlarged (Geden et al., 2008). Development of the virus within the fly leads to a suppression of gonad development in 95% of infected females (Coler et al., 1993, Lietze et al., 2007). The virus is naturally found within field populations of flies and is maintained within the population once it is introduced (Lietze et al., 2012b). However, increasing the prevalence of the virus within a population does not result in a collapse in the population numbers (Lietze et al., 2012a). Instead the virus levels slowly decreases until the average levels of between 1 to 10% are reached. To control and maintain low numbers of flies in rural areas, it would be necessary to have repeated applications of the virus in a specific area.

The virus is only infectious to adults so it does not prevent larvae that are already in development from completing their lifecycle (Geden et al., 2008). In addition, the virus does not affect females that have already developed eggs but have yet to deposit them at a breeding site (Lietze et al., 2007). Repeated applications would again be required to ensure that the next generation of emerging flies were also affected by the virus.

Entomopathogenic fungi are another option for biologically controlling flies(Geden et al., 1995). They infect through the gut wall of ingestion or through damage in the cuticle and can attack several stages of development; both adults and larvae. Temperature and humidity affect infection rates so

seasonal variation is important when planning control with this method (Anderson et al., 2011). Infection with entomopathogenic fungi is decreased at high temperatures though so the success rate of this control method in tropical countries would probably be quite low. Evidence also suggests that the fungi are not self-sustaining in the field which means that multiple applications of the fungi would be required to have a continual effect on fly populations. The entomopathogenic fungus *Beauveria bassiana* (Geden et al., 1995, Kaufman et al., 2005, Lecuona et al., 2005, Anderson et al., 2011, Mishra et al., 2011, Sharififard et al., 2011) and the adult specific mycopathogen *Entomophthora muscae* (Mullens et al., 1987) can be used to control populations of Muscidae. In controlled experiments under lab conditions showed 94-100% mortality (Geden et al., 1995).

Current investigations of larval predators of *Musca* species include the beetle *Carcinops pumilio* (Kaufman et al., 2000) and the black dump fly *Hydrotaea aenescens* (Geden et al., 1986, Carlson et al., 2001, Lysyk, 2004, Geden and Kaufman, 2007, Geden and Moon, 2009). Pupal parasitoids include: (Hymenoptera) Pteromalidae; Muscidifurax*,* Nasonia and Spalangia (Rueda et al., 1997, Kaufman et al., 2001, Skovgard and Nachman, 2004, Geden and Hogsette, 2006, Kaufman et al., 2012, Machtinger et al., 2015).

Bacillus thuringiensis is a microbe that produces endotoxins which show insecticidal activity. Studies have been conducted that show the effectiveness of toxins produced by the bacteria against the larvae of sheep blow fly *Lucilia cuprina* (Gough et al., 2005). The different strains have a range of efficacies against houseflies. Spray and feed studies conducted to ascertain the effectiveness of this microbial insecticide against *M. domestica* larvae in poultry houses have shown a 50% reduction in levels of larvae collected during a 6-week sampling period compared to the control when spraying and 80% reduction after a 2-week period when added to poultry feed (Mwamburi et al., 2011). It is suggested that multiple spraying applications would be required over a short period to achieve the levels of control required for operational programs. The spores may be affected by exposure to high

temperatures, like those seen in the tropics. It appears to be much more effective to add the microbial insecticide to animal feed (Gingrich, 1965, Miller et al., 1971, Ruiu et al., 2008).

1.6.3 Chemical control

Insecticides have a proven track record in controlling populations of vectors, including synanthropic flies (Gratz and Jany, 1994). There are 5 different classes of insecticides including: organochlorines (e.g. DDT), organophosphates (e.g. malathion), carbamates (e.g. bendiocarb, methomyl), pyrethroids (e.g. permethrin, cypermethrin, lambdacyhalothrin, deltamethrin) (Paine and Brooke, 2016) and neonicotinoids (e.g. imidacloprid) (Uragayala et al., 2015). Lambdacyhalothrin and cypermethrin can be used as thermal fogs against houseflies (Lim and Visvalingam, 1990). Lambdacyahlothrin is highly effective when used in a fog at low doses and has good residual activity as well, up to 15 months in some cases (Mazariego-Arana et al., 2002). It is a broad spectrum insecticide with only moderate acute mammalian toxicity (Lim, 1990). Permethrin and deltamethrin are effective against flies in some areas and are very effective at quickly reducing populations of flies when either used as a spray or as a fog (Sukontason et al., 2005). Spinosad is produced by the fermentation process from a soil actinomycete, *Saccharopolyspora spinosa* and is found to be very effective insecticide when used against synanthropic flies in the lab (Scott, 1998). It takes longer to knockdown but has higher eventual mortality than imidacloprid and methomyl. Spinosads lack cross-resistance with other insecticides and have a good safety profile. The disadvantage is that they do not work as favourably when exposed to high temperatures (White et al., 2007). Pyriproxyfen is compatible for use with other biological control agents and due to possibility of resistance development to other insecticides can be used as part of an integrated vector control program (Geden and Devine, 2012). A combination of pyriproxyfen and the parasitoid *Dirhinus himalayanus* was the most effective in controlling housefly populations within Pondicherry when compared with using pyriproxifen or parasitoids alone and compared with the control (Srinivasan and Amalraj, 2003).

Ultra low volume (ULV) spraying may be considered to be the most effective of fly interventions due to the large reduction in adult flies almost immediately following spraying and the reduction in flies visiting households (Chavasse et al., 1999). The large reduction in flies also impacts on the incidence of diarrhoeal disease within communities. The study by Chavasse et al. (1999) in Pakistan showed that ULV spraying reduced the incidence of diarrhoeal disease by 23%, directly attributable to the fly control measures that were in place (Chavasse et al., 1999). ULV spraying is effective but is can be an expensive form of protection, requiring frequent applications to have a continual impact on the numbers of flies. In many places, long term sustainability is not possible although the use of ULV spraying specifically during epidemics can be recommended (Chavasse et al., 1999, Emerson et al., 1999b, Emerson et al., 2004b).

1.6.4 Environmental control

Reduction and elimination of breeding sites by preventing fly access to faeces, through the construction of latrines and sewage systems, can be long lasting and the most sustainable of all fly control methods. In a setting where sewer and water connections have not been established, the best option is the construction of a cistern or pour flush latrine (Busvine, 1982) providing a seal that prevents flies from accessing the faeces contained in the pit below the pan. Pit latrines have been found to be effective at reducing the numbers of *M. sorbens* caught by 30% compared with controls in The Gambia (Emerson, Lindsay et al. 2004). They also demonstrated that *M. domestica* and *M. sorbens* do not preferentially breed in latrines, suggesting that latrine construction could result in a reduction in both of these populations (Emerson, Lindsay et al. 1999, Emerson, Simms et al. 2005). Latrines need maintenance and are only truly effective at preventing the spread of disease if they are used (Emerson et al., 2004a). Allowing the latrine and pit to fall in to disrepair or supplying vent pipes without screens provides an opportunity for flies and mosquitoes to gain access to breeding sites, rendering latrines as a method of control ineffective (Curtis and Hawkins, 1982, Huttly, 1998).

1.7 Lymphatic filariasis

In 2010, an estimated 120 million people were infected with lymphatic filariasis (LF) in 81 countries, 65% of whom reside in South East Asia (WHO, 2010a). Of those, approximately 40 million people experienced severe symptoms consisting of debilitating swelling of the limbs, known as elephantiasis. In 2015, 947 million people were at risk of developing LF, down from an estimated 1.3 billion in 2010 (WHO, 2016). With the help of the Global Programme to Eliminate Lymphatic Filariasis (GPELF), mass drug administration (MDA) with Diethylcarbamazine citrate (DEC) plus albendazole or Ivermectin was estimated to have prevented 97 million cases (WHO, 2016).

1.7.1 Distribution

Lymphatic filariasis is attributable to three filarial worms. Most infections (90%) are caused by the parasite *Wuchereria bancrofti*, while the remaining 10% are attributable to *Brugia malayi* (Cano et al., 2014). The final causative agent of LF is *Brugia timori*, a species that has only been found on a few eastern Indonesian islands (Partono et al., 1977, Supali et al., 2002). Humans are the sole reservoir of the parasite, so elimination of the disease through MDA is a realistic target (WHO, 2016). Transmission is only through a mosquito vector, but different species of mosquito are responsible for transmitting different LF parasites. Bancroftian filariasis has a wide distribution and can be found in South America, sub-Saharan Africa, Asia and many Pacific Islands (Cano et al., 2014). Brugian filariasis has a more restricted distribution to South East Asia.

1.7.2 Parasite life cycle

When an infected mosquito takes a blood meal, infective larvae (L3) can enter the body through the bite puncture wound. When they do, they migrate to the lymphatic system and develop into sexual maturity in the lymph nodes or ducts, through a fourth larval stage and eventually to adults (Reddy et al., 2004). This can take up to 1 year depending on the species (Paily et al., 2009). Adults can survive within the lymph system for 8 years or more (Pfarr et al., 2009). Female worms are ovivivipparous and can produce up to 50,000 microfilariae (Mf) a day. Females are typically fecund for approximately 5 years (Bain et al., 1985). Microfilariae move to the blood stream and can survive

there for up to a year. Microfilaria periodically move to the peripheral blood vessels close to the surface of the skin. Night, sub periodic and diurnally periodic forms exist (Paily et al., 2009). In India, *W. bancofti* shows a nocturnal periodicity and move to the surface of the skin at night where they appear in large numbers (Pfarr et al., 2009). Maximum nightly density was reported between the hours of 10pm and 2am and this generally corresponds with the feeding pattern of local mosquito vectors (Das et al., 2011). During the day Mf retreat into the deeper blood vessels. Infection of the mosquito occurs when the mosquito take blood meal from a human host, ingesting any circulating Mf (Paily et al., 2009).

Ingested microfilariae shed their sheaths in the mosquito midgut before passing through the peritrophic membrane of the gut wall of the mosquito, into the haemocoel and develop through three larval stages to L3 over the period of a 7- 13 days (Paily et al., 1995, Gad et al., 1996). The first stage larvae migrates to the thoracic muscles. The second stage larvae moults and grows in length while the third stage larvae, L3, migrates to the proboscis ready for infecting the next human host (Zielke, 1977). L3 larvae can remain alive for as long as the mosquito survives (46-50 days) (Paily et al., 1995) (Figure 1.2).

Transmission through the mosquito vector requires several factors for success. Some species of mosquito possess a cibarial armature that ingested larvae must pass through to reach the gut (Gad et al., 1996). By limiting the number of microfilariae surviving to reach the gut, a greater proportion survive to be transmitted, known as facilitation (Krishnamoorthy et al., 2004). In some species of mosquito, where the cibarial armature is less effective or not present at all, a reverse process called limitation can occur. Higher densities of Mf ingested in a bloodmeal serve to limit the development and increase mortality of the larvae within the mosquito (McGreevy et al., 1978).

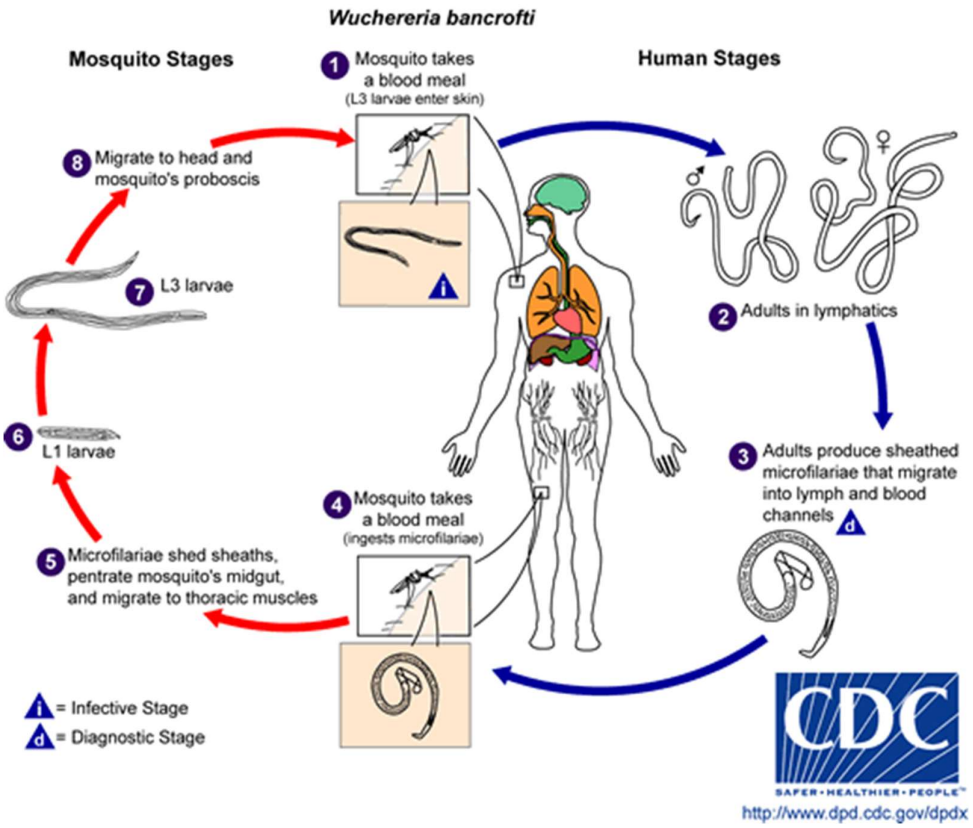

Figure 1.2. *Wuchereria bancrofti* lifecycle through humans and mosquitoes. https://www.cdc.gov/parasites/lymphaticfilariasis/biology_w_bancrofti.html

1.7.3 Clinical Disease

People of all ages are susceptible to infection although infection rates tend to increase with age up 30 years and then level off. In most endemic areas, Mf rates are usually higher in men than women (Cartel et al., 1992). It can take anywhere between 8-16 months for chronic clinical manifestations to appear after infection but only a small portion of those infected will demonstrate clinical symptoms (Tayel Sel et al., 2011, Nutman, 2013). People with filariasis can be categorised as follows: 1) asymptomatic amicrofilaremia - no clinical manifestations and blood not positive for presence of Mf. This usually occurs immediately after initial infection when the L3 larvae have yet to mature in the lymph nodes (Nutman, 2013). 2) Asymptomatic microfilaremia - no clinical manifestations but Mf detectable in blood samples. This occurs soon after initial infection when the adults have had chance to mature and produce microfilariae but before adults cause significant blockages in the lymph

nodes (El-Shazly et al., 2009). 3) Acutely symptomatic - acute inflammation in lymph nodes and surrounding vessels with symptoms of fever, lymphangitis, lymphadenitis and lymphoedema. This occurs when granulomas begin to form in the lymph nodes on death of the adult worm (Pfarr et al., 2009). 4) Chronically symptomatic - due to continual fever, permanent damage has caused fibrosis and obstruction of lymph vessels with symptoms of hydrocele, elephantiasis and chyluria (Nutman, 2013).

1.7.4 Treatment and Control

GPELF was established in 2000 with the aim to remove LF as public health problem by the year 2020. The primary strategy used to further this aim was the initiation of MDA campaigns, stopping the spread of infection by annually treating a large area of people where the infection is endemic.

Treatment and preventative chemotherapy consists of albendazole, DEC or ivermectin (Budge et al., 2018). In many cases, combinations of the drug are the most effective means of treating and the current recommendation is for the combination of 3 (Irvine et al., 2017). When conducted over a period of 4-6 years, the 2-medicine regimen of DEC and albendazole has successfully interrupted the transmission cycle (Ave et al., 2018). However, with the goal of 2020 in mind to eliminate LF as a public health problem, it is thought that the 2-regimen strategy is not sufficient to achieve this aim worldwide. Current modelling shows that a three-medicine regimen could interrupt transmission in some areas within a few weeks instead of years (Irvine et al., 2017, Stolk et al., 2018).

Problems with contraindications mean that DEC cannot be used in areas where onchocerciasis is endemic (Fischer et al., 2017) and ivermectin cannot be used where loiasis is endemic (Kelly-Hope et al., 2018). The treatment suppresses the production of mf in adults in the lymph system and supresses the mf density in the blood stream therefore reduces transmission. In countries without onchocerciasis and loiasis, the following regimen is now recommended: ivermectin (200mcg/kg) with DEC (6mg/kg) and albendazole (400mg). For areas with loiasis, albendazole alone twice a year is

recommended and for areas with onchocerciasis, ivermectin with albendazole (Bockarie et al., 2000, Kelly-Hope et al., 2018).

The continued application of MDA has resulted in 11 countries being certified as eliminating LF as a public health problem (Cambodia, The Cook Islands, Egypt, Maldives, Marshall Islands, Niue, Sri Lanka, Thailand, Togo, Tonga and Vanuatu) with a further 9 under surveillance before being certified (WHO, 2016).

Vector control, while not a primary strategy, is recommended alongside MDA (Bockarie et al., 2008). The benefits are not limited to interrupting LF transmission alone since they will impact other overlapping vector-borne diseases. Depending on the situation, the use of long lasting insecticide treated bed nets (LLIN), indoor residual spraying (IRS) and personal protection is advocated (WHO, 2002a). The scale up of LLIN distribution in sub-Saharan Africa has had an impact on the transmission of *W. bancrofti* by the primary vectors *Anopheles gambiae* and *An. funestus*. Integrated vector management (IVM) can a be a useful strategy to reduce disease risk from multiple vectors and if integrated with environmental awareness can have a much larger impact on health issues (Chanda et al., 2013, Lizzi et al., 2014).

1.7.5 Diagnosis

Traditionally diagnosis in humans was done by taking a blood sample at night, preparing a thick blood film slide and examining for Mf under a microscope. This is not a very sensitive method of detection. Indirect methods can detect filarial specific antibodies (Ab) or circulating filarial antigen (Ag) (Rocha et al., 2009). Antibodies are detected using either the Indirect immunofluorescent test (IFAT) or enzyme linked immunosorbent assays (ELISA) (Noordin et al., 2003). The disadvantage of this method is that antibodies (Abs) can remain high for a very long time after the infection is cured. Antigen (Ag) can be detected by using the Immunochromatographic card test (ICT) or Ag specific ELISAs. The ICT is a rapid diagnostic test (RDT) that is very simple to use, requiring only a drop of blood and can be performed at any time of day, giving results within 15 minutes. It is expensive to

perform when compared to other diagnostic tests and is not sensitive in areas with low endemicity (Rocha et al., 2009).

In mosquitoes, xenomonitoring can be done to determine the level of infection within the vector population. Traditionally, this was done by dissection and microscopy to detect larvae (Goodman et al., 2003). Dissection can identify the specific life stage present within the mosquito but is a very time consuming method and has largely been replaced by molecular xenomonitoring (MX) (Farid et al., 2007). Until recently conventional polymerase chain reaction (C-PCR) was the recommended and most sensitive of methods that could confirm mosquito infection with filarial worms (Williams et al., 2002, Vasuki et al., 2003). However, with advances in real time PCR (qPCR), that has become the preferred method for MX. Real time PCR is much more sensitive to the detection of mosquito infection (Rao et al., 2006, Laney et al., 2010). Testing using MX methods means that mosquitoes can be pooled together from sampling locations, providing a much faster method of determining infection rates (Helmy et al., 2004). This is also especially important in areas with low prevalence rates where more mosquitoes are required to be tested before an estimation of prevalence can be made (Brito et al., 1997, Brito et al., 1998).

1.7.6 Vectors of lymphatic filariasis

The predominant vector of bancroftian filariasis are *Culex* mosquitoes, often associated with polluted water and often found in urban and semi urban areas (Simonsen and Mwakitalu, 2013). *Culex quinquefasciatus* is the primary vector in South America as well as South Asia. It is also a vector on the eastern coast of sub Saharan Africa (Simonsen and Mwakitalu, 2013). *Anopheles* mosquitoes are also vectors of bancroftian filariasis but primarily in Africa, especially in rural areas (de Souza et al., 2012). *Aedes* mosquitoes are the primary vectors in Pacific Islands (Erickson et al., 2009). Brugian filariasis is primarily transmitted by *Mansonia* mosquitoes, associated with aquatic plants in rural areas in South Asia (Ughasi et al., 2012). *Anopheles* are also responsible for some transmission of Brugian filariasis.

1.8 *Culex quinquefasciatus*

Culex quinquefasciatus is a sub species belonging to the *Culex pipiens* complex (Smith and Fonseca, 2004). The other 4 sub species comprising the complex are; *Cx. pipiens pipiens*, *Cx. pipiens pallens*, *Cx. australicus* and *Cx. globocoxitus*. They are morphologically indistinguishable and can only be identified by molecular analysis or behavioural characteristics (Cornel et al., 2003, Farajollahi et al., 2011).

1.8.1 Distribution

Culex quinquefasciatus is a tropical and sub-tropical mosquito. It is found throughout the Americas: from Argentina and Brazil (Morais et al., 2010), through Central America (Kent et al., 2010, White et al., 2018), but the northern limitation in the Americas appears to be the southern states of the US (Moise et al., 2018). Its distribution also extends through almost the entirety of sub-Saharan Africa (Victor et al., 2017, Dida et al., 2018), India and South East Asia (Sadanandane et al., 2018, Thongsripong et al., 2018) and large parts of Northern Australia (Philippe-Janon et al., 2015). So far the vector has not spread into Western Europe but is currently present in Turkey (Morcicek et al., 2018). Modelling has shown that with increases in global temperatures, the distribution of the mosquito is expected to expand in to previously unsuitable areas in Northern Africa and Europe (Samy et al., 2016).

1.8.2 Biology

Gravid females of *Cx quinquefasciatus* travel at night to lay eggs on nutrient rich, still water. Eggs will hatch one to two days after they are deposited. The exact number depends on the temperature and the source of the adult female bloodmeal, but averages 150 (Richards et al., 2012). The larvae feed on organic material in the water, such as bacteria, and require between five to eight days to complete development at 30°C (Rueda et al., 1990). The larvae pass through four larval instars and eventually moult to produce pupae. The 4th stage larvae stop feeding before moulting and the pupae continue without feeding. After approximately 36 hours at 27°C adults emerge (Rueda et al., 1990).

Adults are ready to mate within 72 hours. After mating, the female will seek a bloodmeal in order to complete the egg development process (Richards et al., 2012). Females, on average, lay three rafts of eggs over a lifetime but in certain locations are known to lay up to 8 times throughout the lifespan (Chandra et al., 2009). Females frequently lay eggs in organically contaminated water: ditches, pit latrines and septic tanks. Adults live a month on average, although temperature can effect this (Vrzal et al., 2010).

Males and females feed on sugar water, predominantly from plants but females require a blood meal to complete maturation of eggs (Vrzal et al., 2010, Richards et al., 2012). *Culex quinquefasciatus* has shown a preference for biting avians but will frequently feed on humans (Farajollahi et al., 2011). They are known to feed on a wide range of other mammals including dogs, cats, rodents and rabbits. Feeding is most likely to be opportunistic, with the availability of hosts determining the bloodmeal taken (Richards et al., 2012).

1.8.3 Medical Importance

In addition to *W. bancrofti*, *Cx. quinquefasciatus* is responsible for the transmission St Louis encephalitis (Diaz et al., 2013), Western equine encephalitis (Wang et al., 2012), West Nile Fever (Diaz-Badillo et al., 2011) and potentially Zika virus (van den Hurk et al., 2017). It is also a vector of *Plasmodium relictum,* a malaria parasite of birds (Fryxell et al., 2014).

1.8.4 Control of *Culex quinquefasciatus*

Control of *Cx. quinquefasciatus* targets either the larvae or the adult. Methods for adult control include: ultra-low volume spraying (ULV), or thermal fogging, and Indoor residual spraying (IRS). An advantage of both of these methods is that they are non-specific to the target insect, so will help to reduce multiple populations of disease vectors, and they can also cover a large area in a small amount of time (Srinivasan and Kalyanasundaram, 2006, Lothrop et al., 2007). Unfortunately, *Cx. quinquefasciatus* has demonstrated widespread resistance to a range of available insecticides that hamper traditional control methods (Kumar et al., 2011, Salim-Abadi et al., 2017).

Perhaps a more effective way to control *Cx. quinquefasciatus* is to directly target the larvae. Breeding sites preferences are well known so it is relatively to identify target locations. Methods of controlling larvae come in the form of aquatic biological control agents, non-toxic essential oils, larvicides and Expanded polystyrene (EPS) beads (Shetty et al., 2013, Anogwih et al., 2015, Benelli et al., 2018).

Expanded polystyrene beads (EPS) are used to prevent the emergence of mosquito larvae from pit latrines and prevent females laying eggs on the surface of the water. In Zanzibar, the reduction in light trap catches due to EPS beads was 98.3% (Maxwell et al., 1990). In India, reduction in *Culex* collections due to use of EPS beads in latrines and larvivorous fish in wells was over 90% (Sunish et al., 2007). In urban Dar es Salaam, Tanzania, Chavasse et al. (1995) found that EPS beads (in latrines/septic tanks) and pyriproxyfen (in blocked drains, flooded land) reduced *Culex* populations by 76.7% and 46.2% in the two study areas.

1.9 Latrines and *Culex quinquefasciatus*

The species of particular interest when studying mosquitoes breeding in latrines is *Cx. quinquefasciatus* (Irving-Bell et al., 1987). It is possible that the construction of latrines as part of WASH campaigns will reduce populations of mosquitoes in a similar way to EPS used in pit latrines and contribute to the elimination of LF, as was done in many countries including China, Australia and Brazil (Bockarie et al., 2009, Rebollo and Bockarie, 2017). On the other hand, the construction of latrines could also contribute to an increase in populations of the disease vector (Hiscox et al., 2015).

It has been shown that latrines are a risk factor for the presence of *Cx. quinquefasciatus* with decreasing risk the further away a latrine was situated from the house (Kirby et al., 2008, Collinet-Adler et al., 2015). Constructing latrines in a population that does not currently have any sanitation facilities will increase the breeding sites available through the creation of faecal pits. In theory, if the

latrines are maintained so that access to the pit by mosquitoes is impossible, latrines should not pose as a risk factor. However, latrines are infrequently built to a sufficient standard and are not regularly maintained (Hiscox et al., 2015). They therefore increase risk from vectors that breed in organically rich or faecal material. Conversely, improving latrines, from pit to pour flush latrines could potentially reduce populations of mosquitoes. Pit latrines have no protection against the entry and breeding of mosquitoes unless a control method is specifically used, like EPS beads (Nathan et al., 1996). Pour flush latrines on the other hand, use a water seal that prevents entry to the pit. However, pour flush latrine also introduce more water therefore making the latrine pit a more attractive breeding site. Prevention of access is also dependent on the regular maintenance and sufficient initial construction.

1.10 Study Rationale

A large collaborative randomised control trial study involving three large institutes, (London School of Hygiene and Tropical Medicine, Xavier Institute of Management and WaterAid India) was designed to evaluate the effects of pour-flush latrines in 100 villages in Odisha State, India (Clasen et al., 2014). To complement this study, the overall aim of the present PhD was to measure the impact of latrine construction on populations of synanthropic flies and *Cx. quinquefasciatus* in a subset of rural villages in Odisha state, India. Fifty villages were kept as controls, and fifty villages were provided with pour-flush latrines. The present study collected data over a three-year period in sixty-four villages from a selection of hundred, thirty-two control and thirty-two intervention villages. The abundance and distribution of potential vectors and their capability of transmitting diseases to the population in the region was monitored.

A preliminary collection of flies was performed in a sample of villages and to assess methods for sampling fly and mosquito populations. Data collected were used to inform methods for monitoring the effect of latrines on insect populations and to determine the household variation in fly densities in order to calculate the sample size for the main study. Pilot study data were collected during the

first year of latrine construction. Due to the protracted amount of time taken for the latrines to be constructed, some primary data for comparing the effect of latrine construction took place while continued latrine construction was still ongoing.

1.11 Hypothesis and Objectives

Villages with pour flush latrines will have fewer synanthropic flies and those flies will have carry fewer diarrhoeal disease-causing bacteria than villages without latrines.

Villages with pour flush latrines will have fewer *Culex quinquefasciatus* than villages without pour flush latrines.

1. To determine the best methodology for quantifying synanthropic fly densities
2. To identify the impact pour flush latrines have on populations of synanthropic flies by comparing fly densities in intervention villages with control villages
3. To study the impact of latrines on the transmission of diarrhoea causing bacteria by synanthropic flies
4. To determine whether gravid traps or light traps are best for xenomonitoring *Cx. quinquefasciatus*.
5. To monitor the impact of latrines on population densities of *Cx. quinquefasciatus*
6. To evaluate the impact of latrine contruction on *W. bancrofti* prevalence in *Cx. quinquefasciatus*

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Chapter 2: Comparing trap designs and methods for assessing density of synanthropic flies in Odisha, India.

2.1 Abstract

There are many different traps available for studying fly populations. The aim of this study was to find the most suitable trap to collect synanthropic fly populations to assess the impact of increased latrine coverage in the state of Odisha, India.

Different baits were assessed for use in sticky pot traps (60% sucrose solution, 60 g dry sucrose, half a tomato and a non-baited control), followed by different colours of trap (blue versus yellow) and finally different types of trap (baited sticky pot trap versus sticky card traps). The experiments were undertaken in a semi-urban slum area of Bhubaneswar, the capital of Odisha. The first experiment was conducted in 16 households over 30 nights while experiments 2 and 3 were conducted in 5 households over 30 nights.

The traps predominantly caught adult *Musca domestica* and *M. sorbens* (78.4, 62.6, 83.8% combined total in experiments 1-3 respectively). Non-baited traps did not catch more flies (median 7.0, interquartile range (IQR) 0.0-24.0) compared with baited traps (sucrose solution: 6.5, 1.0-27.0; dry sucrose: 5.0, 0.5-14.5 ; tomato: 5.0, 1.5-17.5). However, there were significantly more flies collected on blue sticky pot traps, which caught nearly three times as many flies as yellow sticky pot traps (incidence Rate Ratio (IRR) =2.91; 95%CI=[1.77-4.79]; $p<0.001$). Sticky card traps (27, 8-58) collected significantly more flies than the non-baited sticky pot traps (10, 1.5-30.5).

Blue sticky card traps can be recommended for the capture of synanthropic fly species as they are non-intrusive to residents, easy to use, readily allow for species identification, and collect sufficient quantities of flies over 12 hours for use in monitoring and control programmes.

2.2 Introduction

Synanthropic (or filth) flies are commonly found in and around human dwellings (Greenberg, 1973). These non-biting flies present a public health problem through their habit of flying between faecal matter and households, facilitating the transmission of enteric diseases by regurgitation, defecation or mechanical transference (WHO and UNICEF, 2009, Greenberg, 1973). Outbreaks of diarrhoeal disease and trachoma are often closely associated with increases in fly numbers, usually during the wet season, and at times when sanitary conditions and hygiene are absent or reduced (Graczyk et al., 2005). Fly control has been found to be protective against the transmission of enteric infections (Emerson et al., 1999), but there is insufficient evidence that the reduction in fly numbers limits disease transmission.

Monitoring synanthropic fly populations can help determine whether programs that increase latrine coverage are effective. They also give an indication of the specific times when human populations might be at most risk from an increase in diarrhoeal diseases. Fly population monitoring programmes commonly use either sticky cards or baited traps, depending on the purpose and location (external or internal) for sampling populations. Most commonly, sticky cards have been used for indoor populations (Cohen, 1991, Hogsette et al., 1993) and baited traps have been utilised for outdoor populations (Pickens, 1994, Boonchu et al., 2003). Monitoring house flies, *Musca domestica,* in household kitchens of rural villages and urban slums is a particular challenge due to the variety of places where the kitchen can be located; indoors or outdoors.

Baited traps allow capture of large numbers of flies and can be classed as a control measure due to the high quantity captured. From the purposes of population density monitoring, these traps allow the identification of species, and monitoring of numbers to measure the effect of control methods on a targeted population. The disadvantage lies in the inability to catch flies individually and prevent flies from contaminating one another for the purpose of testing for bacteria transmission. Baited traps can be too expensive for daily use if needed for continual surveillance performed in multiple

villages. Furthermore, their use may be objectionable to residents; baits shown to be attractive for flies, for example, human faeces, rotting vegetation and fish for the capture of *M. domestica* (Pickens, 1995, Geden, 2005), are foul smelling to some people.

The Scudder grill is ideal for providing a measure of the relative abundance of flies in a given area (Scudder, 1947, Srinivasan et al., 2009). They can be moved to assess the concentration of flies in different locations. However, grill counts only provide a brief window to assess density of fly populations and are dependent on a variety of factors, such as: time of day, weather conditions, user ability, and position of the grill (Raybould, 1966a, Raybould, 1966b, Scudder, 1947).

Sticky cards are relatively cheap, easy to acquire, easy to transport and minimally intrusive to residents (Kaufman et al., 2005, Geden, 2005). They can be left for weeks at a time if monitoring fly numbers only, for instance on cattle farms in the United States of America (Geden, 2005, Geden, 2006). For studies monitoring bacteria in households, they can successfully collect large numbers of flies within 12 - 24 hrs although, the more flies are caught, the ability to trap more flies is reduced as the surface area diminishes (Hogsette et al., 1993). However, it is unknown to what extent the sticky glue could interfere with identification of caught species, or trap dust and other substrates that could hinder the effectiveness of trapping insects and contribute to fly bacterial contamination.

There are many methods that have been used for the capture and assessment of synanthropic flies but there is little uniformity in techniques used (Geden, 2006, Geden, 2005, Cohen, 1991, Hogsette et al., 1993, Kaufman et al., 2005). It is known that flies are sensitive to differing wavelengths of light (Hardie, 1986) and that varying colours from the spectrum may be used to improve trap catches . Although one study undertaken in the field suggested that colour did not have an effect on trap catches (Hanley, 2009), there are several studies that suggest the opposite (Hecht, 1970, Chapman et al., 1999, Geden, 2006, Diclaro et al., 2012). Hall *et al.*, 2003 showed that there can be significant variation in numbers of flies caught on different colours of traps between species as well as within species [22].

Few recent studies have explored the use of traps to assess species and bacterial carriage as well as population densities but these studies were not conducted on a large scale within houses (Talley et al., 2009, Lindsay et al., 2012a). Most experiments have either focused on the species and bacterial load or the population density and species.

The objective of the present study was to assess a variety of trapping methods to determine the best design and method for quantifying *M. domestica* densities in household kitchens in order to evaluate the impact of a sanitation randomised control trial on populations of synanthropic flies [23-24]. This was achieved by: 1) comparing different baits using a sticky pot trap design, 2) finding the best colour for a sticky card trap; blue and yellow colour sticky card traps and 3) comparing the baited pot trap collections with the non-baited sticky card trap method.

2.3 Methods

2.3.1 Study site

Sampling was undertaken in households in a semi urban slum in Bhubaneswar, Odisha, Eastern India (Latitude, longitude: 20.27°N, 85.84°E). Latrine coverage in the slum was low and there were many open defecation sites throughout the area: a report in 2008 stated that 77% of households in urban slums throughout Bhubaneswar did not have access to latrine facilities so open defecation is common (Rout, 2008). Open defecation sites were located in areas of the slum easily accessible by residents and were surrounded by houses. In addition, other sources of faeces, breeding sites for flies, are derived from large numbers of cattle, pigs and chickens that are freely wandering through the slum and surrounding area during the day but were tethered or penned close to the owner's house at night. The houses within the slums of Bhubaneswar are of mixed construction, either concrete or mud. Trapping was undertaken between July 2011 and April 2012, covering a monsoon, winter and summer season.

2.3.2 Sampling methods

There were no data relevant to the area on fly abundance and density prior to this study. The sample size required to detect a significant difference between treatment groups was, therefore, calculated based on fly counts obtained from Scudder grills placed in 10 houses over the course of three days, useful for ascertaining numbers although not for detecting differences in species. From the Scudder grill work, assuming an arithmetic mean of 63 and standard deviation (SD) of 47, the sample size was calculated using the formula by Smith, Morrow and Ross to compare the difference between two means [26]. This resulted in a minimum sample size of 57 traps nights per treatment to detect a 66% difference in effect with 80% power and a significance level of 0.05. As the sample size was based on random sampling methodology with a Scudder grill to collect the data, it was decided to increase the trap nights for each treatment group to 150 allowing for a large error margin in the Scudder grill counts when compared to the sticky card or baited pot traps.

2.3.3 Experimental Designs

Each experiment consisted of two different treatments, with the exception of the first experiment, which contained four different treatment groups. Using STATA 11 (Statacorp, USA), a random mixture of 10 households were selected to participate in the second and third experiment, with 16 being chosen for the first experiment. Different households were used for each of the three experiments. The position of traps was randomised around the houses based using a Latin square design; resulting in 4 houses per treatment per night for the first experiment and 5 houses per treatment per night for the second and third experiments. Experiments were conducted over a 30-day period resulting in 120 trap nights for the first experiment with four different treatment groups and 150 trap nights for the two subsequent experiments looking at two different treatment groups.

Households were initially mapped and then assigned a number for the purposes of identification and randomisation. Fly traps were set in the kitchen area of a house, often a courtyard area shared by

several houses, where preparation and consumption of food usually took place. Traps were set between 10:00 to 12:00 hrs and collected 24 hours later.

2.3.4 Experiment 1: determine the best bait to be used in a baited pot trap

Traps were based on a design by Bristow (Bristow, 2010). A plastic pot (top diameter 150 mm, bottom diameter 100 mm, height 70 mm) with lid (diameter 150 mm) was used to hold the bait and trap the flies. A hole was cut out of the lid (diameter 30 mm) and a circle of nylon mesh (3 mm gauge) attached to the inside of the lid to prevent flies from accessing the bait (Figure 2.1 A). Yellow sticky card (Product code 10271, Suterra Ltd, UK) was cut to the size and shape of the lid. The card was sticky on both sides; one side was used to attach the card to the lid and one side was used to trap flies (Figure 2.1 B).

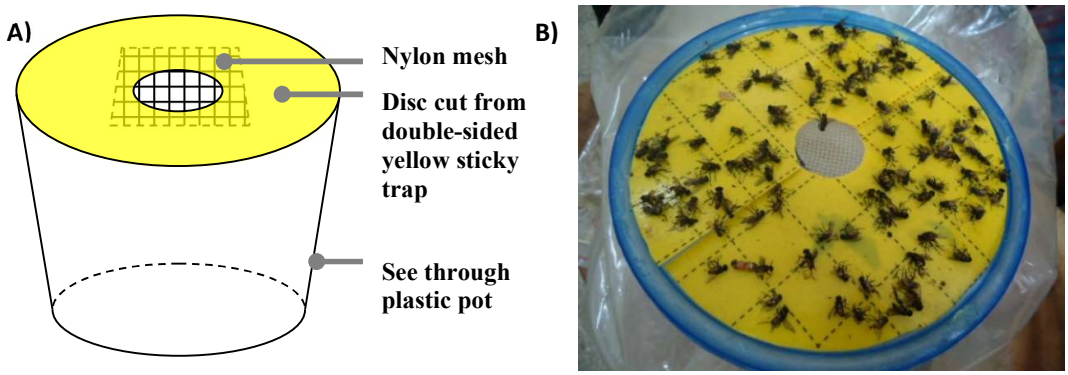

Figure 2.1. A) Schematic diagram of the pot trap design and B) a top-down picture of the pot trap in use. Diagram courtesy of Julie Bristow.

Baits were selected based on previous research advocating different types of fruit and vegetables, and sugars that are needed by *M. domestica* for survival and are readily available (Pickens, 1995, Pickens, 1994, Chapman et al., 1998, Hogsette et al., 2002). The three baits used in the experiment were: 1) no bait (control), 2) sugar water (60% solution: 60 g of locally available sucrose dissolved in 100 ml tap water), 3) sugar (60 g of dry sucrose) and 4) half a tomato (Figure 2.2 A). The baits were prepared before use and changed daily. The experiment was conducted in the monsoon season, July to August 2011.

2.3.5 Experiment 2: determine the best colour to use in a sticky card trap

Two colours of sticky card, yellow and blue (Product codes 10271 and 10303, Suterra Ltd, UK) were used as a non-baited fly trap. According to the manufacturers, in all aspects e.g. material type/thickness, dimensions and catch glue, the traps were the same, the only change between the yellow and blue sticky traps was the colour of the base material (Figure 2.2 B). The sticky card was placed on pot traps as in the first experiment. The sticky traps were changed daily and flies counted. The experiment was conducted in the dry winter season, November to December 2011.

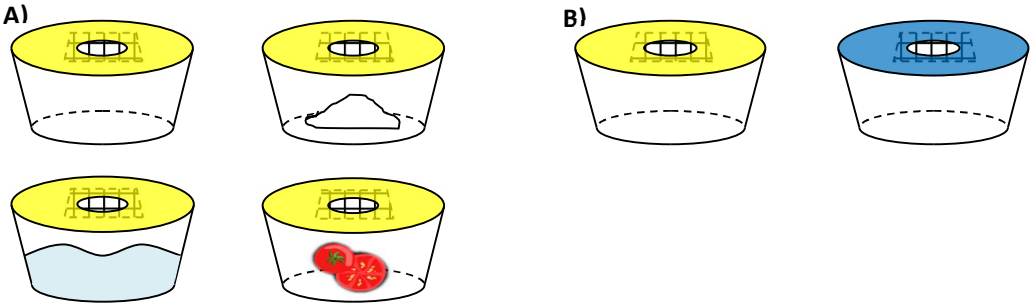

Figure 2.2. A) Schematic diagram of the 4 baited sticky pot traps, showing baits used in the first experiment, clockwise from the top left: control without bait, sugar, half a tomato and sugar water and B) schematic diagram showing the different colours, yellow and blue used in the second experiment.

2.3.6 Experiment 3: determine the best trap to use, either sticky card traps or baited pot traps

Baited sticky pot traps, using sucrose solution and blue sticky card, were compared with non-baited blue sticky card traps, each measuring 200 mm x 245 mm. The baited sticky pot traps were placed on the floor so the horizontal sticky surface was 7 cm from the floor, and the sticky card traps were supported at a 45° angle, using a stick, on the floor with both sticky surfaces exposed to enable capture of flies flying at heights of up to 23 cm from the floor (Figure 2.3 A and 2.3 B). The experiment was conducted over the dry summer season, March to April 2012.

A)  B) 

Figure 2.3. A) A picture of the sticky card trap in location in a sheltered kitchen area and B) a picture after trap collection showing the synanthropic flies caught.

2.3.7 Analysis

Flies were carefully removed from the traps using sterilised forceps, then counted and identified to species using the *Fauna of British India* series keys: Diptera volume 6: Muscidae, 7: Calliphoridae and 10: Sarcophagidae (Aubertin and Smart, 1940, Van Emden, 1965, Nandi, 2002). Fly densities were analysed using STATA 11 (Statacorp, USA). Data were tested for normality and, if necessary, log transformed. Data that were skewed, despite log transformation, were analysed using a negative binomial regression model. The data were over-dispersed and so the untransformed data was analysed using a negative binomial regression model. Total synanthropic fly densities and differences between the main synanthropic species caught, *M. domestica* and *M. sorbens*, also were analysed.

2.3.8 Ethics approval and consent to participate

The study was approved by the ethics committees of Xavier Institute of Management, Bhubaneswar (ref no. 31052010) and the London School of Hygiene and Tropical Medicine (ref no. 5562). Each resident was approached, given a detailed verbal overview of the project in addition to an information sheet, and asked if they would like to participate in the study. Household consent was obtained before any work was undertaken.

2.4 Results

2.4.1 Experiment 1: baited pot traps

In total, 1882 flies were captured including 884 *M. domestica* (46.9% of total catch) and 594 *M. sorbens* (31.5% of total catch). No other synanthropic fly species of public health importance was captured during the course of the experiment. However, 34 mosquitoes were collected of which 10 were identified as *Culex quinquefasciatus* and 7 were *Mansonia annulifera.* The remaining 17 could only be identified to the family Culicidae due to glue covering distinguishing marks making further identification impossible. The traps also captured 5 Phlebotominae (Psychodidae). Remains of flies (178 in total) that lacked an abdomen and/or thorax but obviously comprised of at least a pair of wings, head and/or legs were counted towards the final total. It was not possible to identify 27 flies to species, although sufficient characteristics were available to identify the flies as belonging to the family Muscidae. These also were included in the final analysis.

The final analysis included 1478 synanthropic flies and fly remains. The median number of flies collected per trap/night for each treatment group is shown in table 2.1. None of the baits used in the experiment caught significantly more flies than the control trap without any bait. Neither were there significant differences between the various baits (sucrose solution vs. dry sucrose; IRR=0.65; 95%CI=[0.26-1.59]; p=0.341, sucrose solution vs. tomato; IRR=0.59; 95%CI=[0.24-1.44]; p=0.245, dry sucrose vs. tomato; IRR=0.91; 95%CI=[0.37-2.23]; p=0.833) (Figure 2.4 A).

When the primary synanthropic species captured were analysed; *M. domestica* were not caught more frequently on the baited sticky pot traps when compared with the control trap (Table 2.1). The trap containing 60% sucrose solution caught 27% fewer flies than the control trap and the trap containing dry sucrose caught 33% fewer flies. There were 64% fewer flies captured on the traps containing tomato when compared with the control traps (control vs. tomato; IRR=0.36; 95%CI=[0.14-0.93]; p=0.034). The results for *M. sorbens* were similar to those of *M. domestica*. Baited traps caught similar numbers of *M. sorbens* as the control traps; all of them caught fewer

flies, with the exception of 60% sucrose solution baited trap, although this was not significant (control vs. sucrose solution; IRR=2.18; 95%CI=[0.77-6.15]; p=0.143) (Figure 2.4 B). When analysed to see whether there were any sex-specific differences between the numbers of flies caught between different traps, the only significant differences detected were between control and tomato traps, where significantly fewer flies were captured for both sexes when analysed separately (*M. domestica* male; control vs. tomato; IRR=0.37, 95%CI=[1.48-0.95]; p=0.038, *M. domestica* female; control vs. tomato; IRR=0.32; 95%CI=[0.11-0.96]; p=0.043).

Table 2.1. Comparison of synanthropic flies, *Musca domestica* and *Musca sorbens* collected from baited pot traps.

	N	***Synanthropic flies (Median, IQR)***	***Difference (IRR)***	***95%CI***	***P-value***
Control – non-baited pot trap	428	7.0 (0.0-24.0)	Ref.		
Sucrose solution	470	6.5 (1.0-27.0)	1.10	[0.45-2.69]	0.838
Dry sucrose	304	5.0 (0.5-14.5)	0.71	[0.29-1.74]	0.455
Tomato	276	5.0 (1.5-17.5)	0.64	[0.26-1.58]	0.338
M. domestica					
Control – non-baited pot trap	320	3.5 (0.0-18.5)	Ref.		
Sucrose solution	235	4.5 (0.5-12.5)	0.73	[0.29-1.88]	0.519
Dry sucrose	214	3.0 (0.0-9.0)	0.67	[0.26-1.71]	0.401
Tomato	115	2.0 (0.5-8.5)	0.36	[0.14-0.93]	0.034
M. sorbens					
Control – non-baited pot trap	108	0.5 (0.0-5.5)	Ref.		
Sucrose solution	235	2.0 (0.0-14.0)	2.18	[0.77-6.15]	0.143
Dry sucrose	90	1.0 (0.0-5.5)	0.83	[0.29-2.39]	0.734
Tomato	161	1.5 (0.5-4.5)	1.49	[0.53-4.23]	0.453

2.4.2 Experiment 2: yellow vs. blue as an attractive colour

A total of 2105 flies were caught of which 356 (16.9%) were *M. domestica* and 963 (45.7%) were *M. sorbens*. Nearly twice as many males (64% of the total collection) as females (36% of the total collection) *M. sorbens* were caught. Similarly, more than twice as many *M. domestica* males (68%) were caught when compared with females (32%). Other synanthropic flies caught included 92 *Musca pattoni*, 1 *Chrysomya megacephala* and 6 Sarcophagidae. Other fly species captured were 140 mosquitoes, predominantly *Culex quinquefasciatus* (128) but also *Armigeres kuchingensis* (11) and *Aedes albopictus* (1), and 2 Phlebotominae. The remains (wings, head, and legs) of 278 flies were found and identified to Muscidae family. Due to the lack of other distinguishing characteristics, it was not possible to identify to genus or species. These were included in the final analysis. It was not possible to identify 201 flies to species belonging to the family Muscidae due to glue obscuring distinguishing marks, although it was possible to identify the sex. These also were included in the final analysis.

In total, 1890 synanthropic flies were captured over 150 trap nights. The total number of synanthropic flies caught on the yellow traps was 483 (Median, IQR) (0, 0-2) and on the blue traps 1414 (1, 0-11). Blue traps caught almost three times as many flies as the yellow traps (IRR=2.91; 95%CI=[1.77-4.79]; p<0.001) (Figure 2.4 C).

Musca domestica and *M. sorbens* were the dominant synanthropic species caught, comprising 99% of the collection. When these species were analysed by trap, 3.26 more *M. domestica* were caught on the blue traps compared with the yellow traps (Table 2.2). A similar difference was seen when comparing *M. sorbens* on blue traps and yellow traps (Table 2.2). For both species, males and females were caught more frequently on blue traps compared with yellow traps (Table 2.2 and Figure 2.4 D).

Table 2.2. Comparison of male and female *Musca domestica* and *Musca sorbens* collected from yellow and blue sticky traps.

	Total	***Total no. (Median, IQR)***	***Difference (IRR)***	***95%CI***	***P-value***
M. domestica					
Yellow trap	94	0 (0-1)	Ref.		
Blue trap	262	0 (0-2)	2.79	[1.62-4.80]	<0.001
M. domestica male					
Yellow trap	57	0 (0-0)	Ref.		
Blue trap	186	0 (0-1)	3.26	[1.78-5.97]	<0.001
M. domestica female					
Yellow trap	37	0 (0-0)	Ref.		
Blue trap	76	0 (0-1)	2.05	[1.11-3.79]	0.021
M. sorbens					
Yellow trap	233	0 (0-1)	Ref.		
Blue trap	730	0 (0-4)	3.13	[1.70-5.78]	<0.001
M. sorbens male					
Yellow trap	148	0 (0-0)	Ref.		
Blue trap	467	0 (0-3)	3.16	[1.69-5.89]	<0.001
M. sorbens female					
Yellow trap	85	0 (0-0)	Ref.		
Blue trap	263	0 (0-1)	3.09	[1.55-6.19]	<0.001

2.4.3 Experiment 3: sticky card traps vs. sucrose baited pot traps

A total of 12227 flies were caught of which 9161 were *M. domestica* (74.9%) and 1100 were *M. sorbens* (8.9%). Three times as many males as females were caught of both species: for *M. domestica,* 77% were male and 23% were female, and for *M. sorbens*, 75% were male and 25% were female. Other synanthropic flies caught included 1 *Chrysomya megacephala,* 7 *Stomoxys calcitrans* and 2 Sarcophagidae. Other species of interest captured were 79 Phlebotominae and 44 *Culex quinquefasciatus*. It was possible to identify all flies to family using fly remains (wings, head, and legs) of 887 flies, and these belonged to the family Muscidae but, without any other identifiable

characteristics, it was not possible to determine their species. All flies were included in the final count.

In total, 11158 synanthropic flies were captured, over 150 trap nights. Baited pot traps caught a median of 10 synanthropic flies (IQR=[1.5-30.5]) and sticky traps a median of 27 synanthropic flies (IQR=[8-58]). Sticky traps caught more than twice the number of synanthropic flies than baited sticky pot traps (IRR=2.16; 95%CI=[1.59-2.93]; $p<0.001$) (Figure 2.4 E).

Twice as many *M. domestica,* were caught on the sticky trap than were caught on the baited pot trap. The results were similar when the traps were analysed for *M. sorbens* only. Baited pot traps caught more *M. sorbens* than sticky traps. For both *M. domestica* and *M. sorbens,* twice the number of males and females were caught on the sticky traps as opposed to the baited pot traps

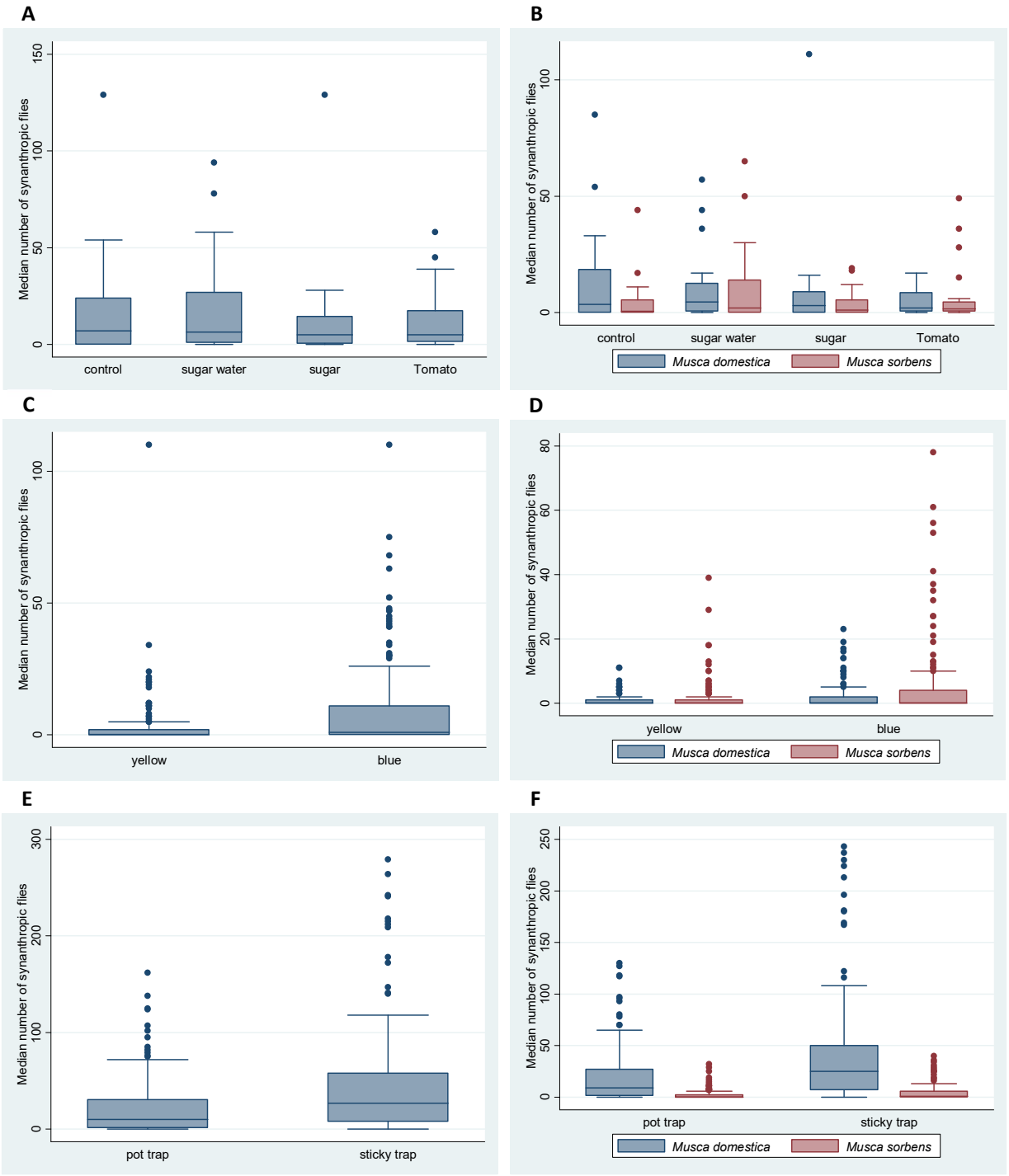

Figure 2.4. **Comparison of traps in experiments designed to find the highest capture rates for synanthropic flies.** Median number and interquartile ranges, with outliers, of synanthropic flies, *M. domestica* and *M. sorbens* (**A**) Synanthropic flies captured from baited traps compared with control traps (480 trap nights), (**B**) *M. domestica* and *M. sorbens* captured from the baited experiment (480 trap nights), (**C**) Synanthropic flies captured from yellow and blue sticky traps (150 trap nights), (**D**) *M. domestica* and *M. sorbens* captured from yellow and blue sticky traps (150 trap nights), (**E**) Synanthropic flies captured from pot and sticky traps (150 trap nights) and (**F**) *M. domestica* and *M. sorbens* captured from pot and sticky traps (150 trap nights).

Table 2.3. Comparison of male and female *Musca domestica* and *Musca sorbens* from pot and sticky traps.

	Total	***Total no. (Median, IQR)***	***Difference (IRR)***	***95%CI***	***P-value***
M. domestica					
Pot trap	2753	9 (1.5-27)	Ref.		
Sticky trap	6408	25 (7-50)	2.19	[1.62-2.96]	<0.001
M. domestica male					
Pot trap	2101	7 (1-18)	Ref.		
Sticky trap	4936	19 (6-39)	2.21	[1.64-2.98]	<0.001
M. domestica female					
Pot trap	652	1 (0-4)	Ref.		
Sticky trap	1472	5 (1-15)	2.12	[1.49-3.02]	<0.001
M. sorbens					
Pot trap	362	0 (0-2.5)	Ref.		
Sticky trap	738	1 (0-6)	1.92	[1.24-2.97]	0.003
M. sorbens male					
Pot trap	263	0 (0-2)	Ref.		
Sticky trap	555	1 (0-5)	1.99	[1.27-3.10]	0.003
M. sorbens female					
Pot trap	99	0 (0-0)	Ref.		
Sticky trap	183	0 (0-1)	1.74	[1.02-2.98]	0.043

2.5 Discussion

The study helped identify a suitable trap for collecting Muscid flies in India. The best design for trapping synanthropic flies of interest in the transmission of diarrhoeal diseases was a non-baited, blue coloured sticky trap.

The numbers of flies collected during the present experiments were lower than previous studies conducted in animal farms in the USA (Gerry et al., 2011, Geden, 2006, Geden, 2005) but comparable to similar field studies conducted in small rural villages in Africa and Asia (Lindsay et al., 2012b, Nurita et al., 2008). Unlike experiments conducted on farms in the USA, where the only breeding, resting, mating sites and source of food is the farm; in Asia and Africa there are many

alternative sites that can compete with the baited and sticky traps, such as open defecation sites and rubbish deposits, which may reduce the numbers of flies caught. The unexpected low numbers of flies caught as part of the experiment comparing baits could have contributed towards the lack of difference in fly numbers caught on the baited traps when compared with the non-baited control trap.

There were restrictions regarding the types of baits that could be used for experimentation. For example, although flies are known to be attracted to human faeces (Lindsay et al., 2012a), it could not be used as an attractant in the baits for cultural sensitivity. Alternative baits, including different meats, fish and chemical attractants (Amin et al., 1998, Boonchu et al., 2003) were unsuitable for the following reasons: (1) meat, while suitable for smaller studies, would have been difficult to access in high quantities for a larger trial; (2) fish, while usually available in large quantities, was variable in supply throughout different times of the year; (3) although commercially produced chemical baits containing imidacloprid or spinosad have been shown to be effective (Kaufman et al., 2010, White et al., 2007, Butler et al., 2007), the cost associated with buying these and shipping them to India was prohibitive. The lack of any overwhelming stimulus that would attract flies to the trap, distinguishing the traps from alternative local sources and could further explain why there were low numbers of flies caught. In a study by Geden *et al*. 2006, it was recorded that strong olfactory cues often overwhelmed any visual stimuli (Geden, 2006). In the case of the slum where there were many attractive odours to filth flies present, it is possible that the individual baits were not competitive enough and the sticky trap provided a convenient resting place, despite not having olfactory cues.

During the second experiment comparing different colours of sticky card on a non-baited pot trap, a much higher proportion of *M. sorbens* were captured (46%) when compared with the first (32%) and third experiment (9%). Despite the lack of bait being the key difference between the first and second experiment, it is unlikely that the increase *M. sorbens* captured were due to the lack of baits, suggesting a possible repellent effect of the baits used. The reason for the increased numbers caught

is unknown but probably due to the differences between the houses used to trap the flies, rather than the trap design. When the numbers of flies caught, were disaggregated by house, some houses caught substantially more flies than others. This suggests that factors external to the trap in some houses contribute to the increase in fly numbers caught, presence of rubbish or open defecation sites close to the house for example.

Studies that have used some form of coloured trap for capturing synanthropic flies have used either blue (Geden, 2006, Hanley, 2009, Khan et al., 2013) or yellow (Pickens, 1994, Emerson et al., 2005, Gerry et al., 2011) as the attractive colour. It is known that muscid flies are visually sensitive to wavelengths: 1) 490 nm (blue) 2) 570 nm (yellow) and 3) 330-350 nm (ultraviolet) (Hardie, 1986). Other species of flies including Calliphoridae, have shown an attraction for wavelengths of darker colours (blue, black, etc.), such as tsetse flies to black and blue targets (Lindh JM, 2009) and stable flies to blue traps (Geden, 2006). It is possible that the darker blue provides a stronger contrast to the surrounding environment and vegetation than the yellow traps (Hecht, 1970, Howard and Wall, 1998). There was a significant difference in the numbers of muscid flies caught on the blue trap as opposed to the yellow traps, mirroring results seen in an experiment by Dicarlo *et al.,* 2012 (Diclaro et al., 2012). They found that blue traps were attractive to *Musca domestica*, yellow traps were repellent, and blue traps with black lines increased attractiveness.

A previous study had shown that sticky cards are able to capture larger flies than muscids such as Calliphorids (Sulston et al., 2014), and that no flies managed to escape the trap once they had landed on the glued surface. However, personal observation and comments by other researchers, have suggested that the glue on sticky traps is not sufficient and that larger flies can escape even if they land directly on the glue. Despite this, few Calliphorids were observed around houses and the absence of any caught on the traps in the present study is probably due to the lack of those flies around kitchens and households in this area of India in contrast to studies conducted elsewhere in Asia and Africa (Lindsay et al., 2012a, Nurita et al., 2008, Sukontason et al., 2007).

Other factors that affected the trap catches included heavy rain. During the second experiment comparing colours of sticky trap, traps placed in outdoor kitchens on days with heavy rain, became soaked and, while the glue was still sticky underneath the water, droplets would form, obscuring the surface. Therefore, although rainfall has been correlated with an increase in fly populations (Srinivasan et al., 2009, Murvosh and Thaggard, 1966, Ngoen-klan et al., 2011), it is possible that trapping success may be reduced during heavy periods of rainfall using sticky traps, if traps are exposed. During the dry season, both summer and winter, dust was present in the kitchens of households that were swept daily. This could result in the partial obscuring of the trap surface reduction in stickiness, and therefore limit the potential number of flies caught.

During the final experiment, comparing a non-baited sticky pot trap with a sticky trap a much larger number of flies were caught. Almost five times as many flies were caught during this experiment than the previous two experiments. Considering that all three experiments used the same trapping methods, it is unlikely that the traps themselves were the cause of the surge in numbers caught. One main difference is in the season collected; the final experiment was conducted in the dry summer season just before the advent of the monsoon season. During the monsoon season, collections were possibly not as high as could be expected due to heavy rainfall obscuring the trap. The cooler season could be less conducive to the development of young larvae. In comparison, during the hot season, there is nothing to inhibit or slow population growth, resulting in higher numbers of flies captured.

Throughout each experiment, it was observed that at least double the number of males than females were caught. It is generally assumed that the male: female ratio at emergence is 1:1 (Izumi et al., 2008). This ratio can be altered by chemicals, factors affecting female survival rates, the position of the trap or the baits used in the trap. This is not the first study to record a bias toward males over females (Gerry et al., 2011, Geden, 2005) but it is uncommon, as the majority of studies see the reverse (Akbarzadeh et al., 2012, Emerson et al., 2005, Ngoen-klan et al., 2011). The most likely explanation is the location of the trap was responsible for catching more males than females.

The traps were placed inside or adjacent to houses and away from primary breeding sites, such as open defecation areas, animals, abundant oviposition sites. Despite this, the ability to transmit diarrhoeal diseases is not limited to the female alone, unlike other medically important arthropods. Both males and females visit areas where diarrhoeal disease causing bacteria like *Escherichia coli* and *Salmonella* could adhere to the external surface of the fly, to be dislodged when next visiting a human food source (Graczyk, 2001).

2.6 Conclusions

The non-baited blue sticky card trap collected more flies than the yellow baited sticky pot traps or yellow non-baited sticky card traps. The primary synanthropic fly collected during the third experiment was *M. domestica* and it was captured in greater numbers on the blue sticky card trap compared with the yellow sticky card trap. *Musca sorbens* were collected in high numbers throughout the experiment. While the sticky trap collected larger fly numbers than any other trapping method, there were limitations. The placement of the traps could potentially select for some species over others in comparison with a sweep net method of capture, which can indiscriminately collect species at a variety of locations at differing time points, with the drawback being labour intensive. In field sites such as Odisha, where baited traps might be competing with equally or more attractive odours, non-baited blue sticky card traps provide a strong visual stimulus to induce landing and are a simple way to collect large numbers of synanthropic flies of interest as they are easy to place and less intrusive to the residents than baited traps. Despite the potential limitation of positional bias, these traps could be useful in the context of large monitoring programs to assess fly densities. If combined with an odour attractant that could compete with local odours, this trap could be used as a method of direct fly control using an attract and trap/kill technique, due to the large numbers of flies captured.

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SECTION A – Student Details

Student	Melissa Joanne Bell
Principal Supervisor	Mary Cameron
Thesis Title	The impact of latrine construction on densities and pathogen infection rates of syanthropic flies and Culex quinquefasciatus mosquitoes in Odisha, India

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?			
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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	PNAS
Please list the paper's authors in the intended authorship order:	Melissa Bell, Thomas Clasen, Sophie Boisson, Parimita Routray, Soumya Nayak, Wolf-Peter Schmidt, Belen Torondel, Seth Irish, Soosai Peppin, Mrutyunjay Suar and Mary Cameron
Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I made major constributions to the inception, design, field work, analysis and write-up of the experiments

Student Signature: [redacted] Date: 20/8/18

Supervisor Signature: [redacted] Date: 20/8/18

Chapter 3: Effect of Latrine Coverage on fly densities and pathogenic bacterial loads in rural Odisha, India: a cluster randomised controlled study

3.1 Abstract

In 2015, India accounted for approximately 21% of the global burden of diarrhoea, a major killer of children under the age of 5 years old. Synanthropic flies are potential vectors of faecal pathogens, and fly control has been shown to reduce diarrhoea. We undertook this study to assess whether pour flush latrines, built as part of a scale up in sanitation coverage by the Total Sanitation Campaign of India, were effective in reducing fly populations and carriage of pathogenic faecal bacteria. Using sticky traps to capture synanthropic flies, population densities were monitored in a random sample of 9 houses in each of 32 intervention and 32 control villages. Population densities were compared between intervention and control villages using sticky traps. A subsample of captured flies (1331) were cultured for the presence of four pathogenic bacterial species (*Escherichia coli*, *Salmonella* sp., *Vibrio cholerae* and *Shigella* spp). The bacteria cultured, and virotypes of bacteria with various toxigenic strains, were confirmed by PCR. Overall, *Musca domestica* (58%) and *M. sorbens* (41%) were the most abundant species found in food preparation areas; 62% were positive for one or more of the bacterial species of interest. There was no statistical difference in the population density of synanthropic flies between control and intervention villages (IRR=0.89; 95%CI=[0.76-1.03]; p=0.131). Neither was there a difference in the carriage of bacteria: *E.coli* O157: control (45%), intervention (44%) (OR=0.94; 95%CI=[0.76-1.17]; p=0.582); *Salmonella* spp.: control (19%), intervention (17%) (OR=0.88; 95%CI=[0.67-1.17]; p=0.382); *Shigella* spp.: control (8%), intervention (7%) (OR=0.87; 95%CI=[0.58-1.31]; p=0.509); and *V. cholerae*: control (25%), Intervention (21%) (OR=0.80; 95%CI=[0.62-1.04]; p=0.090). The construction of pour flush latrines did not result in a decrease in the numbers of synanthropic flies or a difference in the carriage of pathogenic bacteria, in a setting where 63% used latrines.

3.2 Introduction

In the 15 years (2000 to 2015) of working on the millennium development goal for improving access to sanitation and drinking water, India has achieved a 31% reduction in the proportion of the population practicing open defecation (UNICEF, 2015). Despite this, access to improved sanitary facilities has changed very little since 1990, especially in rural areas, and more than half of India's population still practise open defecation (UNICEF, 2008, UNICEF, 2015). A series of campaigns launched by the Indian government to improve the situation has increased the quantity of households with access to latrines (WHO, 2010, JMP, 2012, UNICEF, 2015). From 1990 until 2010 the coverage of latrines in India almost doubled (from 18% to 31%), but there was still a long way to go before over 50% of the population had access to latrines (WHO, 2010). The Total Sanitation Campaign (TSC) (subsequently renamed the Nirmal Bharat Abhiyan) and later the Swachh Bharat Mission (SBM) have largely been responsible, in the last decade, for the construction of pour flush latrines (Jha, 2003) and subsidies to build improved sanitary facilities in households below the poverty line (BPL) (Hueso, 2013). In 2004 in Odisha, less than 10% of the population had access to sanitation facilities despite most of Odisha being BPL and eligible for government subsidiaries to fund construction (Pattanayak et al., 2009).

In India, diarrhoeal diseases cause a mortality rate of 84.2/100,000 in children under five years old (GBD, 2017). Combined with Nigeria, India shares 42% of the global burden of diarrhoeal diseases in children under the age of five years old. The leading cause of diarrhoeal diseases within India is shigellosis (11.1%) (Purwar et al., 2016). Overall, pathogenic bacterial infections account for 65.7% of diarrhoeal cases, followed by viral infections (22%), parasitic infections (16.3%) and finally *Candida* spp. (5.6%) (Purwar et al., 2016). In Odisha, 762 out of every 100,000 people suffer from diarrhoea per year (Division, 2014). The National and Family Health Survey (NFHS) conducted in 2005-6 indicated that 28% of children under 5 years in Odisha had diarrhoea in the preceding two weeks, among which 4.5% suffered from bloody diarrhoea (NFHS-3, 2005). Despite a significant reduction in

burden, 14% of children still suffered from diarrhoeal diseases in 2013 (Division, 2014) and this had reduced to 10% in the 2 weeks before the most recent NFHS in 2015-6 (NFHS-4, 2015). The bacteria mainly responsible for diarrhoeal cases admitted to hospitals in Odisha are pathogenic *Escherichia coli* (75.5%), *Vibrio cholerae* (17.3%), *Shigella* spp. (4.5%) and *Salmonella* spp. (0.7%) (Samal, 2008).

Non-biting synanthropic flies are of public health importance due to their habit of flying between faecal matter and households thereby transmitting pathogenic bacterial fauna and enteric pathogens by regurgitation, defecation or mechanical transmission (Greenberg, 1973). Studies have shown that certain pathogenic bacteria, *E. coli* and *Salmonella* for instance, can be carried on the exoskeleton of the fly and remain viable for up to several hours (Khin Nwe et al., 1989, Nazni et al., 2005, Sukontason et al., 2007). Similarly, the crop and gut of the fly can be ideal environments for the maintenance of bacteria ingested during feeding. These pathogenic bacteria can then be transferred through the deposition of vomit drops, a mixture of enzymes to aid in food digestion, during the next meal or faecal spots. Through vomit drops and carrying on the exoskeleton, both the inside and the outside of the fly are important considerations in the study of pathogenic bacterial transmission.

Outbreaks of diarrhoeal disease are often closely associated with increases in fly numbers, usually during the wet season, and at times when sanitary conditions and hygiene are absent or reduced (Graczyk et al., 2005). Transmission of pathogenic bacteria by flies has been shown to be a major contributor in maintaining diarrhoeal disease in Israel (Cohen, 1991), and interventions designed specifically to control flies (e.g, fly traps) have been shown effective in reducing diarrhoea (Levine, 1991, Chavasse et al., 1999, Emerson et al., 1999). To date, however, there are no reports of experimental studies to assess the impact of sanitation interventions to prevent diarrhoea by reducing fly density and their transmission of pathogens. It has been proposed that a standard pour-flush latrine, if well-constructed and sufficiently maintained, may lead to a decrease in fly populations or their vectoring patterns through safe disposal of faeces and reducing open defecation

practices (Clasen et al., 2012). On the other hand, poor sanitation may aggravate exposure to faecal pathogens by increasing breeding sites (if the latrines are not well constructed) and by bringing them closer to living areas than more distant open defecation sites. (Fletcher et al., 1956, Curtis, 1980, Curtis and Hawkins, 1982, Emerson et al., 2005).

This study aimed to determine the exposure of households in rural India to populations of flies, to assess the carriage of pathogenic faecal bacteria and the potential for mechanical transmission from vector to host. It was conducted during a large cluster randomised control (cRCT) trial examining the effects of increased, improved sanitation coverage (Clasen et al., 2014).

3.3 Methods

3.3.1 Study site

The vector surveillance was undertaken as part of a large cRCT. The design, study setting, participants, intervention and measurement of health outcomes have been described elsewhere (Clasen et al., 2012). Briefly, the cRCT aimed to assess the impact of the Government of India's TSC program to promote construction of latrines in rural villages among all below poverty level households. The TSCs directive was to increase coverage of latrine within villages to above 70% (WSP, 2011). The study compromised 100 villages, 50 randomly allocated to intervention (latrines constructed) and 50 to control in a parallel study design.

The study was carried out in Puri district in Odisha, India from January 2012 – October 2013 (Figure 3.1). The Vector surveillance study was carried out in a subset of 32 control and 32 intervention villages of the cRCT. The estimated sanitation coverage prior to the start of the intervention was below 10% with over 90% of households practicing open defecation (DLHFS-3, 2008, India, 2011). Over 50% of the population at the time of study were classified as living below the poverty line (DLHFS-3, 2008).

Most of the houses in the village were compound houses. This frequently consists of a block of houses, in square, situated around an open, uncovered courtyard in the centre. Often, latrines and water sources are shared between the houses that comprise the compound. Agriculture was the main source of income for villagers, while most households owned livestock, with 66% of households owning at least one cow (Schmidt et al., 2016). Sheep, goats and buffalo also were found frequently in villages (Figure 3.2).

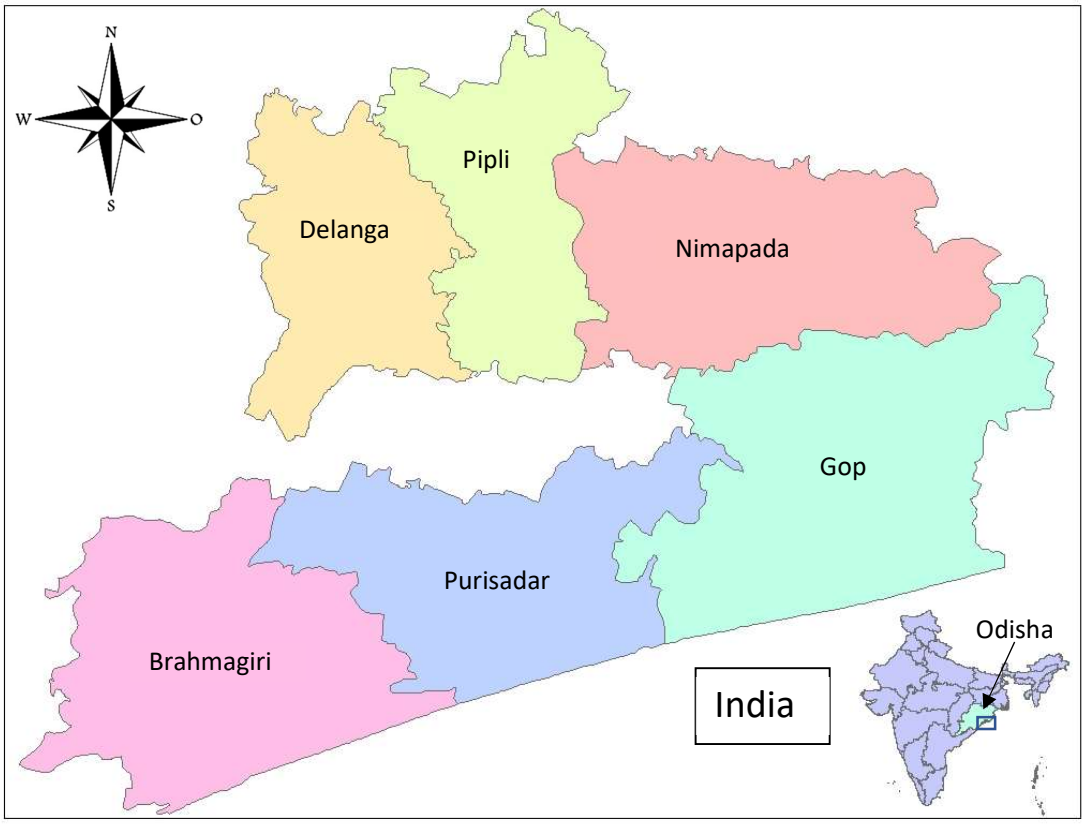

Figure 3.1. Showing the 7 blocks of Puri district where the study villages were located.

There are three seasons in the study area: the wet or monsoon season running from July to September, characterised by frequent heavy rainfall and average daily temperatures of 29°C; a cooler dry season from October to February with average daily temperatures of 24°C but occasionally dropping down to lows of around 10°C for short periods of time in December and a hot dry season from March to June with daily temperatures averaging 32°C, including frequent peaks where the temperature was around 45°C (WorldWeatherOnline.com).

Figure 3.2. An example of the study villages and latrines.

3.3.2 Sample size

Preliminary fly trapping, using sticky card traps, between April 2011 and May 2011 conducted in Puri district caught an average of 46.7 flies (Standard deviation (SD) = 58) over a 24-hour period (Chapter 2). Previous trials investigating the impact of interventions in different settings on fly populations have shown a reduction ranging from 20 to 80% (Cohen, 1991, Chavasse et al., 1999). In order to perform a sample size calculation, a conservative estimate of 30% reduction was deemed appropriate for a large study area using a sanitation intervention, considering estimates provided in a previous study (Emerson et al., 2004). Assuming an intra-class correlation (ICC) of 0.44, 80% power and 5% level of significance, a total sample size of 534 (267 houses sampled in the intervention and 267 in the control arm) would have power to detect a 30% difference. Entomological monitoring took place in a randomly selected subset of 64 villages; 32 intervention and 32 control. Based on the sample size of 534, eight households per village would need to be sampled. In order to build tolerance into the design for potential trap damage or loss to follow up, it was decided to increase the number of households sampled by 1 per village resulting in 9 households per village sampled for a total of 576 houses overall and 288 per arm (Figure 3.3). Each house was sampled for three consecutive nights to estimate average household populations, resulting in 1728 trap nights over the course of the study.

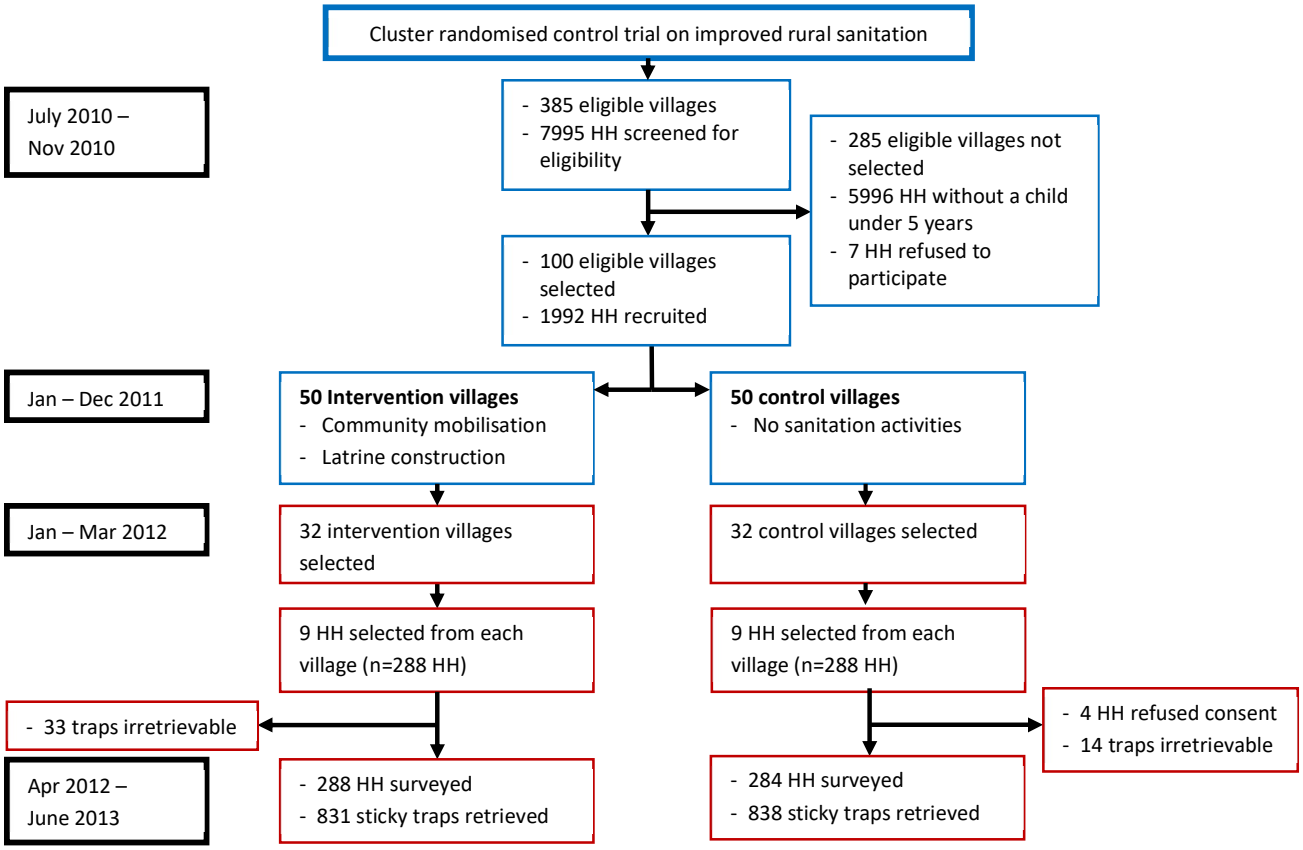

Figure 3.3. Trial profile with the sections highlighted in blue; the design of the overall cRCT and the sections highlighted in red; the design of the flies and bacteria study.

3.3.3 Randomisation

Villages were selected at random from the main study pool of 100 villages using a random number generator in Stata 11 (stataCorp, USA). Distance between the villages ranged from a minimum of 1km up to 40km. While *M. domestica* has been tracked flying up to 20 miles, this is considered exceptional and the accepted average is approximately 1-2 km. Households were randomly selected from the pool of eligible houses recruited by the cRCT; children <5 years permanently resident in the house and houses a minimum distance of 10m apart to reduce interference of trap catches between neighbouring households. In the case that a house that was randomly selected belonged to a compound house, all other houses in that compound were removed from the randomisation selection process. Shared facilities that were used by the all members of the compound houses were used as indicators of presence of latrine and water source, if each house did not separately own

individual facilities. An information sheet in the local language, Oriya, giving a detailed overview of the study, was provided to every household and written consent was procured prior to fly collection.

3.3.4 Fly collection

3.3.4.1 Sticky traps

Sticky traps, comprised of a 200 x 245 mm blue card, coloured to attract synanthropic flies (Chapter 2), were placed in the randomly selected households. The surface of both sides of the card was coated in a 'dry touch, no mess insect glue' (product code 10303, Suterra ltd, UK). The sticky traps were protected by a film of plastic to prevent adherence to the trap before placement. This was removed prior to use but retained to cover the traps when they were collected to prevent additional flies from being caught and increased exposure to environmental cross contamination of pathogenic bacteria on movement after collection. Traps were placed on the floor at an approximate 45° angle, supported by an available piece of wood or branch, within the kitchen or cooking area of the household, a minimum of 0.5m away from an open source of flame, between 09:00 and 11:00 and collected 24 h later. In most households, traps were placed in a position to collect flies that might be involved in contamination of food while not interfering with the daily activities of the household. Traps were preferentially placed in the kitchen or food preparation areas. Climatological data; such as precipitation (mm), humidity and average temperature (°C high and low) also were recorded for the duration of the trapping rounds using the India meterological department website based on readings taken from the closest (straightline) meteorological station to the village where trapping was taking place; either Bhubaneswar or Puri (IMD, 2010).

3.3.4.2 Sweep Net

A sample of flies was collected from 5 randomly selected households per village, between 08:00 to 11:00 h, on the last day of collection in each village, using a sweep net. Sweep net catches were conducted in areas of the house and surrounding area, particularly from around the latrine and open defecation sites if in proximity to the house. The sweep net comprised of woven chiffon, 70 x 70

mesh per inch (product code 7212FA, BioQuip, USA). The net was used to catch individual flies and was sterilised between uses by dipping the net in 95% ethanol and drying before the next use. Sweep netting was done 1 - 2 m off the ground or where a swarm of flies was noted. Up to 5 passes of the sweep net was made to ensure that a sufficient sample of flies had been captured. Once caught, the flies were placed into individual sterile Eppendorf tubes.

3.3.5 Synanthropic fly identification

Following trap collections, flies were transported to a laboratory at Loyola hospital, approximately 5km from the centre of Bhubaneswar, 10 - 50km from the villages, for identification and bacterial testing. Due to the distance of different villages being sampled throughout Puri district from the laboratory, and poor road condition in some areas, the time between collection and laboratory testing varied from 2 - 5 h. On arrival, traps were immediately placed in a -20˚C freezer for 20 mins to knockdown flies before identification. All flies that were caught using either the sticky trap or sweep net were identified using *The Fauna of British India* keys, Diptera volume 6: Muscidae, 7: Calliphoridae and 10: Sarcophagidae (Aubertin and Smart, 1940, Van Emden, 1965, Nandi, 2002).

3.3.6 Sample processing

3.3.6.1 Pathogenic bacterial culture

One fly was selected at random from each sticky trap for pathogenic bacterial testing and using sterile forceps was placed within a sterile 15ml Eppendorf tube. A 1x1 cm transparent numbered grid was overlaid onto the sticky card and a random number generator was used to select a grid. If no flies were present in the grid selected, the random number generator was used until a fly was present to select. If multiple flies were present in the selected grid, a fly was chosen based on ease of removing from the sticky card. The fly was crushed, using an fresh autoclaved pestle for each fly, in 1 ml 0.9% saline solution which was then added to 9 ml sterile H_2O. The solution was lightly shaken by vortex to ensure homogenisation of the fly parts within the liquid. Homogenised flies could not be directly applied to the agar plates and cultured for bacteria due to the overwhelming

growth witnessed after 24 h, resulting in the inability to quantify colony-forming units (CFU) of bacteria. To achieve a quantifiable range of CFU, the homogenised flies in liquid suspension in saline solution were diluted. One ml of the homogenised solution was added to an additional 9ml sterile H_2O. As the fly homogenate was suspended in liquid, the DelAgua method of membrane filtration for thermotolerant choliforms was used. This method is the most efficient and simplest way of culturing and identifying diarrhoeagenic bacteria from liquid samples. Samples were filtered using a sterile 0.45 µm membrane filter (Millipore Corporation, USA) and processed according to the DelAgua manufacturer's instructions using the membrane filtration method for thermotolerant coliforms (DelAgua, 2015). Modification of the DelAgua protocol included incubating the membrane on one of three media: Xylose lysine deoxycholate agar (XLD agar), Thiosulfate citrate bile salts sucrose agar (TCBS agar) and MacConkey agar (MAC) (HiMedia Laboratories, Mumbai, India) instead of on Membrane Lauryl Sulphate Broth (MLSB). All plates were incubated at 37˚C for 24 hours. Plates were examined for growth and morphologically suspect CFUs were recorded on plates with an overabundance of growth recorded as too numerous to count (TNTC). A 20% random sample of flies processed were isolated and stored for further identification.

Two flies per household caught by sweep net were randomly chosen to be cultured for pathogenic bacteria. One fly was processed by homogenisation as described above. The other fly was stored, live, in a sterile Eppendorf tube until it could be exposed to the agar plates. Lives flies were transferred by sterile forceps and allowed to walk unhindered, individually, on the three different types of agar (XLD, TCBS and MAC) for a total of one minute per agar. The number of CFUs on each plate was counted and recorded. The fly was then knocked down at -20˚C and identified as described previously.

3.3.6.2 Molecular analysis

DNA was extracted from the same fly homogenate used to culture the bacteria on agar, to confirm the initial identification and, in the case of *E. coli*, differentiate the pathogenic strains. A 1 ml sample

of the homogenate in saline solution was pipetted into fresh Eppendorf tubes and centrifuged at 13,000 rpm for 5 mins. A total of 200µl DNA was extracted from 200 µl homogenate using a modified DNeasy extraction protocol for blood and tissue (Qiagen Inc, USA). As the PCR was performed on the bacteria that had been contained in or on the fly rather than using cultured cells, it was felt that the additional steps would be necessary to improve the final DNA yield and quality. The modified protocol consisted of: 1) an additional wash step using AW1 buffer to ensure that all additional contaminants that could potentially interfere with the PCR process had been removed before beginning, and 2) an additional elution step to increase DNA yield. All samples were checked using a nanodrop (Thermo Fisher Scientific Inc, USA) to ensure there was a large enough quantity of DNA to begin PCR and that the DNA was of sufficient quality to produce a result.

3.3.6.2.2 PCR amplification of Escherichia coli ChuA gene and Shigella spp. ipaH gene

Confirmation of *E. coli* culture was carried out as described elsewhere (Clermont et al., 2000) using primers ChuA.1 (5'- GACGAACCAACGGTCAGGAT -3') and ChuA.2 (5'- TGCCGCCAGTACCAAAGACA -3') (Thermo Fisher Scientific, India). Confirmation of *Shigella* spp. culture was carried out using a different procedure (Cunningham et al., 2010) using primers SHIG 172F (5'- ATAGAAGTCTACCTGGCCT -3') and SHIG 172R (5'- GGGAGAACCAGTCCGTAA -3'). Amplification was achieved in a final reaction volume of 25 µl including 1 µl of each primer (20 pmol/µl each), 7.5 µl nuclease free water, 3 µl DNA template added to 12.5 µl PCR Mastermix (Bioline) containing 0.625 units of thermo prime *Taq* DNA polymerase, 75 mM Tris-HCL, 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_{2,}$ 0.01% (v/w) Tween 20 and 0.2 mM dNTPs. Cyclic amplification was performed using a thermocycler (Life Technologies Corp., USA). PCR reactions were as follows: Initial denaturation step for 10 minutes at 94°C, 30 amplification cycles (94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds) and a final 7-minute elongation step at 72°C.

3.3.6.2.3 PCR amplification of diarrheagenic Escherichia coli

Identification of *E. coli* virotypes on *E. coli* PCR positive samples was carried out using previously published methods and primers (Nguyen et al., 2005) (Table 1). Amplification was achieved in a final reaction volume of 25 µl including 1 µl of each primer (20 pmol/µl each), 8.5 µl nuclease free water, 2 µl DNA template added to 12.5 µl PCR Mastermix (Bioline) containing 0.625 units of thermo prime *Taq* DNA polymerase, 75 mM Tris-HCL, 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_{2,}$ 0.01% (v/w) Tween 20 and 0.2 mM dNTPs. Cyclic amplification was performed using a thermocycler (Life Technologies Corp., USA). PCR reactions were as follows: Initital denaturation step for 4 minutes at 96°C, 30 amplification cycles (94°C for 20 seconds, 55°C for 20 seconds and 72°C for 10 seconds) and a final 7-minute elongation step at 72°C.

Table 3.1 The five main virotypes of *E. coli* and the target genes for PCR amplification with the size of fragment targeted and the primer sequence required.

Bacteria	Target gene	PCR size	Primer sequence (5'-3')
EAEC – Enteroaggregative *E. coli*	pCVD	630	CTGGCGAAAGACTGTATCAT CAATGTATAGAAATCCGCTGTT
EIEC – Enteroinvasive *E. coli*	*ial*	320	CTGGTAGGTATGGTGAGG CCAGGCCAACAATTATTTCC
ETEC – Entertoxigenic *E. coli*	*eltB*	322	TCTCTATGTGCATACGGAGC CCATACTGATTGCCGCAAT
	estA	147	GCTAAACCAGTAGGGTCTTCAAAA CCCGGTACAGGCAGGATTACAACA
EHEC – Enterhaemorrhagic *E. coli*	*eaeA*	376	CACACGAATAAACTGACTAAAATG AAAAACGCTGACCCGCACCTAAAT
	vt1	130	GAAGAGTCCGTGGGATTACG AGCGATGCAGCTATTAATAA
	vt2	298	ACCGTTTTTCAGATTTTGCACATA TACACAGGAGCAGTTTCAGACAGT
EPEC – Enteropathogenic *E. coli*	*bfpA*	367	TTCTTGGTGCTTGCGTGTCTTTT TTTTGTTTGTTGTATCTTTGTAA

3.3.6.2.1 PCR amplification of Salmonella spp. invA gene

Confirmation of *Salmonella* spp. culture was carried using a standard protocol (Dione et al., 2011) with two primers: invA.F (5'- CTGGCGGTGGGTTTTGTTGTCTTCTCTATT -3') and invA.R (5'-AGTTTCTCCCCCTCTTCATGCGTTACCC -3') (Thermo Fisher Scientific, India). Amplification was

achieved in a final reaction volume of 25 µl including 1.25 µl of each primer (20 pmol/µl each), 9.5 µl nuclease free water, 0.5 µl DNA template added to 12.5 µl PCR Mastermix (Bioline) containing 0.625 units of thermo prime *Taq* DNA polymerase, 75 mM Tris-HCL, 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_{2,}$ 0.01% (v/w) Tween 20 and 0.2 mM dNTPs. Cyclic amplification was performed using a thermocycler (Life Technologies Corp., USA). PCR reactions were as follows: initial denaturation step for 3 minutes at 94°C, 30 amplification cycles (94°C for 2 minutes, 55°C for 1 minute and 72°C for 1 minute) and a final 5 minute elongation step at 72°C.

3.3.6.2.4 PCR amplification of Vibrio cholerae hlyA gene

Confirmation of *V. cholerae* culture was carried out as described previously (Vinothkumar et al., 2013) using primers Vc hlyA-F (5'- CAATCGTTGCGCAATCGCG -3') and Vc hlyA-R (5'- TAATAAGCGAGCGGTACG- 3') (Thermo Fisher Scientific, India). Amplification was achieved in a final reaction volume of 25 µl including 1 µl of each primer (50 pmol/µl each), 6.5 µl nuclease free water, 4 µl DNA template added to 12.5 µl PCR Mastermix (Bioline) containing 0.625 units of thermo prime *Taq* DNA polymerase, 75 mM Tris-HCL, 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_{2,}$ 0.01% (v/w) Tween 20 and 0.2 mM dNTPs. Cyclic amplification was performed using a thermocycler (Life Technologies Corp., USA). PCR reactions were as follows: Initital denaturation step for 5 minutes at 94°C, 30 amplification cycles (94°C for 30 seconds, 62°C for 45 seconds and 72°C for 1 minute) and a final 7-minute elongation step at 72°C.

3.3.6.2.5 PCR amplification of toxigenic Vibrio cholerae ctxA gene

Identification of toxigenic *V. cholerae* on PCR positive samples was carried out using a standard method (Hoshino et al., 1998) and the following primers: VCT1 (5'- ACAGAGTGAGTACTTTGACC -3'), and VCT2 (5'- ATACCATCCATATATTTGGGAG -3'). Amplification was achieved in a final reaction volume of 25 µl including 1 µl of each primer (50 pmol/µl each), 7.5 µl nuclease free water, 3 µl DNA template added to 12.5 µl PCR Mastermix (Bioline) containing 0.625 units of thermo prime *Taq* DNA polymerase, 75 mM Tris-HCL, 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_{2,}$ 0.01% (v/w) Tween 20 and 0.2 mM

dNTPs. Cyclic amplification was performed using a thermocycler (Life Technologies Corp., USA). PCR reactions were as follows: Initial denaturation step for 5 minutes at 94°C, 35 amplification cycles (94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute) and a final 7 minute elongation step at 72°C.

3.3.6.2.6 Controls

At the time of DNA extraction, an extra negative extraction was performed to control for any potential contamination. All PCR runs contained the negative extraction control as well as a negative control (distilled water) and a positive control. Positive samples for *V. cholerae*, *Salmonella*, *Shigella* and the various toxigenic types of *E. coli* were provided by KIIT University, Bhubaneswar.

3.3.6.2.7 Visualisation

Amplified products were determined by gel electrophoresis. A 10 µl sample of PCR product was loaded with 3 µl blue juice on a 2% agarose gel containing 500 ng/µl ethidium bromide and run at 90V for 1 hour in 1X TBE buffer. A 100 bp ladder was run on either side of the gel to assess fragment size. Products were visualised under UV light and photographed using a BioRad system.

3.3.7 Statistical analysis

The primary outcome was the number of synanthropic flies captured in houses and was analysed on an intention to treat basis. Results were analysed using Stata 12.0 (Stata Corp, USA). Synanthropic fly numbers were checked for normality. If the data were not normally distributed, even after log transformation, then catches were analysed using negative binomial regression model adjusted for clustering at the village level with random effects to account for repeat samples at the household level. Due to the skew towards trap catches with few or no flies, and a small number with high catches, a negative binomial regression model was used to analyse the data with aggregation of counts at the village level. The effect of intervention, season and climatological data were tested separately using univariate analysis. Variables found to have an effect (P=<0.1) on the numbers of synanthropic flies were retained for multivariate analysis and mean ratios of flies were defined for

control and intervention arms. Microbiological data were over-dispersed due to samples with no bacterial growth, exhibiting contiguous growth or being too numerous to count (TNTC). Data collated were log transformed and analysed using ordered logistic regression with robust standard errors to account for village level clustering, calculating odds ratios.

3.3.8 Ethical consideration

The study was approved by the Ethics committee of the London School of Tropical Medicine (5561), amendment no. 1, the Xavier Institute of Management, Bhubaneswar and the Asian Institute of Public Health. The CRT is registered with ClinicalTrials.gov (Registration No. NCT01214785).

3.4 Results

3.4.1 Fly collection

Four rounds of fly surveillance were completed between April 2012 and June 2013. A total of 2902 households from 100 villages met the eligibility criteria throughout the trial of which the target was to survey a sub section 576 houses (19.8%) from 64 villages; of those houses, 572 gave consent. Of the 1716 sticky traps set, it was not possible to retrieve 47 due to the absence of the owner or removal of the trap. Therefore, the total number of sticky trap collections analysed was 1669. Latrine coverage in the intervention arm throughout survey rounds was approximately 63%, with a further 10% under construction. In the control arm, approximately 12% of houses had access to latrines and a further 1% were under construction (Table 3.2)

Table 3.2 Household characteristics of the 572 synanthropic fly surveillance houses

Characteristics	Control		Intervention	
	N	%	N	%
Kitchen Location				
Indoors	70	25	72	25
Outdoors	49	17	47	16
Outdoors (sheltered)	165	58	169	59
Fuel Source				
Wood	99	35	125	43
Dung	77	27	62	22
Straw	102	36	96	33
Gas	6	2	5	2
Water Source				
Piped water	7	3	9	3
Tube well	233	82	232	81
Open well	12	4	17	6
Pond/canal/river	32	11	30	10
Water source distance				
In compound	195	69	202	70
Outside compound	89	31	86	30
Defecation sites used				
Open defecation	62	22	31	11
Pit latrines	190	67	99	34
Pour flush latrines	32	11	158	55
Presence of Household latrine				
Yes	43	15	190	66
No	241	85	98	34
Latrine distance				
<50m	79	28	180	62
>50m	205	72	108	38
Own animals				
Yes	226	80	235	82
No	58	20	53	18
Wall material				
Mud	113	40	100	35
Brick	95	33	127	44
Concrete	62	22	55	19
mixture	14	5	6	2
Roof Material				
Thatch	144	51	158	55
Corrugated iron	75	26	63	22
Tiles	0	0	2	1
Concrete	65	23	65	22
Polish walls/floor with cow dung				
Yes	155	55	160	56
No	129	45	128	44
Garbage disposal distance				
<50m	236	83	253	88
>50m	48	17	35	12

A total of 90,401 Diptera were collected during the 4 rounds of surveillance of which 98.5% were Muscidae. Diptera belonging to the Muscidae and Calliphoridae families, known for their synanthropic habits were identified to species. *Musca domestica* comprised 57.8% of the catch of which 39% were male and 19% were female. *Musca sorbens* comprised 41.3% of the flies collected of which 27% were male, compared with 14% female caught. Only 2 *Chrysomya megacephala* were caught. Other synanthropic families and genera that were not possible to identify to species were Phoridae (0.3%), Sphaeroceridae (0.3%), Sepsidae (0.001%) and *Phaonia* (0.1%) belonging to the family Muscidae (Table 3.3). It was not possible to identify 0.3% of Muscidae flies to species due to damage removing flies from sticky traps or glue obscuring important characteristics necessary for identification. The remains of flies; head, legs and/or wings, were found on 262 traps. While it was not possible to confirm with any certainty whether body parts corresponded to the same fly or not, approximately 11000 fly carcases were collected (head with no body, body with no head, just legs and wings).

Table 3.3 Total number, means and standard deviations (SD) of each major family of flies (Diptera) caught on sticky traps placed in cooking areas of households in 64 villages of a cluster randomised control trial.

Family of fly	Control Total	Mean (sd)	Intervention Total	Mean (sd)
Muscidae	50655		38415	
M. domestica	29558	34.7 (73.0)	21984	25.4 (52.0)
M. sorbens	20764	24.4 (60.9)	16052	18.6 (51.7)
Stomoxys calcitrans	161	0.2 (1.9)	153	0.2 (1.9)
Phoania spp.	26	0.0 (0.3)	64	0.1 (0.8)
Unknown Muscidae	146	0.2 (1.3)	162	0.2 (0.9)
Psychodidae	271	0.3 (5.6)	349	0.4 (6.8)
Sphaeroceridae	35	0.0 (0.9)	215	0.3 (4.9)
Sepsidae	1	0.0 (0.0)	0	0.0 (0.0)
Phoridae	260	0.3 (7.4)	27	0.0 (0.4)
Calliphoridae	0	0.0 (0.0)	2	0.0 (0.0)
Culicidae	103	0.1 (0.9)	73	0.1 (0.8)
Phlebotominae	3	0.0 (0.1)	4	0.0 (0.1)
Chironomidae	2	0.0 (0.1)	35	0.0 (1.2)

In total, 66 (3.95%) of the 1669 trap retrieved were covered in some form of solid particulate or liquid that partially or completely obscured the trap which may have affected adhesion of flies.

3.4.2 Intervention arm vs. control arm fly catches

The number of trap nights collected was 838 in the control arm 831 in the intervention arm. In total, 50951 flies were caught in control villages, compared with 38659 in intervention villages. A total of 138 traps (8.3%) were retrieved without any damage or visible tampering but did not catch any flies. There was no statistically significant difference between control and Intervention villages when analysing the capture rates of synanthropic flies.

Important variation in the density of flies was seen between the *Rounds* performed in different seasons. When *Round* was tested as a variable it was found to be a significant predictor of the total number of synanthropic flies present in either control or intervention arms (IRR=0.65; 95%CI=[0.59-0.71]; p<0.001). Between *Round* variation was seen in both the intervention and control arms, although to a much greater extent in the control arm. The highest density of flies was observed in the monsoon season, the second *Round*, where the temperature was consistently warm and wet, between June and August. Several villages in the control arm showed extremely high densities in this season. There were no statistically significant differences between the control and intervention arms when analysed by *Round*, except for the monsoon season (Table 3.4).

Table 3.4 The median, IQR, Incidence rate ratios (IRR) and 95%CI of the total number of synanthropic flies collected in Intervention and control arms in the 4 different seasonal rounds.

Synanthropic flies		Median (IQR)	IRR	95% CI	p-value
Overall	Control	13 (3-57)	Ref.		
	Intervention	12 (2-43)	0.89	[0.76-1.03]	0.131
Round 1	Control	16 (3-47)			
	Intervention	11 (2-34)	0.85	[0.63-1.14]	0.279
Round 2	Control	65 (12-202)			
	Intervention	25(6-88)	0.60	[0.45-0.81]	<0.001
Round 3	Control	11 (4-32)			
	Intervention	13(2-38)	1.11	[0.84-1.47]	0.448
Round 4	Control	6 (0-18)			
	Intervention	5(0-24)	1.10	[0.78-1.55]	0.593

It was not possible to analyse for species-specific affects for the most synanthropic species of interest due to such low numbers captured throughout the study. However, it was possible to analyse the difference between control and Intervention for *M. domestica* and *M. sorbens.* There was no statistically significant difference between control and Intervention arms for either *M. domestica* or *M. sorbens.* Similarly to the overall synanthropic fly counts, *Round* was a predictor of total *M. domestica* (IRR=0.69; 95%CI=[0.63-0.75]; p<0.001) and *M. sorbens* (IRR=0.63; 95%CI=[0.56-0.70]; p<0.001) found in control and Intervention *Rounds* (Table 3.5) (Figure 3.4).

Table 3.5 The medians, IQR, IRR and 95%CI of the total number of synanthropic flies and specifically *M. domestica* and *M. sorbens* collected in Intervention and Control arms in the 4 different seasonal rounds.

		Median (IQR)	IRR	95% CI	p-value
M. domestica					
Overall	Control	8 (2-34)	Ref.		
	Intervention	7 (1-27)	0.91	[0.78-1.07]	0.252
Round 1	Control	8 (2-23)			
	Intervention	5 (1-17.5)	0.95	[0.70-1.29]	0.759
Round 2	Control	53 (9-119)			
	Intervention	13 (2-52)	0.54	[0.40-0.73]	<0.001
Round 3	Control	9 (2-24)			
	Intervention	10 (2-32)	1.09	[0.83-4.45]	0.530
Round 4	Control	3 (0-10)			
	Intervention	2 (0-12)	1.24	[0.88-1.75]	0.219
		Median (IQR)	IRR	95% CI	p-value
M. sorbens					
Overall	Control	2 (0-16)	Ref.		
	Intervention	2 (0-15)	0.87	[0.71-1.08]	0.207
Round 1	Control	5 (0-20)			
	Intervention	3 (0-14)	0.75	[0.53-1.06]	0.101
Round 2	Control	10 (0-79)			
	Intervention	5 (0-53)	0.69	[0.43-1.09]	0.115
Round 3	Control	1 (0-4)			
	Intervention	1 (0-5)	1.19	[0.79-1.79]	0.411
Round 4	Control	0 (0-6)			
	Intervention	1 (0-9)	0.96	[0.61-1.51]	0.853

When the species were analysed by sex, although there was a 12% difference between control and interventions arms for *M. domestica* males, it was not significant and no difference was observed for *M. domestica* females. Similarly, there was an insignificant difference (15%) with *M. sorbens* males between control and intervention arms and a 9% difference for *M. sorbens* female (Table 3.6).

Table 3.6 The medians, IQR, IRR and 95%CI of the total number of synanthropic flies and specifically *M. domestica* and *M. sorbens* male and female collected in Intervention and Control arms in the 4 different seasonal rounds.

		M. domestica male				*M. domestica* female			
		Median (IQR)	IRR	95% CI	p-value	Median (IQR)	IRR	95% CI	p-value
Overall	Control	7 (1-26)	Ref.			1 (0-8)	Ref.		
	Intervention	5 (0-18)	0.88	[0.75-1.02]	0.099	1 (0-6)	1.01	[0.84-1.22]	0.883
Round 1	Control	6 (1-17)				1 (0-4)			
	Intervention	3 (0-14)	0.86	[0.64-1.16]	0.330	1 (0-4)	1.32	[0.93-1.88]	0.126
Round 2	Control	35 (6-74)				16 (0-42)			
	Intervention	10 (1-36)	0.54	[0.40-0.73]	<0.001	1 (0-18)	0.53	[0.36-0.78]	<0.001
Round 3	Control	6 (2-18)				2 (0-8)			
	Intervention	7 (1-19)	1.07	[0.81-1.42]	0.617	3 (0-8)	1.14	[0.83-1.56]	0.430
Round 4	Control	2 (0-9)				0 (0-2)			
	Intervention	2 (0-10)	1.20	[0.86-1.69]	0.289	0 (0-2)	1.36	[0.89-2.08]	0.158
		M. sorbens male				*M. sorbens* female			
		Median (IQR)	IRR	95% CI	p-value	Median (IQR)	IRR	95% CI	p-value
Overall	Control	1 (0-11)	Ref.			0 (0-5)			
	Intervention	1 (0-10)	0.85	[0.69-1.06]	0.147	0 (0-4)	0.91	[0.73-1.14]	0.412
Round 1	Control	3 (0-14)				1 (0-5)			
	Intervention	2 (0-10)	0.78	[0.54-1.12]	0.175	1 (0-4)	0.67	[0.48-0.94]	0.022
Round 2	Control	0 (0-53)				1 (0-25)			
	Intervention	2 (0-33)	0.68	[0.43-1.09]	0.110	1 (0-19)	0.70	[0.44-1.12]	0.140
Round 3	Control	0 (0-2)				0 (0-1)			
	Intervention	0 (0-3)	1.11	[0.72-1.73]	0.631	0 (0-2)	1.33	[0.83-2.14]	0.241
Round 4	Control	0 (0-4)				0 (0-2)			
	Intervention	0 (0-6)	0.90	[0.56-1.45]	0.655	0 (0-2)	1.14	[0.72-1.81]	0.580

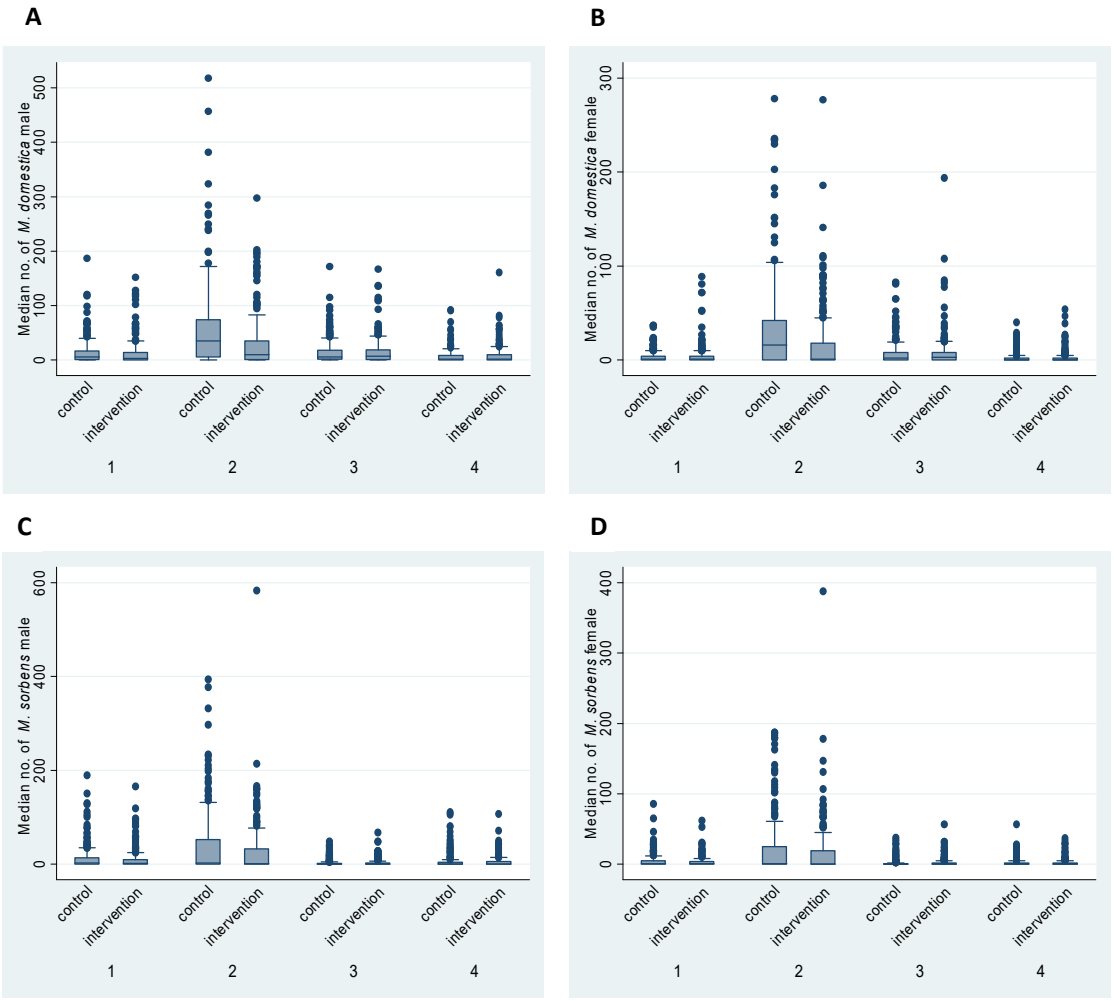

Figure 3.4. The Median range and IQR of synanthropic flies, split by main species captured and sex captured in control and intervention villages over 4 rounds of seasonal trapping; Round 1: Dry, hot, Round 2: Monsoon, Round 3: dry, cool and Round 4: dry, hot. A) *M. domestica* male B) *M. domestica* female C) *M. sorbens* male and D) *M. sorbens* female.

3.4.3 Factors affecting fly catches

Climatological information, including daily precipitation (mm), average high temperature (°C) and low temperature (°C) and relative humidity was analysed for an effect on fly numbers. Temperature both high (p=0.193) and low (p=0.391) did not have a significant effect on the numbers of flies caught in trap catches (Table 3.7). There was a significant correlation between the amount of rainfall recorded, relative humidity and the number of flies caught per month (p=<0.001) (Figure 3.5). The peak of the monsoon season in *Round* 2, when rainfall and humidity was highest, was associated with the largest catches of synanthropic flies and conversely with the lowest catches when there was less rainfall.

Table 3.7 Negative binomial regression model estimating effect size of climatic factors: Average high temperature, average low temperature, relative humidity and total precipitation on trap catches of synanthropic flies including standard error (SE) and 95% confidence intervals (95%CI).

	Effect size	SE	95%CI	p-value
High Temperature (°C)	-0.148	0.11	[0.37-0.75]	0.193
Low Temperature (°C)	0.103	0.12	[0.13-0.34]	0.391
Relative Humidity (%)	0.089	0.02	[0.05-0.13]	<0.001
Precipitation (mm)	0.005	0.001	[0.002-0.008]	<0.001

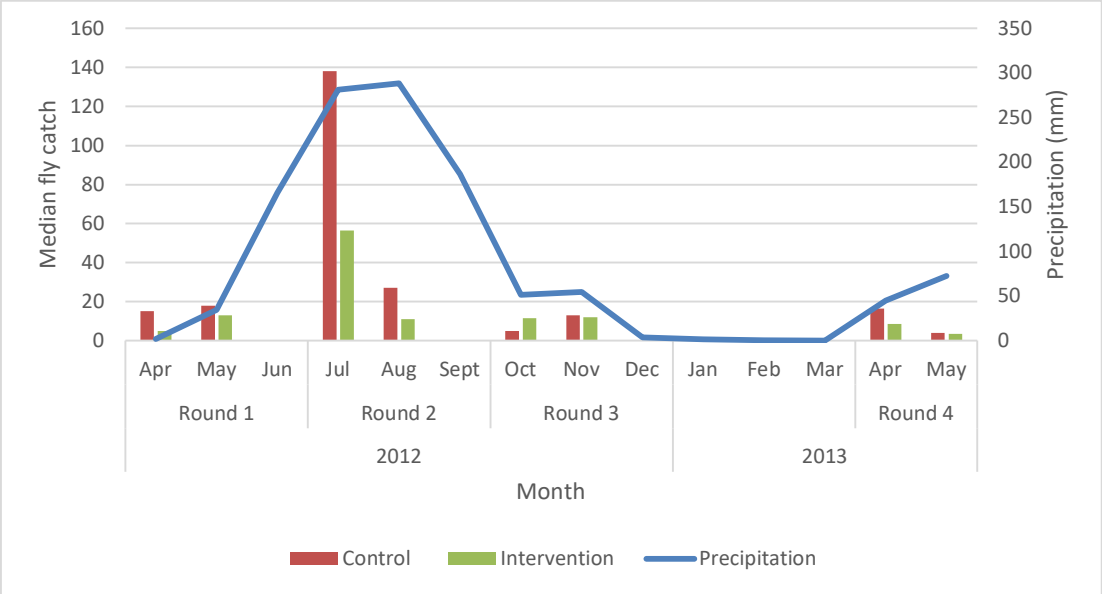

Figure 3.5. The median synanthropic fly catches by month and the mean precipitation (mm).

A comparison of fly numbers between the 4 *Rounds* showed significant differences in the numbers caught from the second *Round*, (monsoon) (Median=44.5; IQR=[8-154]), when compared with the other three *Rounds*. Over three times as many flies were caught during the second *Round* (monsoon) compared with the first *Round* (dry, hot) (Median=13; IQR=[3-41]) (IRR=3.48; 95%CI=[2.81-4.31]; p<0.001). This was even greater when the second *Round* (monsoon) compared with the third *Round* (dry, cool), when more than 4 times as many flies were caught (Median=11; IQR=[3-35]) (IRR=4.19; 95%CI=[3.38-5.20]; p<0.001). There was only a 17% difference in the number of flies caught in the third *Round* (dry, cool) when compared with the first *Round* (dry, hot). The fewest number of flies were surveyed during the fourth *Round*, once again in the dry, hot season in the following year.

3.4.4 Sweep net catches

A total of 400 flies from sweep collections were caught around human habitation, latrines and open defecation sites. Of these, 276 (69%) were *M. domestica* and 124 (31%) were *M. sorbens*. No other species of fly was collected using the sweep net.

3.4.5 Pathogenic bacterial culture

A total of 1331 flies caught using sticky traps were cultured for pathogenic bacteria, using selective media for the growth of *V. cholerae, E. coli, Salmonella* spp. and *Shigella* spp. Of these, 499 flies (39.7%) were negative for the bacteria of interest, 497(37.5%) cultured one of the bacteria, 268 (21.3%) two bacteria, 61 (4.9%) three bacteria and 6 (0.47%) cultured all four of the bacteria of interest. *Vibrio cholerae* was detected from 23% of flies, 46% were positive for *E. coli,* 19% were positive for *Salmonella* spp. and 6 % positive for *Shigella* spp.

Flies caught using a sweep net (200) also were cultured and examined for pathogenic bacteria. The results were comparable with the results from the flies collected using sticky traps. *Vibrio cholerae* was detected on 17% of flies, *Salmonella* spp. on 12%, *E. coli* on 36% and 2% of flies were positive for *Shigella* spp. From live flies that were allowed to walk on agar to ascertain numbers of viable pathogenic bacteria; a much lower percentage of the flies were positive for bacteria by culture (22%) compared with the homogenisation method (62%).

It was possible to test 670 flies and 661 flies from control and intervention villages, respectively. The presence of each bacteria tested on flies was analysed using logistic regression to test whether there was a significant difference between intervention and control villages. There was no significant difference between flies testing positive for any of the pathogenic bacteria of interest: *E. coli* O157, *Salmonella* spp., *Shigella* spp. or *V. Cholerae* in intervention villages when compared with control villages (Table 3.8).

Table 3.8 The OR and 95%CI of the number of synanthropic flies positive for 4 different pathogenic bacteria of interest, collected in Intervention and Control arms in the 4 different seasonal rounds.

		OR	95% CI	p-value
E. coli O157				
Overall	Control	Ref.		
	Intervention	0.94	[0.76 – 1.17]	0.582
Round 1	Control			
	Intervention	1.28	[0.82 – 2.00]	0.271
Round 2	Control			
	Intervention	0.64	[0.42 – 0.99]	0.047
Round 3	Control			
	Intervention	0.74	[0.47 – 1.17]	0.197
Round 4	Control			
	Intervention	1.17	[0.73 – 1.87]	0.521
Salmonella spp.				
Overall	Control	Ref.		
	Intervention	0.88	[0.67 – 1.17]	0.382
Round 1	Control			
	Intervention	0.71	[0.37 – 1.37]	0.308
Round 2	Control			
	Intervention	0.88	[0.55 – 1.41]	0.600
Round 3	Control			
	Intervention	1.03	0.54 – 1.97]	0.929
Round 4	Control			
	Intervention	0.83	[0.48 – 1.45]	0.512
Shigella spp.				
Overall	Control	Ref.		
	Intervention	0.87	[0.58 – 1.31]	0.509
Round 1	Control			
	Intervention	1.04	[0.42 – 2.59]	0.922
Round 2	Control			
	Intervention	0.72	[0.38 – 1.34]	0.293
Round 3	Control			
	Intervention	0.80	[0.27 – 2.36]	0.688
Round 4	Control			
	Intervention	1.06	[0.42 – 2.70]	0.901
V. cholerae				
Overall	Control	Ref.		
	Intervention	0.80	[0.62 – 1.04]	0.090
Round 1	Control			
	Intervention	0.76	[0.42 – 1.39]	0.368
Round 2	Control			
	Intervention	0.62	[0.38 – 1.00]	0.049
Round 3	Control			
	Intervention	0.83	[0.45 – 1.50]	0.531
Round 4	Control			
	Intervention	0.92	[0.57 – 1.49]	0.739

3.4.6 Molecular analysis

Of the flies cultured for pathogenic bacteria, regardless of whether the result was positive or negative, 20% (260) were selected at random and confirmed using PCR. In this way, PCR confirmed that 25% were positive for *V. cholerae*, 62% for *E. coli*, 19% for *Salmonella* spp. and 6% for *Shigella* spp, comparable with the results gained from culture (Table 3.9) (Table 3.10). All the *V. cholerae* and *Shigella* spp. samples that were positive for culture were positive by PCR and those negative by culture also were negative by PCR, resulting in 100% specificity and sensitivity. Four *E. coli* samples positive by culture were negative by PCR and 3 samples negative by culture were positive by PCR; resulting in sensitivity and specificity rates of 97%. Two *Salmonella* spp. samples positive by culture were negative by PCR, resulting in a sensitivity of 96%. All *Salmonella* spp. samples negative by culture also were negative by PCR, resulting in a specificity of 100%.

Table 3.9 Pathogenic bacteria cultured from synanthropic flies after homogenisation, caught using sticky traps

Overall								
	E. coli		*Salmonella* spp.		*Shigella* spp.		*V. cholerae*	
Cultured	No. positive (tested)	%	No. positive (tested)	%	No. positive (tested)	%	No. positive (tested)	%
Round 1 (dry, hot)	131 (329)	40	41 (329)	13	20 (329)	6	51 (329)	16
Round 2 (monsoon)	215 (349)	62	97 (349)	28	46 (349)	13	93 (349)	27
Round 3 (dry, cool)	103 (374)	28	41 (374)	11	14 (374)	4	50 (374)	13
Round 4 (dry, hot)	145 (279)	52	65 (279)	23	19 (279)	7	108 (279)	39
Overall	**594 (1331)**	**45**	**244 (1331)**	**18**	**99 (1331)**	**7**	**302 (1331)**	**23**
PCR confirmation	165 (259)	62	50 (255)	19	15 (255)	6	64 (260)	25

All the samples positive for *E. coli* by PCR (165) were tested for the presence of diarrhoeagenic strains. Of those, 34 (20%) were positive for one of the five strains: ETEC (9/165, 5.5%), EHEC (1/165, 0.6%), EAEC (19/165, 11.5%), EIEC (1/165, 0.6%) and EPEC (4/165, 2.4%). PCR positive samples of *V. cholerae* were tested for the presence of the toxigenic gene 01 and 0139. Of the 64 samples tested,

none were positive for the 0139 gene. However, 9 (14%) tested positive for the toxigenic 01 gene, equivalent to an overall population prevalence rate of 3.5%.

Table 3.10 Pathogenic bacteria cultured from synanthropic flies in control and intervention arms after homogenisation, caught using sticky traps

	Control							
	E. coli		*Salmonella* spp.		*Shigella* spp.		*V. cholerae*	
Cultured	No. positive (tested)	%	No. positive (tested)	%	No. positive (tested)	%	No. positive (tested)	%
Round 1 (dry, hot)	62 (168)	37	24 (168)	14	10 (168)	6	29 (168)	17
Round 2 (monsoon)	115 (172)	67	50 (172)	29	26 (172)	15	54 (172)	31
Round 3 (dry, cool)	59 (194)	30	21 (194)	11	8 (194)	4	28 (194)	14
Round 4 (dry, hot)	68 (136)	50	34 (136)	25	9 (136)	7	54 (136)	40
Overall	**304 (670)**	**45**	**129 (670)**	**19**	**53 (670)**	**8**	**165 (670)**	**25**
PCR confirmation	90 (146)	62	27 (145)	19	7 (145)	5	36 (169)	21
	Intervention							
Round 1 (dry, hot)	69 (161)	43	17 (161)	11	10 (161)	6	22 (161)	14
Round 2 (monsoon)	100 (177)	57	47 (177)	27	20 (177)	11	39 (177)	22
Round 3 (dry, cool)	44 (180)	24	20 (180)	11	6 (180)	3	22 (180)	12
Round 4 (dry, hot)	77 (143)	54	31 (143)	22	10 (143)	7	54 (143)	38
Overall	**290 (661)**	**44**	**115 (661)**	**17**	**46 (661)**	**7**	**137 (661)**	**21**
PCR confirmation	75 (113)	66	23 (110)	21	8 (110)	7	28 (132)	21

3.5 Discussion

This study suggests that the primary fly species of interest in the transmission of diarrhoeal disease in Odisha were *M. domestica* and *M. sorbens* due to their presence and abundance in food preparation areas of homes. There was no statistical difference between sticky trap collection numbers in control and intervention villages over four seasonal rounds of trapping. The four different bacteria (*E. coli, V. cholera, Salmonella* spp. and Shigella spp.) primarily responsible for diarrhoeal disease outbreaks in India were successfully cultured and confirmed by PCR on flies

present in both control and intervention villages. Almost 40% of the flies cultured were positive for at least one of the bacteria of interest. However, there was no difference in the numbers of positive flies between the control and intervention villages.

3.5.1 Fly collections

The large numbers of *M. domestica* and *M. sorbens* collected during the present study by sticky traps support results reported previously stating that this widespread genus is the foremost synanthropic fly in villages in India (Srinivasan et al., 2009, Collinet-Adler et al., 2015). The results from the sticky trap catches are comparable with catches from the sweep net performed at various locations within and surrounding village areas, primarily open defecation sites and around latrines. Sweep net catches did support the results of the sticky trap catches: that *M. domestica* and *M. sorbens*, are likely to be the principal vectors of diarrhoeal diseases within India villages due to their population densities in both kitchen areas and around open defecation sites. While Calliphoridae and Sarcophagidae are present within India, in abundance, they do not appear to be the species most closely associated with food preparation areas in India villages. For control and surveillance it is important to know the species compositions in a given study area because the life cycle and behaviour of houseflies varies from that of the blow flies so collection methods require specific traps and lures that exploit the different visual and olfactory cues they use (Hardie, 1986, Hall et al., 2003, Hanley, 2009).

Although overall there was a difference of 25% in the synanthropic flies caught in intervention villages compared with control villages, this was not significant. Previously, a similar study performed in The Gambia looked at the effect of pit latrine provision on populations of *M. sorbens* and found a reduction in fly numbers of 88% (Emerson et al., 2004). Most other studies, looking at control tools to reduce fly populations have used either baited traps or chemical control methods. These have been successfully deployed resulting in a reduction in the numbers of flies caught. For example, in Pakistan ULV spraying reduced the incidence of diarrhoeal disease by 23%, directly

attributable to the fly control measures that were in place (Chavasse et al., 1999). Similarly, baited fly traps in army camps in Israel resulted in a 42% reduction in diarrhoeal cases and an 85% reduction in shigellosis cases, compared with the control camps (Cohen, 1991). While the upfront cost of latrine construction can be expensive, almost several hundred pounds in some instances, long-term maintenance usually requires far less expenditure.

Animal faeces, primarily from cattle, can be found frequently in the environment surrounding Indian villages and while open defecation persists alongside latrines, human faeces will also be present. Combining latrine access with use reduces open defecation and, in addition to moving animals away from close proximity to households, removes primary and alternative synanthropic fly breeding sites. Simple control methods, like sticky or baited traps, can then be employed around the house to reduce remaining flies. However, achieving this, in reality, would not be so simple and would most likely require supplemental control to target adult populations of flies, along with emphasis placed on ways of reducing flies and their breeding sites and maintaining latrines to prevent the creation of breeding sites close to the home.

3.5.2 Bacteriology

The findings of the present study support published work showing that flies can carry a range of diarrhoeal pathogens, with 60% of the flies tested caught in household kitchens, carrying one or more pathogenic bacteria of interest. *Musca* spp. are attracted to a range of food and can be found on cooked rice, vegetables and meat (Pickens, 1994, Geden, 2005), leading to the likelihood of foodstuffs becoming contaminated. Contaminated raw food is not commonly seen as a problem because the bacteria are destroyed through cooking but salad and fruit that are not cooked can be susceptible to contamination. However, handling contaminated raw food and then not washing hands after, or leaving cooked food without some means of preventing fly access, means that the potential of bacterial transference to food is still a possibility.

Escherichia coli and *Salmonella* spp. are found in both humans and animals and can survive in the environment for long periods of time outside of a host (van Elsas et al., 2011, Finn et al., 2013). High levels of both *E. coli* and *Salmonella* spp. were unsurprising in the villages where the randomised trial was undertaken. Open defecation is more common than latrine use in most villages of the study (Barnard et al., 2013, Routray et al., 2015), despite there being an average of 63% latrine coverage in the intervention villages (Clasen et al., 2014). Close proximity of animals, especially cows and chickens to the household, specifically in the cooking and eating area, facilitate the transmission of pathogenic bacteria between humans and animals and it was expected that high numbers of pathogenic bacteria would be present (Delahoy et al., 2018).

Shigella spp. are only found within humans. Infection can, therefore, only be gained through contact with the bacterium from another person who is suffering from the disease. *Shigella* is highly transmissible by the faecal oral route, while much less so by contaminated food or water (van Elsas et al., 2011). Despite the lack of an animal reservoir and host, the low levels of *Shigella* spp. present are sufficient to cause disease as an inoculum as low as 10 bacteria can cause dysentery. The number of flies isolated with *Shigella* spp. within this trial are comparable with other published papers that have recorded *Shigella* spp. as being carried by flies. A study performed in 1978 in Beirut showed that of 156 flies tested, 10 were positive for *Shigella* spp. (Bidawid et al., 1978). Isolation of pathogenic bacteria in Thailand from *C. megacephala* found that 9.55% of those tested were positive for *Shigella* and 5.69% of *M. domestica* tested positive (Chaiwong et al., 2014). One study in Africa found that a much lower percentage of flies tested positive for *Shigella* spp., 1.4% (Lindsay et al., 2012).

A surprising result from this trial has been the high levels of *Vibrio* spp. cultured. *Vibrio* spp. are typically associated with an aquatic environment and with transmission via contaminated water. Similar to *Shigella* spp., humans are the only host for *V. cholerae* and it is expected that high levels cultured from flies would correlate with an outbreak within the study villages. Despite this, there

have been a few studies that indicated the possibility of flies providing a suitable environment for the transmission of the bacterium. *Vibrio cholerae* bacteria have been shown to readily adhere to the chitin and pulvilum, although not the wings (Fotedar, 2001, Broza et al., 2005). Chironomids have been shown to disseminate *V. cholerae* between different pools of clean water (Broza et al., 2005). Recently, laboratory experiments, have shown that *V. cholerae* can inhabit the midgut of *M. domestica*, although the experiments showed that the proportion of CFUs isolated was directly dependent on the amount ingested (El-Bassiony et al., 2016). The bacterium has also been isolated from *M. domestica* that were allowed to feed on a liquid bait with *V. cholera* contained within (Yap et al., 2008). Based on this evidence is it reasonable to hypothesise that synanthropic flies could allow high transfer of the pathogenic bacteria from once source to another (Tang et al., 2013).

Escherichia coli and *V. cholerae* samples that were confirmed positive by PCR were subject to additional analysis to determine the pathogenic capability of the bacteria, as not all strains are pathogenic to humans. Due to the abundance with which some of these non-pathogenic strains can be found naturally occurring in the environment, it is necessary to ascertain whether the positive samples in the study could result in disease. Although *V. cholera*e serogroup O139 is not as prevalent as O1, it is responsible for occasional severe outbreaks of the disease. In the present study, none of the samples were O139, all were O1, consistent with published papers that cholera outbreaks in India are predominantly attributable to O1 (Hanumanthappa and Rajagopal, 2000, Shah et al., 2012). Of the samples tested, only 3.5% were the toxigenic strain, highlighting the importance of confirming the strain of bacteria present. A similar lower level of pathogenic bacteria was seen when the *E. coli* samples that were tested.

Bacterial samples positive by culture were confirmed through PCR testing. Although, only 20% of the cultured flies were additionally tested, the sensitivity and specificity rates seen in this trial were comparable with the published literature (Nguyen et al., 2005) (Hoshino et al., 1998) (Cunningham et al., 2010) This was a validation of the culture methods used to ascertain bacterial carriage by flies.

3.5.3 Study limitations

It proved very difficult to standardise the location of the sticky trap as each household had a different layout; whether it was indoors, outdoors, how much furniture was present in the kitchen or how large the area was. The trap was placed on the ground and supported at an approximate 45° by a stick that was found at the location. The characteristics of households in Odisha meant that the traps were exposed to daily sweeping, cooking and cleaning activities and at risk of coverage by dust, sand or water. They were sometimes knocked in the process of cleaning. Hanging the traps or placing them at a consistent height on a wall may have negated the impact of dust exposure, although the inconsistent nature of the walls surface, or lack of walls, would also have contributed towards a lack of standardisation in trap location.

After 18 months of construction, the average latrine coverage in intervention villages was 63% compared to 12% in control villages (Clasen et al., 2012, Barnard et al., 2013). Significantly, however, use of latrines remained low, possibly due to little promotion of latrine use, lack of water at the latrine site, and the poor quality and functionality of the latrines (Barnard et al., 2013, Routray et al., 2015) possibly affecting the ability of this study to detect a difference between the intervention and control villages. In addition, even amongst houses where latrine use was higher than 50%, children under 5 years old were not allowed to use the latrines, for fears over their safety and the child faeces inevitably ended up being disposed of near the property.

The impact of reducing breeding sites would not be noticeable or immediate as the impact seen from insecticide spraying. Reducing breeding sites limits the propagation of the population whereas the insecticide spraying kills adults most within a few hours. Ideally, a successful control methodology targets both the adults, larvae as well as reducing breeding sites. Given this, latrines on their own will not be sufficient to control the fly population and transmission of pathogenic bacteria.

3.6 Conclusions

The difference in numbers of synanthopic flies between the control and intervention rounds was not significant so it was not possible to prove that the provision of latrines reduced the densities of flies. It may be that environmental factors such as distance to water, garbage disposal sites and presence and proximity of animals present greater risks of exposure to diarrhoea. However, it will be far harder to interpret and prove convincingly that reducing one such factor will have a great impact alone, as they are often reliant on other linked socio-economic factors. More work needs to be done to understand the effect of sanitation on the impact of fly densities and the carriage of pathogenic bacteria and promote the active control of flies through additional methods controlling contact with food and faeces. Overall, there is so much faecal bacteria in the environment that even reducing fly numbers has no effect on diarrhoeal incidence. Therefore, fly control through installing latrines needs to be added to a number of other environmental interventions. Given the tendency of some houses to be the source of the majority of flies, effective control would limit community wide fly populations as most flies do not disperse further than 1-2 km from the initial breeding site.

3.7 Acknowledgements

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Chapter 4: Evaluation of traps for the collection of vectors of lymphatic filariais in Odisha, India.

4.1 Abstract

There are a range of traps for studying populations of mosquitoes. The aim of this study was to find the most suitable trap to collect *Culex quinquefasciatus* to assess the impact of increased latrine coverage in the state of Odisha, India.

Densities of *Culex quinquefasciatus* captured were compared in gravid traps, without an organic or chemical attractant, and light traps. The experiment was conducted in 8 households over 15 nights in a semi-urban slum area of Bhubaneswar, the Capital of Odisha. Collected mosquitoes were identified to species, sex and gonotrophic stage.

In total 7893 insects were caught from 20 species or species groups. Both gravid and light traps predominately caught *Culex quinquefasciatus*. Gravid traps caught more mosquitoes, *Cx quinquefasciatus* and female gravid *Cx quinquefasciatus* than light traps. Many mosquito specimens were found damaged in both gravid and light traps. The proportion of males to females collected was approximately the same for both gravid and light traps. Gravid traps also collected more Phlebotmine sand flies than lights traps.

The results demonstrate the advantage of using gravid traps over lights traps for assessing densities of female gravid *Culex quinquefasciatus* and suggest it might be a suitable trap for lymphatic filariasis monitoring in India.

4.2 Introduction

The predominant vector of bancroftian filariasis are *Culex* mosquitoes, often associated with dirty water like stagnant drains, septic tanks and cesspits or organically polluted water, where larvae develop rapidly (Simonsen and Mwakitalu, 2013). *Culex quinquefasciatus* is the primary vector of bancroftian filariasis in South America as well as South Asia and on the eastern coast of sub Saharan Africa (Simonsen and Mwakitalu, 2013). *Culex quinquefasciatus* is also the vector of several other zoonotic diseases, including western equine encephalitis, St Louis encephalitis and West Nile fever.

In order to estimate population densities of *Cx. quinquefasciatus* several traps have been used. BG Sentinel, CDC Gravid traps, CDC light traps can all without or without Carbon dioxide or additional attractants to increase the number of mosquitoes caught while searching for ovipositing sites or blood meals (de Azara et al., 2013, Sukumaran et al., 2016, Asigau et al., 2017, Degener et al., 2019). These traps vary in terms of function and design but the most commonly used to collect *Cx. quinquefasciatus* are CDC Gravid and light traps. Each has advantages and disadvantages.

Gravid traps traditionally use an infusion, usually of organic material, to attract ovipositing mosquitoes. A battery-powered fan is used to suck mosquitoes into the collection bag once they have approached the infusion. Gravid trapping is an effective method for the collection of gravid *Cx quinquefasciatus*, among other species, and an established method of xenomonitoring for western equine, St Louis and West Nile viruses (Reiter et al., 1986, Muturi et al., 2007). Gravid traps collect mostly gravid females. Ovipositing mosquitoes attracted to the traps have fed at least once (Irish et al., 2015b). Due to the advantages of testing mosquitoes which have taken a blood meal, gravid traps are used successfully to collect mosquitoes for pathogen testing including filariasis detection (Williams and Gingrich, 2007a, Farid et al., 2007).

The disadvantage of using gravid traps is the preparation of grass infusion commonly used as an oviposition bait in the trap. It was shown that 4L of grass infusion per gravid trap is optimal in the collection of gravid female *Cx. quinquefasciatus*. The amount of infusion required daily for an

experiment involving 10 traps would be 40L. This amount is impractical in terms of both storage and transportation when a large-scale monitoring program is considered. In addition, standardising the infusion is difficult, where the organic infusion is made from local vegetation. Temperature and bacteria content can change the composition of the infusion and add variation that cannot be controlled for. Alternatives to local vegetation include chemical lures like nonanal, Chemtica or AtrAedes (Irish et al., 2013, Irish et al., 2014, Irish et al., 2015a). These lures are effective for attracting gravid female mosquitoes but are more expensive to acquire than an organic infusion using local vegetation. The cost involved in an expansive monitoring program could potentially be prohibitive. Water is not as attractive as using an organic or chemical lure but is much easier to acquire. In areas where water is not restricted, tap water can be acquired directly from the house where the gravid trap is to be placed. However, without any additional attractant, water would not be as competitive, especially during the monsoon season, reducing the efficacy of the trap.

Light traps are routinely used for collecting mosquitoes indoors (Amusan et al., 2005, Kweka et al., 2008a, Maxwell et al., 1990). Compared with the numbers of mosquitoes caught using gravid traps, light traps catch greater numbers from a wider range of species. In the study by Williams and Gingrich 2007, light traps collected 60,201 mosquitoes from 28 species and gravid traps collected 3,195 from 19 species, over 48 trap nights (Williams and Gingrich, 2007b). While the benefit of using gravid traps to gather vectors for pathogen testing has been documented (Reiter et al., 1986, Williams and Gingrich, 2007b, DiMenna et al., 2006), the volume of mosquitoes caught by light traps can provide more information about mosquito species densities and infection rates.

Light traps using carbon dioxide can catch far larger quantities of mosquitoes than other traps. However, the use of carbon dioxide adds considerable cost and several difficult logistical considerations to routine trapping, such as where to source, produce or store CO_2 and how to transport it to trapping sites. While light traps can catch large quantities of mosquitoes, due to their

positioning inside houses next to potential blood meal host, the majority of these mosquitoes are unfed. For the purposes of monitoring disease transmission, light traps can be less suitable.

In India, CDC light traps are routinely used for the regular sampling of adult anophelines and phlebotomine sand flies. The use of gravid traps has been more limited and confined to LF transmission monitoring (Rao et al., 2016, Subramanian et al., 2017). In Odisha, mosquito collection has been done by the labour and time intensive method of indoor resting collection (Sukhvir et al., 2008). Not only does this require a skilled labour force but often collects significantly fewer mosquitoes than the equivalent number of houses/nights that can be sampled using traps.

The objective of the present study was to compare the trapping efficiency of gravid traps and light traps to determine the best method for quantifying *C. quinquefasciatus* densities, in order to evaluate the impact of a sanitation randomised control trial on populations of *C. quinquefasciatus* and the potential for *W. bancrofti* transmission. This was achieved by comparing gravid traps and light traps in household locations in the state of Odisha, India.

4.3 Methods

4.3.1 Study site

The study was performed in a semi urban slum in Bhubaneswar, India (Latitude, longitude: 20.27°N, 85.84°E)(Figure 4.1). The houses are of mixed construction, either permanent structure made from either bricks/concrete or temporary, usually consisting of plastic sheeting for the walls and roof. Water is accessed through two main sources: water pumps from tube wells and open ponds. Tube wells can be found in some of the older and more permanent residential locations and in some communal areas. Residential tube wells are normally shared between several houses within a small area or compound. Tube wells in communal areas are accessible to all however, these are not well distributed and travel time to one can be up to half an hour. Alternatively, small ponds, predominately used for washing, are also used for drinking water. Water is kept in metal containers after collection for use throughout the day. Most houses have drainage channels dug into the

ground to contain water for disposal after washing utensils and food. This water frequently sits in small puddles without ever fully draining away as it is refilled once or twice a day. Livestock, usually cattle, pigs or chickens were found throughout the slum during the day and at night are tethered or penned closer to the owners' house.

Figure 4.1. An example of houses in Salia Sahi

4.3.2 Sampling methods

There was no data available on the abundance of *C. quinquefasciatus* in the area prior to the study. The sample size required to detect a significant difference between the treatment groups was, therefore, calculated on published capture rates of *Culex pipiens* from Williams and Gringich 2007 (Gravid traps: mean 15.8, standard error = 2.9; Light traps: mean = 3.6, Standard error = 1.72), the most closely comparable species of interest to *C. quinquefasciatus.* Using the formula by Smith, Morrow and Ross to compare the difference between two means, it was calculated 27 trap nights would be required per trap to detect a 25% difference between gravid and light trap captures with

80% power and a significance level of 0.05. As the sample size was derived from data collected in a different country and environment, the trap nights were doubled to 60 trap nights per trap to add a significant margin of error.

4.3.3 Experimental design

The slum was mapped, and houses were assigned a number and marked using GPS coordinates for the purposes of identification and randomisation. A selection of 8 houses were randomly chosen from the population using STATA 11 (Statacorp, USA). Traps were randomised between houses using a Latin square design. Each night, 4 light traps and 4 gravid traps were set in 8 households. The experiment was conducted over a period of 15 days for a total of 120 trap nights. Traps were set late in the afternoon between 16:00 - 17:00 hrs in the evening and collected the following morning between 09:00 - 11:00 hrs. Trapping was conducted in the month of March 2011 during the summer season.

4.3.4 Traps

CDC gravid traps (John W. Hock Co., USA) (Figure 4.2) were placed outside of the house or, where issues of security or space outside the property were a problem, inside the house but close to the , approximately 4L of water was used in the gravid traps. The use of water simplified the methodology for using the gravid traps by significantly reducing the preparation time from several days to approximately 5 mins. Both of the traps were run on 6v, 12 Amp batteries that were recharged daily. CDC light traps (Bioquip products, Inc, USA) were placed indoors no more than 1 metre from the sleeping area of residents. Wooden roof supports were usually used to hang the light trap. These bamboo poles were between 1.5 and 2 metres from the floor. Traps were hung between 1 and 1.5 metres from the floor. Residents were provided with an insecticide treated bed net on consenting to participate in the study and were asked to sleep underneath the bed net for the duration of the experiment.

Figure 4.2. Showing a CDC gravid trap (left) and a CDC light trap (right). Image courtesy of http://www.johnwhock.com/

4.3.5 Identification

Trap nets were taken to Loyola hospital laboratory where mosquitoes were killed in a -20°C freezer. Mosquitoes were sorted, physiological status was recorded and identified to species using 'The Fauna of British India Keys' (Christophers, 1933, Barraud, 1934).

4.3.6 Analysis

Densities of *C. quinquefasciatus* were analysed using STATA 11.0 (Statacorp, USA). Data were tested for normality and if necessary, log transformed. Data that was still skewed despite log transformation were analysed using a negative binomial regression model. All trap captures of *Cx. quinquefasciatus* were over-dispersed and so the untransformed data were analysed using a negative binomial regression model. Densities of gravid female *Cx. quinquefasciatus* and differences between physiological status also were analysed.

4.3.7 Ethical approval

Residents were approached and given a verbal overview and a written information sheet before being asked if they would like to participate in the study. Residents written consent was obtained before any work was undertaken. The study was approved by the ethics committees of Xavier Institute of Management, Bhubaneswar (ref no. 31052010) and the London School of Hygiene and Tropical Medicine (ref no. 5562).

4.4 Results

Approximately 13,043 insects were collected during the experiment of which 7893 (61%) were undamaged and could be identified to species or species group (Table 4.1). A large proportion of insects were damaged as they entered the trap and passed the fan. Head, thorax and abdomen parts were routinely found and not counted in the final analysis, as it was difficult to identify parts to one insect. In total, 7208 whole mosquitoes were captured, accounting for 91% of all insects caught. *Cx. quinquefasciatus* comprised 86% of all insects captured and 94% of all mosquitoes captured (7208). Other fly species captured were Phlebotminae (538), Psychoda spp. (59) and Psychodidae (77) (Table 4.1).

Table 4.1 Total number of whole, undamaged insect species captured during the comparison between gravid and light traps.

Species	Gravid trap N (median, IQR)	Light trap N (median, IQR)	Total
Aedes albopictus	36 (0, 0-1)	7 (0, 0-0)	43
Aedes linneatopennis	2 (0, 0-0)	0 (0, 0-0)	2
Anopheles aconitus	1 (0, 0-0)	0 (0, 0-0)	1
Anopheles fluviatilis	0 (0, 0-0)	1 (0, 0-0)	1
Anopheles maculatus	1 (0, 0-0)	0 (0, 0-0)	1
Anopheles minimus	1 (0, 0-0)	0 (0, 0-0)	1
Anopheles vagus	3 (0, 0-0)	3 (0, 0-0)	6
***Anopheles* sp.**	3 (0, 0-0)	3 (0, 0-0)	6
Armigeres kuchingensis	171 (4, 0-6)	120 (3, 0-5)	291
***Armigeres* sp.**	4 (0, 0-0)	27 (0, 0-0)	31
Cx. quinquefasciatus	5284 (110, 58-191)	1486 (18, 10-25)	6770
Cx. vishnui	13 (0, 0-0)	19 (0, 0-1)	31
Cx. whitmorei	2 (0, 0-0)	17 (0, 0-0)	19
Mansonia annulifera	0 (0, 0-0)	2 (0, 0-0)	2

Mansonia uniformis	0 (0, 0-0)	4 (0, 0-0)	4
Musca domestica	0 (0, 0-0)	3 (0, 0-0)	3
M. sorbens	0 (0, 0-0)	6 (0, 0-0)	6
Phlebotominae	424 (0, 0-0)	114 (0, 0-0)	538
***Psychoda* spp.**	16 (0, 0-0)	43 (0, 0-0)	59
Psychodidae	11 (0, 0-0)	66 (0, 0-0)	77
Stomoxys calcitrans	0 (0, 0-0)	1 (0, 0-0)	1
Total	**5971**	**1922**	**7893**

In total, raps caught 2326 female gravid *Cx quinquefasciatus*, over 119 trap nights. On one occasion, it was not possible to collect a gravid trap catch from one house, during the experiment because the resident was absent when visiting. The final analysis consisted of 59 gravid trap nights and 60 light trap nights. Although the proportion of males to females was similar for both the light and gravid traps (Table 4.2), gravid traps caught more than 3 times as many female *Cx. quinquefasciatus* than light traps ((IRR=3.51; 95%CI=[2.26-4.45]; p<0.001).When *Cx. quinquefasciatus* was analysed by status; gravid traps caught more female gravid *Cx. quinquefasciatus* (2164, median 26, IQR 17-90) compared with light traps, (162, median 2, IQR 1-5). Altogether gravid traps caught over 13 times as many female gravid *Cx. quinquefasciatus* as light traps (IRR=13.36; 95%CI=[8.67-20.57]; p<0.001).

Table 4.2 Total numbers and percentage of whole *Cx. quinquefasciatus*, including sex and status, captured by gravid and light traps, as a proportion of all mosquitoes captured.

	Gravid trap N (median, IQR)	**Light trap N (median, IQR)**	**Total**
All mosquitoes	5520 (118, 68-191)	1688 (25, 12-32)	7208
Cx. quinquefasciatus			
Total	5284 (110, 58-191)	1486 (18, 10-25)	6770
Male	2061 (44, 28-68)	567 (9, 5-13)	2628
Female	3223 (47, 25-132)	919 (9, 3-16)	4142
Unfed	941 (17, 7-30)	733 (5, 2-8)	1674
Blood-fed	104 (2, 0-4)	19 (0, 0-1)	123
Semi gravid	14 (0, 0-0)	5 (0, 0-0)	19
Gravid	2164 (26, 17-90)	162 (2, 1-5)	2326

4.5 Discussion

Gravid traps caught significantly more *Cx. quinquefasciatus* than light traps in this study which is similar to other studies that have compared light with gravid traps in East Africa (Muturi et al., 2007, Irish et al., 2015b). Furthermore, in all cases, the proportion of gravid mosquitoes was higher in the gravid traps than the light traps (Irish et al., 2015b). Somewhat surprising was the higher total numbers of other mosquitoes captured by the gravid traps compared with the light traps, there were few exceptions to this (*Cx. whitmorei*, *Armigeres* sp. and Psychodidae). Higher totals of *Cx. quinquefasciatus* were also seen in gravid traps for every physiological status as well. Also surprising was the high proportion of damaged insects that were found in both gravid and light traps.

The reason for the higher numbers of *Cx. quinquefasciatus* collected in gravid traps than light traps may be due to the trap position. Unlike gravid traps, light traps are generally placed indoors, rather than outdoors, and used to collect mosquitoes from within households (Kweka et al., 2008b, Farid et al., 2007). Due to ethical restrictions of trap studies, residents are requested to sleep under an insecticide impregnated bed net. It is possible, although unlikely, that the presence of insecticide deterred mosquitoes from the area or they killed before entering the trap. However, high levels of insecticide resistance within populations of *Cx. quinquefasciatus* make this an unlikely scenario. The presence of bed nets is also a possible reason for the difference in proportions in the status of female *Cx. quinquefasciatus*. Light traps are less likely to catch bloodfed mosquitoes if the bed nets are used correctly (Pedersen et al., 2009). This reduces the chances of finding filarial parasites within the mosquito but does not eliminate it as mosquitoes may have fed on an infected person a week earlier. Since gravid traps are specifically designed to catch gravid mosquitoes, which have already taken a bloodmeal, it should increase the chance of finding infection within the mosquito. However, studies have shown that infection rates are sometimes higher in light traps (Irish et al., 2015b). Alternative methods to trapping include collecting indoor and outdoor resting mosquitoes (Sadanandane et al., 2004). This should result in a higher catch of bloodfed mosquitoes, due to the habit of females resting after blood feeding. However, this is an extremely labour-intensive process

and not feasible for use in a situation where large numbers of mosquitoes are needed such as quantifying the prevalence of *W. bancrofti* infection.

In the comparison between gravid and light traps, it is also worth pointing out that gravid traps caught a greater variety of mosquitoes compared with the light traps, including a larger number of *Anopheles* species. While the principal vector of *W. bancrofti* in India is *Cx. quinquefasciatus*, *Anopheles* is a secondary vector and can also be tested for the presence of *W. bancrofti*.

Ideally, a comparison between the infection rates of mosquitoes captured in light traps and gravid traps would have been undertaken in this study. This would have better informed which trap to use in the entomological surveillance of the RCT since it is known that there is a difference in the infection rates between mosquitoes caught in light traps and gravid traps (Irish et al., 2015b). A substantial portion of the mosquitoes that entered the traps were damaged when passing through the fans into the collection bag. The light trap damaged the highest proportion of mosquitoes when compared to the gravid trap.

4.6 Conclusions

Gravid traps caught significantly more *Cx. quinquesfaciatus* and, although the infection rates of the mosquitoes captured was not explored, the difference in numbers captured of gravid mosquitoes between the types of traps suggests that the gravid trap would be the better trap to use for quantifying infection rates. As the trap also collected far fewer mosquitoes and specifically *Cx. quinquefasciatus* than the gravid traps, it cannot be recommended for use in lymphatic filariasis monitoring in India.

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SECTION A – Student Details

Student	Melissa Joanne Bell
Principal Supervisor	Mary Cameron
Thesis Title	The impact of latrine construction on densities and pathogen infection rates of syanthropic flies and Culex quinquefasciatus mosquitoes in Odisha, India

If the Research Paper has previously been published please complete Section B, if not please move to Section C

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Where was the work published?			
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Please list the paper's authors in the intended authorship order:	Melissa Bell, Soumya Nayak, Sophie Boisson, Parimita Routray, Wolf-Peter Schmidt, Belen Torondel, Seth Irish, Soosai Peppin, Mrutyunjay Suar, Thomas Clasen and Mary Cameron.
Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I made major constributions to the inception, design, field work, analysis and write-up of the experiments

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Supervisor Signature: [redacted] Date: 20/8/18

Chapter 5: Effect of Latrine Coverage on *Culex quinquefasciatus densities* and *Wuchereria bancrofti* transmission in rural Odisha, India: a cluster randomised controlled study

5.1 Abstract

India accounts for 42% of the global burden of lymphatic filariasis (LF). As part of a cluster randomised control trial, the effect of the scale-up of pour-flush latrines among rural communities was assessed for its effect on mosquito populations. Using gravid traps to capture gravid female *Cx. quinquefasciatus*, the local vector of lymphatic filariasis, population densities were measured in a random sample of 8 houses from each of 32 control and 32 intervention villages (512 houses total) and the results analysed to ascertain any differences between the two arms. Captured gravid female *Cx. quinquefasciatus* were tested by PCR for the presence of *W. bancrofti* as a means of estimating prevalence of parasite infection in mosquito populations. Female *Cx. quinquefasciatus* dominated mosquito trap captures (78%). Despite the collection of 12% fewer female *Cx. quinquefasciatus* in control villages than in intervention villages, there was no significant difference between the villages surveyed (IRR=0.88; 95%CI=[0.74-1.06]; p=0.178). Seasonal variation had a large effect on captures between different trapping *rounds*. A very low proportion of pools tested for *W. bancrofti* were positive (16 of 1984, 0.8%) and there was no difference between control and intervention villages prevalence rates (p=0.808). The construction of pour-flush latrines did not facilitate an increase in the number of *Cx. quinquefasciatus* trapped. Low usage of latrines in the intervention villages could have contributed to this.

5.2 Introduction

Lymphatic filariasis (LF) is a worldwide disease with approximately 947 million people at risk. In 2000 the World Health Organisation (WHO) started the Global Programme for the Elimination of Lymphatic Filariasis (GPELF) as an initiative to eliminate the disease in endemic countries through the distribution of antihelmenthic drugs, treating and curing infected cases and controlling the vector (WHO, 2010b) (Rebollo and Bockarie, 2013). The amount of people requiring mass drug administration (MDA) as preventative chemotherapy to prevent acquisition of the parasite has reduced from 1.4 billion in 2011 to 947 million in 2015 (WHO, 2016). Severe symptoms consist of elephantiasis, hydrocoeles and lymphodaema, potentially debilitating conditions resulting from infection with one of the three main causative filarial parasites. *Wuchereria bancrofti* is responsible for over 90% of infections; *Brugia malayi* and *Brugia timori are* responsible together for the remaining 10% (WHO, 2016). These parasites are transmitted by the bite of an infected mosquito. The major vectors of the disease are *Culex quinquefasciatus* and *Anopheles gambiae* as well as *Aedes polynesiensis* (WHO, 2013). Since 2011 the population requiring MDA has decreased from 1.4 billion people to 947 million in 2015 due in large part to the number of countries once considered endemic having successfully implemented a mixture of the three methods advocated by GPELF and reduced the number of infected individuals (WHO, 2010a).

India accounts for 42% of the global burden of LF (Ramaiah and Ottesen, 2014). Within India, MDA with diethylcarbamazine (DEC) and albendazole is promoted throughout endemic states for the elimination of the disease. In southern India, seven years of MDA resulted in a 14% decrease in patients suffering from lymphoedema (Yuvaraj et al., 2008). After completing 10 rounds of MDA, transmission was interrupted and microfilariae (mf) prevalence continued to decline during 6 years of post-surveillance (Ramaiah and Vanamail, 2013). Reports from 2015 showed that MDA coverage in affected areas in India was almost at 100% (WHO, 2016). However, the situation is not heterogeneous across India with the difference between coverage and compliance proving a difficult

obstacle to overcome in many states (Babu and Babu, 2014). One study within Odisha testing children aged between 5-18 years found that 20.5% of 3055 children were either positive for Microfilariae (Mf) (n=154) or Og4C3 antigen positive (n=480) (Barnard et al., 2013). In Odisha, compliance has been low since the MDA coverage programme started in 2004 (Sukhvir et al., 2008, Walsh et al., 2016). Studies assessing the progress of MDA in Odisha have found there was a 25% difference between reported coverage rates and the actual compliance rates with only approximately 42% of people covered by MDA actually consuming the tablets (Babu and Kar, 2004, Cassidy et al., 2016). Mostly because people did not understand what the drugs were and feared side effects (Hussain et al., 2014). In addition to the MDA programmes in place, Odisha has also been implementing vector control programmes using ULV fogging with deltamethrin with the aim of reducing the possibility of transmission of LF and other prevalent diseases in the area; malaria and dengue.

Culex quinquefasciatus is the most important vector of LF in India and in Odisha (Raghavan, 1957). *Culex quinquefasciatus* typically breed, and immature stages develop, in organically rich water such as latrines and septic tanks (Curtis and Hawkins, 1982, Irving-Bell et al., 1987). It is possible that the construction of latrines as part of Water, Sanitation and Hygiene (WASH) campaigns will increase populations of mosquitoes. Pit latrines rarely prevent access to the pit contents, providing an ideal aquatic habitat. Improving pit latrines and transforming them into pour flush latrines, in theory would result in a decrease in mosquito populations in a similar way to Expanded polystyrene beads (EPS) used in pit latrines that has contributed to the reduction or elimination of LF as part of integrated vector control management strategies (Maxwell et al., 1990, Al-Kubati et al., 2011, Sunish et al., 2016). A pour-flush latrine introduces a water block between the septic tank and provides a barrier to easy access for the mosquito. If there is water in the u-bend of the foot pan, *Cx. quinquefasciatus* should not be able to gain entrance to potential breeding sites. In addition, pit contents are frequently buried. Any soil coverage above the concrete rings of the cesspit would prevent direct access to the contents. However, the major limiting factor in the continued impact of

WASH programmes as a control method is latrine upkeep. If not well maintained or poorly constructed, cracks or uncovered ventilation pipes can allow easy access to the contents of the pit. If poorly constructed latrines result in a multiplication of breeding sites, pour flush latrine construction has the potential to lead to an increase in mosquitoes present in villages (Hiscox et al., 2015).

In this study, the effect of pour-flush latrine coverage on populations of *Cx. quinquefasciatus* was monitored in a randomly selected group of villages in Odisha. This was achieved by assessing the densities of *Cx. quinquefasciatus* populations and the infection rates with the parasite *W.* bancrofti. This was conducted in the context of a large cluster randomised control trial assessing the effect of increased sanitation coverage (cRCT)(Clasen et al., 2014).

5.3 Methods

5.3.1 Study Site

The mosquito surveillance study was conducted in Puri district, Odisha, India from April 2012 to June 2013 in the context of a cluster randomized controlled trial (cRCT). The design and study setting of the cRCT have been described elsewhere (Clasen et al., 2012). In brief, the aim of the study was to assess the impact of community latrine construction and subsequent population coverage under the

Figure 5.1. The faecal pit with concrete lid and inside of a pour flush latrine constructed as part of the Total sanitation campaign in Odisha, India.

Government of India's Total Sanitation Campaign (TSC). The villages, households, average temperature and rainfall recorded during the study are described in Chapter 3.

The estimated sanitation coverage in the district, as judged by each families access to a latrine, was under 10% (DLHFS-3, 2008, India, 2011) before the start of the trial. The aim of the TSC was to mobilize residents, classified as being below the poverty line, defined as living on less than $1.90 a day, to construct pour-flush latrines until village level coverage was at or above 70% in accordance with the government of India's guidelines (Figure 5.1)(WSP, 2011). Village level eligibility criteria for our trial were established as the sanitation coverage less than 10%, having an improved water supply with no other water, sanitation or hygiene interventions planned for the upcoming 30 months (Clasen et al., 2014). The implementation of the TSC increased the latrine coverage in intervention villages to an average of 63% (Clasen et al., 2012, Barnard et al., 2013).

5.3.2 Sample size

Mosquito trapping, using CDC gravid traps with tap water, conducted as part of a pilot study in non-cRCT villages between September – November 2011, caught an arithmetic mean of 17.6 (SD 2.24) gravid females of *Cx. quinquefasciatus* over a 24 hour period per trap per house from 8 houses over 32 days (256 trap nights in total). Previously published trials investigating the impact of interventions focused on supressing populations of *Cx. quinquefasciatus* within latrines have shown a reduction in densities of between 50% and 98% (Curtis and Hawkins, 1982, Maxwell et al., 1990). Therefore, a 30% difference in effect for a larger study, using pour flush latrines as the intervention, would be a conservative estimate. Assuming an intraclass correlation coefficient (ICC) of 0.08, 80% power and 5% level of significance and using the mean of 17.6 mosquitoes/trap/night, resulted in a sample size of 446 houses in total; 223 in control and 223 in intervention villages, required to detect a 30% difference between treatment arms. The study was conducted in a subset of 64 cRCT villages, 32 control villages with latrine coverage <10% and 32 intervention villages where latrines had been built recently with coverage averaging approximately 63% (Clasen et al., 2014). As the sample size

required to detect a difference between the intervention and control villages for *Cx. quinquefasciatus* was 446, a minimum of 7 households in each village would need to be sampled. In order to build in tolerance to the sample size for potential battery failure with the traps or missing data, the sample size was increased to 8 households in each village resulting in a total of 512 households, with 256 households in the control and 256 in the intervention arm (Figure 5.2). Households were selected at random from the eligible pool of households using a random number generator in Stata 11 (StataCorp, USA). In order to estimate average household populations, each household was sampled for three consecutive nights resulting in 1536 trap nights over the course of the study.

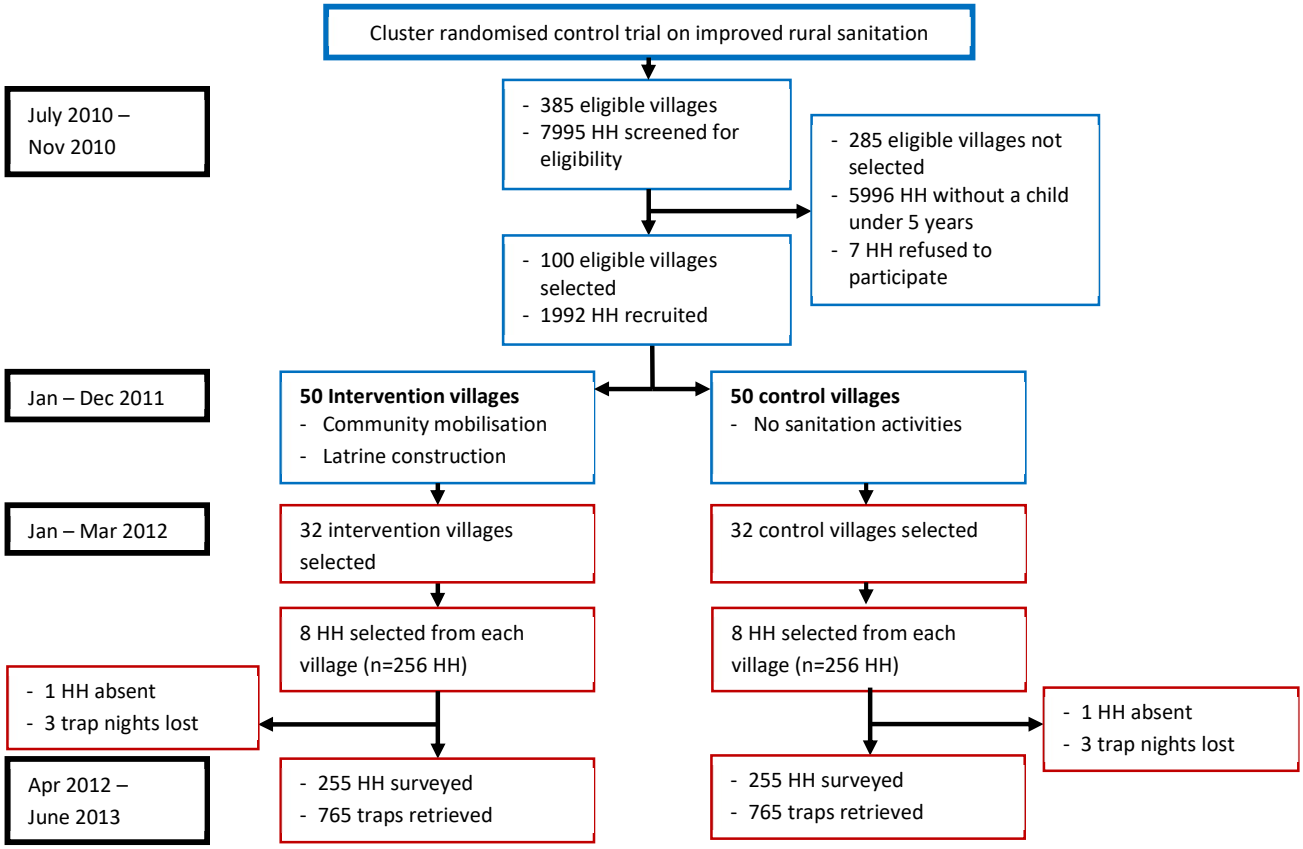

Figure 5.2. Trial profile with the sections highlighted in blue; the design of the overall cRCT and the sections highlighted in red; the design of the flies and bacteria study

For the determination of the prevalence of *W. bancrofti* infection in *Cx. quinquefasciatus* based on the calculations by Katholi *et al.* 2006 (Katholi and Unnasch, 2006), a sample size was calculated

from the estimated prevalence of infection within the vector population and the expected population of mosquitoes. The prevalence of infection within the vector population in Odisha was estimated to be 1.3% based on the most recent papers published at the time (Sukhvir et al., 2008). An average of 17.6 mosquitoes per trap night were collected in previous experiments in the area. On this basis, a minimum of 140 trap nights in total, 70 trap nights per treatment arm were needed to detect a 30% difference in vector LF infection prevalence between control and intervention arms.

5.3.3 Randomisation

Using Stata, households were randomly selected using STATA 11.0 from those available to the cRCT and meeting the eligibility criteria; having children under the age of five years and residing permanently in the village, with the additional criteria that they were a minimum distance of 10m apart, the approximate distance between separate house blocks, to reduce interference of trap catches between neighbouring households. Data collection tools were translated into the local language, Odisi, and piloted before use. An information sheet giving a detailed overview of the study was provided to every household and written consent was gained prior to mosquito collection.

5.3.4 Mosquito collection

CDC gravid traps were used for the collection of *Cx. quinquefasciatus* (John W. Hock Co., Gainesville, FL) (Figure 5.3). The traps were run on 6v batteries, recharged each day before use. Where possible the traps were placed outside under the eaves of the house. However, in some cases, if there was insufficient room outside, concerns about security or rain, traps were placed indoors on the ground. The traps were set each day between 08:00 and 10:00 and collected 24 hours later. Approximately 4L water was added to the gravid trap instead of a grass infusion or synthetic alternative. The use of water was preferable to smelly infusions for indoor use and simplified the methodology: the time required to prepare grass infusion is extensive and the quantity needed for the trial was significant, approximately 300g of grass daily and more than 2 days to prepare each infusion (Irish et al., 2012, Irish et al., 2013b). Alternative, synthetic chemical attractants such as Nonanal or AtrAedes were

considered but these were not readily available in country and the quantity needed again was prohibitive (Irish et al., 2013a, Irish et al., 2014, Irish et al., 2015a). Although significantly more *Cx. quinquefasciatus* have been collected in traps with grass infusion (Irish et al., 2013a), substantial numbers can also be collected from water only gravid traps (Irish et al., 2014).

Figure 5.3. CDC gravid traps in location at houses used to collect of mosquitoes, in particular gravid female *Cx. quinquefasciatus.*

5.3.5 Mosquito identification

On completion of trap collection, mosquitoes were transported to a laboratory at Loyola Hospital for identification. On reaching the laboratory, traps were immediately placed within a -20˚C freezer to kill mosquitoes before identification and preserve any *W. bancrofti* DNA. Species, sex and physiological status (unfed, bloodfed, gravid and semi-gravid) were recorded for each mosquito. Mosquitoes were identified to species using The Fauna of British India keys (Christophers, 1933, Barraud, 1934). Other Diptera were identified to family or order (Aubertin and Smart, 1940, Van Emden, 1965). Some mosquitoes were damaged while passing through the fan of the gravid trap so identifiable body parts were recorded where possible. For the detection of *W. bancrofti* DNA, gravid females of *Cx. quinquefasciatus* were transported to KIIT university. They were pooled in groups of 20 by household and, if insufficient numbers were present in a household, by village and then

Round. Any remaining mosquitoes were pooled together in a single tube resulting in unequal numbers of mosquitoes in pools.

5.3.6 Lymphatic filariasis detection

5.3.6.1 DNA extraction

Mosquitoes were tested for the presence of *W. bancrofti* DNA. DNA was extracted using a modified DNeasy extraction protocol for blood and tissue (Qiagen Inc, USA). Additional steps were taken to improve the final DNA yield and quality including the following: 1) an additional wash step using AW1 buffer to ensure that all additional contaminants that could potentially interfere with the PCR process had been removed before beginning, and 2) an additional elution step to increase DNA yield. All extraction groups were accompanied by a negative extraction control to ensure that contaminants had not inadvertently been included during the extraction process. All samples were checked using a nanodrop (Thermo Fisher Scientific Inc, USA) to ensure there was a sufficient quantity and quality of DNA to begin PCR and that the DNA was of sufficient quality to produce a result. The extracted DNA was kept in -20°C until PCR assays.

5.3.6.2 PCR amplification of the Wuchereria bancrofti SspI

Detection of *W. bancrofti* 188bp was carried out as described by Rao *et al.* (Rao et al., 2006) using primers NV1: 5'CGTGATGGCATCAAAGTAGCG 3' and NV2: 5' CCCTCACTTACCATAAGACAA 3'(Thermo Fisher Scientific, India). Amplification was achieved in a final reaction volume of 50 µl: 1 µl of each primer (10 pmol/µl each), 22 µl nuclease free water, 1 µl DNA template added to 25 µl PCR Mastermix (Bioline) containing 0.625 units of thermo prime *Taq* DNA polymerase, 75 mM Tris-HCL, 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.01% (v/w) Tween 20 and 0.2 mM dNTPs. Cyclic amplification was performed using a thermocycler (Life Technologies Corp., USA). PCR reactions were as follows: initial denaturation step for 15 minutes at 95°C followed by 54°C for 5 minutes, 35 amplification cycles (72°C for 30 seconds, 94°C for 20 seconds and 54°C for 30 seconds) and a final 5 minute elongation step at 72°C. In addition to the samples, all PCR runs also contained a negative extraction control, a

negative control (distilled water) and a positive control (DNA obtained from Regional Medical Research Centre, Bhubaneswar from a mosquito fed on a patient with LF that tested positive).

5.3.6.3 Visualisation

Amplified products were determined by gel electrophoresis. 10 µl PCR product was loaded with 3 µl blue juice on a 2% agarose gel containing 500 ng/µl ethidium bromide and run at 90V for 1 hour in 1X TBE buffer. A 100 bp ladder to assess fragment size. Products were visualised under UV light and photographed using a BioRad system.

5.3.7 Statistical analysis

Results were analysed using Stata 11.0 (Stata corp, USA). Mosquito numbers were checked for normality. *Cx quinquefasciatus* numbers were checked for normality. If the data were not normally distributed, even after log transformation, then catches were analysed using negative binomial regression model adjusted for clustering at the village level with random effects to account for repeat samples at the household level. Due to the skew towards trap catches with few or no mosquitoes, and a small number with high catches, a negative binomial regression model was used to analyse the data with aggregation of counts at the village level. The effect of intervention, household characteristics and latrine use, were tested in a univariate analysis. Variables found to have an effect (P≤0.1) on the numbers of *Cx. quinquefasciatus* were retained for multivariate analysis. The results of pooled samples of gravid female *Cx. quinquefasciatus* tested for the presence of microfilariae was analysed using unequal pool sizes selection in Poolscreen 2.0 for the maximum likelihood estimate of infection prevalence within the population (Helmy et al., 2004).

5.3.8 Ethical consideration

The study was approved by the Ethics committee of the London School of Tropical Medicine (5561), amendment no. 1, the Xavier Institute of Management, Bhubaneswar and the Asian Institute of Public Health. The CRT is registered with ClinicalTrials.gov (Registration No. NCT01214785).

5.4 Results

5.4.1 Mosquito collection

Altogether, 58,753 intact and damaged (head and thorax) mosquitoes were caught in all villages during this study (Table 5.1) of which 78.2% (46,000) were female *Cx. quinquefasciatus.* Female *Cx. quinquefasciatus* were separated by sex and physiological status and only gravid females were kept for further analysis. Approximately 13,357 insects belonging to dipteran families other than Culicidae were also caught in the course of the trial. Other families included but were not limited to: Psychodidae (6412) of which 2259 were Phlebotominae, Muscidae (298), Limoniidae (165), Ceratopogonidae (89), Phoridae (63) and Chironmidae (18). A proportion of mosquitoes entering the gravid trap were damaged as they passed the fan. Thorax, wings and head were identifiable as belonging to one mosquito in most cases. In instances where parts were not identifiable as belonging to one mosquito, body parts were counted as unidentified. Mosquitoes that were not possible to identify to genera, using the keys available, were also classed as unidentifiable.

5.4.2 Intervention vs. control villages

Mosquito surveillance was successfully carried out in 64 villages. A total of 510 of 512 households eligible and chosen to participate in the study were used for trapping; 2 households initially gave consent but were then absent for the remaining period of trapping. Of the remaining 510 households, it was possible to complete three nights' worth of consecutive trapping in all of the remaining households. Consequently, 1530 trap nights were analysed. The number of trap nights in the control and intervention arm was equal (765).

In both the control and intervention arm in each *Round*, gravid female *Cx. quinquefasciatus* dominated the trap catches, comprising 67.5% of the mosquitoes (39,646) (Table 5.2). In the control arm, 21,090 (median 13, IQR 0-33) gravid females of *Cx. quinquefasciatus* were captured; whereas 18,556 (13, 0-31) were captured in the intervention arm. The highest density of gravid female *Cx. quinquefasciatus* was seen during *Round* 2, the monsoon season between June and August, where it

rained daily and average daily temperatures were 31°C. Seasonal variation was seen in both the control and intervention arms.

Table 5.1 Species of Culicidae other than *Cx. quinquefasciatus* captured with CDC gravid traps in Odisha during a cRCT on sanitation including intact and damaged mosquitoes (head and thorax).

Species	***Round* 1 (dry, hot)**	***Round* 2 (Monsoon)**	***Round* 3 (dry, cool)**	***Round* 4 (dry, hot)**
Aedes albopictus	45	251	144	41
Ae. linneatopennis	0	0	1	0
Anopheles annularis	30	14	270	22
An. culicifacies	0	0	2	0
An. culiciformis	0	0	1	0
An. fluviatilis	0	4	0	0
An. hyrcanus	0	30	0	0
An. maculatus	51	4	856	39
An. minimus	0	2	0	0
An. ramsayi	0	2	0	0
An. stephensi	0	0	12	0
An. subpictus	25	28	95	15
An. subpictus/vagus	54	72	149	38
An. sundaicus	0	3	0	0
An. vagus	12	119	65	8
An. spp.	17	42	57	23
Armigeres subalbatus	105	127	227	87
Cx. gelidus	0	479	0	0
Cx. sitiens	0	2	0	0
Cx. tritaeniorhynchus	0	3	0	0
Cx. vishnui	1	693	43	1
Cx. whitmorei	70	609	119	58
Culex spp.	1	10	2	5
Mansonia annulifera	78	577	126	69
Ma. uniformis	385	369	539	383
***Mimomyia* sp.**	0	2	0	0
Uranotaenia luteola	1	1	2	0
Ur. recondita	0	1	0	1
Unidentifiable	18	31	20	19

The *Round* of trapping was found to be a significant predictor of the total number of gravid female *Cx. quinquefasciatus* captured in either the control or the intervention arm, so was added to the final multivariate model. Overall, there was no difference in the number of mosquitoes caught in the intervention arm when compared with the control arm (IRR=0.88; 95%CI=[0.74-1.06]; p=0.178).

Table 5.2 Total numbers of intact and damaged mosquitoes (head and thorax) captured and proportion of gravid female *Cx. quinquefasciatus* captured using CDC gravid traps in Odisha during a cRCT on sanitation.

	***Round* 1 (dry, hot)**	***Round* 2 (monsoon)**	***Round* 3 (dry, cool)**	***Round* 4 (dry, hot)**
All mosquitoes				
Mosquitoes	11126	22048	10722	14857
Of which damaged (%)	387 (3.5)	709 (3.2)	576 (5.4)	401 (2.7)
Cx. quinquefasciatus				
Total (%)	10233 (92.0)	18573 (84.2)	7992 (74.5)	14048 (94.6)
Of which damaged (%)	372 (3.6)	303 (1.6)	305 (3.8)	326 (2.3)
Female (%)	9065 (88.6)	16746 (90.2)	7309 (91.5)	12880 (91.7)
Not including damaged				
Unfed %	13.9	8.8	20.5	9.8
Blood-fed %	1.5	1.6	2.5	1.1
Semigravid %	0.3	0.2	0.8	0.2
Gravid (%)	7643 (84.3)	14977 (89.4)	5568 (76.2)	11458 (89.0)

Table 5.3 The medians, interquartile ranges (IQR), incidence rate ratios (IRR) and 95% confidence intervals (95%CI) of the total number of gravid female *Cx. quinquefasciatus* collected in intervention and control arms in the 4 different seasonal Rounds: Round 1, dry hot; Round 2, monsoon; Round 3, dry cool and Round 4, dry hot from a negative binomial regression model.

Culex quinquefasciatus		(Median, IQR)	IRR	95%CI	p-value
Overall	Control		Ref.		
	Intervention				
Round 1	Control	10.5 (0-24.5)			
	Intervention	13 (0-28)	1.05	[0.77-1.44]	0.747
Round 2	Control	15 (0-48)			
	Intervention	18.5 (0-49)	0.89	[0.59-1.33]	0.570
Round 3	Control	5 (0-19)			
	Intervention	9 (0-24)	1.56	[1.07-2.27]	0.020
Round 4	Control	24.5 (3-49)			
	Intervention	15 (0-28.5)	0.57	[0.41-0.78]	0.001

When the difference between intervention and control arms was analysed by *Round*, only *Round* 3 (dry cool season) and *Round* 4 (dry hot season) showed significant differences (Table 5.3). In *Round* 3 there was a 56% increase in the number of mosquitoes seen in the intervention arm when compared with the control arm (IRR=1.56; 95%CI=[1.07-2.27]; p=0.020). This was reversed when the data were analysed for *Round* 4; when significantly fewer mosquitoes were captured in the intervention arm when compared with the control arm, a difference of 43% (IRR=0.57; 95%CI=[0.41-0.78]; p=0.001).

5.4.3 Lymphatic filariasis detection

All the gravid females of *Cx. quinquefasciatus* were tested for the presence of *W. bancrofti*. Altogether, 1984 pools of mosquitoes were tested of which 16 were positive (0.8%) (Table 5.4). The maximum likelihood estimate of infection prevalence within the population of gravid *Cx. quinquefasciatus* was 0.0004. The 95% confidence intervals based on the likelihood ratio is 0.0002 – 0.0006. A logistic regression model was used to compare the mosquito infection rates between control and intervention villages. There was no statistical significance in the difference between the two groups (z=-0.24, p=0.808).

Table 5.4 Total numbers of gravid female *Cx. quinquefasciatus* tested and pools positive by PCR for *W. bancrofti* after capture by CDC gravid traps during a cRCT on sanitation in Odisha.

Trial arm	Total *Cx. quinquefasciatus*	Number of pools tested (20 mosquitoes/pool)	No. of positive pools	% positive pools
control	21090	1056	9	0.85
intervention	18556	928	7	0.75

5.5 Discussion

There was no difference in numbers of gravid female *Cx. quinquefasciatus* between intervention and control arms, and prevalence of LF infection in gravid female *Cx. quinquefasciatus* was similar for both intervention and control arms.

5.5.1 Mosquito collections

In contrast to catches using gravid traps in Africa, far fewer mosquitoes were collected during this trial (Mboera et al., 2000, Irish et al., 2015b). However, the numbers of mosquitoes caught do appear to be comparable or slightly less than other experiments conducted in similar ecological regions and environmental conditions within the Indian subcontinent; Indoor resting catches have previously caught between 0-25 *Culex*/trap/day (WHO, 2006). Gravid traps with oviposition bait used in Sri Lanka caught approximately 28,700 from 600 trap nights (Rao et al., 2016) and gravid

traps with oviposition bait in Tamil Nadu caught 5012 *Culex* from 207 households in one site and 5311 mosquitoes from 231 households at a second site (Subramanian et al., 2017).

Reduced numbers caught during this trial in comparison to published literature may be due to the lack of infusion used to attract mosquitoes to the gravid trap. Unlike other studies where infusions of organic material have been used to attract *Cx. quinquefasciatus*, specifically gravid females, this study used tap water in the trap. Other types of oviposition lures that can be used to attract mosquitoes to the gravid trap are nonanal, Chemtica or AtrAedes lures (Irish et al., 2012, Irish et al., 2014, Irish et al., 2015a). Despite these studies showing the relative attractiveness of these lures to gravid mosquitoes, they have not been tested in large monitoring programmes. The lack of lure notwithstanding, this study captured a substantial number and high proportion of gravid mosquitoes (75-95% of all captures) with just water. The household collection of *Culex* mosquitoes by gravid traps proved an efficient way to gather large numbers for the purposes of xenomonitoring populations of mosquitoes that are capable of transmitting LF. The trapping efficiency was not dependent on the season in this trial.

Culex quinquefasciatus was the dominant species collected, in all seasons, in both the control and intervention arms of the trial. Similar numbers of gravid female *Cx. quinquefasciatus* were seen in both the control and intervention villages. Previous trials that studied methods of controlling *Cx. quinquefasciatus* focused on ways to block the mosquitoes from breeding in the septic tanks of the latrines (Curtis, 1980, Maxwell et al., 1990, Curtis, 1991, Clements, 1999). Expanded polystyrene (EPS) beads have been used to prevent the emergence of mosquito larvae and egg laying from pit latrines. In Zanzibar, the reduction in light trap catches due to EPS beads was 98.3% (Maxwell et al., 1990). In India, reduction in *Culex* collections due to use of EPS beads in latrines and larvivorous fish in wells, was over 90% (Sunish et al., 2007). In urban Dar es Salaam, Tanzania, Chavasse et al. (1995) found that EPS beads (in latrines/septic tanks) and pyriproxyfen (in blocked drains, flooded land) reduced *Culex* populations by 76.7% and 46.2% in the two study areas. The disadvantage of EPS

beads is the potential effect on the environment. EPS beads are not biodegradable and should only be used in secure containers.

Sites with organically rich waster, such as cess pits, septic tanks and drainage ditches are favoured oviposition sites of *Cx. quinquefasciatus* (Boakye et al., 2007, Bockarie et al., 2009). An important factor potentially affecting oviposition site availability was that the rate of latrine use was much lower than the rate of latrine coverage. Latrine coverage in control villages was <10%, compared to 63% in the intervention villages. However, a cross sectional study of individuals (n=1933) within 20 of the study villages found that 37% of people never used a latrine, irrespective of latrine coverage (Clasen et al., 2012, Barnard et al., 2013). This might mean that the latrines were not providing convenient oviposition sites for mosquitoes close to houses. In addition, the study showed that 53% of 321 latrines surveyed were classified as not functional, due to either insufficient wall height (under 5 meters), a broken or blocked pan, a non-functional pit pipe connection or no entryway cover (Barnard et al., 2013). Non-functional latrines were dry and lacked faecal material for breeding. In several villages in this trial, the very top of the pit was above ground. Where the top of the pit was uncovered, the means of preventing mosquito access to the pit was through a concrete lid. In some cases, this was not sealed and would have provided easy access for the mosquitoes (Hiscox et al., 2016).

5.5.2 Xenomonitoring

Due to the large number of gravid female *Cx. quinquefasciatus* caught and the low infection rates expected, it was necessary to pool them for *W. bancrofti* infection testing. Despite the large numbers of pools assessed, only 0.80% of the pools were infected resulting in a very low population prevalence estimate and there was no significant difference between the control and intervention arms. Surveys in Tamil Nadu, India, before MDA coverage, put the estimated vector infection prevalence rate at between 2.2-2.7% and post MDA coverage at between 0.6-1.2% (Subramanian et al., 2017). The most recent estimate of vector infection rate before this trial in Puri district, Odisha was 1.3% with an infectivity rate of 1.4% (Sukhvir et al., 2008). However, criticism of the local

governments MDA programme shortly before the trial began had resulted in an increase in frequency of implementation, the most recent MDA *Round* being in June 2010 (NVBDCP, 2010). In areas where *Cx. quinquefasciatus* limitation occurs, it is possible to reduce the prevalence rates to minimal levels but difficult to eradicate without both MDA and vector control (Brito et al., 1998, Sunish et al., 2007). It has been observed in parts of India that transmission ceases when the overall infection rate falls below 1% (WHO, 2006).

It is possible that the use of conventional PCR (C-PCR) resulted in lower estimates of prevalence than if real time PCR (qPCR) had been used. Comparisons between the two methods have been undertaken and found that qPCR is a much more sensitive xenodiagnostic tool than C-PCR, although with re-testing of samples that produced different results, the agreement rate between qPCR and C-PCR results were similar (Rao et al., 2006). Optimisation of the methodology and decreasing prices of reagents, mean that the relative value of the two methods are comparative in terms costs (Vasuki et al., 2016). However, availability of the equipment required and the costs involved in maintaining the instruments mean that it is not always practical to run the a qPCR in smaller regional laboratories in comparison to national reference laboratories (Rao et al., 2006). Another disadvantage of C-PCR is the detection of all life stages within the mosquito (microfilariae – L3 larvae). For the purposes of xenomonitoring, only the detection of L3 larvae, the stage that is transmitted to humans, is required (Laney et al., 2010). Despite this, C-PCR is still the more sensitive and efficient method of xenomonitoring when compared to detection by dissection and microscopy (Vasuki et al., 2003).

5.6 Conclusions

In conclusion, the lack of difference in gravid female *Cx. quinquefasciatus* between control and intervention arms overall suggests that the pour-flush latrine coverage in general and more specifically improper construction of the pour flush latrines, in some villages, did not alter the population levels of *Cx. quinquefasciatus*. Despite the increase in latrine coverage, however, open defecation continued by many residents due to poor latrine quality and functionality or the absence

of a change in behaviour (Barnard et al., 2013, Routray et al., 2015).WASH programmes should ensure, where the emphasis is placed on latrine construction and coverage, that they are built to a standard that decreases, not increases the risk of breeding *Cx. quinquefasciatus*. The significant difference in numbers caught in the control and intervention arms caught in *Round* 3 and *Round* 4 provides evidence of the variable nature of focusing on only one method of control and that multiple methods of control, EPS beads or ULV fogging should be considered to successfully supress the vector population.

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Chapter 6: Discussion

Evaluating the impact of interventions that affect vector populations is extremely important. The construction of latrines is part of sustainable development goal 6; aiming to provide access to sufficient sanitation facilities in all human populations worldwide by 2030. However, few studies have measured the health impact of building latrines (Patil et al., 2014) and even fewer have demonstrated the impact on fly populations (Islam et al., 2018). Improving access to sanitation removes faeces from the environment and should contribute to controlling three out of the four pathways of faecal contamination; from food, water and flies.

The initial experiments focused on surveillance methods for both synanthropic flies and *Culex quinquefasciatus*, finding that blue sticky traps for flies and gravid traps for mosquitoes were the best for monitoring population densities. These traps were used in the RCT to find that latrine construction had no impact on the density of flies or mosquitoes within and around households. The carriage of bacteria on flies was not different between intervention and control villages, neither was the prevalence of *W. bancrofti* in mosquitoes. The majority of synanthropic flies caught were either *Musca domestica* or *M. sorbens*.

6.1 Rationale and limitations of fly trap selection for entomological surveillance of the RCT

In addition to acceptability to residents, the type of trap to be used to complement the RCT needed to be able to catch a sufficient number of flies to determine the relative population densities of different species of interest, and collect them in a suitable condition to determine their relative bacterial loads for different species of bacteria It was found in the pilot study that a non-baited blue sticky trap, placed in the kitchen, collected the largest amount of synanthropic flies compared with a baited sticky pot trap or non-baited yellow sticky trap; in particular *M. domestica* and *M. sorbens*. Several different trap types were used in the pilot trial before coming to this conclusion each with advantages and disadvantages as described below.

The pilot study excluded baits that are primarily used for the capture of synanthropic flies in areas where humans are not always resident, because they were not acceptable for use around households due to the unpleasant smell of the baits For example, faeces has been used effectively for many years for the capture of synanthropic flies (Pickens, 1994, Pickens, 1995) because they are oviposition attractant for flies (Emerson et al., 2001). However, it is unacceptable to place faeces in an area of human habitation, primarily due to reasons of disgust from the odour. Furthermore, besides the difficulty of obtaining a sufficient quantity of faecal material required for a large scale trial, volatile attractants released from faeces vary based on the diet ingested previously by the animal source and are difficult to reproduce consistently between replicates (David et al., 2014).

Instead, baits that are more suitable for domestic use, e.g. sources of sugar, were tested in the pilot study. Sugars have also been used for a long time as a trap attractant for flies, mosquitoes and other vector species (Hogsette et al., 2002, Muller et al., 2008, Muller and Schlein, 2008, Kaufman et al., 2010, Muller et al., 2010). Sugar and water are essential for fly survival as they contain many necessary nutrients missing when adult flies emerge from pupae (Greenberg, 1959). Similarly, other vegetables and fruits, including tomatoes, contain nutrients that are necessary for the continued survival of the fly. It is common to see flies clustered around natural sugar sources; such as rubbish sites, bruised fruit or kitchens where food is being prepared (Pickens, 1994, Pickens, 1995). Around human habitation, there are many competing odours. A trap that does not use an odour more attractive than natural sources is unlikely to catch a large representative sample of the synanthropic fly population. This was seen in the pilot bait experiments conducted before the main trial. None of the baits tested caught significantly more than the un-baited control trap, suggesting that they were not sufficiently competitive. Since non-baited traps caught sufficient flies for testing, the evidence showed that odour baited traps are not always required for entomological surveillance.

The use of additional visual cues, beyond selecting a blue versus a yellow sticky surface, were also considered prior to opting for the trap used for entomological surveillance in the RCT. For example,

it was shown that flies tend to cluster in a previous trial (Chapman et al., 1999). Inducing one fly to land on a trap provides a visual stimulus for other flies to land subsequently. Clustering behaviour has been exploited in one trap design which included a dark spot, to simulate a landed fly, in an attempt increase trap catches (Chapman et al., 1999). Chapman *et al.* found the addition of clustered spots, together with pheromone, greatly increased the trap catches. However, Hanley *et al.* found no such increase when using spots alone. The combination of olfactory and visual cues, the addition of cuticular hydrocarbons for example, may be necessary to improve trap catches (Hanley, 2009). Since no synthetic attractants with broad spectrum activity for the range of flies of interest were available, and because the unbaited sticky traps caught adequate numbers of flies for the purposes of entomological surveillance for the RCT, no additional visual cues were used. The sticky traps were only used once and so a continuous supply of traps were required, over 500 throughout the course of the trial and initial experiments. Traps cost approximately $0.50. Any modification would have been expensive and time consuming, taking away from the simple, cheap method of monitoring.

The largest limitation of sticky traps was the surface area available to catch flies. The surface area of the pot trap used in the pilot was approximately 177cm$^{2.}$ In contrast, the sticky trap used for entomological surveillance of the CRT was 490cm^2, almost three times the size of the pot trap. In the experiment comparing the two traps directly, almost three times the number of flies were caught on the sticky card trap when compared with the sticky pot trap. This was not necessarily an indication that the trap was better in terms of its attractiveness to the synanthropic flies of interest but that the large surface area enabled more flies to be caught. Other studies that have analysed surface area of traps have also found that increasing surface areas results in a statistically significant increase in trap catches (Lee et al., 2013, Oloo et al., 2014). It is possible that a trap that was three times the size of the sticky trap would catch three times as many flies. However, a trap three times the size would be more inconvenient to the residents and more likely to suffer accidental damage.

One problem of having a sticky substrate was that, on several occasions comparing baits, colours and pot traps and sticky traps, geckos were stuck on the traps. Geckos were presumably attracted to the captured flies; a readily available source of food. The presence of geckos did not appear to reduce the number of flies by either predation or diverting additional flies from landing on the traps; although these potential factors were not analysed statistically. The carcasses of flies, including legs, wings and heads, were seen on many traps. It was assumed that geckos or other fly predators had successfully eaten the flies, without themselves being caught, leaving their remains behind. There are few reports in the literature mentioning similar problems with sticky traps except for one study comparing different oils to use for capturing phlebotomines (Asimeng, 1988). It is possible that the position of the trap near to the ground, instead of hanging from rafters, facilitated gecko capture.

Although the blue sticky card trap was selected as the optimal trap for entomological surveillance of the RCT, questions remain as to whether this type of trap design would work in other environments. For instance, in areas where *M. domestica* and *M. sorbens* are not the most abundant species. The glue used to capture *M. domestica* and *M. sorbens* may not be strong enough to restrain larger flies like Calliphoridae or Sarcophagidae. Legs and wings attributed to *M. domestica* and *M. sorbens* were found on numerous traps throughout initial experiments and main trial. It is possible that these smaller diptera also escaped from the restraining glue. However, without witnessing this first hand, it was impossible to state whether it was due to this or other reasons, for example due to feeding geckos. Another limitation is that it proved very difficult to standardise the location of the trap as each household had a different layout; whether it was indoors, outdoors. Other factors, inclusing how much furniture was present in the kitchen or how large the area was also affected trap placement.

For fly surveillance, more work needs to be done to determine the best height to set the trap. Fly ribbons (spiral sticky traps) are hung up, usually from a roof beam or rafter, between 1-2 m off the ground (Rady et al., 1992). The present study used sticky traps set on the ground. It was determined

that this was sufficient for population density monitoring but its possible trap catches could have been increased if the traps had been set higher. Lindsay *et. al.* 2012 conducted similar experiments when designing a trap to catch *Chrysomya putoria* (Lindsay et al., 2012). In these experiments, it was found that traps placed lower to the ground and caught significantly more flies. Lid colour did not increase the capture of flies (Lindsay et al., 2012). While this experiment was conducted in a different environment and capturing a different fly, the results suggests that the placement of the trap on the ground was optimal for fly capture.

6.2 Population density of synanthropic flies and bacterial load in the RCT

The entomological surveillance conducted within the RCT found that there was no difference between the density of flies in both intervention and control villages or in the carriage of the four bacteria of interest Unlike trials using ULV spraying or insecticides to control fly populations, this trial did not demonstrate a difference between the villages with latrine coverage and villages with low latrine coverage (Chavasse et al., 1999).

In comparison to other control methods, latrine construction would not be termed an active control measure, as there is no direct killing mechanism. This is because the construction of latrines is not directly targeted at the control of *M. domestica* but could provide an added benefit if the latrines are constructed properly and used correctly (Collinet-Adler et al., 2015). Unlike ULV or insecticides, latrine construction prevents access to breeding sites rather than killing adults or larvae (Lothrop et al., 2007). However, the latrines can also increase populations if it is damaged or not well constructed (absence of a roof or permanent walls) (Irish et al., 2013a). There would be a long-term impact on fly densities from reducing breeding sites. However, spraying with insecticide has an immediate, if short-term effect, on fly densities. (Emerson et al., 2004).

It proved challenging to strike a balance between needing to know about population densities and bacterial carriage. Traps were left for 24 hours in the field, primarily because synanthropic flies are diurnal (Hertz et al., 2011). In order to accurately capture a full active day, the trap was left in place

from sunrise to sunset. Furthermore, from a logistical point, leaving traps for 24 hours meant fewer visits to households so was less obtrusive to occupants residents. However, the longer the traps were left in place, the greater the risk of bacterial contamination from sources external to the fly or between flies (van Elsas et al., 2011). A working kitchen within a household has a lot of movement and there are many sources of potential contamination, for example dust from the floors being swept.

Despite the logistical benefits, homogenising the entire fly caught on a sticky trap, as used in the present study, can over-represent the bacterial transmission potential of the fly. Whole fly homogenates, contain bacteria from the gut of the fly which would not survive the process through the digestive tract and so may not be representative of those deposited in vomit drops or faecal spots while feeding (Lindsay et al., 2012). Studies using spot cards to collect the faecal and vomit drops from flies can provide evidence regarding the amount of bacteria that could be viable after transmission via this route but cannot be used to identify which flies are responsible for transmission (Brazil et al., 2007). A study by Nazni et al. (2005) used an autoclaved and sterile glass slide containing a few drops of sugar solution to collect faecal and vomit drops left from a feeding fly (Nazni et al., 2005). This method seems superior as it allows the capture of internal as well as external bacteria. However, it suffers from the same draw back as spot cards in that, unless watched closely or the fly is captured immediately, it is impossible to tell the species of fly responsible for the bacteria. Furthermore, as with sticky traps, environmental contamination could be an issue when the slides are exposed to the environment for a long time (van Elsas et al., 2011). Swabs of surfaces from around the trap area would help to identify potential environmental bacterial contaminants.

Therefore, in an attempt to confirm whether the results seen from cultures produced from homogenized flies caught on sticky traps was representative of the actual bacteria transmission potential, sweep nets catches were made to obtain live flies that were either cultured using homogenized whole flies or live flies released onto agar. As it was much more labour intensive to

catch live flies and transport them for culture, far fewer were caught and used in the experiment than those collected using sticky traps. The sweep net capture and live fly agar culture found comparable proportions of flies were positive as obtained by whole fly homogenisation. The low sample size of live flies available for testing may explain the disparity of results using this method compared to the published literature with sticky trap caught flies where a lower percentage of each bacteria was isolated (Sukontason et al., 2007, Lindsay et al., 2012). The assumption is that not all the bacteria being carried by flies will survive the journey through the fly gut, or if viable, will actually be transmitted by the fly (Greenberg, 1969, Talley et al., 2009).

Escherichia coli and *Salmonella* were the most commonly isolated bacteria in this study, comparable with other studies (Forster et al., 2009, Chaiwong et al., 2014). These bacteria are considered to be more robust than *Shigella* and *V. cholera* as they survive much better in hostile; more acidic, less oxygen rich, environments (van Elsas et al., 2011, Finn et al., 2013) suggesting that they will frequently be isolated from flies.

PCR is a very sensitive way to detect a multitude of bacterial and viral pathogens transmitted by flies. The disadvantages are that primers are needed for every type of bacteria or virus of interest and PCR cannot differentiate between inactive or viable bacteria; possibly leading to an over estimation of transmission potential (Rogers et al., 2010). However, PCR was shown to be more sensitive to multiple bacterial infections compared with culture methods (Aydemir et al., 2014). Agar culture is less expensive than PCR, although is a more time intensive methodology since agar plates need to be incubated overnight. The advantage of PCR is that samples can be pooled. A caveat is that PCR is not suitable for an assessment of multiple sources of faecal transmission as specific primers are required for detection of pathogens.

Not all diarrhoeal causing pathogens were studied in the entomological surveillance in the RCT. The aetiological agent most responsible for diarrhoea within India is rotavirus (Tate et al., 2014). However, it was decided to limit pathogen detection to just bacteria, and specifically the four

studied, based on the results of main causes of diarrhoeal related hospitalisation from hospital records in the region of Odisha (Samal, 2008). The inherent flaw is that not every person that gets sick will be able or willing to visit a hospital, due to the costs, distance or feeling that the illness is not severe enough to warrant a visit to hospital (Roy et al., 2015). It is possible that there is under-reporting of the main causes of diarrhoeal disease within the region and only focusing on the four bacteria limited the scope of this trial. However, they were sufficient to act as markers to ascertain the relative decrease or increase in diarrhoeal disease transmission.

6.3 Rationale for and limitations of mosquito trap selection for entomological surveillance of the RCT

The three most common traps used for collecting mosquitoes are BG-sentinel traps, light traps and gravid traps. The BG sentinel trap is designed to catch ovipositing *Aedes* mosquitoes (de Azara et al., 2013). Gravid traps are designed specifically for catching ovipositing *Cx. quinquefasciatus* mosquitoes and light traps are designed to catch species of phototropic mosquitoes with the exception of *Aedes aegypti* (Chen et al., 2011, Irish et al., 2013c). The pilot study focused on comparing gravid and light traps to determine the best means of gathering large quantities of mosquitoes for xenomonitoring (MX) of *Cx. quinquefasciatus.* Gravid traps caught significantly more *Cx quinquefasciatus* than light traps and, although the proportion of captured males to females was the same for both light and gravid traps, caught a much higher proportion of gravid mosquitoes while light traps caught a much higher proportion of unfed mosquitoes.

Logistical constraints, in addition to collection ability, should also be taken into consideration when selecting a trapping method for the entomological surveillance of an RCT or for a xenomonitoring system. Light traps, using light as the sole attractant for mosquitoes, are easier to transport than gravid traps and require fewer resources to set up. Gravid traps usually require an oviposition attractant to function efficiently (Irish et al., 2013b, Irish et al., 2014, Irish et al., 2015a), such as a

grass or organic material infusion. The preparation required to create this infusion is considerable: a minimum of 2 days, a significant quantity of grass on a daily basis and a large quantity of water (Irish et al., 2012). The standardisation of such an infusion is difficult to achieve, especially in the context of a monitoring programme where a large quantity of traps and the resources needed to make the traps function are required. It has been shown that water works to attract *Cx. quinquefasciatus* but generally the trap catches are smaller with a lower proportion of gravid mosquitoes than using water combined with an oviposition attractant (Irish et al., 2013b). Despite gravid traps collecting significantly more *Cx. quinquefasciatus* in the present study, the proportion of males to females was the same for both of the traps. By using an attractant, in addition to water, it is probable that not only the quantity of *Cx. quinquefasciatus* caught would increase but also the proportion of gravid females for testing in the context of a MX programme (Irish et al., 2013c, Irish et al., 2015b).

In one study, light traps collect a larger proportion of blood-fed females compared to gravid traps (Irish et al., 2015b), but this was not found to be the case in the present study where gravid traps caught significantly more gravid mosquitoes compared with the light traps and the proportions of blood-fed mosquitoes were similar for the two different types. Blood fed mosquitoes are more likely to be caught in light traps when the population does not use or sleep under insecticide treated bed nets. Blood-fed mosquitoes are more likely to be infected with parasites that have survived bloodmeal digestion. As has been shown, infection rates in mosquitoes caught using CDC light traps can be much higher than in gravid traps, potentially giving a much better estimation of transmission potential (Irish et al., 2015b).

Differences in the positioning of the two traps can also affect the eventual estimation of infection within the mosquito population. Light traps are set within houses and close to where people sleep at night. This means that the proportion of blood-fed mosquitoes is usually higher than in the gravid traps that are usually placed outside of the house, although what has been shown is that light traps

next to sleeping areas can provide a better estimate of infectivity as the mosquitoes captured are intending to bite humans (Irish et al., 2015b). Whereas gravid traps catch a higher proportion of gravid mosquitoes, these mosquitoes could have fed on animals as well as humans within the vicinity. Insecticide treated bed nets were given to every household as part of the ethical approval for the work in houses. Although it was noted that the majority of residents did not use bed nets, it is possible that the presence of the insecticide had a negative effect on the light trap catches compared to the gravid traps.

6.4 Population density of *Culex quinquefasciatus* and *Wuchereria bancrofti* prevalence in the RCT

This study found that there was no difference in the number of *Cx. quinquefasciatus* captured between control and intervention villages and that there was no difference in the prevalence of *W. bancrofti* between intervention and control arm. Previous estimates of the filarial prevalence in Cx. quinquefasciatus captures in Odisha were around 1.3% (Sukhvir et al., 2008). The lower prevalence rate of 0.8% found in the present RCT is consistent with pooled results from a LF programme in Tamil Nadu that was conducted after sustained MDA campaigns in the region (Subramanian et al., 2017).

t is important to ensure that reasonably defined clusters are used for sampling because areas that have low or no persistence of mf infection in mosquitoes can dilute areas that have high infection rates (hotspots), distorting the conclusions of the survey (Farid et al., 2007b). Conversely the sample size to calculate the infection rates needs to be large enough to capture accurate rates of infection and houses need to be sampled randomly to get an overall estimate of prevalence. (Irish, 2018). Problems testing *Cx. quinquefasciatus* for the presence of *W. bancrofti* can arise if there are ongoing, supplementary vector control programs running alongside MX assessments. The results from Sri Lanka highlight the difference between the Transmission assessment survey (TAS) results, the marker for elimination of lymphatic filariasis, and MX results (Rao et al., 2016). The TAS results showed that the area had reached a level of prevalence low enough for MDA to stop. However, the

MX showed that there were hotspots present. It was suggested that, since MX is a sensitive tool for the assessment of LF prevalence, it should be used alongside TAS in determining whether MDA has been successful and LF is reduced to a sufficiently low level of risk for the population (Moustafa et al., 2017).

There are limitations with MX relating to seasonality. For example, Irish *et al.* did not manage to collect sufficient quantities of *Cx. quinquefasciatus* based on the sample size calculations and one of the reasons suggested was lack of information before starting the study on the seasonal distribution of *Cx. quinquefasciatus* which varied significantly according to trapping season (Irish 2018). There was a massive increase in mosquito populations during the monsoon, due to the abundance of aquatic habitats available, which is probably the best period to undertake MX surveys. In contrast, far fewer mosquitoes were captured during the dry hot and dry cool seasons, correlating with the decrease in populations usually seen when the temperatures increase above 40°C and below 20°C average (Rueda et al., 1990), suggesting that these would be poor season to perform MX surveys. The higher temperatures can kill L3 in the mosquito. Increased mortality of mosquitoes at high temperatures will also result in decreased L3. The overall estimation of prevalence of mf within the vector population was so low in the intervention and control arms that it was decided not compare the difference between seasons. However, if the overall prevalence had been higher within study, it would have been interesting to see if there were any changes in the prevalence of infection related to the impact of temperature and rainfall on the mosquito populations.

Probably the biggest barrier to successful uptake of MX, as standard after or with TAS and post MDA, is the operational capacity of regional centres. While the mosquito trapping itself is labour intensive, training can easily be given on the best positions to locate traps to maximise capture. Species identification, while more specialised and requiring experience, does not require technological capacity unlike the final pooling and testing by PCR for mf presence. In Sri Lanka, night blood surveys were performed to determine the prevalence of mf within the human population. Recent work by

Rao (2016) showed that MX surveys were more sensitive in detecting *W. bancrofti* in areas with residual filariasis activity post MDA, especially in the detection of hotspot areas (Rao et al., 2016). Checking blood samples is more labour intensive than setting gravid traps and the latter is less intrusive overall to the household being tested.

Two sub-species of the *Cx. pipiens* complex, *Cx. quinquefasciatus* and *Cx pipiens*, are almost morphologically indistinguishable. The females can usually be distinguished by differences in wing venation and males definitely distinguished by differences in the structure of the genitalia (Mattingly PF, 1951). It is possible to differentiate between the two by PCR but this adds to the overall costs involved of a RCT and for any LF elimination programme (Smith and Fonseca, 2004). Published literature suggests that *Cx. quinquefasciatus* is the predominant species of the complex within India and Odisha (Shaikevich et al., 2016). This study did not use molecular methods to confirm whether the species captured was *Cx. quinquefasciatus* due limited budget and the expense required to test thousands of mosquitoes.

Pathogen detection was only tested in *Cx. quinquefasciatus*, recognised as being the primary vector of LF within India (Chandra et al., 2009). There were several other potential vectors caught throughout the trial however, that could also have been tested for the presence of LF, i.e. *Anopheles* and *Mansonia*. The fact that such low levels of infection were detected with gravid *Cx. quinquefasciatus* pools suggests that extremely low levels of infection would probably have been found within the secondary vector populations. It is possible that as more control methods are focused specifically on *Cx. quinquefasciatus*, the importance and role of secondary vectors which are not targeted increases in comparison (Pichon, 2002). Future studies in the area should consider testing all potential vectors, given sufficient time and resources to do so.

Despite the possibility of using MX as a tool to ascertain *W. bancrofti* transmission, Farid *et al.* 2007 suggested that there is little correlation between human and mosquito infection rates (Farid et al., 2007a). This was based on comparing infection status of household residents and parasite status in

mosquitoes captured from the same houses. A study by Subramanian, 2017 that similar trends were seen in the human and mosquito populations (Subramanian, 2017). In this study, no human testing was performed to detect prevalence of mf within the population. It is impossible to say whether the low levels of *W. bancrofti* detected in the vector meant that there was very little active infection within the human population or risk of acquiring the infection in the future. There are a variety of factors that can affect the transmission of *W. bancrofti* from the vector to the human population (Mahanta et al., 2001). Based on the vectorial capacity formula, the man biting rate, the parasites extrinsic incubation period, the daily survival of the vector population and the ratio of mosquitoes to humans, all contribute to pathogen transmission (Macdonald, 1956). *Wuchereria bancrofti* infection rates within the vector can be influenced by the methods used to catch the vector as well as the physiological status of the vector tested for infection (WHO, 2002).

In addition to testing for *W. bancrofti* infection in mosquitoes, it might be useful to conduct a bloodmeal analysis of any blood-fed mosquitoes. While *Culex* might be anthropophilic, they are also known feed on birds and other mammals (Samuel et al., 2004). While it was assumed they were feeding primarily on humans in the area, the abundance of other animals present means that humans might not be their only food source. As the use of insecticide treated bed nets and insect repellents increases, it is possible that a shift in feeding habits will result in a decrease in pathogen transmission potential despite high mosquito populations (Meyers et al., 2016).

6.5 Limitations of entomological surveillance in relation to the RCT

6.5.1 Sample Size

Whereas the pilot studies determined a suitable trapping method for flies to be used for entomological surveillance in the RCT were performed within the urban slums of Bhubaneswar the RCT was performed in rural villages. However, an urban slum and a rural village in India, while having some similar aspects, are different in terms of the environmental setting. Open defecation occurs much closer to habitation and in specific areas in urban slums compared with rural villages where

defecation usually takes place in surrounding fields. Although the ownership of animals and the quantity owned is much higher in villages, they are allowed to roam more freely over a wider area than in the urban areas.

Fly populations are subject to a variety of factors other than the availability of breeding sites. High and low temperatures, rainfall, readiness of food sources as well as abundance of breeding sites, affect the complicated life cycle of flies. A study by Ngoen-Klan (2011) found that the population densities of *M. domestica* increased with humidity, temperature and rainfall (Ngoen-klan et al., 2011). More *M. domestica* were captured in rural areas in the study, compared with urban ones, although primarily in residential areas, suggesting that factors other than presence of humans contribute to the abundance of flies in the area, like presence of animals (Collinet-Adler et al., 2015).

6.5.2 Rural sanitation control programme

The trial presented issues: the level of coverage achieved in intervention villages was not as high as was expected. Latrine construction was delayed or was still ongoing in many villages during the trapping rounds, and the actual estimate of latrine usage was far lower than the coverage, all of which may have explained the lack of impact (Clasen et al., 2014). Low use of latrines varied from men to women. For women, the custom is to go in groups to defecate for security but more importantly, this would form part of a social ritual with other women, away from household chores. For men, it is often more convenient to open defecate as they work away from the home in fields where latrines are not readily available. For both groups, seasonality effects open defecation as it is dependent on the availability of water for anal cleansing (Routray et al., 2015).

6.5.3 Limitations of RCTs

Randomised control trials are the gold standard of collecting evidenced based research. Despite this, there are limitations inherent in the design of RCTs. The information gathered is difficult to apply to different ecological or environmental settings (J. Bulpitt, 1983). When the nature of the data is specific a particular setting, as is the case with many vector borne diseases, it is hard to extrapolate

how a similar intervention will function if the environment is different. At minimum, 2 RCTs are required in order to produce data that can be generalised and scaled in settings other than where the RCT was undertaken. Vector control methods can also be specific to an ecological region or require behaviour change from the study population to maintain control. Willingness to maintain control methods is dependent on the local government and population and varies in different countries and regions. The effectiveness of interventions can lessen when the researchers monitoring the impact leave and motivational campaigning ceases (Luby et al., 2009). In the case of controlling diarrhoeal diseases by improved sanitation; behaviour change is key to long term sustainability. Without educational messaging, latrines will not be used even if constructed and open defecation will continue, leading to the ongoing risk of flies transmitting diarrhoeal pathogens. An alteration in how rubbish is stored and disposed of is also required but there are potential external factors that can effect the success of such measures. If there are no safe disposal sites or businesses willing to clean faecal sludge from pits to maintain the effectiveness of a sanitation intervention, the entire system of changes can collapse. Continued support from the government as well as household investment and understanding of the need for sanitation and hygiene services are key to promoting latrine usage.

6.6 Future Work

6.6.1 Flies and bacteria

There is now a lot of data to show that flies carry bacteria (Greenberg, 1973, Khin Nwe et al., 1989, Sukontason et al., 2007, Chaiwong et al., 2014). There is little data to show to what extent they are responsible for the incidence of diarrhoea episodes and that controlling flies reduces transmission (Esrey, 1991, Emerson et al., 1999). This can only be fully demonstrated by an RCT. Ideally, a dose response relationship needs to be observed between the numbers of flies, carriage and quantity of bacteria and diarrhoea cases. If the relationship between bacterial contamination on flies and episodes of diarrhoea within a community can be established, it might be possible, for future

intervention campaigns, to determine a percentage reduction in flies that would be needed in order to have an impact on fly borne diarrhoeal incidences. Using all of the information on the effect of control measures of flies, a model could be developed to calculate thresholds for fly reduction to eliminate the fly transmission of diarrhoeal pathogens (Stauch et al., 2014).

It is also possible to source the origins of bacteria using microbial source tracking (MST); whether they are from human or animals and this would allow a more targeted approach when considering control measures for fly populations and the potential pathways of bacterial contamination (Odagiri et al., 2016).

Despite being a disease that has not been reported in Odisha previously, there are a large number of blindness cases within the state of Odisha and it is possible that a few have been misdiagnosed and could be the result of trachoma (Chidambaram et al., 2018). A large portion of the synanthropic flies captured was *M. sorbens*. It would be interesting in future work to study whether *Chylamydia trachomitis,* the bacteria responsible for trachoma, is present in the flies.

6.6.2 *Culex quinquefasctisus* and lymphatic filariasis

For MX to be widely used and effective, the cost benefit of its use and the best point in an LF campaign for it to be used need to be ascertained (Rao et al., 2016). As MDA campaigns reduce the parasite burden in humans and interrupt transmission, larger samples of mosquitoes will be needed to detect infection within the mosquito population (Farid et al., 2007a). With the ability to pool mosquitoes, this reduces time and labour for the molecular analysis but capturing more mosquitoes requires either more traps or more efficient methods of collection, all of which increase the cost. The best time to use MX is when the levels of transmission are low (WHO, 2002). The benefit of using MX would then be two-fold; it would avoid the need to test the human population that would be certified free of LF after a successful MDA campaign but would allow the possibility of monitoring reinfection from neighbouring regions that have not had effective MDA campaigns (Subramanian et

al., 2017, Mehta et al., 2018). To take full advantage of MX, the issue of the best but most cost effective attractant to use in the gravid traps should be resolved.

6.6.3 Integration of vector control and monitoring methods

The study of diarrhoeal pathways and LF is not one that has been considered in the same body of work before. However, the construction of latrines can have an effect on multiple diseases and their transmission pathways. Many control programmes are attempting to integrate their methodologies in an attempt to reduce the costs of control, the effectiveness and to prevent unnecessary overlap (Chanda et al., 2013). This would allow communities to benefit from interventions without the ongoing disruption that multiple, follow-on programmes would have. Programmes that focus on WASH should consider integrating control methods for both flies and other disease vectors. Integrated vector management involves using several different control methods against one particular vector that can be responsible for multiple diseases. In this case, the effective use of water and maintenance of latrines combined can have a control effect on multiple vectors (WHO, 2016).

6.7 Conclusions

Incorporating vector surveillance into a latrine construction RCT is not simple. Most vector trials usually focus on what impact a control method will have directly on the populations of vectors. This trial was designed specifically to investigate the impact of latrine construction on the health of the village communities, relating to diarrhoeal disease, a more passive control method designed to reduce breeding sites compared with using an insecticide to actively kill flies. The vector work was conducted to ascertain whether there was an effect on vector populations. A 30% reduction in fly numbers, while not definitive would have shown strong evidence of the effect of latrines on fly numbers. However, due to the complexity of the transmission routes and the level of interdependency each route has on another it was not possible to show a reduction in synanthropic fly numbers.

The lack of effect of latrine construction on the fly populations and their bacterial load, should not prevent further attempts at exploring the importance of fly control in diarrhoeal disease prevention. The routes of transmission for faecal contamination are complex. Only focusing on one pathway will not reduce transmission when compared with controlling all the pathways of contamination. Water treatment, cooking food properly, hand hygiene and adequate sanitation are interlinked and only focusing on sanitation allows the other pathways to continue acting as sources of contamination.

AWith regards to flies, other sources of contamination can contribute to high levels of bacteria and abundance of flies. While having human faeces as preferential breeding sites, animal faeces and garbage disposal sites also affect fly densities. Latrines need maintenance and are only truly effective at preventing the spread of disease if they are used. Similarly, allowing the latrine and pit to fall in to disrepair or supplying vent pipes without screens provides an opportunity for flies and mosquitoes to gain access to breeding sites, potentially rendering latrines as a method of control ineffective (Curtis and Hawkins, 1982, Huttly, 1998, Emerson et al., 2004, Emerson et al., 2005). Interventions that integrate multiple methodologies should be the most effective; fly control through spraying or larvicides, water filters or chlorine tablets, soap and handwashing is needed to gain the most impact (Mishra et al., 2011, Chanda et al., 2013, Lizzi et al., 2014, Anogwih et al., 2015). Without health education to supplement the building of latrines, low usage can lead to situations where they are not as effective as they otherwise could be (Belmekki, 2004).

6.8 References

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Appendix 1: Ethical Approval

1.1 Ethical approval application LSHTM

London School of Hygiene & Tropical Medicine

Keppel Street, London WC1E 7HT *(Version 07 May09)*

Ethics Committee

Application to conduct a study involving human participants

For use of Ethics Committee only	Application No.	5561

Title of Project ASSESSING IMPROVED SANITATION FOR PREVENTING DIARRHOEA AND HELMINTH INFECTION	
Name of Principal Investigator (PI) at LSHTM	Thomas F. Clasen, JD, PhD
Appointment Held (or Research student)	Senior Lecturer
Unit/Department	DCVB/ITD
Medically qualified	NO
Are you a member of a medical protection organisation?	NO
Other personnel involved	Sophie Boisson, Wolf Schmidt, Jeroen Ensink, Adam Biran, Steven Sugden, Sandy Cairncross
Signature of Principal Investigator	[redacted]

Name and email of investigator dealing with correspondence if different from Principal Investigator	thomas.clasen@lshtm.ac.uk
If Research student: Name, signature and approval of Supervisor	
Grant reference/RCA reference code	

Is this a DrPH Professional attachment ?	NO
Is this study using anonymised secondary datasets only	NO
Fastrack — Applications in these categories will be dealt with by Chair's action	

Does this study involve the taking of blood and/or any other tissue?	NO

I approve this project scientifically.	Approval
Signature of Head of Department Date	Signature of Chair of Ethics Committee
Received by Ethics Committee	
Signature of Ethics Committee Administrator Date	Date

	Give an outline of the proposed project. Sufficient detail of the protocol must be given to allow the Committee to make an informed decision without reference to other documents. (Additional material should only be attached if considered absolutely necessary). *Max 300 words* **DO NOT ATTACH FULL PROTOCOL. APPLICATIONS WILL BE RETURNED.**
Answer: Expand box to fit	WaterAid India plans to undertake an assessment of its sanitation programme in Orissa. The assessment is primarily designed to evaluate the effectiveness of the programme and to learn lessons that may help optimize the delivery and uptake of the intervention. At the same time, WaterAid India and its affiliate, WaterAid UK wish to take advantage of the assessment in order to help fill the evidence gap about the effectiveness of sanitation interventions in preventing diarrhoea and helminth infection. They have therefore asked LSHTM to work with Indian partners to design and undertake the assessment in a manner that provides the most rigorous information about the effect of the programme on these outcomes. In order to minimize the impact of the trial current programmatic activities, the assessment will follow a step-wedge design. The sanitation intervention will be rolled out to randomly among the eligible villages. While all of the participating villages will receive the intervention when the programme reaches them over 12-24 months, the villages who have not yet received the intervention will serve as the controls for those that have received latrines. This step-wedge design allows us to continue to make progress on the overall project while collecting necessary data in a manner designed to meet the rigorous methodological requirements of a randomized controlled trial (RCT).
	State the intended value of the project. If this project or a similar one has been done before what is the value of repeating it?. Give details of overviews and/or information on the Cochrane database. *Max 300 words* *This area is of increasing importance – please ensure you give a full response.*
	Clasen and colleagues have recently submitted a Cochrane review that shows that there is little rigorous evidence from intervention studies that demonstrates the health impact of improved sanitation. This lack of evidence may be one reason for the fact that sanitation is the farthest off-track of all the MDGs. This assessment would result in the most rigorous evidence to date on the potential for improved sanitation to prevent diarrhoeal diseases and intestinal helminth infections. In addition to assessing the programme, the assessment seeks to provide policy makers and stakeholders with a reliable estimate of the effect of sanitation. It will also yield valuable evidence on the cost of the intervention, providing a basis to assess cost-effectiveness. The assessment will also provide an important opportunity to closely monitor use, maintenance and acceptability of improved sanitation—factors that may affect health impact, uptake and long-term sustainability. The study will also be designed to investigate anthropometric and non-health outcomes such outlays for disease treatment, lost days at work and school absenteeism. The proposed assessment will also provide the opportunity to study the epidemiology of diarrhoeal diseases and helminth infections in relation to

	sanitation in general, and will therefore be embedded in a number of related research activities aimed at corroborating the results and producing further epidemiological insights.
	Specify numbers, with scientific justification for sample size, age, gender, source and method of recruiting participants for the study. *Max 300 words*
	We estimated the sample size based on our primary outcome, diarrhoeal disease morbidity. We assumed a baseline mean longitudinal prevalence of diarrhoea of 1.7% among individual of all ages (1.7 days with diarrhoea per 100 days of observations). Based on previous studies in Asia, we estimated an intracluster correlation coefficient of 0.04 and a design effect of 7 to account for clustering at the village level (Van de Hoek, 2001). After adjusting the sample size for intermittent sampling, lost to follow-up, and loss of power due to the stepped wedge design, we calculated that 24 clusters per arm would be required to allow detecting a reduction of diarrhoea by 30% with 80% power and 5% level of significance.
	State the likely duration of the project, and where it will be undertaken.
	Following a 3-month period of formative research and piloting of the data collection tools, the assessment will be launched; it will continue for a minimum of 12 months and a maximum of 24 months. The actual length of the assessment will be determined by the rate of coverage. It will be terminated at any time after 12 months when latrine coverage reaches 90%, but will not go beyond 24 months (unless coverage is under 60%, at which point we would consider an extension and apply for further ethics clearance for the extension). The study will take place in eligible villages in the Puri district, state of Orissa, India. In Puri, WaterAid India currently focuses on five blocks (Puri, Pilili, Gope, Nimapada and Brahmagiri) with a plan to cover more blocks, in line with the government target to declare Puri an open defecation free district by 2012.
	Specify the procedures, including interviews, involving human participants with brief details of actual methods. *Max 500 words*
	The main purpose of the assessment is to determine whether sanitation reduces all-age diarrhoeal and parasitic diseases at the community level. As children are particularly vulnerable to these diseases, the study will be powered to allow separate analysis for children under the age of 5 years. The specific objectives are (i) to test the effectiveness of implementing improved sanitation (via pit latrines) on reducing diarrhoeal disease morbidity (primary objective); (ii) to test the effectiveness of implementing improved sanitation on reducing diarrhoeal disease morbidity in children under the age of 5 years; (iii) to study the effectiveness of improved sanitation on reducing intestinal nematode infections, (iv) to study the effectiveness of improved sanitation on weight gain in children under the

	age of 5 years; (v) to assess the effectiveness of the intervention on school attendance and performance, and (vi) to study changes in the distribution of diarrhoeal diseases and intestinal nematodes over the intervention period. In the context of the trial, it will also be useful to assess the cost of implementing the intervention (in order to assess cost-effectiveness) and actual use of the sanitation facility as well as economic, educational and other outcomes that are potentially associated with having access to improved sanitation. WaterAid India and its implementing partners will prepare a list of eligible villages where they plan to introduce the intervention (improved latrines, hygiene instruction and solid/liquid waste management) over the ensuing 24 months. After receiving full details and providing their informed consent, a baseline survey will be conducted and among all householders in the eligible villages. During the baseline, we will ask 2000 participants from 400 randomly selected households to provide stool samples for baseline helminth assays; this group will then be offered anthelmintics (distributed under the supervision of the Chief Medical Officer of Puri District) to establish a baseline for helminth infection. The order in which the intervention Locally-hired field investigators trained and supervised by a LSHTM trial manager will then visit all participating households once every month to obtain information on diarrhoea during the previous seven days. They will also collect economic (outlays for drugs and treatment), educational (absences at school) and other information, and will observe, survey and conduct small group discussions among a sample of intervention group clusters to obtain information on use, acceptability and maintenance of the sanitation improvement.
	State the potential discomfort, distress or hazards that research participants may be exposed to (these may be physical, biological and/or psychological). What precautions are being taken to control and modify these? Include information on hazardous substances that will be used or produced, and the steps being taken to reduce risks.
	Apart from the time taken at each visit to collect the information (and, if applicable, a stool sample), no discomfort or distress will be imposed on study participants. No special hazards are presented by the trial. Antihelminthics are recommended by the Secretary of Health for the State of Orissa, but are rarely available. Stool samples will be collected, prepared and analyzed in accordance with WHO and India guidelines.
	LSHTM risk assessment procedures are set out at http://intra.lshtm.ac.uk/safety/ (Travel Safety). Please confirm that all necessary procedures will be completed for all staff before fieldwork commences.
	YES

	Specify how confidentiality will be maintained with respect to the data collected. When small numbers are involved, indicate how possible identification of individuals will be avoided. *See guidance notes at:* http://intra.lshtm.ac.uk/committees/ethics/
	During the initial survey, information will be obtained on households and householders residing therein. Thereafter, all households and householders will be identified only by an assigned code without any names or other identifying references. No identifying information will be made available during or at any time following the conclusion of the trial.
	State the manner in which consent will be obtained and supply copies of the information sheet and consent form. Written consent is normally required wherever possible. Where not possible, a detailed explanation of the reasons should be given and a record of those agreeing kept. *See guidance notes at:* http://intra.lshtm.ac.uk/committees/ethics/ If research is on human tissue samples, PIs must refer to guidance notes at http://intra.lshtm.ac.uk/committees/ethics/ If any photographs are to be taken, whether for teaching or research purposes, ensure that the participant's consent to their use has been given in line with the provisions in *British Medical Journal, 1998,* ***316,*** *1009-1011.*
	Following an initial visit during which the purpose of the study will be discussed and all questions answered, community members will be asked whether they wish to participate. Those who agree to participate will be provided with a written form of consent using a prescribed form translated into the local language (attached). Heads of household (male and female) will be asked to sign two copies of the form. The first copy will be provided to the participant and the second copy kept by the investigators.
	State the personal experience of the applicant and of senior collaborators in the study in the field concerned, and their contribution to the study.
	Dr. Clasen has conducted household-based studies assessing the microbiological assessment and/or health impact of water quality interventions in Ethiopia, Vietnam, Peru, Bolivia, the Dominican Republic, Colombia, Bangladesh and India. He has also investigated emergency water treatment practices for the WHO in India, Sri Lanka and Indonesia. He will assist in study design, supervision, analysis and reporting. Dr. Wolf-Peter Schmidt, a clinical research fellow with experience in epidemiology and biostatistics has helped design the study and will oversee the analysis of the data. Sophie Boisson, an epidemiologist with field experience in India, Peru, Ethiopia and the Dominican

	Republic, will manage the study on site. Dr. Jeroen Ensink, whose expertise includes soil transmitted helminths, will oversee the helminth infection aspects of the study. Dr. Adam Smith and Mr. Matt Freeman will handle certain qualitative data collection and analyze information on school attendance. Steven Sugden will provide technical support on the latrine intervention and use. Dr. Richard Rheingans, a health economist from Emory University, will assist in connection with the economic analysis of the data. The host organisations will provide necessary logistics, staff, transportation and other on-site resources and support. We will receive local research support by the National Institute for National Institute of Cholera and Enteric Diseases, Kolkata and the Human Development Foundation of Bhubaneswar.
	State what medical supervision is available and its location in relation to the participants.
	Field investigators who observe any circumstances that present possible health issues during their household visits will, after discussing the same with the householder and obtaining their consent, refer the matter to local health workers.
	Will equivalent service or support to participants be available after the study ends? If NO, give details and describe steps to minimise loss of service or support.
	YES. The entire study population will be offered the intervention.
	If the aim of the study is to improve treatment or management indicate how successful treatment would be continued or expanded. See Guidance notes at http://intra.lshtm.ac.uk/committees/ethics/
	Not Applicable
a)	Does the project involve pre-marketing use of a drug/appliance or a new use for a marketed product?
	NO
b)	Does the company producing or providing any drug/appliance (whether pre-marketed, new use for marketed product or licensed use of marketed product) agree to abide by the guidelines on compensation for non-negligent injury of the Association of the British Pharmaceutical Industry (ABPI) ?

	If YES, a written statement from the company to this effect should be attached.
	Not Applicable
	Does this study involve the taking of blood samples and/or any other tissue?
	NO
a)	If YES Please list samples which will be taken
	Not Applicable
b)	Please confirm that you have undertaken the on-line training programme available at http://intra.lshtm.ac.uk/support/research/humantissueact.html and that you will ensure that any staff involved in the procedures for taking consent will also have undertaken an agreed training programme.
	Not Applicable
c)	If samples are taken overseas, will the samples be brought back to the UK
	Not Applicable
a)	Is the study a clinical/intervention trial of a medicine or any other form of treatment or management ? If YES please note the policy being applied by the International Committee of Medical Journal Editors (from 1/7/2005) that all trials must be registered before the enrolment of the first patient. Registers currently in operation are www.prsinfo.clinicaltrials.gov operated by NIH and www.biomedcentral.com/clinicaltrials *Lancet Vol 365 no 9474 1827-1831*
	NO
b)	Does the trial comply with Good Clinical Practice (GCP)?
	Not Applicable
c)	For clinical trials of medicines in the UK or EU please give details of CTA (Certificate of Clinical Trial Authorization).
	Not Applicable

d)	For clinical trials outside the UK or EU, please give details of regulatory approval.
	Not Applicable
e)	Is there a Data Monitoring & Safety Committee in place?
	Not Applicable If NO, please explain reason
	Will payments be made to participants? These should usually not be for more than travelling expenses and/or loss of earnings and must not represent an inducement to take part. If YES give details and justification.
	NO
a)	Where the research is to take place overseas, the Principal Investigator **must** seek ethical approval, through his/her overseas collaborators, in the country(s) concerned. Approval from the LSHTM Committee is dependent on local approval having been received. Please list the countries where research is being undertaken and arrangements being made to obtain local ethical approval:
	We will seek ethics approval from, and comply with any necessary requirements of, the Indian Council for Medical Research or one of their affiliated research institutes authorized to consider and act on applications to conduct research in India.
b)	Where the research is taking place in the UK, please list other UK Committees from which approval is being sought.
	Not Applicable
	Please give details of research sponsor. This is not necessarily the funding body. The sponsor is responsible for the initiation and management of the study. All clinical trials should have an identified sponsor. For LSHTM-sponsored studies, please contact the Clinical Trials QA Manager for further information.

	WaterAid India and WaterAid UK are funding the assessment. Each is a registered charity it its respective country.
	Any other relevant information including ethical issues not already addressed.
	None

APPENDIX A

Information Sheet and Consent Form

[To be translated into local language and read aloud at village meeting.]

As you may know, diarrhoea and worm infection are very serious problems in India, causing a lot of illness and death, particularly in young children. Often these diseases are caused by poor sanitation leading to widespread open defecation. As part of the Total Sanitation Campaign in India, WaterAid India and its partners will be working with your community to implement a sanitation intervention here aimed at having each household construct and use a latrine as an alternative to open defecation. They have asked us to undertake a study designed to monitor and assess the project and to determine its effectiveness in preventing certain diseases. We are with the London School of Hygiene & Tropical Medicine, and will be responsible for the assessment. Our local collaborators here in India are with the National Institute of Cholera and Enteric Diseases, Kolkata and the Human Development Foundation, Bhubaneswar.

For the next 30 months we would like to test whether the construction and use of latrines impacts on the health of your community. In order to do that, we will need the help of everyone in the community. If you agree to take part, we will ask you to do the following things:

-First, we will ask you questions about you and your family members. These questions will take no more than 30 minutes. A random sample of participants will be asked to provide a stool sample that we can analyze for worm infection. Those who provide stool samples will be will be given tablets that will certain kill any worms that may be infecting them from exposure to faeces.

- During the course of the 24-month intervention period, we will be visiting participating households once each month to obtain information about diarrhoea among family members during the previous week. We will also collect information on other symptoms of gastrointestinal illness, such as stomach pain, vomiting, etc. We will also collect information on latrine use, school attendance, and health care expenditures. During each of these monthly visits, we will also weigh and record the weight of each child under 5 years of age.

- Following the end of the 24-month intervention period, those participants who provided stool samples and received anti-worm tablets will be asked to provide another stool sample. This will allow us to determine if they have been re-infected with worms.

Participation in this study is not expected to present any risk or hazard to you or your family. Your decision to take part is completely up to you. If at any time during the study you decide that you no longer wish to take part, you may withdraw with no penalty. All information you give us will be

kept confidential. The names of you or your family members will not appear on any report of this project.

Your taking part may be of help to many other communities by determining if boiling is the best available option for treating water at home. Following the completion of the study, we will have a meeting with all the participants, and anyone else in the community, to explain the results of our study.

Do you have any questions? If at any time during the project you have questions about the project or about your rights as a person in a research project, you may speak to any of the investigators. Would you like to take part in this project? [If yes]: I am now going to read you a statement, and if you agree to it, please will sign this paper to confirm that.

Agreement to Participate

The above description of the research project was read to me. Anything I did not understand was explained to me, and any questions I had were answered. I voluntarily agree to participate in this project.

Name of Person Giving Consent: _____

(print)

_____/_____

(Signature of Person Giving Consent) Date

Name of Person Obtaining Consent: _____

(print)

_____/_____

(Signature of Person Obtaining Consent) Date

Witnessed: _____/_____

1.2 Ethical approval LSHTM

LONDON SCHOOL OF HYGIENE
& TROPICAL MEDICINE

ETHICS COMMITTEE

APPROVAL FORM

Application number: **5561**

Name of Principal Investigator	**Thomas F. Clasen**
Department	**Infectious and Tropical Diseases**
Head of Department	**Professor Simon Croft**

Title: **Assessing improved sanitation for preventing diarrhoea and helminth infection**

This application is approved by the Committee.

Chair of the Ethics Committee ..... [redacted]
.....

Date .....4 August 2009.....

Approval is dependent on local ethical approval having been received.

Any subsequent changes to the application must be submitted to the Committee via an E2 amendment form.

1.3 Ethical amendment application LSHTM

London School of Hygiene & Tropical Medicine

Keppel Street, London WC1E 7HT

Ethics Committee

Application to amend or extend a study which has received LSHTM Ethics Committee approval

Title of Project **ASSESSING IMPROVED SANITATION FOR PREVENTING DIARRHOEA AND HELMINTH INFECTION**	
LSHTM Ethics Committee Reference number	5561
Date approved	4 August 2009
Name of Principal Investigator (PI) at LSHTM	Thomas F. Clasen, JD, PhD
Appointment Held (or Research student)	Senior Lecturer
Unit/Department	DCVB/ITD
Signature of Principal Investigator	[redacted]
If Research student: Name, signature and approval of Supervisor	

Does this study involve the taking of tissue and/or blood samples	NO

Received by Committee:	
Approved by Chair:	
Date:	

	Give an outline of the proposed amendment/extension. Sufficient detail must be given to allow the Committee to make an informed decision.
	The Bill & Melinda Gates Foundation have offered additional funding for the study to (i) increase the sample size in order to detect an impact of the sanitation intervention on children <5 and not just all ages as initially contemplated by the study design. They have also agreed to provide funding to monitor the impact of the sanitation intervention on selected mosquitoes (biological vectors of lymphatic filariasis) and flies (mechanical vector for diarrhoea). As a result, we propose to amend the study in the following respects: 1. Powered the study up to assess diarrhoeal disease in <5s as the primary objective. This increased the sample size to 100 villages (50/50). See sample size calculation below.) 2. Revised the surveillance strategy to 6 approximately tri-monthly visits (from 18 monthly visits) and reduced the number of households to be visited to 35/village (rather than 75/village). These changes were allowed because of the increase in the number of villages. 3. Increase the number of villages that must be included in the sanitary surveys to 100, and increase the number of stool samples to be collected and assayed, from 4000 to 8000. Again, these changes were occasioned by the increase in the sample size. 5. Include vector monitoring in villages and households in the study population to include (i) setting traps in selected households to quantify the number of vectors and to

	capture a sample of vectors for further testing, (ii) testing of samples of the *Culex* sp. mosquitoes for *Wuchereria bancrofti* (the parasite responsible for lymphatic filariasis) using PCR, and (ii) testing of samples of flies *for E. coli, V. cholerae, Shigella* spp., *Salmonella* spp and *Aeromonas* spp., the most common agents associated with diarrhoeal disease in Orissa. We assumed a baseline mean longitudinal prevalence of diarrhoea of 7% among <5s in the control group and 5.25% in the intervention group. In making this estimate, we also had the benefit of data from University of California, Berkeley, Sumeet Patil's data from the Pattanayak (WHO Bull. 2009) study of sanitation in Orissa and other studies. For the cluster-level variability, we assumed 0.47, representing the midpoint of studies in Tamil Nadu and observations in large national or state-level studies (SHEWA-B, WSP MP). After adjusting the sample size for intermittent monthly sampling with a 3 day recall (Schmidt, submitted) and 15% loss to follow-up, we calculated that 40 clusters per arm or 80 villages in total would be required to allow detection of a 25% reduction of diarrhoea with 80% power and 5% level of significance. To build in a margin of error given the uncertainty of diarrhoea prevalence and inter-cluster variability, we have elected to target 100 villages (50 intervention/50 control) for the study. In all other material respects, the study will proceed in accordance with the original LSHTM ethics approval. Local ethics approval for the study in India was obtained on 29 May 2010.
	Does this amendment/extension involve the taking of blood samples and/or other tissue?
	NO
2.1	If YES – Does this change arrangements already notified in the original application?
	Not Applicable
2.2	If YES - List samples which will be taken

	Not Applicable
2.3	Please confirm that you have undertaken the on-line training programme available at http://intra.lshtm.ac.uk/support/research/humantissueact.html and that you will ensure that any staff involved in the procedures for taking consent will also have undertaken an agreed training programme.
	Not applicable
2.4	If samples are taken overseas, will the samples be brought back to the UK?
	Not applicable

1.4 Ethical Amendment LSHTM 1 & 2

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

ETHICS COMMITTEE

APPROVAL FORM Application number: A180 5561

Name of Principal Investigator	**Thomas Clasen**
Department	**Infectious and Tropical Diseases**
Head of Department	**Professor Simon Croft**

Title: ASSESSING IMPROVED SANITATION FOR PREVENTING DIARRHOEA AND HELMINTH INFECTION

Amendments to this application have been approved by the Ethics Committee.

Chair of the Committee [redacted]

Date .....6 August 2010.....

Approval is dependent on local ethical approval having been received.

Any subsequent changes to the application must be re-submitted to the Committee.

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

ETHICS COMMITTEE

APPROVAL FORM Application number: A289 5561

Name of Principal Investigator **Tom Clasen**

Faculty **Infectious and Tropical Diseases**

Head of Faculty **Professor Simon Croft**

Title: ASSESSING IMPROVED SANITATION FOR PREVENTING DIARRHOEA AND HELMINTH INFECTION

[redacted] Chair of the Committee

Amendments to this application have been approved by the Ethics Committee.

Date .....25 November 2011.....

Approval is dependent on local ethical approval having been received.

Any subsequent changes to the application must be re-submitted to the Committee.

1.5 Ethical approval application XIMB

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
UNIVERSITY OF LONDON
Department of Infectious and Tropical Diseases
Disease Control and Vector Biology Unit
Keppel St., London WC1E 7HT UK
+1 44 (0)20 7927 2916

10 May 2010

Institutional Ethics Committee

Xavier Institute of Management, Bhubaneswar

751013 Bhubaneswar, Orissa

India

Dear Committee Members:

Re: Application for Ethics Clearance

The London School of Tropical Medicine and Hygiene has partnered with WaterAid UK and its affiliate, WaterAid India, to undertake an assessment of its sanitation programme in Puri District, Orissa. Thomas Clasen, JD, PhD, is a Senior Lecturer at LSHTM and is the principal investigator of the study. Professor S. Peppin and colleagues at the Xavier Institute of Management, Bubhanewsar (XIMB) are the proposed Indian research partner in this assessment. This study has been authorized by the Principal Secretary of the Rural Development Department of the State of Orissa, who is also the Member Secretary of the Orissa State Water and Sanitation Mission. We have also obtained ethical clearance for the study by the Ethics Committee of LSHTM (Approval #5561). The purpose of this letter is to request clearance of the study by the Institutional Ethics Committee (IEC) of XIMB.

A. Background

Excreta-related diseases, including as diarrhoea, soil-transmitted helminth infection and trachoma, impose a heavy health burden worldwide, especially among young children. Diarrhoeal diseases cause an estimated 1.8 million deaths per year and are responsible for 17% of all deaths in children under 5 years in developing countries. Schistosomiasis affects 190 million people worldwide, killing 280,000 annually and leaving others with impaired physical and mental development. The major soil-transmitted intestinal helminth infections include

ascaris (1.2 billion people infected), trichuris (795 million) and hookworm (around 740 million). Unsanitary excreta disposal is also believed to contribute to the transmission of trachoma (around 6 million cases of blinding annually) by increasing the abundance of flies acting as disease vectors.

Diarrhoeal disease is an important health problem in Orissa. The National and Family Health Survey conducted 2001-2 indicated that 28% of children under 5 had diarrhoea in the preceding two weeks, among which 16% suffered from bloody diarrhoea (NFHS-3). However, diarrhoea is highly seasonal, being more prevalent during the rainy seasons from July to October (RDLHFS). Soiltransmitted helminths infections are common. A study carried out in 2001 among school aged children in Puri district revealed the following prevalence figures: Ascaris 16%, hookworm 8% and Trichuris 4.5%.

Despite progress on the MDG water targets, sanitation coverage continues to fall behind with 2.6 billion people still lacking access to even basic sanitation. India has been identified as one of the countries in which coverage is lowest. Despite substantial efforts, only 28% of the Indian population has the benefit of improved sanitation (WHO 2010). The sanitation coverage in Orissa for 2005 was estimated at 10% and 60% for rural and urban settings respectively. In Puri district, sanitation coverage was estimated at 23% overall and 15% in rural areas (DLHFS).

B. The Need for a Rigorous Study of Sanitation

Governments and funding organisations appear hesitant to invest in improved sanitation, especially in rural populations, partly due to the lack of evidence on the effectiveness of sanitation interventions to demonstrate a substantial health impact through large-scale coverage and consistent, sustained use. The assumption that sanitation is effective in preventing a wide range of conditions is based predominantly on plausibility and a number of observational studies, often of poor quality. In a recent systematic review, we found evidence that improvements in sanitation facilities reduces diarrhoea and may also be effective in preventing trachoma, cholera and intestinal helminth infections. However, because the interventions mainly include improvements in water supplies, hygiene promotion or other measures, it was not possible to ascribe the reduction in risk solely to improvements in excreta disposal. To date, there has not been a single randomised controlled trial on the effect of improved sanitation to prevent diarrhoea and intestinal infections.

In the light of the poor evidence, WaterAid, a leading implementer of sanitation interventions in low-income settings, have partnered with the London School of Hygiene and Tropical Medicine, a world leader in public health research, to undertake a cluster-randomised trial to assess the effectiveness of improved sanitation to improve health. It seeks to provide policy makers and stakeholders with a reliable estimate of the effect of sanitation, not only among those who adopt the intervention but also on their neighbours. It will also yield valuable

evidence on the cost of the intervention, providing a basis to assess cost-effectiveness to help governments and health economists evaluate sanitation interventions in the context of other health priorities. The trial will also provide an important opportunity to closely monitor use, maintenance and acceptability of improved sanitation—factors that are likely to affect health impact, uptake and long-term sustainability. The study is also designed to investigate anthropometric, nutrition and non-health outcomes such outlays for disease treatment, lost days at work and school absenteeism. Finally, the trial will provide the opportunity to study the epidemiology of diarrhoeal diseases and helminth infections in relation to sanitation in general, and will therefore be embedded in a number of related research activities aimed at corroborating the trial results and producing further epidemiological insights.

C. Proposed Study in Puri District, Orissa

With the cooperation of WaterAid, LSHTM have already completed most of the preliminary planning for the proposed trial. This includes (i) working with the WaterAid to agree on study objectives, the actual sanitation intervention and key parameters of study site; (ii) identifying and visiting potentially suitable sites to in Puri District, Orissa and meeting with district water and sanitation authorities to confirm suitability and measure key parameters; (iii) designing the trial and developing a study protocol based on conditions prevailing at site; (iv) developing a schedule, estimating local staffing requirements for data collection, and preparing a budget for study costs; (v) securing LSHTM ethics approval; and (vi) securing funding for the study from the UK Department for International Development (DFID). We have also identified XIMB as our Indian research partner. XIMB brings particular experience and expertise in the social sciences and economics, two areas of considerable value in the study. XIMB will also engage and work with Loyola Hospital in Bhubaneswar who will conduct the stool assays in their pathology lab.

The proposed setting for the trial is in costal district of Puri in the eastern Indian state of Orissa. About 50% of the population lives below the poverty line; scheduled castes and tribes make up a significant proportion the population. While water supply coverage is extensive, the government's Total Sanitation Campaign has only yielded about 10% latrine coverage in the district to date; open defecation is still widely practiced. The National and Family Health Survey conducted in 2001-2 indicated that 28% of children under 5 had diarrhoea in the preceding two weeks, among which 16% suffered from bloody diarrhoea. Soil-transmitted helminth infections are also common: a study carried out in 2001 among school-aged children in Puri district revealed a high prevalence of ascaris (16%), hookworm (8%) and trichuris (4.5%).

In 2008, WaterAid India and the government of Orissa launched a district-wide initiative in Puri. The initiative focuses primarily on rural areas since 80% of the population of Orissa is rural. It seeks to declare Puri as an open defecation free district by 2012. Government subsidies cover labour (local mason), concrete rings for pit lining, water-sealed

pan, slab, pipes but does include the cost of the superstructure. Households are provided with information about different technology options for different price ranges. A supply chain for construction material is established through construction centres and rural sanitary marts. These resources, together with WaterAid's mobilization campaign have been effective at securing coverage.

With the assistance of the State Department of Rural Development and the District water and sanitation authorities, we will select a total of 54-60 villages (comprising approximately 4,200 to 4,600 households) for participation in the study. Following enrolment of households and completion of the baseline survey, half of the villages will be randomly allocated to an intervention group and half to a control group. In October 2010 (following the end of the rainy season), latrine construction will commence by the three implementing partners (SSUD, TAS and MWA), with each completing coverage in at least 90% of its allocated 9-10 villages by March 2011; meanwhile, with the understanding of Puri authorities, latrine construction will be postponed in the control villages until 2 years later (October 2012). Commencing in April 2011, when latrine coverage is expected to be high in interventions and low (<10%) in control villages, monthly surveillance will be undertaken to assess reported all age diarrhoea and WAZ for <5. Enumerators will also report on the condition and use of the latrine (reported use by each family member supplemented by objective indicators and "smart latrine monitoring devices). We will also investigate the impact of the intervention on water quality, flies and mosquito populations, and open defecation practices. Sub-studies include investigations of the impact of the intervention on gender issues, school attendance, and productivity, and will collect detailed information cost and cost savings to determine cost effectiveness. Following the end of a 21 month surveillance period (April 2011 to December 2012), which includes the 2011 and 2012 rainy seasons, surveillance will be completed and construction of control villages. Thereafter construction of the control village latrines will commence and be completed by December 2012 to comply with the overall goal of total sanitation coverage by 2012.

D. Additional Benefits of the Study

The study should provide a number of important benefits to the Government of India, the State of Orissa, XIMB and a variety of stakeholders. It will provide valuable information about (i) disease prevalence, (ii) the effectiveness of sanitation interventions to prevent disease and infection and promote normal growth and development, (iii) the acceptability and use of the intervention, (iv) the impact of the intervention on school attendance and lost days at work, and (v) cost-effectiveness of sanitation and the potential savings to the State in averting health care costs by preventing disease and infection. XIMB will be a full partner in the study, creating an opportunity to contribute their expertise to the project and to build capacity in programme assessment and epidemiology through international collaboration with LSHTM and to co-publish the results of the assessment. WaterAid's implementing partners in Orissa will also benefit by building capacity and by having available rigorous documentary evidence of the effectiveness of their programmatic activities to be used in support of applications for

future funding. Local workers will benefit, since the assessment will involve the hiring of dozens of full-time staff for the full duration of the study to be trained to conduct necessary surveillance and sample collection. The study will also contribute by hiring local laboratories for microbiological assays of stool samples.

However, the most important beneficiaries of this research will be the 2.4 billion who are waiting patiently to participate in the health gains and personal dignity of basic sanitation. By establishing the evidence base for sanitation, we hope to raise the profile of this basic necessity, and attracting the political will and financial investment necessary to put the MDG sanitation target within reach.

E. Ethics Review

This study constitutes an evaluation of a programme that WaterAid is authorized to undertake in India. Accordingly, it not subject to formal ethics review under the Ethical Guidelines for Biomedical Research on Human Subjects (IMRC 2006). Nevertheless, we plan to comply with all the requirements of such Guidelines. Moreover, insofar as XIMB will be a research partner in this study, we hereby request that an Institutional Ethics Committee (IEC) of XIMB review and authorize the study.

We believe main ethical issues are (i) whether participation voluntary and consent informed, (ii) whether the study population is put at increased risk as a result of participation in this study, (iii) whether there is undue infringement on their time, and (iv) whether the study minimizes any potential confidentiality issues. We do not believe that this study presents any significant issues on any of these fronts. We also believe that the benefits of a rigorous assessment of this programme outweigh these minor disadvantages.

First, participation is completely voluntary. Each participant will be provided complete details about the study and, only if they consent in writing to participate, will be enrolled. They will also be advised that they may withdraw at any time.

Second, this is an assessment of an ongoing sanitation programme approved by the Government of India and the State of Orissa, not a new intervention that we are investigating for research purposes only. As a result, there is no additional exposure that the study is presenting to study participants. The only possible additional exposure from participation in the study is that approximately 2000 study participants will be asked to participate in a sub-study in which they will provide stool samples and then be provided with deworming tablet so that we can monitor rates and intensity of re-infection. However, this is a WHO and government of India-approved deworming tablet used by UNICEF in Orissa when there is sufficient funding. Thus, this is more a potential benefit (deworming) than any kind of risk.

Third, the information statement details the time and steps required for participation, and we take all steps to minimize the time for participation. While participants to not receive any compensation for participation in the study, they and their communities can potentially benefit by having regular surveillance to assess diarrhoea and weight of their children <5. Moreover, the communities and state will benefit by having a rigorous assessment of the programme in order to improve its effectiveness.

Finally, we will take all reasonable steps to maintain the confidentiality of study participants. We will use codes instead of names of study participants to record and analyze our data from the households, and we will not report, present or publish any information that would reveal the identity or any personal information on any study participant.

If you have any questions regarding this request, please contact Prof. Peppin or the undersigned. We would be grateful to obtain a letter from the IEC as soon as possible and before the end of May 2010 confirming its review of the ethics issues presented by this study and confirming that ethics clearance of same.

Sincerely,

Thomas F. Clasen, JD, PhD
Senior Lecturer and Principal Investigator

S. Peppin
Professor and Co-Investigator

1.6 Ethical approval XIMB

Ethics Committee

APPROVAL FORM

Application Number : 310510

Name of the Co-Investigator* : **Soosai Peppin**

Department/Area : **Rural Management**
Head of Department/Area : **Prof. C. Shambu Prasad**
Coordinator

Title: **Assessing Improved Sanitation for Preventing Diarrhoea and Helminth Infection**

The amendment as specified in the letter dated 31st October 2011 is approved.

Chair of the Ethics Committee: Fr. P.T. Joseph, S.J [redacted]

Date:---8/11/11---

Note: Any subsequent material changes to the application must be submitted to the Committee and the approval must be approved.

**The Principal Investigator is Thomas F.Clasen, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, University of London-*

1.7 Ethical amendment XIMB

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Ethics Committee

APPROVAL FORM

Application Number : 310510

Name of the Co-Investigator* : **Soosai Peppin**

Department/Area : **Rural Management**
Head of Department/Area : **Prof. C. Shambu Prasad**
Coordinator

Title: Assessing Improved Sanitation for Preventing Diarrhoea and Helminth Infection

The amendment as specified in the letter dated 15th March 2012 is approved

Chair of the Ethics Committee: Fr. P.T. Joseph, S.J: [redacted]

Date: 31st March 2012

Note: Any subsequent material changes to the application must be submitted to the Committee and the approval must be approved.

**The Principal Investigator is Thomas F.Clasen, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, University of London-*

Appendix 2: Informed Consent Forms

2.1 Fly population survey

Information sheet and Consent form

As you may know, flies can be a considerable annoyance around the house. Flies are not only a nuisance though; they can contribute to the transmission of diarrhoeal diseases that can lead to illness and death, particularly in young children. In order to monitor the flies that can cause such problems, traps have been designed specifically to catch them. These traps can catch up to 100 flies a night. The traps work by being placed in areas where flies are at their highest concentration and can easily fly into the trap. The flies then become attached to the trap and because of the glue cannot free themselves. We are with the London School of Hygiene and Tropical Medicine and, as part of a study looking at how latrines impact on health, we have been asked to monitor flies that contribute to disease transmission in Orissa. This is an important part of a larger sanitation trial being conducted across Orissa in collaboration with XIMB assessing the effect of latrines on fly populations.

For the next few days we would like to set traps in your house to catch flies. In order to do this we will need the help of your household. If you agree to take part we would ask the following things:

First we would you questions about your household and the surrounding area. These questions will take no longer than 5 minutes.

Second we would set a trap each morning in your house for a total of 3 days, these will be changed daily. These traps will be placed inside or outside the house, depending on where food preparation or consumption takes place and where the fly concentrations seem to be highest. We would ask that you do not move these traps while they are in place. These traps will be set and collected each morning.

Participation in this study is not expected to present any risk or hazard to you or your family. Your decision to take part is completely up to you. If at any time during the study you decide that you no longer wish to take part, you may withdraw with no penalty. All information you give us will be kept confidential. The names of you or your family members will not appear on any report of this project.

Do you have any questions? If at any time during the project you have questions about the project or about your rights as a person in a research project, you may speak to any of the investigators. Would you like to take part in this project? [If yes]: I am now going to read you a statement, and if you agree to it, please will you sign this paper to confirm that.

Agreement to Participate

The above description of the research project was read to me. Anything I did not understand was explained to me, and any questions I had were answered. I voluntarily agree to participate in this project.

Name of Person Giving Consent: _____
(print)

_____/_____

(Signature of Person Giving Consent) Date

Name of Person Obtaining Consent: _____
(print)

_____/_____

(Signature of Person Obtaining Consent) Date

Witnessed: _____/_____

ସୂଚନା ପତ୍ର ଏବଂ ସ୍ୱୀକୃତି ପ୍ରଣାଳୀ

ଯେପରି ଆପଣ ଜାଣନ୍ତି, ମାଛି ଗୁଡିକ ଆମକୁ ଆମ ଗୃହ ଚତୁପାର୍ଶ୍ୱରେ ବିରକ୍ତିଭାବ ସୃଷ୍ଟି କରିଥାନ୍ତି । ମାଛି ଗୁଡିକ କେବଳ ହଇରାଣ କରନ୍ତି ନାହିଁ, ସେମାନେ ମଧ୍ୟ ହଇଜାଭଳି ମାରାତ୍ମକ ରୋଗର ମୂଳକାରଣ ହୋଇଥାନ୍ତି ଯାହାକି ଆମକୁ, ବିଶେଷକରି ଶିଶୁମାନଙ୍କୁ ଶାରୀରିକ ଅସୁସ୍ଥତା ଏବଂ ମୃତ୍ୟୁ ମୁଖରେ ପକାଇଥାଏ ଏହି ରୋଗରୁ ରକ୍ଷାପାଇବାର ଖୁବ୍ ସହଜ ମାଧ୍ୟମ ଅଛି । ମସାରିର ବ୍ୟବହାର ଏକ ସହଜ ଉପାୟ ଅଟେ ଯାହା ମସା ମାନଙ୍କୁ ଅବରୋଧକରିଥାଏ । ଏହା ଛଡା ମସା ମାନଙ୍କୁ କମ୍ କରିବାର ଆନ୍ୟ ଏକ ଉପାୟ ଯାହାକି ତାଙ୍କୁ ଆଉ ସୃଷ୍ଟି ନକରିବା ସେଥିପାଇଁ ମାଛିମାନଙ୍କ ଦ୍ୱାରା ଘଟୁଥିବା ଏହିସବୁ ସମସ୍ୟାର ଦୂରପଯୋଗ କରିବା ପାଇଁ ମାଛି ଧରିବା ଯନ୍ତାର କୌଶଳ ଅବଲମ୍ବନ କରାଯାଇଛି । ଏହି ଯନ୍ତାଗୁଡିକ ଗୋଟିଏ ରାତିରେ ୧୦୦ ପର୍ଯ୍ୟନ୍ତ ମାଛି ଧରିପାରିବ । ଯେଉଁ ସ୍ଥାନରେ ଅତ୍ୟଧିକ ମାଛି ଦେଖାଯାନ୍ତି, ସେହି ସ୍ଥାନରେ ଏହି ଯନ୍ତାଗୁଡିକ ଅବସ୍ଥାପିତ କରାଯିବ ଏବଂ ସେମାନେ ସହଜରେ ଯନ୍ତା ଭିତରକୁ ପଶିପାରିବେ । ତାପରେ ମାଛି ଗୁଡିକ ଯନ୍ତା ଗୁଡିକ ସହ ଲାଗିଯିବେ ଏବଂ ଯନ୍ତାରେ ଦିଆଯାଇଥିବା ଅଠା ଯୋଗୁଁ ସେମାନେ ମୁକ୍ତ ହୋଇପାରିବେ ନାହିଁ । ସେହିଭଳି ମାଛିମାନଙ୍କୁ ଧରିବା ପାଇଁ ବିଭିନ୍ନ ପ୍ରକାରର ଯନ୍ତା ବ୍ୟବହାର କରାଯାଇପାରେ । ଆମେ ଲଣ୍ଡନ ସ୍କୁଲ ଅଫ୍ ହାଇଜିନ୍ ଏବଂ ଟ୍ରପିକାଲ ମେଡିସିନ୍ ସହିତ ଏବଂ ଏହି ଅଧ୍ୟୟନର ଏକ ଅଂଶ ଭାବେ ଜାଣିବାକୁ ପାଇଛୁ ଯେ, ମାଛି ହିଁ ଓଡିଶାରେ ସଂକ୍ରମଣ ଜନିତ ରୋଗର ମୁଖ୍ୟକାରଣ ଏବଂ ଏକ ପରୀକ୍ଷଣ ଭାବରେ ଜାଣିଲୁ ଯେ, ଯନ୍ତା ଗୁଡିକ ହିଁ ମାଛି ନିରାକରଣ ସର୍ବଟ ।ମ ମାଧ୍ୟମ । ଏହା ସ୍ୱାସ୍ଥ୍ୟ ଉନ୍ନତି ବିଧାନରେ ଗୁରୁତ୍ୱପୂର୍ଣ୍ଣ ଅଧ୍ୟାୟ ଯାହାକି ଏକ୍.ଆଇ.ଏମ୍.ବି ର ସହାୟତାରେ ଓଡିଶାରେ ଏକ ପରୀକ୍ଷା ମୂଳକ ଭାବରେ ପରିଚାଳନା କରାଯାଉଛି ଏବଂ ତାହାଦ୍ୱାରା ଅତ୍ୟଧିକ ମାଛିମାନଙ୍କ ଗହଣରେ ଶୌଚାଳୟର ପ୍ରଭାବକୁ ମଧ୍ୟ ଲକ୍ଷ୍ୟ କରାଯାଉଛି ।

ପରବ ୀ ମାସରେ ଆମେ ଦେଖିବାକୁ ଚାହୁଁଛୁ ଯେ କେଉଁ ଯନ୍ତା ସବୁଠାରୁ ଅଧିକ ମାଛି ଅଧିକାର କରିଛି । ଏହି ସବୁ ଉଦ୍ୟମ ପାଇଁ ଆମ୍ଭେମାନେ ଆପଣଙ୍କ ଗୃହର ସହାୟତା ଲୋଡିବୁ । ଯଦି ଆପଣ ଏଥିରେ ଏକମତ ତେବେ ଆପଣଙ୍କୁ ନିମ୍ନପ୍ରଦ ବିଷୟରେ ଉ ର ଦେବାକୁ ଅନୁରୋଧ ।

ପ୍ରଥମେ ଆମେ, ଆପଣ ଏବଂ ଆପଣଙ୍କ ପରିବାର ବିଷୟରେ ପ୍ରଶ୍ନ ପଚାରିବୁ । ଏହି ପ୍ରଶ୍ନଗୁଡିକ ୫ ମିନିଟ୍ ସମୟରୁ ଅଧିକ ହେବ ନାହିଁ । ଆମେ ଆପଣଙ୍କୁ ପଚାରିବୁ ଯେ ଆପଣ ଅଧ୍ୟୟନର କାର୍ଯ୍ୟକାଳ ସମୟରେ, ରାତିରେ ଶୋଇବାବେଳେ ମଶାରୀ ବ୍ୟବହାର କରୁଛନ୍ତି କି ? ପରୀକ୍ଷଣ ସମୟରେ ଆପଣ ମାଛିଙ୍କ ଠାରୁ ନିଜକୁ ନିବାରଣ କରିବାପାଇଁ ଏହା ଏକ ଉଦ୍ୟମ । ଆମ୍ଭେମାନେ ଆପଣଙ୍କୁ ଏକ ମଶାରୀ ପ୍ରଦାନ କରିବୁ । ପ୍ରତିଦିନ ସକାଳେ ଆମେ ଆପଣଙ୍କୁ ପଚାରିବୁ ଯେ ପୂର୍ବରାତିରେ ଆପଣ ମଶାରୀ ବ୍ୟବହାର କରିଥିଲେ କି ନାହିଁ ?

ସାଧାରଣତଃ ୩ ପ୍ରକାର ଯନ୍ତାକୁ ନେଇ ଆମେ ପରୀକ୍ଷା କରୁଛି ଯେ ସେଗୁଡିକ କେତେ ମାଛିଙ୍କୁ ଧରିପାରୁଛି । ପ୍ରତି ସଂଖ୍ୟାରେ ଏହି ଯନ୍ତାଗୁଡିକ ଅନ୍ୟ ଗୃହରେ ଆବ ନ ପ୍ରକ୍ରିୟାରେ ଅବସ୍ଥାପିତ ହେବ । ଏହି ଯନ୍ତାଗୁଡିକ ଘର ଭିତରେ କିମ୍ବା ଘର ବାହାରେ, ଯେଉଁଠାରେ ଅଧିକ ମାଛି ଥାନ୍ତି, ସେହିଠାରେ ବସାଯାଇ ପାରିବ ।

ଏହି ଅଧ୍ୟୟନରେ ଅଂଶଗ୍ରହଣ କରିବା ପାଇଁ ଆପଣଙ୍କୁ କିମ୍ବା ଆପଣଙ୍କ ପରିବାରକୁ କୌଣସି କ୍ଷତି କିମ୍ବା ବିପଦ ସହିବା ପାଇଁ ପଡିବ ନାହିଁ । ଯଦି ଆପଣ, ଏହି ଅଧ୍ୟୟନର କୌଣସି ସମୟରେ ବି ନିର୍ଣ୍ଣ ନେବେ ଯେ, ଆପଣ ଏଥିରେ ଅଂଶଗ୍ରହଣ ପାଇଁ ଇଚ୍ଛୁକ ନୁହନ୍ତି, ତେବେ ଆପଣ ବିନା ଜୋରିମାନା ସହ ପ୍ରତ୍ୟାହାର କରିପାରିବେ । ଆପଣ ଦେଇଥିବା ସମସ୍ତ ତଥ୍ୟ ଗୋପନ ରଖାଯିବ । ଆପଣଙ୍କ ନାମ କିମ୍ବା ଆପଣଙ୍କ ପରିବାର ସଦସ୍ୟଙ୍କ ନାମ, ଏହି ଅଧ୍ୟୟନର ବିବରଣୀରେ ପ୍ରକାଶ କରାଯିବ ନାହିଁ ।

ଆପଣଙ୍କର କୌଣସି ପ୍ରଶ୍ନ ଅଛି କି ? ଯଦି, ଅଧ୍ୟୟନ ସମୟରେ ଆପଣଙ୍କ ମନରେ ଅଧ୍ୟୟନ କିମ୍ବା ଅଧିକାର ସମ୍ବନ୍ଧୀୟ କୌଣସି ପ୍ରଶ୍ନ ଉଠେ, ତାହେଲେ ଆପଣ ଯେକୌଣସି ଅନୁସନ୍ଧାନକାରୀଙ୍କୁ ପଚାରିପାରିବେ । ଆପଣ ଏହି ଅଧ୍ୟୟନରେ ଅଂଶ ଗ୍ରହଣ କରିବାକୁ ଚାହାନ୍ତି କି ? {ଯଦି ହଁ } ମୁଁ ଆପଣଙ୍କ ସମ୍ମୁଖରେ ଏକ ବିବୃତି ପଢିବାକୁ ଯାଉଛି, ଆପଣ ଯଦି ଏଥିରେ ଏକମତ ଦୟାକରି ନିଶ୍ଚିତ ହେବାପାଇଁ, ଆପଣଙ୍କ ଦସ୍ତଖତ ଦେବେ କି ?

ଯୋଗଦାନର ଚୁକ୍ତି

ଉପରୋକ୍ତ ବର୍ଣ୍ଣନୀୟ ଗବେଷଣା ଜନିତ ବିବରଣୀକୁ ପଢିଲି । ଯେଉଁ ଉକ୍ତି ଗୁଡିକ ମୁଁ ବୁଝିପାରିନଥିଲି, ତାହା ମୋତେ ବର୍ଣ୍ଣନା କରାଗଲା ଏବଂ ଯେଉଁ ପ୍ରଶ୍ନ ଗୁଡିକର ଉ ର ମୁଁ ଦେଇନଥିଲି, ତାହାର ଉ ର ମଧ୍ୟ ମୁଁ ଜାଣିଗଲି । ମୁଁ ସ୍ୱଇଚ୍ଛାରେ ଏହି ଗବେଷଣାରେ ଯୋଗଦେବା ପାଇଁ ସଂପୂର୍ଣ୍ଣ ଭାବରେ ଏକମତ ।

ସ୍ୱୀକୃତି ଦେବା ବ୍ୟକ୍ତିଙ୍କ ନାମ .....

(ସ୍ୱୀକୃତି ଦେବା ବ୍ୟକ୍ତିଙ୍କ ସ୍ୱାକ୍ଷର) (ତାରିଖ)

ସ୍ୱୀକୃତି ଉପାର୍ଜନ କରିବା ବ୍ୟକ୍ତିଙ୍କ ନାମ .....

(ସ୍ୱୀକୃତି ଉପାର୍ଜନ କରିବା ବ୍ୟକ୍ତିଙ୍କ ସ୍ୱାକ୍ଷର) (ତାରିଖ)

ସାକ୍ଷ୍ୟ: ...../ .....

2.2 Mosquito population survey

Information sheet and Consent form

As you may know, mosquitoes can be a considerable annoyance around the house, biting especially during the evening and night. The bites of these mosquitoes can not only cause significant irritation but also illness and death, through diseases like malaria and elephantiasis, particularly in young children. These diseases can easily be prevented by the use of a bed net that stops the mosquitoes biting and transmission of disease. In order to monitor the mosquitoes that can cause such problems, traps have been designed specifically to catch them. These traps can catch up to 1000 mosquitoes a night. The traps work by attracting the mosquitoes to them and then drawing them into the trap by means of a fan. We are with the London School of Hygiene and Tropical Medicine and as part of a study looking at the mosquitoes that transmit diseases in Orissa, we have been asked to undertake a trial to monitor mosquitoes. This is an important part of a larger sanitation trial being conducted across Orissa in collaboration with XIMB assessing the effect latrines have on mosquito populations.

For the next few days we would like to set traps in your house to catch mosquitoes. In order to do this we will need the help of your household. If you agree to take part we would ask the following things:

First we would you questions about you and your family. These questions will take no longer than 5 minutes.

Second we would set a trap each morning in your house for a total of 3 days, these will be changed daily. These traps will be placed outside the house. We would ask that you do not move these traps while they are in place. The base of this trap will be filled with water. These traps will be set and collected each morning.

There are three different traps that we are testing to see the how many mosquitoes they catch. Each evening the traps will be rotated between different households. The first of these traps will be positioned outside of the household on the ground. The second trap will be hung outside and the final trap will be hung from the ceiling inside the house next to a bed net. The traps will be set in the evening and collected the following morning.

Participation in this study is not expected to present any risk or hazard to you or your family. Your decision to take part is completely up to you. If at any time during the study you decide that you no longer wish to take part, you may withdraw with no penalty. All information you give us will be kept confidential. The names of you or your family members will not appear on any report of this project.

Do you have any questions? If at any time during the project you have questions about the project or about your rights as a person in a research project, you may speak to any of the investigators. Would you like to take part in this project? [If yes]: I am now going to read you a statement, and if you agree to it, please will you sign this paper to confirm that.

Agreement to Participate

The above description of the research project was read to me. Anything I did not understand was explained to me, and any questions I had were answered. I voluntarily agree to participate in this project.

Name of Person Giving Consent: _____
(print)

_____/_____

(Signature of Person Giving Consent) Date

Name of Person Obtaining Consent: _____
(print)

_____/_____

(Signature of Person Obtaining Consent) Date

Witnessed: _____/_____

ସୂଚନା ପତ୍ର ଏବଂ ସ୍ୱୀକୃତି ପ୍ରଣାଳୀ

ଯେପରି ଆପଣ ଜାଣନ୍ତି, ମଶା ଗୁଡିକ ଆମ ଚତୁପାର୍ଶ୍ୱରେ ରହି ଆମକୁ ବିରକ୍ତି କରିଥାନ୍ତି, ସାଧାରଣତଃ ସଂଧ୍ୟାବେଳେ ଏବଂ ରାତିରେ ଆମକୁ କାମୁଡିଥାନ୍ତି । ମଶା କାମୁଡିବା ଯୋଗୁଁ କେବଳ, ଆମର ବିରକ୍ତି ଭାବ ବଢିନଥାଏ, ସେମାନେ ମଧ୍ୟ ମ୍ୟାଲେରିଆ ଏବଂ ଗୋଦର ରୋଗ ଭଳି ମାରାତ୍ମକ ରୋଗର ମୂଳ କରାଣ ହୋଇଥାନ୍ତି, ଯାହାକି ଆମକୁ ବିଶେଷକରି ଶିଶୁମାନଙ୍କୁ ଶାରୀରିକ ଅସୁସ୍ଥତା ଏବଂ ମୃତ୍ୟୁମୁଖରେ ପକାଇଥାଏ । ମଶାମାନଙ୍କ ଦ୍ୱାରା ଘଟୁଥିବା ଏହିସବୁ ସମସ୍ୟାର ଦୂରପଯୋଗ କରିବାପାଇଁ ମଶାଧରିବା ଯନ୍ତାର କୌଶଳ ଅବଲମ୍ବନ କରାଯାଇଛି । ଏହି ଯନ୍ତାଗୁଡିକ ଗୋଟିଏ ରାତିରେ ୧୦୦୦ ପର୍ଯ୍ୟନ୍ତ ମଶାଙ୍କୁ ଧରିପାରିବ । ଏହି ଯନ୍ତାଗୁଡିକ ପ୍ରଥମେ ମଶାଗୁଡିକୁ ନିଜ ଆଡକୁ ଆକର୍ଷଣ କରେ ଏବଂ ଫ୍ୟାନ୍ ଦ୍ୱାରା ସେମାନଙ୍କ ଯନ୍ତା ଭିତରକୁ ଟାଣିଥାଏ । ସେହିଭଳି, ମଶାମାନଙ୍କୁ ଧରିବା ପାଇଁ ବିଭିନ୍ନ ପ୍ରକାରର ଯନ୍ତା ବ୍ୟବହାର କରାଯାଇପାରେ । ଆମେ ଲଣ୍ଡନ୍ ସ୍କୁଲ ଅଫ୍ ହାଇଜିନ୍ ଏବଂ ଟ୍ରପିକାଲ୍ ମେଡିସିନ୍ ସହିତ ଏବଂ ଏହି ଅଧ୍ୟୟନର ଏକ ଅଂଶ ଭାବେ ଜାଣିବାକୁ ପାଇଛୁଯେ, ମଶା ହିଁ ଓଡିଶାରେ ସଂକ୍ରମଣ ଜନିତ ରୋଗର ମୂଖ୍ୟ କାରଣ ଏବଂ ଏକ ପରୀକ୍ଷଣ ଭାବରେ ଜାଣିଲୁ ଯେ, ଯନ୍ତାଗୁଡିକ ହିଁ ମଶା ନିରାକରଣର ସର୍ବୋ ମ ମାଧ୍ୟମ । ଏହା ସ୍ୱାସ୍ଥ୍ୟ ଉନ୍ନତି ବିଧାନର ଗୁରୁତ୍ୱପୂର୍ଣ୍ଣ ଅଧ୍ୟାୟ ଯାହାକି ଏକ୍.ଆଇ.ଏମ୍.ବି ର ସହାୟତାରେ ଓଡିଶାରେ ଏକ ପରୀକ୍ଷା ମୂଳକ ଭାବରେ ପରିଚାଳନା କରାଯାଇଛି ଏବଂ ତାହାଦ୍ୱାରା ଅତ୍ୟଧିକ ମଶାଙ୍କ ଗହଣରେ ଶୌଚାଳ୍ୟର ପ୍ରଭାବକୁ ମଧ୍ୟ ଲକ୍ଷ୍ୟ କରାଯାଉଛି । ଏହା ଦ୍ୱାରା ହେଉଥିବା ରୋଗର ପ୍ରଭାବ ଓ ବିସ୍ତାର ବିଷୟରେ ଜାଣିପାରିବୁ । ଏଥିପାଇଁ ଆପଣଙ୍କ ସହାୟତା ଓ ସହଯୋଗ ଖୁବ ଦରକାର । ଆମେ ଆପଣଙ୍କ ଘରେ ଏକ ଯନ୍ତା ଅବସ୍ଥାପିତ କରି ମାଛି ମାନଙ୍କ ଗତିବିଧି ଦେଖିବୁ ।

ପରବ ୀ ମାସରେ ଆମେ ଦେଖିବାକୁ ଚାହୁଁଛୁ ଯେ କେଉଁ ଯନ୍ତା ସବୁଠାରୁ ଅଧିକ ମଶା ଅଧିକାର କରିଛି । ଏହି ସବୁ ଉଦ୍ୟମ ପାଇଁ ଆମ୍ଭେମାନେ ଆପଣଙ୍କ ଗୃହର ସହାୟତା ଲୋଡିବୁ । ଯଦି ଆପଣ ଏଥିରେ ଏକମତ, ତେବେ ଆପଣଙ୍କୁ ନିମ୍ନପ୍ରଦ ବିଷୟରେ ଉ ର ଦେବାକୁ ଅନୁରୋଧ ।

ପ୍ରଥମେ ଆମେ, ଆପଣ ଏବଂ ଆପଣଙ୍କ ପରିବାର ବିଷୟରେ ପ୍ରଶ୍ନ ପଚାରିବୁ । ଏହି ପ୍ରଶ୍ନ ଗୁଡିକ ୫ ମିନିଟ୍ ସମୟରୁ ଅଧିକ ହେବ ନାହିଁ । ଆମେ ଆପଣଙ୍କୁ ପଚାରିବୁ ଯେ, ଆପଣ ଅଧ୍ୟୟନର କାର୍ଯ୍ୟକାଳ ସମୟରେ, ରାତିରେ ଶୋଇବାବେଳେ ମଶାରୀ ବ୍ୟବହାର କରୁଛନ୍ତି କି ? ପରୀକ୍ଷଣ ସମୟରେ ଆପଣ ମାଛିଙ୍କ ଠାରୁ ନିଜକୁ ନିବାରଣ କରିବାପାଇଁ ଏହା ଏକ ଉଦ୍ୟମ । ଆମ୍ଭେମାନେ ଆପଣଙ୍କୁ ଏକ ମଶାରୀ ପ୍ରଦାନ କରିବୁ । ପ୍ରତିଦିନ ସକାଳେ ଆମେ ଆପଣଙ୍କୁ ପଚାରିବୁ ଯେ ପୂର୍ବରାତିରେ ଆପଣ ମଶାରୀ ବ୍ୟବହାର କରିଥିଲେ କି ନାହିଁ ?

ସାଧାରଣତଃ ୩ ପ୍ରକାର ଯନ୍ତାକୁ ନେଇ ଆମେ ପରୀକ୍ଷା କରୁଛି ଯେ ସେଗୁଡିକ କେତେ ମଶାଙ୍କୁ ଧରିପାରୁଛି । ପ୍ରତି ସଂନ୍ଧ୍ୟାରେ ଏହି ଯନ୍ତାଗୁଡିକ ଅନ୍ୟ ଗୃହରେ ଆବ ନ ପ୍ରକ୍ରିୟାରେ ଅବସ୍ଥାପିତ ହେବ । ପ୍ରଥମେ, ଏହି ଯନ୍ତାଗୁଡିକୁ ଘର ବାହାରେ ମାଟିରେ ରଖାଯିବ । ଏହାର ମୂଳ ଭାଗଟି ତରଳ ହୋଇଥିବ ଯେଉଁଥିରେ ଜଳ ସହିତ କିଛି ଘାସର ମିଶ୍ରଣ ହୋଇଥିବ । ଦ୍ୱିତୀୟରେ, ଏହି ଯନ୍ତାଗୁଡିକୁ ଘରବାହାରେ କୌଣସି ସ୍ଥାନରେ ଝୁଲେଇ ରଖାଯିବ ଏବଂ ଶେଷରେ ଏହାକୁ ଘର ଭିତରେ ମଶାରୀ ନିକଟରେ ଝୁଲେଇ ରାଖାଯିବ । ଏହି ସବୁ ଯନ୍ତାଗୁଡିକୁ ସଂଧ୍ୟା ସମୟରେ ସଠିକ୍ ଜାଗାରେ ରଖାଯିବ ଏବଂ ପରଦିନ ସକାଳେ ମଶା ସଂଗ୍ରହ କରାଯିବ ।

ଏହି ଅଧ୍ୟୟନରେ ଅଂଶଗ୍ରହଣ କରିବା ପାଇଁ ଆପଣଙ୍କୁ କିମ୍ବା ଆପଣଙ୍କ ପରିବାରକୁ କୌଣସି କ୍ଷତି କିମ୍ବା ବିପଦ ସହିବା ପାଇଁ ପଡିବ ନାହିଁ । ଯଦି ଆପଣ, ଏହି ଅଧ୍ୟୟନର କୌଣସି ସମୟରେ ବି ନିର୍ଣ୍ଣ ନେବେ ଯେ, ଆପଣ ଏଥିରେ ଅଂଶଗ୍ରହଣ ପାଇଁ ଇଚ୍ଛୁକ ନୁହନ୍ତି, ତେବେ ଆପଣ ବିନା କୋରିମାନା ସହ ପ୍ରତ୍ୟାହାର କରିପାରିବେ । ଆପଣ ଦେଇଥିବା ସମସ୍ତ ତଥ୍ୟ ଗୋପନ ରଖାଯିବ । ଆପଣଙ୍କ ନାମ କିମ୍ବା ଆପଣଙ୍କ ପରିବାର ସଦସ୍ୟଙ୍କ ନାମ, ଏହି ଅଧ୍ୟୟନର ବିବରଣୀରେ ପ୍ରକାଶ କରାଯିବ ନାହିଁ ।

ଆପଣଙ୍କର କୌଣସି ପ୍ରଶ୍ନ ଅଛି କି ? ଯଦି, ଅଧ୍ୟୟନ ସମୟରେ ଆପଣଙ୍କ ମନରେ ଅଧ୍ୟୟନ କିମ୍ବା ଅଧିକାର ସମ୍ବନ୍ଧୀୟ କୌଣସି ପ୍ରଶ୍ନ ଉଠେ, ତାହେଲେ ଆପଣ ଯେକୌଣସି ଅନୁସନ୍ଧାନକାରୀଙ୍କୁ ପଚାରିପାରିବେ । ଆପଣ ଏହି ଅଧ୍ୟୟନରେ ଅଂଶ ଗ୍ରହଣ କରିବାକୁ ଚାହାନ୍ତି କି ? {ଯଦି ହଁ } ମୁଁ ଆପଣଙ୍କ ସମ୍ମୁଖରେ ଏକ ବିବୃତି ପଢିବାକୁ ଯାଉଛି, ଆପଣ ଯଦି ଏଥିରେ ଏକମତ ଦୟାକରି ନିଶ୍ଚିତ ହେବାପାଇଁ, ଆପଣଙ୍କ ଦସ୍ତଖତ ଦେବେ କି ?

ଯୋଗଦାନର ଚୁକ୍ତି

ଉପରୋକ୍ତ ବର୍ଣ୍ଣନୀୟ ଗବେଷଣା ଜନିତ ବିବରଣୀକୁ ପଢିଲି । ଯେଉଁ ଉକ୍ତି ଗୁଡିକ ମୁଁ ବୁଝିପାରିନଥିଲି, ତାହା ମୋତେ ବର୍ଣ୍ଣନା କରାଗଲା ଏବଂ ଯେଉଁ ପ୍ରଶ୍ନ ଗୁଡିକର ଉ ର ମୁଁ ଦେଇନଥିଲି, ତାହାର ଉ ର ମଧ୍ୟ ମୁଁ ଜାଣିଗଲି । ମୁଁ ସ୍ୱଇଚ୍ଛାରେ ଏହି ଗବେଷଣାରେ ଯୋଗଦେବା ପାଇଁ ସଂପୂର୍ଣ୍ଣ ଭାବରେ ଏକମତ ।

ସ୍ୱୀକୃତି ଦେବା ବ୍ୟକ୍ତିଙ୍କ ନାମ .....

(ସ୍ୱୀକୃତି ଦେବା ବ୍ୟକ୍ତିଙ୍କ ସ୍ୱାକ୍ଷର) (ତାରିଖ)

ସ୍ୱୀକୃତି ଉପାର୍ଜନ କରିବା ବ୍ୟକ୍ତିଙ୍କ ନାମ .....

(ସ୍ୱୀକୃତି ଉପାର୍ଜନ କରିବା ବ୍ୟକ୍ତିଙ୍କ ସ୍ୱାକ୍ଷର) (ତାରିଖ)

ସାକ୍ଷ୍ୟ: ...../ .....

Appendix 3: Questionnaire

Entomology Questionnaire

DATE	(dd/mm/yy)
HOUSEHOLD CODE	

	Question	Guidance	Answer
1.	How many children under 5 live in your house?	1. 5 2. 4 3. 3 4. 2 5. 1 6. 0	
2.	Where is the kitchen or cooking area for your house?	1. Indoors 2. Outdoors 3. Sheltered	
3.	What fuel source do you use to cook with?	1. Wood 2. Dung 3. Coal 4. Gas 5. Other (please specify)	
4.	What is the water source for your house?	1. Well 2. Pump 3. Tap 4. Tube well 5. Other (specify)	
5.	How close to your house is the water source? (estimated distance)	1. <10m 2. 11-20m 3. 21-50m 4. 51-100m 5. 100m +	

IF THE ANSWER TO Q6 IS YES PROCEED TO Q7, IF THE ANSWER IS NO: SKIP TO Q8			
6.	Does your household have a latrine?	1. Yes 2. No	
7.	What type of latrine does your house have?	1. Pour flush latrine 2. Pit latrine	

	(If more than one, note latrine used primarily)		
8.	Where do people in your household normally defecate?	1. Latrine 2. Open defecation 3. Other (please specify)	
9.	How close to your house is the latrine/open defecation area? (estimated distance)	1. <10m 2. 11-20m 3. 21-50m 4. 51-100m 5. 100m +	

IF THE ANSWER TO Q10 IS YES: PROCEED TO Q11, IF THE ANSWER IS NO: SKIP TO Q13			
10.	Does your household own any animals? (Include pets and livestock)	1. Yes 2. No	
11.	What animals does your household own? (State number of each animal owned)	1. Cattle	
		2. Pig	
		3. Goat	
		4. Poultry	
		5. Cat	
		6. Dog	
		7. Other (please specify)	
12.	How close to your house are the animals kept? (estimated distance)	1. <10m	
		2. 11-20m	
		3. 21-50m	
		4. 51-100m	
		5. 100m +	

13.	What material are your house walls constructed from?	1. Brick 2. Concrete 3. Daub 4. Other (please specify)	

14.	What material is your roof constructed from?	1. Corrugated iron 2. Thatch/straw 3. Tiles 4. Concrete 5. Other (please specify)	
15.	Do you polish the floors or walls of your house with cow dung? If yes, how frequently?	1. Yes 2. No	
16.	What is the garbage disposal site proximity to your house? (estimated distance)	1. <10m 2. 11-20m 3. 21-50m 4. 51-100m 5. 100m +	
17.	Do you use any fly control measures?	1. Yes (specify) 2. No	

Appendix 4: Household characteristics

Characteristics	Control		Intervention	
	N	Percent	N	Percent
Kitchen Location				
Indoors	66	23.6	76	26.0
Outdoors	49	17.5	47	16.10
Outdoors (sheltered)	165	58.93	169	57.9
Fuel Source				
Wood	99	35.4	125	42.8
Dung	77	27.5	62	21.2
Straw	98	35	100	24.3
Gas	6	2.1	5	1.7
Water Source				
Piped water	7	2.5	9	3.1
Tube well	229	81.8	236	80.8
Open well	12	4.3	17	5.8
Pond/canal/river	32	11.4	30	10.3
Water source distance				
In compound	191	68.2	206	70.6
Outside compound	89	37.8	86	29.5
Defecation sites used				
Open defecation	58	20.7	35	12.0
Pit latrines	190	67.9	99	33.9
Pour flush latrines	32	11.4	158	54.1
Presence of Household latrine				
Yes	39	13.9	194	66.4
No	241	86.1	98	33.6
Latrine distance				
<50m	79	28.2	180	61.6
>50m	201	71.8	112	384
Own animals				
Yes	222	79.3	239	81.9
No	58	20.7	53	18.1
Wall material				
Mud	113	40.4	100	34.3
Brick	95	33.9	127	43.5
Concrete	62	22.1	55	18.8
mixture	10	3.6	10	3.4
Roof Material				
Thatch	144	51.4	158	54.1
Corrugated iron	75	26.8	63	21.6
Tiles	0	0	2	0.7
Concrete	61	21.8	69	23.6
Polish walls/floor with cow dung				
Yes	155	55.4	160	54.8
No	125	44.6	132	45.2

Garbage disposal distance				
<50m	236	84.3	253	86.5
>50m	44	15.7	39	13.4

Household characteristics and fly presence

	N	Synanthropic flies (median, IQR)	difference	95%CI	p-value
Kitchen Location					
Indoors	142	41 (8.5-184.5)	(ref.)		
Outdoors	96	60 (22.5-156)	0.89	[0.62-1.29]	0.543
Outdoors (sheltered)	334	56 (17-139)	0.85	[0.64-1.13]	0.274
Fuel Source					
Wood	224	43 (10-141)	(ref.)		
Dung	139	51.5 (16.5-123.5)	0.71	[0.52-0.95]	0.023
Straw	198	73.5 (24.5-222)	1.18	[0.90-1.54]	0.230
Gas	11	11 (1-20)	0.09	[0.04-0.21]	<0.001
Water source distance					
In compound	397	47 (13-139)	(ref.)		
Outside compound	175	58 (23-202)	1.17	[0.91-1.52]	0.218
Defecation sites used					
Open defecation	93	51 (19-134)	(ref.)		
Pit latrines	289	57 (15-159)	1.48	[1.06-2.07]	0.047
Pour flush latrines	190	48 (16-162)	1.44	[1.00-2.05]	0.023
Presence of Household latrine					
Yes	233	48 (17-152)	(ref.)		
No	339	56 (16-163)	1.03	[0.81-1.31]	0.818
Latrine distance					
<50m	259	43 (11-123)	(ref.)		
>50m	313	63.5 (21-195)	1.36	[1.07-1.72]	0.011
Own animals					
Yes	461	60 (19-184)	(ref.)		
No	111	32 (9-101)	0.84	[0.62-1.13]	0.262
Wall material					
Mud	213	53 (15-134)	(ref.)		
Brick	222	66 (23.5-255)	1.60	[1.22-2.08]	<0.001

Concrete	117	38 (9-116)	0.75	[0.55-1.04]	0.085
Mixture	20	24.5 (16-69.5)	0.50	[0.26-0.96]	0.038
Roof Material					
Thatch	315	57 (18-152)	(ref.)		
Corrugated iron	138	62 (19-219)	1.14	[0.85-1.52]	0.371
Tiles	2	59.5 (17-102)	0.37	[0.05-2.72]	0.332
Concrete	130	38 (9-114)	0.60	[0.45-0.81]	<0.001
Polished walls/floor with cow dung					
Yes	315	57 (19-137)	(ref.)		
No	257	45.5 (12-190.5)	0.97	[0.77-1.23]	0.807
Garbage disposal distance					
<50m	489	51 (14-143)	(ref.)		
>50m	83	73 (32-195)	1.16	[0.83-1.61]	0.398