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Flies as vectors for *Salmonella* spp. and their control in pork butcheries in Kampala, Uganda – A contribution to improve public health

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Martin Heilmann

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Submitted by

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Abbreviations

AIDS	Acquired immune deficiency syndrome
BW	Best-worst
C	Celsius
CE	Choice experiment
CGIAR	Consortium of International Agricultural Research Centers
CO_2	Carbon dioxide
DALY	Disability-adjusted life year
EHEC	Enterohemorrhagic *Escherichia coli*
E.	*Escherichia*
Et al.	*Et alii* – Latin for *and others*
e.g.	Exempli gratia – Latin for *for example*
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
GDP	Gross Domestic Product
GLEAM	Global Livestock Environmental Assessment Model
GPS	Global Positioning System
g	Gram
HACCP	Hazard analysis and critical control points
HIV	Human immunodeficiency virus
h	Hours
i.e.	Id est – Latin for *that is*
ILRI	International Livestock Research Institute
ISO	International Organization for Standardization
kg	Kilogram
LLIN	Long Lasting Insecticidal Nets
m	Meter
µ	Micro
min	Minute
M.	*Musca*

p	P-value
Ref	Reference
S.	*Salmonella*
SD	Standard deviation
sp.	Species
spp.	Latin for multiple species
UGX	Ugandan shilling
UV	Ultraviolet
WHO	World Health Organization
YLL	Years lost to life

1 Introduction

Flies play an important role in the world's biodiversity and the degradation of organic material (Greenberg, 1971). Particularly, synanthropic[1] flies that live close to humans have adapted to the mass of decaying organic matter near human settlements. In their role as epidemiological link between human foodstuff, animal manure and filthy environments, they are known vectors for various diseases caused by bacteria, viruses, helminths and protozoans of public health importance (Greenberg, 1971; Keiding, 1986; Olsen, 1998). Many of these diseases are associated with food (Bidawid *et al.*, 1978; Olsen, 1998; Macovei *et al.*, 2008), which is of emerging importance in developing countries, such as Uganda, where population and food consumption increase, while the implications on public health remain unclear (Roesel *et al.*, 2013).

Foodborne diseases have increasingly become a health concern worldwide (Lukinmaa *et al.*, 2004) and particularly diarrhoeal diseases remain a leading cause of preventable death, as they are the most common illnesses resulting from unsafe food and caused at least half of the global burden of 550 million foodborne diseases in humans in 2010 (WHO, 2015a). *Salmonella* spp. are among the leading causes of diarrhoeal diseases worldwide (Lukinmaa *et al.*, 2004; Velge *et al.*, 2005; WHO, 2015a), and particularly non-typhoidal *Salmonella enterica* in Africa (WHO, 2015a).

Occurrence of diarrheal diseases, especially in developing countries, can be closely related to increased fly numbers, while on the other hand, effective fly control can correlate with a decline of diarrheal diseases (Greenberg, 1973; Echeverria *et al.*, 1983; Olsen, 1998; Graczyk *et al.*, 2001). A closer look at Uganda shows that its pig numbers have increased from 0.19 to 3.2 million within the past three decades (UBOS & MAAIF, 2009) and that it boasted the highest pork consumption of all countries in East Africa in 2011 with 3.4 kg per capita per year (FAOSTAT). The current research approach intends to bring more light into this increasing pork business by aiming at:

[1] From Ancient Greek Anthropos (ἄνθρωπος) for "human"; prefixed with syn (σύν) for "in company with"

1. Epidemiological investigations on *Salmonella* spp. in pork butcheries
2. Abundance of flies in pork butcheries and their potential role as vector for *Salmonella* spp.
3. Impact of an intervention with insecticide-treated fence material to reduce flies
4. People's knowledge, attitudes and practices on food safety

The epidemiological investigations on *Salmonella* spp. in pork butcheries and particularly flies serve as a hazard identification, according to Codex Alimentarius framework[2], to support the pilot of a risk-based management approach with insecticide-treated fence material and a study on people's knowledge, attitudes and practices in a food safety context. The use of insecticide-treated fence material is already known from bed nets to fight malaria (Lengeler, 2000), and to protect livestock from flies and related vector-borne diseases (Bauer *et al.*, 2006; Maia *et al.*, 2010; Rohrmann, 2010; Bauer *et al.*, 2011; Holzgrefe, 2012; Maia *et al.*, 2012). However, its use in managing food safety by controlling flies in pork butcheries at traditional markets in Kampala is new to date.

A casual eye may see that food is more risky at traditional markets than in a supermarket. However, several hundred million people who rank among the world's poorest depend on these informal channels for access to affordable food and income-generating activities. Developments in public health management efforts cannot only ban them, but they also need to recognize and embrace their potential in contributing directly to better life outcomes. Misguided efforts like blunt crack-downs on informal markets only replace one form of poverty by another. In this context, this project is carried out under the banner of the project Safe Food, Fair Food[3], funded by German Federal Ministry for Economic Cooperation and Development, and implemented by ILRI and partners in sub-Saharan Africa:

" […] to improve safety of food in informal markets of sub-Saharan Africa as well as the quality of nutrition and shifting policymaking from knee-jerk reactions to an evidence-based approach."

(Roesel & Grace, 2014)

[2] The Codex Alimentarius was established by FAO and the WHO in 1963 to develop harmonized international food standards, which protect consumer health and promote fair practices in food trade

[3] http://www.safefoodfairfood.wordpress.com

2 Literature review

2.1 Changing pork consumption patterns in Uganda

Over the past three decades, Uganda's pig numbers have increased from 0.19 to 3.2 million (UBOS & MAAIF, 2009). In 2011, Uganda had a pig meat supply of 117,592 tonnes (FAOSTAT). Figure 1 shows the distribution of pigs kept for meat production in different areas of the world in percent according to the Global Livestock Environmental Assessment model (GLEAM[4]). Sub-Saharan Africa seems to have a small pig population with five percent only (48 million) compared to other parts of the world, in particular South-East Asia with 60% of the global population, corresponding with 581 million pigs (GLEAM, 2010).

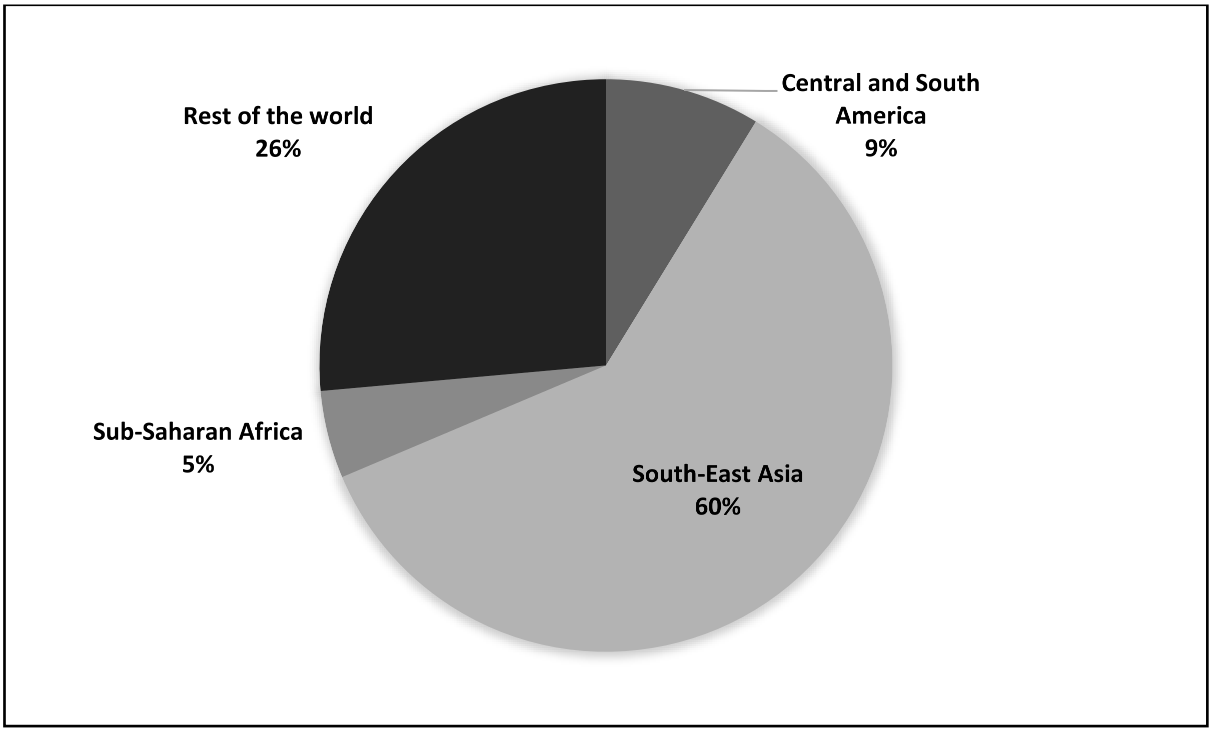

Figure 1 Distribution of pigs kept for meat production worldwide in percent according to regions (GLEAM, 2010)

However, the GLEAM data from 2005 compared with 2010 reveals that the increase of pig numbers in sub-Saharan Africa was 93 percent, far higher than in other regions as shown in Figure 2.

[4] GLEAM is a modelling framework developed at the Animal Production and Health Division of FAO

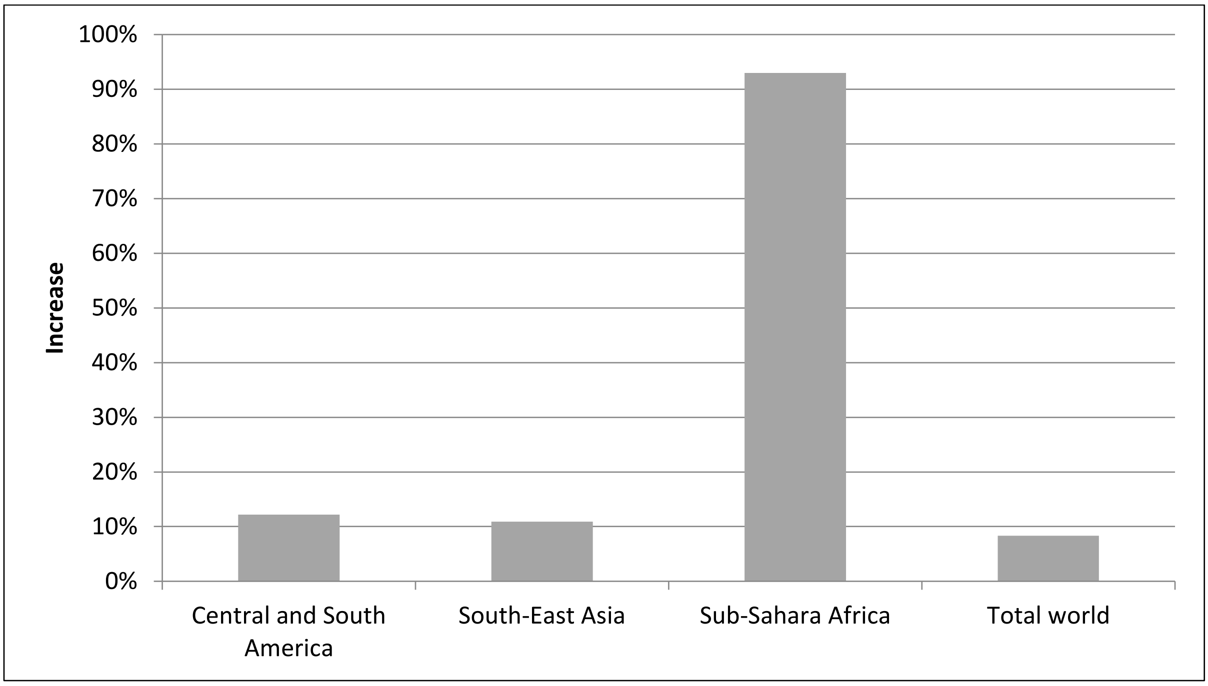

Figure 2 Increase of pig numbers from 2005 to 2010 in percent according to regions (GLEAM, 2010)

Pork is generally a minor component of diets in Africa, and pigs do not figure prominently in farming systems across the continent (Tacher *et al.*, 2000). There may be cultural reasons for this, such as a lack of tradition of pig-keeping and the influence of Islam, as well as production constraints, especially the continued threat of African Swine Fever (ILRI *et al.*, 2011). However, following the Idi Amin[5] years, pig keeping has grown rapidly in Uganda and latest available numbers from 2011 show that the nation boasts the highest pork consumption of all countries in East Africa with 3.4 kg per capita per year[6] (FAOSTAT). Pork is hereby second to beef in terms of meat production, and pork meat accounts for at least one third of the current ten kilogram meat consumption per year (ILRI, 2011). The majority of pigs in Uganda are in the hands of smallholders and contribute to the livelihoods of the poorest (UBOS & MAAIF, 2009). The Consortium of International Agricultural Research Centers (CGIAR) Livestock and Fish research program therefore recognizes small pig production in Uganda as a value chain system with a high potential for poverty alleviation through increased livestock productivity (ILRI *et al.*, 2011; Roesel *et al.*, 2013).

[5] Ugandan president and dictator ruling from 1971 to 1979

[6] Which is still little compared to the annual pork consumption of ca. 40 kg per inhabitant in Germany (Federal Statistical Office *Destatis*, 2013) but also indicates the potential growth in future

However, there is little documentation in Uganda on the context in which pork is produced, marketed and consumed, or on what implications this may have on public health (Roesel *et al.*, 2013). Up to 70 percent of the produced pork is estimated to be consumed in urban and periurban areas (ILRI, 2011) and mainly through informal butcheries and so-called pork joints (Roesel *et al.*, 2013), a combination of a road-side butchery to buy raw pork and a bistro for cooked pork (Figure 3 and 4).

Figure 3 Pork joint in Uganda with the pork hanging in the window © Martin Heilmann, ILRI / Freie Universität Berlin

The close physical contact between raw pork and ready-to-eat food raises questions about food safety, since raw pork is considered unsafe for human consumption (Mwanje, 2012 ; Roesel *et al.*, 2013). Most pork is produced in the rural areas and sold through informal markets where food safety practices such as inspections and the hygiene of sale remises are uncommon (Roesel *et al.*, 2013). Additionally, most of the pork purchased by the butchers comes from pigs slaughtered in backyards or non-gazetted abattoirs without inspection (Heilmann *et al.*, 2015). In the context of ILRI's goal to improve food safety, further investigations are required in order to identify food related risks and to provide appropriate recommendations for prevention and improvements of food safety.

Figure 4 Pork joint in Uganda from the inside © Martin Heilmann, ILRI / Freie Universität Berlin

2.2 Foodborne diarrheal diseases

Foodborne diseases have increasingly become a health concern worldwide (Lukinmaa *et al.*, 2004). Hereby diarrhoeal diseases remain a leading cause of preventable death as they caused 230,000 deaths in humans and represent the most common illnesses resulting from unsafe food causing at least half of the global burden of 550 million foodborne diseases in humans in 2010 (WHO, 2015a). All the more important, forty percent of the foodborne disease burden was among children under five years of age (WHO, 2015a) and accounted for nine percent of deaths within this age band in Uganda in 2014 (WHO, 2015b).

The economic and health impacts are even greater than the figures suggest given that cases of foodborne illness often go unreported (FAO & WHO, 2006) and concern not only early deaths but also the number of years lost due to chronic or acute diseases and disability. Increasingly common in the field of public health is the indicator *disability-adjusted life years* (DALY), which was developed in the 1990s as a measure of the overall disease burden expressed as the number of years lost due to ill-health, disability or early death. The WHO shows that the global burden of foodborne diseases was 33 million DALYs in 2010 (WHO, 2015a).

Most diarrheal diseases are transmitted primarily through the faecal–oral route (Keusch *et al.*, 2006). The transmission may be direct through person to person/animal, or indirect through water or food. The contamination of food or water may itself be through insects, soil, air or other potential pathogen vectors in the environment. The contribution of specific sources (including types of foods) and transmission routes have been gathered under the term *source attribution*. Source attribution becomes an increasingly important tool for identifying and prioritizing effective interventions to prevent and control foodborne diseases (Havelaar *et al.*, 2007). It is critical for delineating foodborne and waterborne diseases, since water is ingested just as food, and particularly related to zoonotic diseases as the water source itself is often contaminated by an animal reservoir, including food-producing animals (WHO, 2015a).

***Salmonella* spp. and its role as foodborne pathogen**

Salmonella spp. are among the leading causes of diarrhoeal diseases worldwide (Lukinmaa *et al.*, 2004; Velge *et al.*, 2005; WHO, 2015a), and particularly non-typhoidal *Salmonella enterica* in Africa (WHO, 2015a).

The current view of *Salmonella* (*S.*) taxonomy assigns the members of this genus into two species ubiquitous pathogenic to humans and animals: *S. bongori* and *S. enterica.* The latter itself is divided into six subspecies, *enterica, salamae, arizonae, diarizonae, indica,* and *houtenae,* also known as subspecies I, II, IIIa, IIIb, IV, and VI, respectively (Popoff & Le Minor, 1997). The nomenclature used in this thesis is based on names for serotypes in subspecies I. For example, *Salmonella enterica* subsp. *enterica* serotype Enteritidis, is shortened to *Salmonella* serotype Enteritidis or *Salmonella* Enteritidis (Brenner *et al.*, 2000).

The natural habitat of members of *Salmonella enterica* subspecies *enterica* is the intestines of warm-blooded vertebrates (Uzzau *et al.*, 2000). They are usually faecal-oral transmitted by ingestion of food or water contaminated by infected faeces (Uzzau *et al.*, 2000). Serotypes, such as *S.* Enteritidis or *S.* Typhimurium generally cause gastrointestinal infections (Velge *et al.*, 2005). The emergence of foodborne human infections caused by *S.* Enteritidis, and aggravated by multiple-antibiotic resistant strains, was shown in various studies around the world, including sub-Saharan Africa (Rabsch *et al.*, 2001; Molbak, 2005; Velge *et al.*, 2005; Callejon *et al.*, 2015; Tinega *et al.*, 2016; Ndoboli *et al.*, *forthcoming*). The Centers for Disease Control and Prevention of the United States classifies drug-resistant foodborne bacteria, including *Campylobacter, Salmonella* and *Shigella* species, as serious health threats. Therefore all potential sources of multi-drug resistant bacteria should be considered to mitigate these threats including strategies devised to reduce their presence in foods (Doyle, 2015)

Situation in Kampala regarding food-associated *Salmonella* spp.

There are only a few epidemiological investigations on food-associated *Salmonella* spp. in Kampala (Nasinyama, 1996; Nasinyama *et al.*, 1998; Bosco *et al.*, 2012; Mugampoza *et al.*, 2014; Tinega *et al.*, 2016). In these investigations significantly ($p<0.05$) more cases of diarrhoeal diseases were reported in low than in high socio-income areas (Nasinyama, 1996). Meat and meat products were hereby the most common source of infection with *Salmonella* spp. It was further reported that having left-over food, pests animals, untreated drinking water, or fewer income earners in the household were positively associated with diarrhoea ($p<0.05$) (Nasinyama, 1996). It could also be shown that *Salmonella* spp. occurred in stools of 8.1 percent of patients with acute diarrhoea in Kampala district and almost 70 percent of these isolates showed multiple antibiotic resistances (Nasinyama *et al.*, 1998). The authors concluded that the epidemiological patterns of *Salmonella* spp. are rather complex and sources of infection include various stages of the food chain. Any attempts to control *Salmonella* spp., therefore, require an understanding of the potential entry points for infection and an integrated approach for intervention, including environmental sanitation entailing both pest control and hygienic disposal of waste (Nasinyama, 1996) .

Bosco *et al.* (2012) found that in Kampala, 76 % of a variety of food from animal origin was positive for *Salmonella* spp. By comparing resistance pattern and plasmid profiles from these isolates and others from humans and farm animals, the majority of *Salmonella* spp. were multidrug-resistant (Bosco *et al.*, 2012).

2.3 Flies and their role as vectors

Flies are cosmopolitan and play an important role in the world's biodiversity and in the degradation of organic material (Greenberg, 1971). Non-biting domestic filth flies, including the families Sarcophagidae (flesh flies), Muscidae (houseflies and latrine flies) and Calliphoridae (blowflies and bottleflies) are specialized to the mass of organic material near human settlements and are therefore considered synanthropic (Greenberg, 1971, 1973; Förster *et al.*, 2009).

2.3.1 Systematics

The dipterans (*two-winged*) comprise a large order, containing more than 200,000 species. Due to their feeding and breeding ecology, synanthropic flies are of particular importance from a food safety perspective. Three main families are relevant in context of this research:

Calliphoridae

Calliphoridae are known as bluebottles, greenbottles or blowflies. Adults are commonly shiny with metallic colouring and commonly found feeding and breeding on meat, fish, dairy products, animal carcasses, garbage, and excrements. Consequently these flies can harbour many agents pathogenic to humans and animals (Zumpt, 1956; Greenberg, 1973; Grella *et al.*, 2014).

Sarcophagidae

Sarco in ancient Greek refers to flesh, which deduces their common name *flesh fly*. Adults are blackish with grey longitudinal stripes, which are never metallic such as in Calliphoridae. These flies differ from most flies since they are ovoviviparous and opportunistically deposit hatched maggots instead of eggs on dung, carrion, decaying material and open wounds of mammals. Pig carcasses are also commonly used as breeding places (Szpila *et al.*, 2015), which can contaminate the food itself but can also lead to intestinal pseudomyiasis to people or animals that have been accidentally ingested dipteran larvae (Zumpt, 1965; Udgaonkar *et al.*, 2012).

Muscidae

This family is commonly known as houseflies due to their synanthropic life close to humans. The family contains almost 4,000 described species in more than 100 genera with its worldwide known representative *Musca (M.) domestica* alias common housefly.

The reproductive habits and feeding ecology of synanthropic flies are very similar (Greenberg, 1973). Since the common housefly is by far the most significant fly around human settlements, its morphology, development and epidemiology will be explained in further detail in the following sections.

2.3.2 Morphology

Adult houseflies are 5 – 8 mm long, have a three segmental body like all insects and one pair of membranous wings (Figure 5). The hind wings are reduced to form club-shaped halteres used as balancing organs during flight. Females are slightly larger than males and compound eyes are set wider apart than in males, which can be used for sex identification. The thorax is yellowish-greyish to dark-greyish with four black length stripes. The abdomen is entirely haired with a dorsal dark middle stripe and a yellowish bottom side. The wings are transparent to smoky with a specific wing venation. These phenotypic characters vary strongly between species and are commonly used for species identification and taxonomy (Taylor *et al.*, 2007).

Figure 5 *M. domestica* on pork meat using its proboscis with the labellum for identifying and liquefying food © Martin Heilmann, ILRI / Freie Universität Berlin

To find and identify foods and breeding places, flies use the labellum, a sponge-like organ at the distal end of the flies' mouthpart (Figure 5 and 6), as their primary taste organ (Shim *et al.*, 2015). They can also use chemosensory bristles on their legs and the third segment of the antennas, called arista, which is sensitive to changes in temperature and moisture due to thermo- and hygroreceptors (Taylor *et al.*, 2007). Houseflies have siphoning-sucking mouthparts with a tank. This structure is called the proboscis and is typical for non-biting insects as they use these tubular mouthparts for feeding and sucking. This adaptation is of major importance for pathogen transmission and contamination (Greenberg, 1973). The labellum at the end is used to suck the food up similar to a sponge. The absorbed food, potentially including bacteria, is initially stored in the fly crop as temporary reservoir, where food can be predigested via salivary carbohydrases and/or regurgitated to liquefy the next meal (Lehane & Billingsley, 1996).

2.3.3 Development

Houseflies, like all dipterans, are holometabolic insects and pass through four distinct stages during their life cycle: egg, larva, pupa and adult. The cycle depends strongly upon temperature and other environmental factors (WHO, 1997). The most important feeding sources to complete this cycle in order to gain enough energy are proteins or proteinaceous secretions such as meat, flesh, wound secret, blood or saliva of warm-blooded mammals (Greenberg, 1971).

Three to four days after insemination the females lay egg packages, containing 100 – 150 white eggs, in organic material such as faeces, cadaver, meat or other food and decomposing material (Ebeling, 1975). Under conditions of 30 – 37°C and a humidity of over 90 percent, development takes up to two or three weeks to be completed. Temperatures below 12°C lead to a dormant state in adult or pupal stages, and temperatures over 45°C cause death for eggs, larvae and pupa (Keiding, 1986).

Assuming that conditions remain favourable, the first larva hatches after 12 – 24 h and develops into the third-stage larva within seven days through two mouldings. Instead of a head, the acephal and legless larvae have a cephalopharyngeal skeleton with mouth hooks to hitch onto the substrates they were dropped into. On their posterior they have paired breathing openings, so called bulla stigmalis, which are also used for species identification.

For pupation, the last larva preferably burrows into mostly dry and cool ground and retreats into a coarctate pupa. After the pupal stage of an average of ten days, the imago hatches. After

36 h, the first insemination can follow. In one life cycle, a female can lay about 500 – 2,000 eggs. In summer or warmer periods, up to 20 generations may occur annually due to flies' short generation time and high reproduction rate. This explains massive infestations of flies; especially in warmer seasons or tropical areas. The life span of flies ranges from two weeks up to two months. However, under suboptimal conditions the cycle may require more time (Greenberg, 1971).

2.3.4 Epidemiology

Houseflies are globally distributed, extending from sub-polar regions to tropical regions. In temperate zones, they usually fly from May to October. Indoors, like stables or houses, and in warmer regions, they are active and can reproduce throughout the winter (Graczyk *et al.*, 2001). Favourable climatic conditions in tropical areas allow fly activity throughout the year, which is of importance in the developing world due to the added harms of the lack of safe drinking water, sanitation and hygiene, as well as poorer overall health and nutritional status (UNICEF/WHO, 2009).

Flies' radius of action is 500 – 800 m but a passive distribution over longer distances is possible and cases of individual flies travelling as far as 32 km have been described (Murvosh & Thaggard, 1966; Graczyk *et al.*, 2001).

Houseflies are mainly diurnal (Nazni *et al.*, 2007) when temperature is above 14°C. They prefer sunny places while they rest on walls, trees or other dark, calm areas during the night. Under colder conditions, the housefly overwinters in either egg, larval or pupal stage under manure piles, soil, crannies or other protected locations.

2.3.5 Diseases

Historically, the first reported investigations on the association of living bacteria within the alimentary canal and the body surface of houseflies were made by Graham-Smith (1910), who experimentally infected flies with pathogens and measured the recovery over time. A study in the 1950s confirmed the flies' role as carriers and further revealed that the internal (gastrointestinal) bacterial load of *M. domestica* was approximately 20 times the number of external bacteria on the exoskeleton (Mcguire & Durant, 1957). However, bacteria harbored externally may dry during flight and lose viability (Yap *et al.*, 2008), and bacteria harbored internally face digestion and defensive responses from gut epithelia (Nayduch *et al.*, 2013).

Not only adult flies, but all trophic levels of houseflies, including larvae, pupae and adults, develop and feed in decaying organic matter such as animal manure, human refuse, open privies, soiled animal bedding, litter, waste and foodstuff, which all are areas teeming with diverse and active microbial communities (Greenberg, 1973; Moon, 2002). As such, flies are commonly contaminated with various microorganisms, which make them potential vectors for pathogens including multidrug-resistant bacteria (Graczyk *et al.*, 2001; Macovei *et al.*, 2008; Gupta *et al.*, 2012; Zurek & Ghosh, 2014). Particularly in areas with both high fly abundance and exposure to human foodstuff, diseases in humans through food contamination are more likely (De Jesus *et al.*, 2004). In this context, seasonal patterns of diarrheal diseases were shown to be enhanced by high temperature as it triggers both a rapid growth of bacteria and an increase of the fly populations (Graczyk *et al.*, 2001). Studies in Thailand, for example, found a connection between seasonal peaks of fly populations associated with outbreaks and cases of food-associated pathogens such as *E. coli, Shigella* spp., *Vibrio cholera* and *Vibrio fluvialis* (Echeverria *et al.*, 1983; Graczyk *et al.*, 2001).

Mechanisms of pathogen transmission

As adult houseflies are highly mobile, they can transport bacteria from septic environments to other substrates via mechanical dislodgement from the exoskeleton (feet, wings, bodies), faecal deposition or regurgitation of crop contents (Moon, 2002).

For transmission of pathogens, the front of the proboscis with the labellum, plays a major role (Greenberg, 1973). This structure consists of many grooves, called pseudotracheae, which sop up liquids and pathogens similar to a sponge (Figure 6). But also the tarsi can contribute to transmission of pathogens through the pulvilli, a pad-like structure between the tarsal claws of the legs, which are coated with an adhesive substance released by glandular hairs (Sukontason *et al.*, 2006). This sticky substance helps flies to stick on surfaces, but also allows microorganisms to stick. Other body parts, such as wings are considered as less important in terms of contamination (Sukontason *et al.*, 2006; Yap *et al.*, 2008).

Kobayashi *et al.* (1999) have shown in experiments that when feeding EHEC to houseflies the ingested bacteria were harboured in the intestine of flies and were continuously excreted for at least three days after feeding. Further, those bacteria actively proliferated in the previously mentioned pseudotracheae of the labellum. However, food masses containing EHEC in the fly intestine were completely surrounded by a peritrophic membrane during digestion and discharged rapidly (Kobayashi *et al.*, 1999). The researchers concluded that the persistence of bacteria in the intestine and faeces is mainly a result of proliferation in the mouthparts and accumulation in the crop (Kobayashi *et al.*, 1999). Other studies revealed that there are also pathogens which can pass through the gastrointestinal track without alteration of their infectivity (Graczyk *et al.*, 1999).

Other stages in the life cycle of flies, such as the maggot stage, are of lesser importance in flies' role as vectors, but are described as carriers as they feed and thrive on substrates potentially containing pathogens (Greenberg & Klowden, 1972). Particularly, secondary infections occur through larvae growing inside the host while feeding on its tissue, known as myiasis (Zumpt, 1965). Maggots have limited mobility, though, and only a few pathogens, if any, survive the skinning process when becoming an adult fly (Greenberg, 1959; Graczyk *et al.*, 1999; Graczyk *et al.*, 2001).

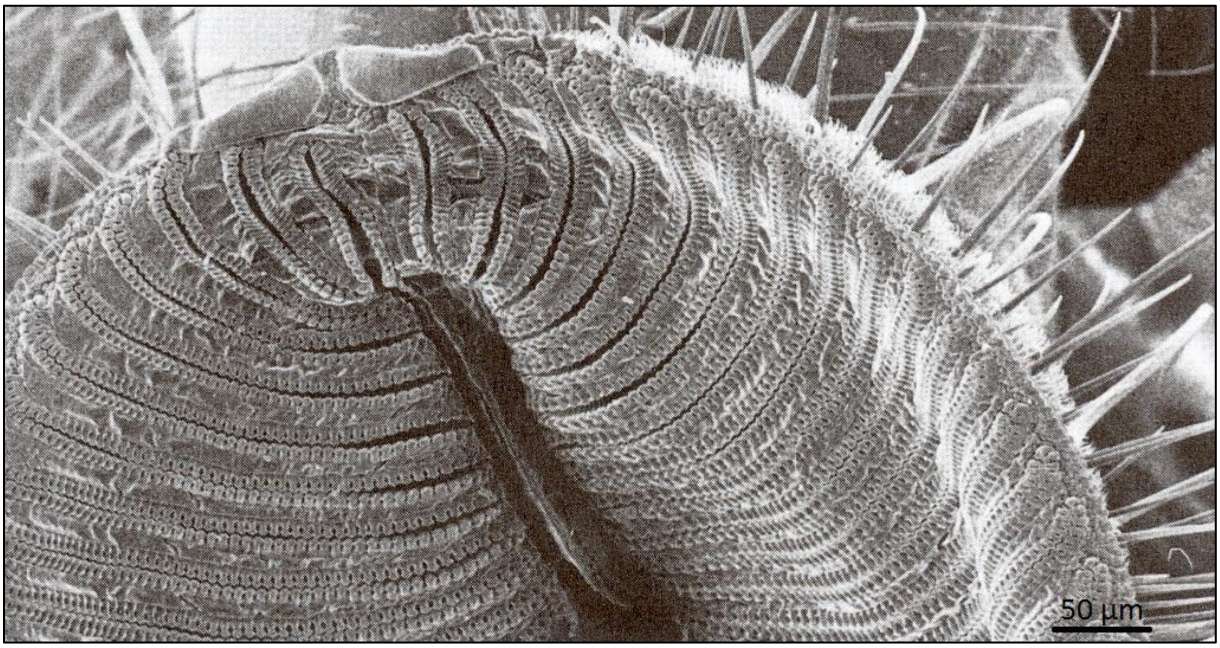

Figure 6 Distal end of housefly's proboscis with labellum and pseudotracheae. Scanning electron micrograph © Prof. Dr. H. Mehlhorn

Pathogens associated with flies

More than 100 pathogens and parasites have been isolated from flies including 65 disease organisms that affect humans and animals (Greenberg, 1971, 1973; Khan & Huq, 1978). Flies have been implicated in the transmission of diseases including anthrax, ophthalmia (including trachoma), typhoid fever, tuberculosis, cholera, infantile diarrhoea and traveller's diarrhoea

(Greenberg, 1965; Keiding, 1986; Alam & Zurek, 2004; Szalanski *et al.*, 2004; Yap *et al.*, 2008). They can further harbour pathogenic bacteria including *Salmonella* spp. (Greenberg, 1971; Bidawid *et al.*, 1978; Mian *et al.*, 2002), *Proteus* spp., *Shigella* spp. (Greenberg, 1971), *Chlamydia* spp., *Campylobacter jejuni* (Shane *et al.*, 1985; Förster *et al.*, 2009), *Klebsiella* sp. (Fotedar *et al.*, 1992), *E. coli* O157:H7 (Kobayashi *et al.*, 1999), *Yersinia pseudotuberculosis* (Zurek *et al.*, 2001) and *Helicobacter pylori* (Li & Stutzenberger, 2000).

Transmission of viruses, helminths, parasitic protozoa or fungi is reported in humans and animals, including poliomyelitis (Zumpt, 1949), foot-and-mouth disease (Hoffmann & Herrmann, 2002), infectious bovine rhinotracheitis (Johnson *et al.*, 1991), *Ancylostoma caninum*, *Hymenolepis* spp., *Taenia* spp., *Trichuris trichiura* (Umeche & Mandah, 1989), *Cryptosporidium parvum* (Graczyk *et al.*, 2003), *Toxoplasma gondii (Wallace, 1971)* and *Candida* spp. (Förster *et al.*, 2009).

Apart from diseases associated with flies, flies can also be a nuisance to humans with negative impacts as the presence of flies is considered a sign of unhygienic conditions (WHO, 1997). In livestock production, flies can also lead to decreased productivity through animal disturbance (Taylor *et al.*, 2012), either directly through biting flies causing skin damages and blood losses or indirectly due to pain and stress as a nuisance factor leading to increased blood cortisol concentrations (Byford *et al.*, 1992).

Studies indicate further that houseflies are the main synanthropic arthropods (among cockroaches, wasps, spiders and ants) carrying the majority of human pathogens in hospital environments (Sramova *et al.*, 1992; Graczyk *et al.*, 2001). In dairy farming for example, flies are associated with teat lesions and high levels of *Staphylococcus aureus* mastitis (Ryman *et al.*, 2013). Recent studies also point out the role of flies as vectors for antimicrobial resistances in Dutch poultry farms (Blaak *et al.*, 2014; Doyle, 2015), in swine farms in the United States (Ahmad *et al.*, 2011), and even widespread dissemination of plasmids with antimicrobial resistance genes between farms (Doyle, 2015; Usui *et al.*, 2015). Similar studies exist for transmission of resistant bacteria at Chinese airports (Liu *et al.*, 2013).

2.3.6 Vector control

An integrated vector control begins with knowledge, awareness and should encompass environmental modifications at early stages, for instance, infrastructural development and sanitation services (Lizzi *et al.*, 2014). The latter includes the building's structure, but also regular cleaning and safe disposal of waste and other sources, which could attract flies to potential breeding and feeding places. Taylor *et al.* (2007) have confirmed that keeping facilities clean and dry can successfully mitigate the development of fly larvae at an early stage. It is shown that synanthropic flies are more frequent in urban areas where unsanitary conditions are present and usually scarce when sanitary conditions are enforced (Graczyk *et al.*, 2001). Furthermore fly control is closely related to fewer cases of enteric diseases (Greenberg, 1971, 1973; Olsen, 1998; Graczyk *et al.*, 2001). Similar results are shown by Echeverria *et al.* (1983) and Khalil *et al.* (1994) where fly control along with education coincided with a significant reduction of gastroenteritis among children in developing countries. Vector control can be complemented by various physical measures hindering flies from entering the facilities by using nets on doors, windows and other entrances or trapping flies directly with sticky-, electric-, UV-light or CO_2- traps. Also air conditions can be taken into account as flies avoid draft (Shiff, 1998).

Biological control

Natural enemies of flies are fungi, mites, parasitic hymenopterans, coleopterans, other flies and their larvae (Kühlhorn, 1983). Since the importance of arachnids, birds and other vertebrates as natural predators of flies is considered negligible (Kühlhorn, 1983), they will not be further discussed.

Within the class of insects, the dump fly, *Hydrotaea aenescens* (syn. *Ophyra aenescens*), is an antagonist of the common housefly. Their larvae use the same breeding substrate and kill housefly larvae to cover their nutritional needs for growth. In laboratory experiments, the hatching rate of houseflies is shown to be around 81 percent without the presence of the *Hydrotea aenescens*. This rate decreased to 2.8 percent when *Hydrotea aenescens* was present (Müller, 1982). Other predators among insects are scorpion wasps (Ichneumonidae), namely *Muscidifurax raptor*, *Spalangia* spp. and *Muscidifurax zaraptor* (Skovgard & Nachman, 2004; Eckert *et al.*, 2008). After releasing those pupal parasites, they lay their eggs in the fly pupae, which results in significant suppressions of housefly populations (Coch, 1981). However, their use as effective tool against flies depends strongly on the time of year and the number of wasps (Coch, 1981). Another example of a biocontrol agent is the entomopathogenic fungus *Entomophthora muscae* (syn. *Empusa muscae*). It infests insects including houseflies, and leads to widespread deaths in fly populations (Kühlhorn, 1983).

The Gram positive bacterium *Bacillus thuringiensis* (Bt), can also be used to control flies as it produces proteinaceous crystals during sporulation that are ingested by flies, causing a release of endotoxins finally destroying the intestinal epithelium (Dean, 1984). *Bacillus thuringiensis* also has an effect on larvae in terms of mortality and pupation rate (Labib & Rady, 2001). However, studies indicate that adult houseflies can develop resistance to the Bt-toxin (Harvey & Howell, 1965; Wilson & Burns, 1968). On top of natural predators, genetic modification such as the *Sterile Insect Technique* have become more common (Raphael *et al.*, 2014). One example is to sterilize male flies by using radio or chemotherapeutics and then to release them in sufficient numbers in natural habitats thereby interfering with the reproductive cycle.

The use of plants and their terpenes as repellents ranging from cloves, nutmegs, juniper, and pines have been described but a major disadvantage is the short persistence of their effects. Kumar *et al.* (2014) used plant monoterpenes such as menthone, menthol, menthyl acetate or limonene against different life stages of housefly. Bioefficacy against housefly adults were highest for menthol (96 percent) and menthone (83 percent). Another study in houseflies showed an insecticide effect of the castor-oil plant (*Ricinus communis*). Mortality was higher as the time of exposure to the extract and a significant reduction of the pupal development in the presence of the extract was observed (Alvarez Montes de Oca *et al.*, 1996).

Chemical control

Insecticides are mainly used in fly control, but should only be an addition to an existing and integrated hygiene management system (WHO, 2006). A good and effective insecticide requires both high selectivity and minimal toxic effect on warm-blooded animals and other non-target insects (Eckert *et al.*, 2008). The insecticide agents are classified depending on the product formulation as a liquid, aerosol or granulates, and the method of absorption in insects through contact, ingestion or respiration. Since pyrethroids constitute a majority of commercial household insecticides (Metcalf, 2000), they will be explained in detail in the following section. Apart from that, other chemical classes such as organophosphates, carbamate, avermectine, chlornicotinyl- and phenylpyrazol-compounds are also important but will not be further described.

Pyrethroids

Pyrethroids are lipophilic compounds, and are mostly used as contact or feed poison. Toxic effects are mediated through preventing the closure of voltage-gated sodium channels in axonal membranes leading to an uncontrolled influx of sodium and therefore to a constant depolarization. Consequently symptoms range from nervous disorders, agitation, paralysis and death depending on dose, mode of application and exposure time (Soderlund *et al.*, 2002).

The predecessors of synthetic pyrethroids are natural pyrethrins, which originate from *Chrysanthemum* plants. Synthetic pyrethroids can be divided into type I without a substitution at the alpha-carbon such as permethrin, and type II with a cyano substitution at the alpha-carbon such as deltamethrin, which is often used in insecticide-treated fence material.

Insecticide-treated fence material

Insecticide-treated fence material is commonly used in many tropical areas as insecticide-treated bed nets to fight malaria and other vector-borne diseases such as dengue, Chagas and African trypanosomiasis (sleeping sickness, nagana) in humans and animals (O'Meara *et al.*, 2010; Bauer *et al.*, 2011; Bauer *et al.*, 2012). While attracted by the odours of targets, such as humans or animals, flies frequently collide with the net, thereby picking up the insecticide deltamethrin through their tarsi. After contact, the insects will become paralyzed within a few minutes and ultimately perish. The use of these interventions has proven to be cost effective (Wiseman *et al.*, 2003) and of great significance in terms of reducing malaria prevalence amongst infants (Leenstra *et al.*, 2003) and pregnant women (Gamble *et al.*, 2006).

The insecticide-treated fences also play a role in protecting livestock from vector-borne diseases: Bauer *et al.* (2006) initiated a study in western Kenya with beta-cyfluthrin-treated mosquito nets to protect dairy cattle. The intervention has led to a reduction of various pest insect species and lower infection rates of trypanosomes in animals. A similar study in Ghana against biting and nuisance flies resulted in consistently lower catches of insects and a considerable reduction of 70 – 80 percent of nuisance and animal disturbance (Maia, 2009; Maia *et al.*, 2010). In 2007, another control trial investigated the protection of pigsties with insecticide-treated mosquito fences in the Ghanaian forest. A reduction of tsetse flies of more than 90 percent was shown in the protected area, while only seasonal variations in the control were observed. Also, the trypanosome prevalence in pigs decreased significantly (Bauer *et al.*, 2011).

A study in Germany investigated the effectiveness of deltamethrin-treated nets to protect cattle stables from midges, by netting doors and windows. A significant reduction of midges' blood meals on animals was shown, but no significant reduction of other pest insects compared to the non-netted control stable. Reasons given for a missing reduction have been the big mesh size of the net, which smaller midges can easily pass through. The unsatisfactory effect of the nets on flies was presumably due to, among other things, the detected insecticide resistance against deltamethrin (Rohrmann, 2010).

Another study in Germany was performed in the grazing season to protect horses from biting and sucking flies with insecticide-treated fence material. After intervention, the density of Muscidae decreased by about 60 percent and the actual infestation on the individual animal by 97 percent (Blank *et al.*, 2005). These results were confirmed by Zaspel (2008) where reductions of 33 percent in flies and 50 percent in tabanids were recorded on two protected horse farms. However, the netting material had also an impact on the number of non-target insects such as Syrphidae, Asilidae, Conopidae, Hymenoptera, Coleoptera, Odonata, Saltatoria, Neuroptera, Mecoptera and others (Zaspel, 2008).

A pilot study in Nairobi, Kenya used insecticide-treated fence material in 30 butcheries over five weeks and looked into the contamination of butcheries with enteric bacteria and the effect of the net on fly density. *E. coli* was the predominant contaminant on flies, meat and butchery surfaces. The net was effective at reducing the fly density in butcheries and has therefore been recommended for use. The study further concluded that trial studies should be done in the dry season when fly numbers are relatively high (Atuhairwe, 2014).

One reason for the development of such new approaches and considerable investments is the development of chemical resistance, due to indoor use of insecticides, particularly spraying, which causes a strong selection pressure on insects (Maia *et al.*, 2012). According to the WHO, resistance is hereby defined as the ability of a species to withstand the effects of an insecticide by becoming resistant to its toxic effects by means of natural selection and mutations (Davidson, 1957). Widespread distribution of pyrethroid-treated bed nets to fight malaria for example has led to resistance in its vectors in sub-Saharan Africa (Weetman & Donnelly, 2015). Similarly, the over-reliance on the most effective molecules, such as pyrethroids, has led to the widespread development of resistance in target species such as *M. domestica* in Germany (Eckert *et al.*, 2008; Jandowsky, 2010; Bauer *et al.*, 2012). Bauer *et al.* (2012) concluded that considering the likelihood of future vector-borne diseases outbreaks and the scarcity of new active ingredients, there is an urgent need to have effective, targeted and environmentally non-hazardous vector control techniques as a first line-defence at hand before an effective vaccine can be developed.

Persistence and ecotoxicology

The application of micro-encapsulated deltamethrin on fence material remained highly effective under field conditions over two years, according to Peters *et al.* (2015). In tropical areas, the nets persisted for at least nine months as shown by Maia *et al.* (2010) despite the net's exposure to high rainfall and intense sunlight. However, indoor use of Long Lasting Insecticidal Nets (LLIN) is expected to last about three years, including about twenty washings (Kroeger *et al.*, 2004). In this context, LLINs have been scaled up across much of Africa through the WHO's Roll Back Malaria global partnership since 1998.

Pyrethroids have less environmental impact on soil and water than earlier classes of insecticides due to their strong chemical bond to soil particles, implicating little risk of leaching appreciably into groundwater, according to the Agency for Toxic Substances and Disease Registry at the United States Department of Health and Human Services (Todd *et al.*, 2003). However, pyrethroids are highly toxic for bees, fish and other non-target and aquatic organisms (Friberg-Jensen *et al.*, 2003; Van Wijngaarden *et al.*, 2005). Deltamethrin particularly does not persist long in the physical environment as it is rapidly processed and degraded through hydrolysis, photolysis and by microorganisms. The structural modifications of deltamethrin entails a strong lipophilicity, high absorbance on hard inorganic material and a low steam pressure, which makes it easy to utilize for impregnations and outdoors. Environmental persistence of the deltamethrin-treated fence material, similar to the one used in this research, was investigated in a study in Germany where dairy cattle were protected with insecticide-treated fence material. Samples were taken before and after the trial but no deltamethrin was detected in the milk, faeces or water samples (Rohrmann, 2010). Soil samples taken directly under the net showed a contamination with deltamethrin of 16,5 µg/kg, while soil samples in a close range[7] had four µg/kg deltamethrin (Frenzel, 2008; Rohrmann, 2010).

[7] *Range* and thresholds have not been specified

2.4 Choice experiment

2.4.1 Best-worst method

Best-worst (BW) method is a special form of a discrete choice experiment (CE) to determine the relative strength of preferences subjects have for a set of attributes. This approach was developed by Louviere and Woodworth (1990) and first published by Finn and Louviere (1992). The method is an extension of paired comparison, which is less demanding than ranking, since subjects need only decide between two options, A and B. Instead of arranging items in pairs, BW presents subjects with subsets of three or four choices from the larger set, and asks them to choose their most and least preferred choice in each subset. In addition, this kind of arrangement decreases the number of subsets necessary and is mostly free of the scale bias known in rating scales (Cohen *et al.*, 2009). In fact, BW overcomes one of the main limitations of scaling methods, namely that scaling is difficult to compare across subject groups. There are proven differences between responses from, for example, Asian countries, where people overwhelmingly avoid extremes, and Western cultures, who are more likely to use the full scale continuum to express hedonic differences (Yeh *et al.*, 1998). Therefore, the BW method is very powerful for cross-cultural research studies (Cohen *et al.*, 2009). The same applies for ranking experiments, which are sometimes exhausting for respondents when the number of items to rank is high. In BW, attributes can be arranged by applying a balanced incomplete block design within choice sets. Each item appears the same number of times across all choice sets, and each pair of attributes is listed precisely once across all the tables. This design reduces the number of choice sets and allows the researcher to obtain a full ranking of the items studied. Reducing the number of choice sets, and therefore the length of the survey, also likely decreases the likelihood that respondents' attention span is maxed out.

BW has recently been used in food and health care research all over the world (Auger *et al.*, 2007; Jaeger *et al.*, 2008; Lee *et al.*, 2008; Lusk & Briggeman, 2009; Dekhili *et al.*, 2011). Depending on the use, there are a range of different analysis methods for BW data like BW scores, standardized scales, importance weights, discrete choice and segmentation models (multinomial logit, latent class, etc.), just to mention a few.

3 Materials and Methods

3.1 Study design

A baseline study was conducted in Kampala with 60 randomly selected pork joints, including interviews with 60 butchers and 240 customers between June and July 2014. Structured questionnaires for butchers (Annex 1) and their customers (Annex 2) were used during face-to-face interviews to assess people's knowledge, attitudes and practices on food safety and key practices of pork butchers in Kampala. Additionally their perception of what is important to their customers when buying pork was assessed using BW method in the questionnaires.

In order to determine contamination with *Salmonella* spp., 60 butcheries from the baseline interviews among with 17 other randomly selected butcheries were sampled for *Salmonella* spp. from June – October 2014. Samples were taken once in all butcheries and included raw and roasted pork, water, tomatoes, cabbage, onions, flies and swabs of butchers' hands and their equipment. Cultural isolation of *Salmonella* spp. was performed according to ISO 6579:2002.

From August – November 2014, an intervention with insecticide-treated fence material took place in pork butcheries to determine the impact on the density of flies. The monitoring included a zero value survey prior to introduction of the fence material in the intervention butcheries. A controlled longitudinal study followed where 23 out of the 60 butcheries from the baseline were purposively selected. Eighteen were allocated as intervention and five as control.

After finishing the monitoring, the intervention arm of 18 butcheries was re-sampled for *Salmonella* spp., according to the protocol used in the baseline sampling.

Ethical considerations

The study involved collecting information and biological specimen from humans, their foods and work places. It was approved by the Research and Ethics Committee of the College of Veterinary Medicine and Biosciences of Makerere University (Ref.: VAB/REC/14/111) and the ILRI Institutional Research Ethics Committee (Ref.: ILRI-IREC2014-07). Prior to questionnaire administration and biological sampling, verbal consent was obtained from the respondents after explanation of the purpose of the study. The delivery of feedback to butchers after the study included sharing of key results and practical advises for food safety and hygiene.

3.2 Study area

The Republic of Uganda is a landlocked country in East Africa. It is bordered in the east by Kenya, in the north by South Sudan, in the west by the Democratic Republic of the Congo, in the southwest by Rwanda, and in the south by Tanzania. The southern part of the country includes a substantial portion (45%) of Lake Victoria, shared with Kenya and Tanzania, situating the country in the African Great Lakes region. The lake is the biggest freshwater body in Africa (68,800 km^2) fed mainly through precipitation and thousands of small streams, whereby the river Nile is the only drain of the Lake. Uganda lies within the Nile basin, and has a varied but generally equatorial climate. The capital Kampala has a total area of 189 km^2 from which 31 km^2 are classified as wetlands that have an important economic and environmental value for wastewater purification and nutrient retention (Emerton, 2003). The project is restricted to pork joints in the urban and peri-urban areas of Kampala as shown in Figure 7 (0° 18′ N, 32° 38′ E).

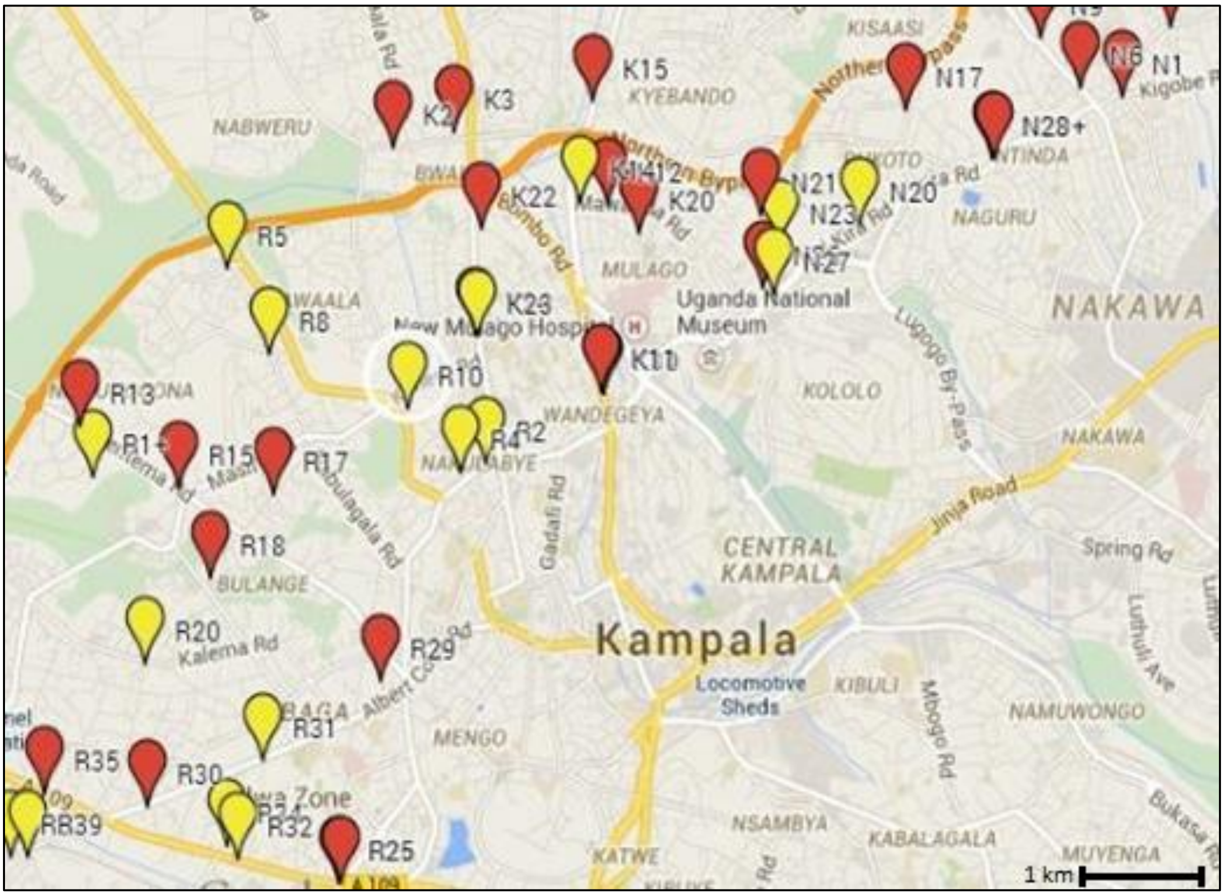

Figure 7 Section of study area with pork butcheries from baseline (dark) and the one of them who took part in the intervention (light) © 2014 Google Maps Engine Lite

Sixty pork joints were randomly selected out of 179 mapped in a previous survey (Kungu *et al., forthcoming*). Based on the information available, three of the five administrative divisions of Kampala were considered for the survey while keeping the same ratio of pork joints between divisions as the study by Kungu *et al.* indicated. This resulted in 26 butcheries in Nakawa, 21 in Rubaga and 13 in Kawempe. Uganda has an administrative system consisting of five levels: District Local Council (LC) 5, County (LC4), Sub-County (LC3), Parish (LC2) and zone/village (LC1) (UN, 2004). The fieldwork was facilitated by the local government (Kampala Capital City Authority) and conducted with support of chairpersons at the LC1 level.

3.3 Interviews with butchers and customers

Between June and July 2014, data was collected by enumerators trained to conduct structured questionnaires for butchers (Annex 1) and their customers (Annex 2). Since two different questionnaires were used, they are described separately in the result section.

The baseline interviews included interviewing 60 butchers and 240 customers with Luganda[8] and English speaking researchers from Makerere University, Kampala. Both researchers independently translated the questionnaires from English into Luganda, and cross-checked the resulting translations to ensure the accuracy of their wording. The questionnaires included data on socioeconomic characteristics, e.g. education, age, business and work experience, as well as market-related characteristics, e.g. building structure, environment, cleanliness, source of pork and storage conditions. Additionally the butchers' questionnaires contained questions concerning attitudes in preparing pork and vegetables as well as an observation list filled by enumerators and a 4-point Likert scale to measure butchers' attitudes to a particular statement.

Data was collected through face-to-face interviews asking one butcher and four customers in each of the 60 butcheries. Female and male customers were interviewed equally at each butchery through consecutive sampling based on agreement in participation and direct purchase of pork meat at the pork joint. Interviews lasted on average thirty minutes per respondent. Obtained data was treated anonymously without using any personal identifiable information. Each paper survey was translated into a corresponding number as a unique identifier for data entry. Data processing was completed without any connection to the original geo-referenced coordinates and names of respondents interviewed.

[8] Luganda is the major language spoken in central Uganda

3.3.1 Choice experiment

An additional component of the butchers' questionnaire was a choice experiment (CE) to assess what butchers consider as the most and the least important attributes to their customers when buying pork. A similar CE was done for customers, but results are not part of this thesis.

In order to determine a list of factors that may influence customers' purchase of pork, pre-tests were conducted in a participatory approach with butchers and customers of four randomly selected butcheries in Kampala ahead of this survey. Earlier research was considered as well in order to complete the list of factors (Oyewumi & Jooste, 2006; Roesel *et al.*, 2013). As a result of this preliminary research, thirteen attributes were chosen as demonstrated in Table 1.

Table 1 Attributes considered relevant for pork purchase used for choice experiment

Attribute no.	Attribute description
1	Price
2	Colour of the meat
3	Bony meat
4	Fat layer of the meat
5	Meat from the same day
6	Butcher wearing coat
7	Cleanliness of the butchery
8	Trust in the butcher
9	Type of building structure
10	Butchery close to main road
11	Pest animals in/around the butchery
12	Presence of flies in the butchery
13	Age of the animal

A balanced incomplete block design was implemented to arrange the thirteen attributes within thirteen choice sets. Respondents could only choose one as most and one as least important item in each choice set presented as a so called *choice card* during the interviews (Figure 8).

Please indicate the attribute you think is most important for your customers and the attribute you think least important for them when buying pork meat

Most important	**Attribute**	**Least important**
☐	Fat layer of the meat	☐
☐	The butcher is wearing a coat	☐
☐	Cleanliness of the butchery	☐
☐	Butchery close to main road	☐

Figure 8 Example for a choice card used in the questionnaire for butchers

Best-worst (BW) data were analysed with BW scores, standardized scales and importance weights. The BW score was calculated for each of the thirteen attributes, according to the number of times the item was chosen as the most or least important. More precisely, the BW score is calculated by subtracting the number of times a given attribute was selected as most important (B) from the number of times a given attribute was selected as least important (W). The resulting difference between these values for a particular attribute is the BW score, whereby a positive number signifies that an attribute was more often selected as the most important attribute than it was chosen as the least important attribute. Conversely, a negative score signifies that an attribute was more often selected as the least important attribute.

The analysis included the standardized (mean) BW score (generally known as BW scores), which was calculated as follows according to Mtimet *et al.* (2014):

$$\textit{Standardized Most – Least Score} = (\textit{No.Most} - \textit{No.Least})/(m \cdot n)$$

No.Most: Number of times the attribute was chosen as most important

No.Least: Number of times the attribute was chosen as least important

m: Number of respondents

n: Number of times the attribute was presented to the respondent

The standard deviation (SD) of the butcher's responses for a given attribute was calculated as it reflects the heterogeneity of responses for a particular feature. To investigate this further, the coefficient of variation was calculated as well showing the extent of variability in relation to the mean (SD/mean).

Another measurement this study considered is the square root (sqrt) of B divided by W. The resulting sqrt(B/W) ratio scale was used for rescaling to a standardized scale, where the most important attribute takes on a value of 100 by multiplying each sqrt-value (*i* = 1, · · · ,n) with a factor given in the following equation from Mueller Loose and Lockshin (2013).

$$Weighting\ factor_{\ ratio\ scale} = \frac{100}{max_i \left(sqrt\,\frac{B}{W}\right)}$$

This rescaling with positive values allows for an easier interpretation of the data (Flynn *et al.*, 2007). Namely, values under 100 can be evaluated as how important an attribute is deemed relative to the attribute with the highest sqrt(B/W).

3.4 Insecticide-treated fence material intervention

Out of the 60 butcheries from the baseline, 23 were purposively chosen according to their compliance, presence of flies, rain proofed building structure and no existing fly controlling. Intervention butcheries were randomly allocated to a group of 18 netted butcheries (treatment) and or five non-netted butcheries (control). The insecticide-treated fence material *ZeroFly*®[9] (M/s. Vestergaard Frandsen Pvt. Ltd, Lausanne, Switzerland) was obtained from a sale store in Kampala (MTK Uganda limited; Nasser road, Kampala). Susceptibility to deltamethrin was assessed before intervention in ten randomly selected pork butcheries using a bioassay (as explained in section 3.4.2) with 100 randomly caught houseflies that showed no signs of resistance towards deltamethrin.

Instructions about proper net handling, potential risks and standard operating procedures in case of physical contact was provided with the consent form.

The nets were adapted individually to the pork joints' physical layout. This generally meant constructing custom-sized wooden frames to meet the unique proportions of each pork joint's windows or doors, where the mesh-covered frames would be installed (Figure 9 and 10). Alternatively, an installation on walls or planks was implemented (Figure 11 and 12) since the window openings often were too small to additionally cover them with a net. This limitation existed since butchers require openings to pass pork and money through.

The nets' locations were selected based on observations of frequent flight routes (e.g. window-food-door) and fly resting spots (dark, windless, etc.). The fixation of the net was done with a commercial hand stapler. On average, 1.5 m^2 netting material were used in each pork joint. Nets were cleaned with a soft brush and rinsed with water once per week by the researchers.

[9] Knitted textile made of 100% polyethylene, black with 4 g deltamethrin per kg textile (270 mg deltamethrin/m2) incorporated into fibre polymer, mesh count of 25 holes/cm^2; roll of 100 m x 1.0 m; weight 70 g/m^2; shelf life 2 years

Figure 9 Pork joint from intervention where net is installed as door frame on the right © Martin Heilmann, ILRI / Freie Universität Berlin

Figure 10 Pork joint from intervention with a net frame at the bottom of a glazed butchery © Martin Heilmann, ILRI / Freie Universität Berlin

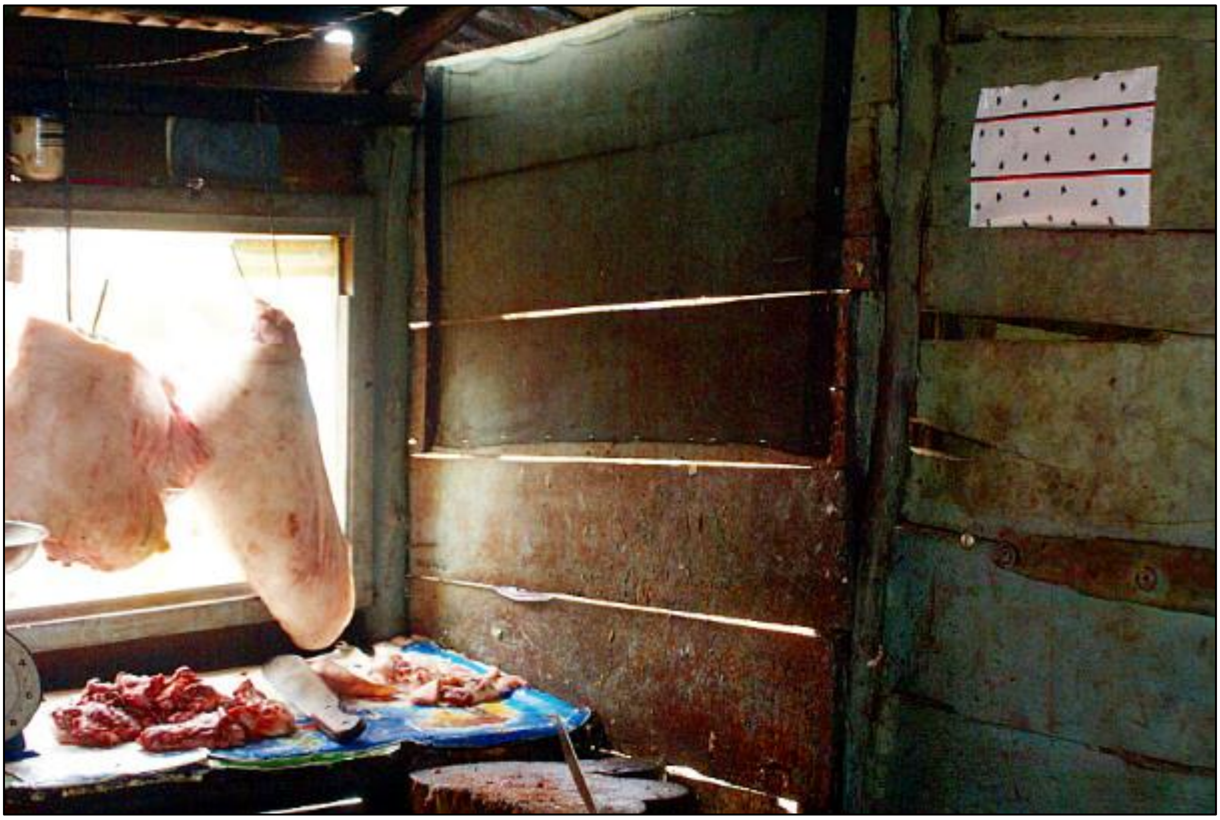

Figure 11 Pork joint from intervention with a net stapled to the wall (sticky trap in the right upper corner for fly monitoring) © Martin Heilmann, ILRI / Freie Universität Berlin

Figure 12 Pork joint from intervention with a glazed cage covered with net from the outside © Martin Heilmann, ILRI / Freie Universität Berlin

3.4.1 Monitoring of fly numbers

Monitoring in the intervention butcheries took place over 15 weeks, two weeks were used as zero value survey prior to the introduction of the insecticide-treated fence material and the subsequently following intervention period of 13 weeks. The units were monitored for the flies' abundance by using non-attractant black-white sticky traps, which were placed within the pork joint once per week for 48 consecutive hours as shown in Figure 13.

Fly catches were identified and counted individually for each butchery to determine families and abundance on a biweekly basis during the zero value survey and on a weekly basis over the intervention period. The spot of the trap and time of installation and collection were kept similar during the whole monitoring and supervised by the researchers. A phenotypical fly identification was carried out according to available identification keys (Zumpt, 1956; Greenberg, 1971; Schaefer, 2002; Couri, 2007; Williams & Villet, 2014). Identification was done using a stereo microscope (*Seben Incognita III binocular*) up to the species level for *M. domestica* and family levels for Sarcophagidae and Calliphoridae.

Figure 13 Pork joint from control group from the inside with sticky trap on the left to monitor fly numbers © Martin Heilmann, ILRI / Freie Universität Berlin

Non-parametric statistics were used for data analysis. The fly catches were compared building the median individually for each butchery before and after intervention. Additionally, the mean of individual weekly medians were grouped into control and intervention arm to allow an insight in the development of catches over time. For displaying the distribution of catches before and after intervention in the netted group, a box plot was used. Two-pair comparison in a Wilcoxon sign rank test were used to assess significance. Results were considered significant when $p < 0.05$.

3.4.2 Net efficacy and environmental persistence

Monthly net samples were collected in five randomly selected butcheries and stored in aluminium foil at 4°C for further investigating net efficacy over time after the study by using a fly bioassay according to previous studies (Maia *et al.*, 2010; Maia *et al.*, 2012). *M. domestica* (Lei-phylum) is recommended from the WHO for use as a deltamethrin sensible test species and was provided by the *Umweltbundesamt Berlin*, Germany. The flies were between three and six days old when tested, so that the chitin cuticle was already hardened. The net samples were cut into pieces of 30 cm x 30 cm and stapled on the inner layer of a fiberboard FlyBox® (Dr. Bauer, Freie Universität Berlin).

About 50 flies were released into the box through a small opening with a sterile 16 cm long tube (Rotilabo®). After an exposure of 10 seconds, flies were released into an observation cage where they were observed at 3, 5, 10, 15, 30 min and 6 h, 24 h intervals. During observation, flies were kept at room temperature and provided with a milk powder-sugar mix and water serving as nutrition. Each net sample was evaluated three times together with an untreated net as a control.

In the last week of intervention, samples of soil and water were taken to investigate potential contamination through run offs of deltamethrin from the insecticide-treated fence material. Three pork joints were selected randomly and 500 g soil and one litre surface water[10] were taken from each pork joint. The samples were transferred to an environmental laboratory in Kampala (Chemiphar (U) Ltd.) and tested for pyrethroids by using gas chromatography with an electron capture detector (Akre & MacNeil, 2006).

[10] Samples of surface water were taken in a range of maximal 2 m from each pork joint

3.5 Microbiological sampling

Seventy-seven pork joints in Kampala were randomly selected out of 179 mapped in a previous survey (Kungu *et al.*, *forthcoming*). These included 60 butcheries from the baseline interviews along with 17 other randomly selected butcheries. *Salmonella* spp. was used as an indicator pathogen due to its high significance for foodborne diseases related to diarrheal diseases (WHO, 2015a).

From June – October 2014, samples were taken once in all butcheries. Samples included raw and roasted pork, water, tomatoes, cabbage, onions and swabs of, butchers' hands and their equipment; as well as one housefly per butchery. Flies have been caught in sterile tubes and paralyzed by cooling to 1°C for about 30 min. They were identified using a stereo microscope, killed by decapitation and dissected with sterile instruments. Based on a protocol used by Förster *et al.* (2009), the mid-guts were removed and transferred into 200 µl sterile physiological sodium chloride solution in 1 ml tubes (Eppendorf) and crushed with sterile instruments. The homogenized solution was incubated for 5 min at room temperature and a sterile swab was used to sample the solution. These swabs were immediately sent to the Central Diagnostic Laboratory of Makerere University, Kampala for further investigations. Cultural isolation and biochemical identification of *Salmonella* spp. was performed according to ISO 6579:2002.

Post-intervention sampling

In the first week after intervention, the netted intervention butcheries were re-sampled for *Salmonella* spp. based on the previously described protocol. Prevalence of *Salmonella* spp. before and after intervention was analysed using a Pearson's chi-squared test in order to determine significant differences.

4 Results

4.1 Interviews

This section is divided in 3 subsections whereby interview results are presented separately first for butchers and second for customers.

4.1.1 Socio-economics

Butchers

The butchers' socioeconomic characteristics are presented in Table 2. The majority of respondents were male, averaging 30 years of age, with more than half of them held a middle school degree or higher.

Table 2 Butchers' socioeconomic characteristics compiled from interviews (n=60)

Characteristics	Definition	Percentage
Sex	Male	93%
	Female	7%
Educational level	Illiterate	13%
	Literate/primary	27%
	Middle school	10%
	Secondary	45%
	University	5%
Position	Owner	12%
	Manager	20%
	Worker	68%
Main business	Yes	77%
	No	23%
Age (in years)	Mean (min-max)	30 (17 – 47)
Experience (in years)	Mean (min-max)	7 (0 – 22)

For 66% of the butchers, the pork joint was the main and only business in which they work. For an additional 11%, the pork joint was their main business, but they also worked in at least one other business. Overall, one third of all butchers were involved in other business activities, including pig/livestock production (17%) and pig/livestock slaughtering (17%). The majority of interviewed butchers were workers (68%), while 20% were managers and 12% owners of the butchery.

Customers

The customers' socioeconomic characteristics are presented in Table 3. Sixty percent of pork was bought by the head of the household, who was male in 73% of cases. Each household consisted of 4.2 persons on average, ranging in ages from 1 to 32. In most households, children were present (68%) in ages ranging from less than 2 years (32%), to 3-6 years (32%), 7 – 12 (19%) and 13 – 18 years (10%). Approximately one third of the respondents had an income less than 150,000 Ugandan Shillings (UGX[11]), 150,000 – 500,000 UGX (28%), 500,000 – 1,000,000 UGX (14%) and more than 1,000,000 (5%). Twenty percent did not provide income information.

Table 3 Customers' socioeconomic characteristics compiled from interviews (n=240)

Characteristics	Definition	Percentage
Educational level	Illiterate	1%
	Literate/primary	16%
	Middle school	3%
	Secondary	53%
	University	27%
Marital status	Single	32%
	Relationship	15%
	Married	52%
	Divorced or Widow	1%
Age (in years)	Mean (min-max)	30 (17 – 70)

[11] 1.00 USD = 2563 UGX at 1 July 2014

4.1.2 Knowledge, attitudes and practices

Butchers

The average price for raw pork at the sampled shops was 8,600 UGX per kg, but prices varied between 6,000 UGX and 15,000 UGX showing a standard deviation (SD) of 1,273 UGX. Half of the butchers bought pork from the local city abattoir; the rest obtained their meat from wholesalers (27%), smaller slaughter slabs (13%) or private farms. In the latter case, the butchers did their own slaughtering on their farms or in their backyards (10%).

According to the butchers' perceptions, raw pork was the most commonly sold form of meat (53%) followed by fried (30%), roasted (15%) and cooked (2%) pork. A high proportion of butchers (87%) stated that they serve cooked pork with raw accompaniments like avocado, onions, tomatoes or cabbage. One fifth of butchers stored pork for more than 1 day usually (20%). More than three quarters of butchers (63%) disagreed or strongly disagreed (15%) on a 4-point Likert scale that "In this working environment, keeping clean is easy". Further results are presented in Table 4, including the medians and distribution of answers.

Table 4 Butcher's answers to statements on a 4-point Likert scale showing the median (M) from 1 to 4 (1=Strongly agree, 2=Agree, 3=Disagree, 4=Strongly Disagree) with distribution of answers in percent including "Don't know"

Statement	M	1	2	3	4	Don't know
Being able to work fast is the most important skill of a good worker.	2	7%	57%	22%	13%	2%
People working in food jobs are more likely to get sick than other people.	3	8%	30%	35%	3%	23%
In this working environment, keeping clean is easy.	3	0%	22%	63%	15%	0%
A little dirt on the clothes or tools will not cause harm.	2	8%	63%	13%	3%	12%
If pork is labelled by an inspector it is always safe to eat.	4	0%	3%	38%	58%	0%
Ensuring hygiene is the responsibility of the management or the owner.	3	5%	28%	30%	35%	2%
If pork is well-cooked then it is safe to eat.	4	0%	0%	27%	72%	2%
Providing high quality products helps generate more profit.	4	2%	0%	20%	78%	0%
I give suppliers advice on how to improve quality.	4	0%	2%	28%	68%	2%

On observation, it was found that more than 3 quarters of butchers interviewed (80%) had "clothing and shoes with visible dirt". A valid certificate of health fitness (usually given by the government) was observed in 21 butcheries (35%), while 42% of butchers did not have a valid one or claimed to the certificate but not presently available for viewing (23%). Furthermore 25% of the cutting surfaces and 22% of the crates and storage equipment had visible dirt on them. Further results from the observations are presented in Table 5, indicating the proportion of the variables that were or were not present during the observation or could simply not be observed at that time (e.g. butchers' certificate of health fitness as mentioned above).

The majority of shops (85%) used wood (usually a wooden stump) as a cutting surface for their pork, and only a few butchers used tiles, metal or concrete. More than half of butchers said that they clean cutting surfaces 3 – 5 times per day. Almost one fifth (18%) stated that they clean these surfaces even more often, and 20% noted cleaning them once a day. Butchers utilized a combination of different ways to clean these areas. The main cleaning methods, with some butchers using multiple methods were water and detergent (57%), scraping surfaces with a large cutting knife (27%), wiping surfaces on butcher's clothes (22%) or rinsing with cold water (20%). In 43% of butcheries, pest animals such as rodents, birds and insects (excluding flies) were present. In addition, flies were observed in 78% of pork joints. 77% of butchers said they noticed flies in their shops. The majority of butchers (92%) believed that flies can transmit diseases. Out of all butchers interviewed, 85% said that they "strongly dislike" flies and almost half of the interviewed butchers associated flies with health and hygiene issues. Eighty-three percent of butchers stated that they would be "willing to buy and install insecticide-treated nets".

Table 5 Observations made in butcheries by enumerators (n=60)

Observation	Yes	No	Not observed
Is there a permanent structure (brick, cement, wood house)?	98%	2%	0%
Is the pork protected from the environment?	80%	20%	0%
Is there a source of electricity?	100%	0%	0%
Is there a refrigerator present?	75%	22%	3%
Is there a freezer present?	28%	68%	3%
Does the retailer have access to running water?	78%	22%	0%
Does the retailer have access to a hand-washing area with soap?	80%	20%	0%
Is the flooring of a material that is easy to clean (i.e. tiles, concrete)?	88%	12%	0%
Is there a facility to dispose rubbish, old pork (i.e. dustbin)?	85%	7%	8%
Are there mosquito nets installed?	15%	85%	0%
Are there windows in the selling area present?	90%	7%	3%
Do workers have clothing and shoes free of visible dirt?	20%	80%	0%
Do the workers wear protective gloves and/or protective clothing?	12%	88%	0%
Do workers have uncovered wounds?	0%	98%	2%
Do workers have any visible signs of communicable diseases?	0%	100%	0%
Are latrines present in area?	85%	3%	12%
Are workers eating, drinking or smoking while handling pork?	3%	97%	0%
Does the butcher have a certificate of health fitness?	35%	42%	23%
Are crates and storage equipment free of visible dirt?	78%	22%	0%
Is the area free from pest animals (i.e. birds, flies)?	53%	43%	3%
Are there flies present?	78%	22%	0%
Is the cutting/selling/processing area free from visible dirt?	73%	25%	2%
Is the storage equipment made of a material that is easy to clean (e.g. metal, plastic)?	85%	15%	0%
Are the tools free from cracks and damage?	72%	28%	0%
Is pork in contact with other food products?	15%	85%	0%
Is there a strict separation between clean and dirty areas?	73%	27%	0%

Customers

Raw pork was the most commonly bought form of meat (48%), followed by fried (38%), roasted (9%) and cooked (5%) pork. When customers were asked to rank the consumption of different meat types at home from 1 (most frequently) to 6 (least frequently), they indicated that pork is the second most common meat consumed at home after beef, as illustrated along with poultry, sheep, goat and fish in Figure 14. For almost all respondents pork joints and butcheries were the main place to buy pork; only 1% stated that they bought pork from pig farmers, at super markets or using other methods.

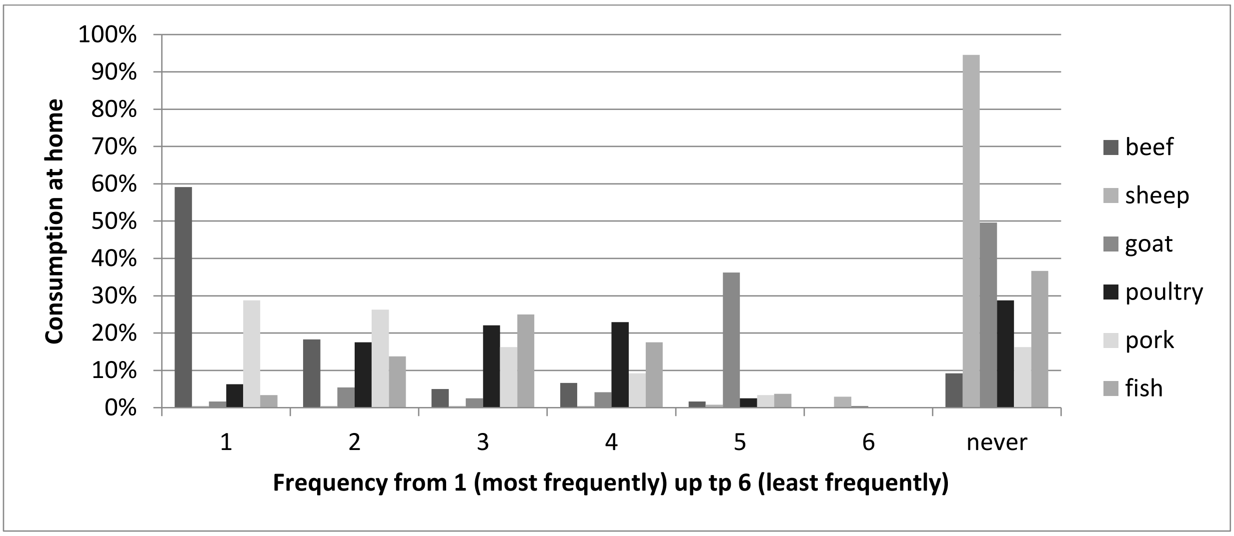

Figure 14 Customers' answers from interviews indicating consumed meat at home from most frequently (1) up to least frequently (6) and "never" respectively

Seventy-seven percent of respondents stated that they mostly to fully trust the butchers to provide hygienically processed pork. Others stated they don't really trust the butchers (20%), or do not trust at all (5%) or provided no answer (5%). One third of households bought raw pork occasionally (30%), "once per two weeks" (20%), "once a week" (20%), a "few times a week" (15%) or every day (15%).

The average price for 1 kg raw pork in pork joints, where the customers bought pork recently, was 8,995 UGX and varied from 6,000 – 15,000 UGX showing a SD of 1,423 UGX. Pork is consumed 2.18 times per week on average. Most people stated to eat 0.25 kg per meal (60%), 0.5 kg (27%), 1 kg (11%) or less than 0.25 kg or more than 1 kg per meal in 2% of cases. Eighty-two percent of customers ate their pork with raw and cooked vegetables including matoke[12] (59%), cassava[13] (51%), tomatoes (9%), cabbage (5%), Irish potatoes (4%), onions (3%), greens (2%) and avocado, carrots or posho[14] (<1%). When asked when they last suffered from vomiting, diarrhoea or stomach pains, most respondents indicated "never" (66%), last year (14%), last month (13%) or last week (7%). Twenty-one percent of customers said they fully trust food certificates or food safety labels. Perceptions differed from "mostly trust" (25%), "no real trust" (22%), "no trust at all" (25%), "don't even look" (1%) to "don't know" (7%).

The main source of information for customers were television (68%), radio (29%), newspaper (15%), internet (10%), friends (9%), colleagues (2%) or others (1%). Health aspects influenced the majority of respondents' purchase decisions a lot (89%) while 9% stated "a bit" or "not at all" (2%). Most of the customers (76%) noticed the presence of flies in pork joints. Three quarters stated they "strongly dislike" flies or disliked them "somewhat" (22%). Two percent felt not affected. Most respondents indicated "health" (59%), "hygiene" (72%), or both (34%) as main reasons for disliking flies. Five percent stated "nuisance" as the main reason.

Almost all customers thought that flies could carry diseases and contaminate the meat (95%). When asked to specify the "disease" carried by flies, they stated mostly diarrhoea (41%), followed by cholera (38%), dysentery (16%), stomach pain (7%), vomiting (4%), Ebola (3%), malaria (1%) or others (<1%).

4.1.3 Choice experiment

Butchers' opinion about the most important attributes to their customers was determined by using the BW method, whose results are presented in Table 6. The first two columns describe how often an attribute was chosen as the most and least important one. The difference between these values are presented in the following columns as the BW score and ordered according to their mean in the fourth row as standardized BW scores, which are generally known as BW score. The attributes in Table 6 are ranked according to their importance from 0.700 down to −0.513 based on the BW score shown with its SD for each attribute. The square root (sqrt) of B/W was used to rescale to a standardized scale, where "Meat from the same day" had a value of 100, with the remaining attributes relative to that attribute as described in the methodology.

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[12] Cooked banana/plantain; common staple crop around Lake Victoria, and staple food in Uganda and Rwanda

[13] Woody shrub; major staple food in the developing world (known as manioc in developed countries)

[14] Dish of maize flour cooked with water to a porridge

Table 6 BW method indicating relative importance of attributes according to butcher's choices from BW method (n=60)

Attribute	Most total	Least total	BW score	Standardized BW score (mean)	Standard deviation	SQRT (B/W)	Standardized ratio scale
Meat from the same day	175	7	168	0.700	1.139	5.00	100.00
Cleanliness of the butchery	153	7	146	0.608	0.769	4.68	93.50
Trust in the butcher	154	14	140	0.583	0.871	3.32	66.33
Colour of the meat	128	51	77	0.321	0.929	1.58	31.68
The butcher is wearing a coat	63	20	43	0.179	0.928	1.77	35.50
Butchery close to main road	36	26	10	0.042	0.764	1.18	23.53
Price	12	30	-18	-0.075	0.514	0.63	12.65
Type of building structure	19	44	-25	-0.104	0.567	0.66	13.14
Bony meat	10	76	-66	-0.275	0.418	0.36	7.25
Presence of flies in the butchery	15	127	-112	-0.467	0.628	0.34	6.87
Fat layer of the meat	2	120	-118	-0.492	0.181	0.13	2.58
Pest animals in/around the butchery	5	127	-122	-0.508	0.279	0.20	3.97
Age of the animal	8	131	-123	-0.513	0.468	0.25	4.94

Altogether, “Meat from the same day” was revealed as the most important attribute. As graphically presented in Figure 15, in the middle of the range are “Colour of the meat”, “The butcher is wearing a coat”, “Butchery close to main road”, “Price”, “Type of building structure” and “Bony meat” according to their standardized BW score.

On the other end of the spectrum of the BW score, “Fat layer of the meat” was chosen as the least important attribute after “Presence of flies in the butchery”, “Pest animals in/around the butchery” and “Age of the animal”.

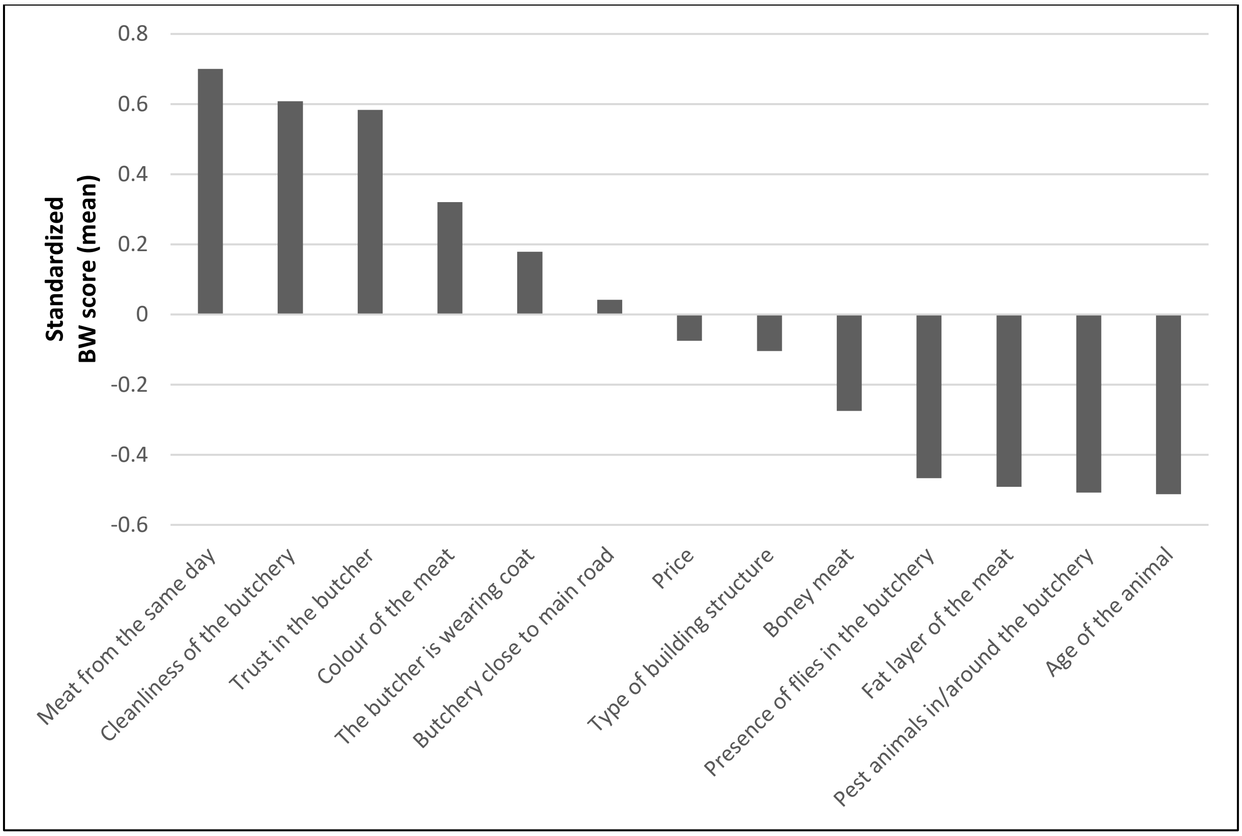

Figure 15 Standardized BW scores of attributes according to butcher’s choices from BW method

The standardized ratio scale in Table 6 indicates the importance of each of the attributes' relative to the most important attribute, "Meat from the same day", which itself is denoted as 100. Figure 16 graphically presents this scale, in order to more easily compare the relative importance of each attribute.

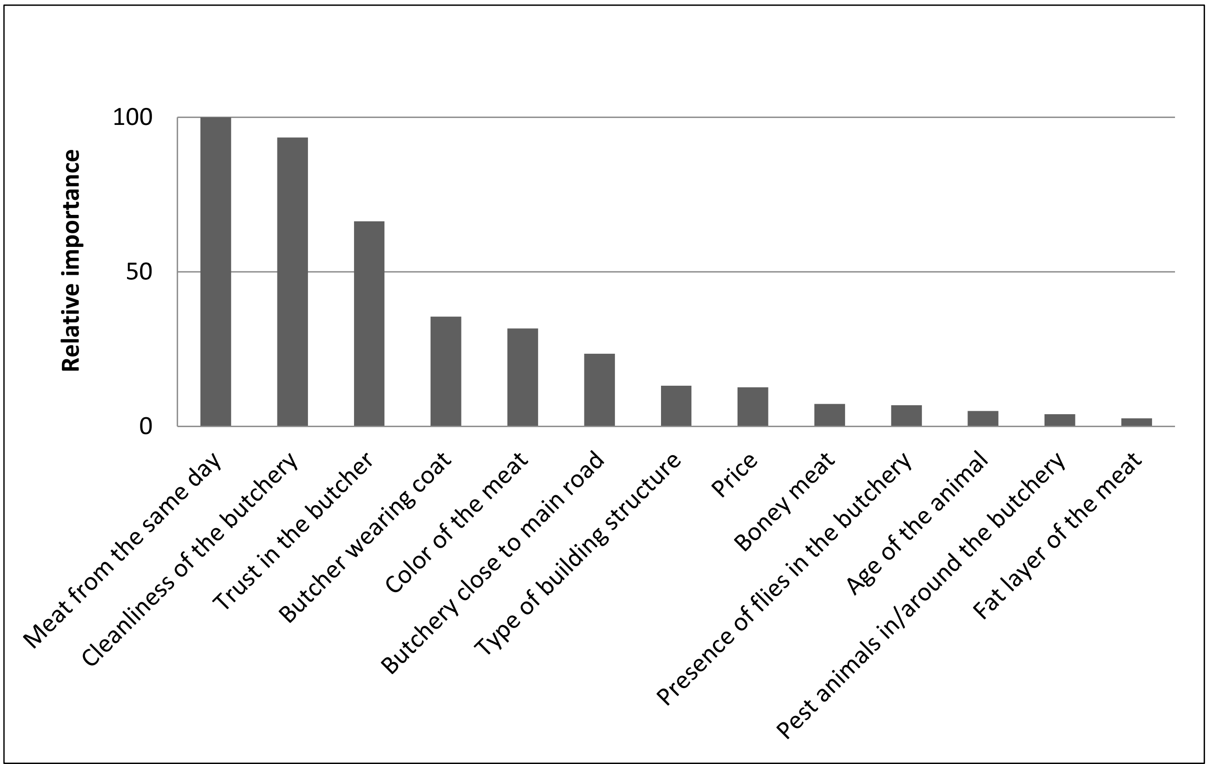

Figure 16 Relative importance on a standardized ratio scale of attributes according to butcher's choices from BW method where the most important attribute is denoted as 100

4.2 Entomological monitoring

During the monitoring from August to November 2014, a total of 7,953 flies were caught with sticky traps. The catches contained 85% *M. domestica*, 14% Calliphoridae and 0.4% Sarcophagidae. The rest were other non-target species like *Dermaptera sp., Nematocera sp., Drosophila sp.,* and coleopteran, ants, spiders or cockroaches.

Fly catches

Total numbers of fly catches are shown in Table 7 individually for each butchery according to their function as control or intervention. Additionally the medians are shown from before (zero value survey) and after intervention (intervention period) including first and third quartiles.

Table 7 Total fly numbers for each butchery (B) and medians with lower and upper quartile (Q) before and after intervention with insecticide-treated fence material

Function	Butchery	Total number	Zero value survey	Intervention period
			Median (Q2) [Q1, Q3]	Median (Q2) [Q1, Q3]
Control	B1	124	6 [4.5, 7.75]	6 [4, 9]
Control	B2	446	16.5 [13.75, 20.25]	26 [14, 34]
Control	B14	753	51 [48.5, 52.25]	41 [32, 54]
Control	B22	129	5 [2.75, 7]	6 [4, 9]
Control	B23	259	12 [11.25, 12.25]	16 [8, 27]
Intervention	B3	398	30 [22.5, 35]	22 [12, 27]
Intervention	B4	383	29 [21.5, 37.75]	16 [8, 29]
Intervention	B5	775	47.5 (40, 53]	48 [33, 57]
Intervention	B6	391	40.5 [36.25, 48.25]	17 [13, 22]
Intervention	B7	95	13.5 [10.5, 15.5]	3 [2, 5]
Intervention	B8	242	31 [27.25, 42.25]	6 [4, 10]
Intervention	B9	163	19 [15.75, 24.25]	6 [4, 7]
Intervention	B10	96	11 [9.5, 17]	2 [1, 3]
Intervention	B11	282	11.5 [10.25, 12.5]	12 [10, 21]
Intervention	B12	317	9 [8.5, 9.5]	15 [10.75, 29]
Intervention	B13	221	44 [41.25, 52]	1 [1, 2]
Intervention	B15	351	34.5 [30, 38]	15 [8, 23]
Intervention	B16	714	32.5 [20.75, 45.75]	38 [27, 53]
Intervention	B17	544	22.5 [15.75, 28.25]	25 [15, 42]
Intervention	B18	567	88.5 [85, 96]	16 [11, 18]
Intervention	B19	99	13 [11.25, 14.5]	4 [1, 6]
Intervention	B20	189	14 [10.5, 20.25]	7 [5, 11]
Intervention	B21	415	33 [29.5, 38.25]	21 [12, 32]
Total		**7953**		

Development of fly catches over time is illustrated in Figure 17 showing medians of the control and intervention group for each mess point. As illustrated, the intervention group has shown numbers varying from 17 to 32 flies per catch during the zero value survey but stabilises over the intervention period with 6.5 to 18.5 flies per catch. The control group however started lower with catches from 9 to 13 flies during the zero value survey and had stronger variances during the intervention period ranging from 6 to 32 flies showing peaks early in September and the end of October with 28 and 32 flies per catch respectively.

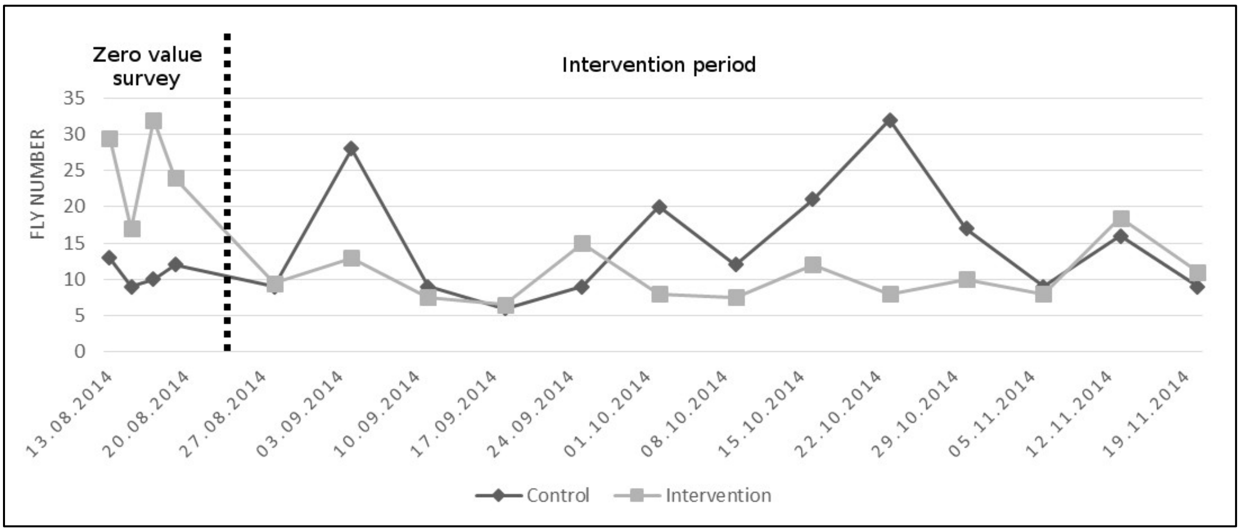

Figure 17 Median of fly numbers over time during zero value survey and intervention period for intervention group (n=18) and control group (n=5) in pork butcheries in Kampala

The individual medians of the netted butcheries resulted in a median of 29.5 flies before and 15 flies after the intervention while the control group had an increase from 12 to 16 flies. Figure 18 illustrates the decrease in fly numbers in the intervention group after netting. Netting reduced fly numbers by 48% (p= 0.002).

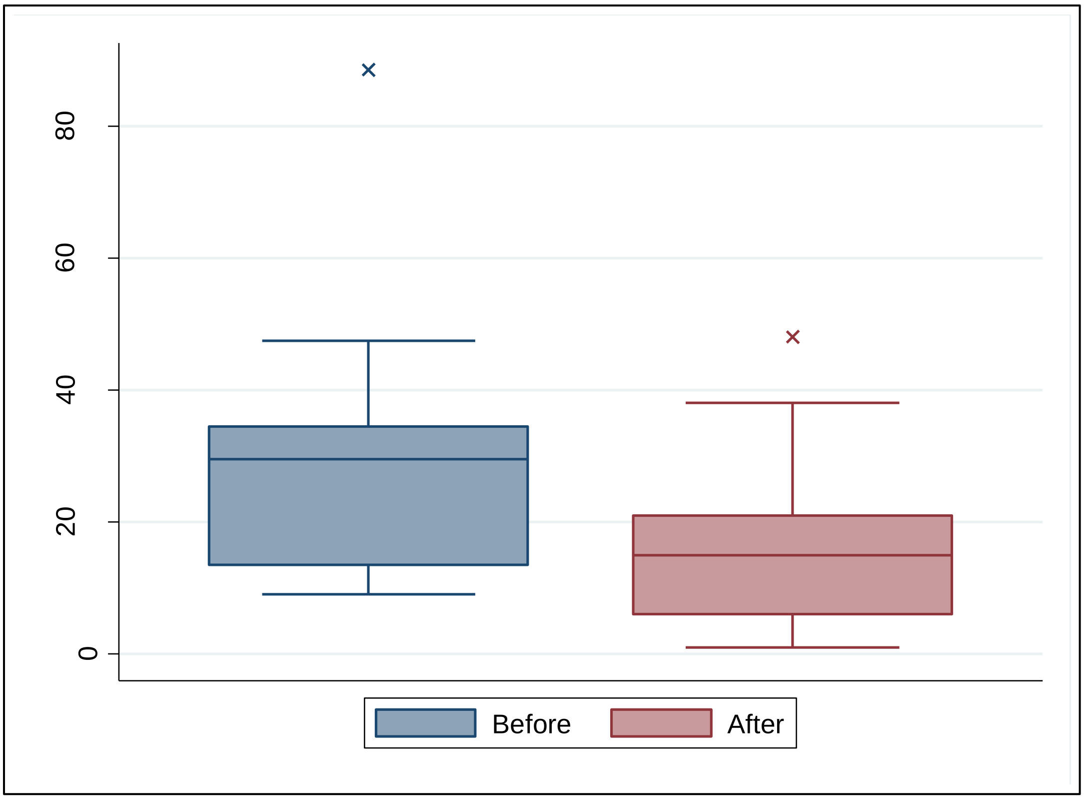

Figure 18 Box plot of the fly numbers before and after intervention for netted butcheries (p=0.002)

4.2.1 Insecticide persistence

Sixteen net samples were assessed for persistence of insecticidal activity using a fly bioassay. Each month 5 samples were collected and tested 3 times each. The average percentage of paralyzed flies after exposure is shown in Table 8 and illustrated in Figure 19.

Over the experimental period, particularly in the first 5 to 10 minutes, an increase of time between exposure and paralysis of flies exposed to the net can be observed. While exposure to samples from July to September resulted in 100% paralysis during 5 – 30 min, it took up to 30 min to 6 h before 100% paralysis was reached for the last sample from October at the termination of the study. After 6 h however, a total paralysis of flies was reached in all samples.

Environmental samples were taken once at the end of the intervention period from soil and water in 3 randomly selected pork joints. Samples were sent to an environmental laboratory in Kampala (Chemiphar (U) Ltd., Uganda) for pyrethroid analysis. No deltamethrin or other pyrethroids were found, or the concentration was below the threshold (50 µg/kg) respectively.

Table 8 Percentage of paralyzed flies (*M. domestica*) after 10 seconds of exposure to insecticide-treated fence material samples taken over intervention period

Time after contact with tested nets	Control (untreated net)	Net exposed in the field (months)			
		1st day	Aug	Sep	Oct
5 min	0%	18%	13%	9%	4%
10 min	0%	74%	70%	63%	52%
15 min	1%	92%	91%	92%	84%
30 min	1%	100%	100%	100%	97%
6 h	3%	100%	100%	100%	100%
24 h	3%	100%	100%	100%	100%

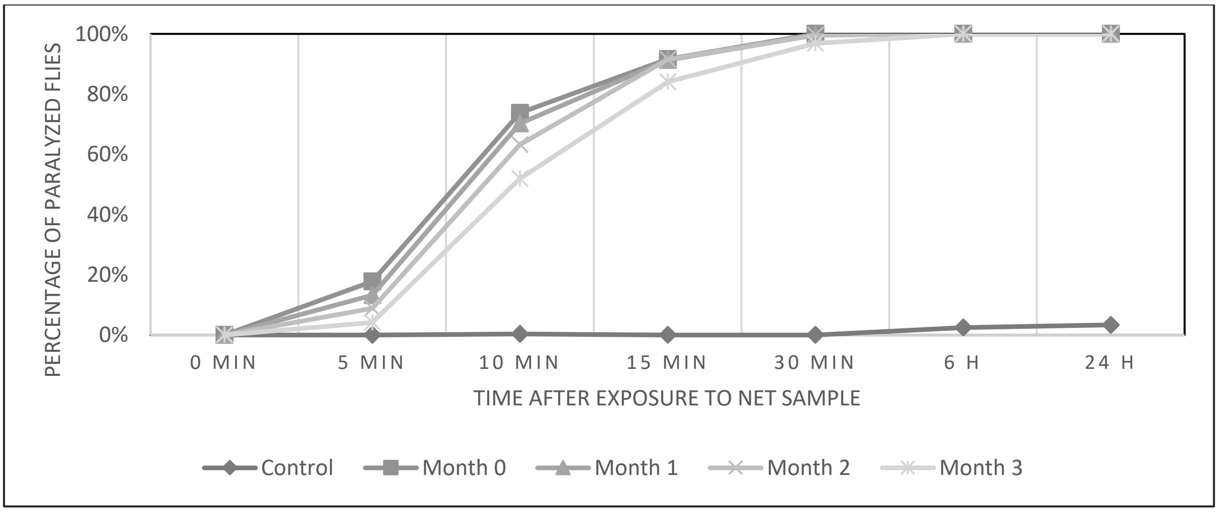

Figure 19 Percentage of paralyzed flies (*M. domestica*) after 10 seconds of exposure to insecticide-treated fence material samples and non-treated nets (control)

4.3 Microbiological sampling

Baseline sampling

Among 693 samples, 8.8% were tested positive for *Salmonella* spp. as indicated in Table 9. Thereby, 7.9% were tested positive for *S*. Enteritidis, including in detail 31.2% for raw pork (24/77), 22.1% for houseflies, 9.1% for water, 5.2% for tomatoes and 3.9% for cabbage. Further, *S*. Gallinarum[15] was found in 0.9% of cases ranging from 2.6% for tomatoes, 2.6% for onions, 1.3% for cabbage and 1.3% for roasted pork. All 154 samples taken from either butchers' hands or their equipment were negative.

Table 9 Prevalence of *Salmonella* spp. in samples taken from pork joints in Kampala during baseline sampling

Sample	**Quantity N**	***S*. Enteritidis N (%)**	***S*. Gallinarum N (%)**
Raw pork	77	24 (31.2)	0
Flies	77	17 (22.1)	0
Water	77	7 (9.1)	0
Tomatoes	77	4 (5.2)	2 (2.6)
Cabbage	77	3 (3.9)	1 (1.3)
Onions	77	0	2 (2.6)
Roasted pork	77	0	1 (1.3)
Butchers' hand surface	77	0	0
Butchers' equipment	77	0	0
Total	**693**	**55 (7.9)**	**6 (0.9)**

Post-intervention sampling with netted pork joints

The 18 netted intervention pork joints were re-sampled after the intervention period. In the baseline sampling, the intervention group had a prevalence of *Salmonella* spp. of 11% (20/144). After intervention, the prevalence decreased to 6% (11/144), but there was no significant difference between the intervention and the change in *Salmonella* spp. prevalence ($\chi^2 = 2.928$, $p=0.087$).

[15] *Salmonella enterica* subsp. *enterica* serovar Gallinarum

5 Discussion

Study participants were afraid and suspicious to some extent at the beginning about our intentions and the aim of such research. One reason for this perception was that media created fear about lack of food safety in the pork business, which might put the informal food sector in bad reputation (Muhairwe, 2012). It was of utmost importance to have support from local councillors and local researchers that introduced us to each market, explained our research interests and helped us thereby undertake the interviews and samplings in a participatory manner. Such a transparent approach was crucial in this context and is therefore considered as the starting point of the following discussion including suggestions for future research.

5.1 Interviews

Among the key findings of this study is that raw pork accounts for half of the total pork sold by pork joints and bought by customers. The other half is served by butchers as cooked pork, usually served with raw vegetables. Interesting in this context is that 87 percent of butchers stated that they serve cooked pork with raw vegetables. To cut the pork and vegetables, 85 percent of pork joints in our survey used a wooden stump as a cutting surface, despite this material being very difficult to disinfect. Given the *Salmonella* spp. prevalence in our samples taken from vegetables, this epidemiological link could be one of the key sources for foodborne disease. To investigate these links further, genome sequencing and drug sensitivity tests of the isolates are envisaged in cooperation with the Institute of Food Hygiene of Freie Universität Berlin, Germany. In this context, it might also be helpful to compare the samples with isolates from other studies taken on the level of primary food production. Research on animal husbandry and on farm level could not only help to identify epidemiological links between the isolates found, but could be a worthy target for future food safety interventions at an earlier stage of the food chain.

Approximately half of the pork purchased by the butchers in this study originated from pigs that were slaughtered in backyards or non-gazetted abattoirs. This implies that this meat is not subject to meat inspection, as is imposed on larger slaughterhouses such as the local city abattoir in Kampala named *Wambizzi*. This is in line with the study of (Kungu *et al., forthcoming*), in which 60 percent of pork in Kampala originated from sources other than the local city abattoir. However, most pork pathogens are not detectable by visual meat inspection and there is evidence that small informal abattoirs produce meat that is less contaminated than in large formal gazetted abattoirs with higher risk for cross-contamination (Mwai, 2011).

Over three quarters of butchers disagreed that “In this working environment, keeping clean is easy” and observations have shown that the same amount of butchers had visible dirt on their clothing and that almost a quarter of butcheries had visible dirt on cutting surfaces and equipment. However, the significance of such findings is limited since the definitions of “dirt” and “cleanliness” are perceived differently among people, particularly from a butcher and researcher perspective.

Almost all butchers believed with certainty that flies can transmit diseases and eighty percent stated that they tried to reduce the amount of flies in their shops, showing that butchers have an interest in controlling flies. This is also reflected in the fact that eighty-three percent of butchers stated that they would be willing to buy and install insecticide-treated nets. In the majority of these butcheries we observed that butchers used old rolled papers, paperboards or rags to scare away flies by waving the objects in circles. This resulted in the material getting in contact with all but the flies it sought to deter. We also observed some other measures of fighting flies such as using salt, Christmas tree branches as repellent, as well as the use of smoke. Particularly the latter one was mostly associated with fireplaces inside the butcheries. The smoke seemed very effective since flies strictly avoid smoke, but the smoking process might also cause adverse effects on human health. However, although we recognise their use and potential for fly control, this study did not follow up on these measures.

5.2 Choice experiment

As part of the interviews we used the BW method to ask butchers what they consider as the most and least important attribute to their customers when buying pork. We discovered that butchers believe that customers care most about “Meat from the same day” and “Cleanliness in of the butchery” while “Pest animals in/around the butchery”, “Presence of flies in the butchery” and “Fat layer of the meat” were the least important attributes. In this context, a customer study could help by comparing both butcher’s and customer’s points of view, in order to get a better understanding of existing risks and understand the psychological constraints faced with implementing food safety measures. Having this data would also allow to share customer demands more effectively with butchers and their customers in order to implement food safety measures accordingly.

Surprisingly “Fat layer of the meat” was chosen as the least important factor by the butchers in this study, despite being listed among the most important factors by Roesel *et al.* (2013). This might be explained by the fact that Roesel *et al.* asked pig farmers in rural areas of Uganda who have other priorities in which the fat layer could be seen as positive value since it can be an indicator for pig’s health while too much fat on the other hand is known to have negative implications on human health. In the middle of the spectrum was the “price of the meat”, which did not seem an important criterion for customers’ choices according to the butchers. This may be due to the fact that pork prices per kg are very homogenous across Kampala as shown in only small variations of pork prices given by butchers and customers during the interviews. On the other end of the spectrum, “Presence of flies in the butchery” was among the least important attributes. This finding underlines the fact that flies seemed to be not that important to customers according to butchers’ opinions, which was not surprising at this early stage of our study. It is rather interesting that the SD of this attribute indicates that there was high heterogeneity in the answers. On the one hand, it is caused by the relatively small sample size, on the other hand, it may be due to a notable number of outliers who may have been more open to recognize the role of flies as a risk factor. This large dispersion might be statistically less significant, but it highlights the potential for changes in butchers’ thinking.

These results of the BW method give insight into butchers’ perceptions of customer preferences at pork joints and are essential to understand the psychological constraints faced with implementing food safety measures. However, just because a butcher perceives something to be important to a customer does not mean that the butcher himself also finds this factor important. Then again, from a supply and demand perspective, since his income depends on customers’ preferences (demand) and ultimately buying choices, the butcher has a clear interest in aligning his practices (supply) with his clients’ preferences. Similar to this approach, Mtimet *et al.* (2014) used the BW method to assess sheep traders’ preferences in Kenya and concluded that providing sheep smallholders with the appropriate animal breed characteristics will enable them match market demand and increase their incomes.

5.3 Sampling

One of our scientific hypotheses included a possible epidemiological link between flies, food contamination, and diarrhoea in pork joints in Kampala. Factors supporting this hypothesis are 1) the high prevalence of *Salmonella* spp. in flies 2) the presence of *Salmonella* spp. in foodstuff and 3) the previously described occurrence of *Salmonella* spp. in stool samples of patients with acute diarrhoea as described in the literature review.

The baseline sampling revealed 7.9 percent tested positive for *S*. Enteritidis. A closer look highlights the unexpectedly high prevalence on raw pork and flies with about 25 to 30 percent. However, the pork usually passes through a cooking process, which limits the risk of contamination substantially. Only one sample of roasted pork was tested positive for *S*. Gallinarum, which was quite surprising to us since this pork sample went through a roasting process and *S*. Gallinarum is a poultry-adapted strain excreted in the faeces of infected birds. However in that case we observed, that a live poultry seller was next door to the pork butchery. Although both butcher and poultry seller confirmed that no direct contact between their customers, poultry or foodstuff was given, we observed flies frequently flying from roasted pork to the chicken's faeces and vice versa.

Almost ninety percent of butchers stated that they serve cooked pork with raw vegetables. This becomes epidemiologically relevant given that the *S*. Enteritidis prevalence was up to 5.2 percent in our samples taken from vegetables. These vegetables are mostly served raw as salad (Kachumbari[16]) or only partly cooked and could be one of the key sources for foodborne disease. This is similar to the prevalence in water, which is a risk factor given that it is used for cleaning equipment, hands and vegetables. However, the samplings of hands were all negative, which might be due to the common practice of hand washing we have observed during the research period in the field. The sources of these positive samples remain to clarify but since subspecies I of *Salmonella*, including *S*. Enteritidis, is associated with disease in warm-blooded animals (Porwollik *et al.*, 2004), there are various entry points to consider along the entire farm-to-fork continuum.

Finally, sampling and investigating epidemiological connections is important, but also need to be viewed in relation with the conditions butchers have in the field. Local infrastructure, poor access to potable water, safe disposal of waste, and the access to raw material from reliable and affordable sources needs to be taken into account when improving food safety.

[16] Fresh salad dish of tomatoes and onions that is popular in East African region

5.4 Intervention

This research served as a pilot study to gain a first insight into practical use, acceptance and impact of insecticide-treated fence material to reduce flies in pork joints. The intervention has shown that insecticide-treated fences can reduce flies in pork joints by about 50 percent. However, the catches differed strongly depending on local conditions in the butcheries, the surrounding environments and the installation of fence material as window frame, on walls or other methods. Further research should focus on identifying the factors influencing the net's efficacy and further investigate the link between fly abundance and the prevalence of *Salmonalla* spp. and other food-related pathogens. Due to the relatively small sample size and very different individual conditions in the butcheries, we were facing large dispersion and high heterogeneity of data. We therefore focused on descriptive statistical analysis and put an emphasis on the practical feasibility in implementing such interventions in the given environment.

Among the most influencing factors we found important were regular cleaning in the butchery, varying compliance of personnel (regarding advises for fence handling, pork storage over night without refrigeration, hygiene) or environmental influences such as floods in close proximity of the butchery. We also obtained weather data (rainfall, temperature, humidity and wind speed) from a local station for the entirety of Kampala over the whole period, but since it was a consistent shared condition for all butcheries it could not explain individual differences in catches. However, we recognise the need of taking these factors into account in a broader study in order to have more accurate data and reduce bias on fly catches. In our case, for example, we have seen that four of five butcheries, which surprisingly showed an increase of total fly numbers during intervention, were among those where the above mentioned factors mostly occurred (no regular cleaning, poor compliance, pork storage over night without refrigeration, floods in close proximity of the butchery). Excluding these four butcheries from the statistical analysis, the fly numbers in the remaining intervention butcheries are reduced by 68 percent compared to the butcheries without nets according to the previously used Wilcoxon signed rank test for two-paired comparisons ($p<0.005$). This reduction suggests that the effectiveness of insecticide-treated fence material might even be higher when influencing factors are properly identified beforehand.

Practical use of fences

Setting up the fences was mostly a question of manual skills individually adapted to spatial conditions in butcheries, since some were made of wooden planks, concrete with or without tiles and glazed windows. When installed properly the fences lasted for the whole intervention period. Both installations, on the wall and within a wooden frame, were options easy to implement for butchers. Installing fences on walls was easier for installation, but the cleaning was more difficult. Since fences were stapled on the planks, they could not be removed easily and most dirt and dust coming through the planks was accumulating in the net. A better way, demanding more manual skills, was the wooden frame individually adapted to the windows. However, it was not always possible covering a whole window with a net as many used these as openings to give money or pork to customers. In this context customers told us during informal conversations that pork looks darker through the net behind a frame. They perceived that as “old pork”, which they considered as poor quality. Therefore, we only partly covered windows so that the pork could still be presented on the uncovered half.

A customized solution was established in a butchery where pork was stored in a cage made of wood and covered by glass (Figure 12). We covered the outer layer of the cage with the fence leaving the front side open so that customers still saw the pork while it could not get in contact with the net since the glass was in-between. In this scenario we had a reduction of flies by 90 percent. The effect of such a cage with the “attractant” inside works like a trap where flies collide frequently with the fence while trying to get inside. This approach could be of interest for further interventions and was seen in many other cases for non-animal sourced street vended food where the glass served as protection against dust and insects while food was still visible and canvassing for customers (author’s observation).

Insecticide contamination

In this setting, we have carefully chosen net locations by keeping distance to foodstuff in order to avoid direct contact with insecticides. However, the importance of butchers attitudes and awareness remains important when using such approaches. Misuse is, for example, described for insecticide-treated bed nets that were used for fishing which implies adverse effect on the environment and fish ecology (Minakawa *et al.*, 2008; McLean *et al.*, 2014). A point has also been made by a study from the 1970s: after extensive treatments of a poultry farm with insecticides, flies died and were not removed. Afterwards, the infection rate of laying hens with the cestode *Choanotaenia infundibulum*, which uses flies as intermediate host, increased exponentially because the hens ate the contaminated dead or paralyzed flies from the ground (Abrams, 1976).

5.5 Feedback

We provided feedback to the butchers after the study. This included a short version of key findings and information on good hygienic practices including *Five Keys to Safer Food* [17], which we have translated into the local language Luganda, and prepared as a laminated poster to display at the butcheries. Another less technical feedback to a wider audience was given through an ILRI research brief giving a short summary of the study methodology, results, implications and an future steps.

Despite the restraint in the beginning, study participants were generally curious about food safety including the role of flies. Apart from our feedback to them, they also provided information and feedback to us in a participatory approach with focus group discussions over the whole research period. This facilitated not only our research but also helped to increase people’s interest over time, as they were demanding more information about food safety, the fences and their use. One of the challenges was that most people could not comprehend the impact of such fences on flies, given that the flies usually collide with the net picking up the insecticide, fly away and die somewhere else. Compared to the sticky traps, which we have used for fly monitoring, the nets seemed to them quite “ineffective and useless”. For this reason, we came up with a demonstration of the fence`s efficacy, which we called the “cup-experiment”. We invited people in butcheries to join a demonstration with a dark plastic cup and a simple rubber strap as well as a small piece of insecticide-treated fence material. Then we caught a few flies with the cup and covered it with the net. Once the flies were in the cup, they collided frequently with the net. Consequently after some minutes flies became visibly paralyzed and died. Seeing this impact, the perception of people about our research changed substantially. These demonstrations facilitated not only our monitoring but also increased butchers’ participation in such interventions to control flies beyond our research.

Individual oral feedback was given to us by a bar owner who sold his drinks next to a pork joint, which is a quite common combination in Kampala. He improvised plastic covers for drinks and

[17] http://www.who.int/foodsafety/publications/consumer/en/5keys_en.pdf

bottles, which were used by customers to avoid “these blue flies coming from the butchery” entering the bottles. Indeed, flies (mostly Calliphoridae and fruit flies) came from the butchery attracted to the drinks and snacks. Hence, there is not only a given risk, there might also be the will of other businesses to control flies. Therein lies a further potential to include sampling of drinks or snacks provided in these bars. Even the role of fruit flies (e.g. spotted wing drosophila) in this context could be of interest since they are considered a major pest in North America (Lee *et al.*, 2011). Also a few fish sellers have expressed strong interest in measures to control flies. According to the author’s personal observations, the fly burden at fish selling areas is much higher compared to pork joints, but mainly dominated by Calliphoridae.

Another effect observed raised attention: people saw cockroaches dying since they mostly rested between the planks and nets where they were permanently exposed to the insecticide. During informal conversations with participants on site, we have seen that many people considered cockroaches major pests. Consequently a high fence efficacy was immediately recognized by people when seeing dead cockroaches. Three points are interesting in this context and remain unclear: First, what is the role of cockroaches as vector in this context? Second, to what extent could a fence installation contribute in controlling other pest insects? Third, thinking of advertising for such fence materials, could there be a greater potential in cockroaches as a “symbol” for vector control. This approach might be a more attractive intervention but needs to be in line with the most effective one. However, given that almost half of customers during our interviews stated that they trust food safety labels, there might be even a potential to combine both in a kind of “pest control” label. This can also be combined with the promotion of other health aspects through public information channels that are frequently used by customers, e.g. television (68%) or radio (29%), to influence purchase decisions and contribute to healthier clients and improved public health.

6 Summary

Synanthropic flies live close to humans and their animals, feed on their food and breed in faeces and other organic material. As such, they are known vectors for *Salmonella* spp., which are among the most common causes for foodborne diseases worldwide, especially in the developing world due to poorer sanitary conditions. Given that pork consumption in Uganda is rapidly increasing, while good food safety practices remain absent, this study aimed to assess the occurrence of *Salmonella* spp. in pork joints[18] and to investigate the impact of insecticide-treated fence material on the number of flies in selected pork joints Kampala, Uganda.

From June – October 2014 a baseline study was conducted in Kampala with 60 randomly selected pork joints, including interviews with 60 butchers and 240 customers. Best-worst (BW) method was used during face-to-face interviews to assess key practices of pork butchers in Kampala and their perception of what's important to their customers when buying pork.

Samples of houseflies, foodstuff and equipment were taken once in all butcheries and tested for *Salmonella (S.)* spp. Cultural isolation was performed according to ISO 6579:2002. After the baseline, a pilot study was done to investigate the impact of insecticide-treated fence material allocating 18 pork joints to an intervention and 5 to a control arm. A biphasic weekly monitoring using sticky traps with pre-intervention and following intervention was done from August to November 2014.

Butchers' answers indicate that raw pork is the most commonly sold form of meat (53%) followed by fried (30%), roasted (15%) and cooked (2%) pork. This is in line with customers' answers which reveal raw pork as the most commonly bought form of meat (48%) followed by fried (38%), roasted (9%) and cooked (5%) pork. Pork is the second most common meat consumed at home after beef.

A high proportion of butchers (87%) stated that they serve cooked pork with raw accompaniments like avocado, onions, tomatoes or cabbage. Half of the butchers buy pork from the local city abattoir; the rest obtain their meat from other sources.

Flies were observed in 80% of pork joints and 43% had other pest animals, such as rodents, birds and other insects according to the enumerator's observation. The majority of butchers

[18] Combination of a road-side butchery to buy raw pork and a bistro for cooked pork

(92%) believed that flies can transmit diseases. The vast majority of butchers (85%) and customers (97%) dislike flies and associate them with health and hygiene issues.

By asking sixty butchers what they consider as the most and the least important attribute to their clients, this study revealed that butchers believe that customers care most about “Meat from the same day” and “Cleanliness in the butchery” while “Fat layer of the meat”, “Pest animals in/around the butchery” and “Presence of flies in the butchery” are the least important attributes.

Among 693 samples, 8.8% were tested positive for *Salmonella* spp. Thereby, 7.9% were tested positive for *S*. Enteritidis. These cases rank from 31.2% for raw pork (24/77), 22.1% for houseflies, 9.1% for water, 5.2% for tomatoes and 3.9% for cabbage. Further, *S*. Gallinarum was found in 0.9% of cases including 2.6% for tomatoes, 2.6% for onions, 1.3% for cabbage as well as 1.3% for roasted pork. All 154 samples from either butchers’ hands or their equipment were negative.

Fly monitoring during intervention has led to a total of 7,953 flies containing 85% *M. domestica*, 14% Calliphoridae and 0.4% Sarcophagidae. Medians of caught flies in the netted group before and after intervention corresponds with a reduction in catches of 48% (p=0.002), while a slight increase in the control group was observed.

Both the high fly abundance and prevalence of *S.* Enteritidis illustrates the need for improving food safety in pork joints. This accounts in particular for raw vegetables given that interviews indicate that they are mostly served raw. According to butchers’ opinions, flies are less important to customers. However, both customers and butchers dislike flies and the majority associate them with diseases. For establishing an effective and affordable implementation on markets social behaviour and butchers’ knowledge, attitudes and practices should be taken into account when improving food safety including vector control. Insecticide-treated fence material provide a practical, affordable and sustainable solution in controlling flies and are therefore recommended as a complementary strategy to food safety and improve public health.

7 Zusammenfassung

Fliegen als Überträger für *Salmonella* spp. und deren Kontrolle in Schweinefleischereien in Kampala, Uganda – ein Beitrag zur Verbesserung der öffentlichen Gesundheit

Synanthropische Fliegen leben nahe Menschen und deren Haustieren. Sie ernähren sich unter anderem von menschlichen und tierischen Nahrungsmitteln und vermehren sich in Fäkalien und anderen organischen Materialien. In diesem Zusammenhang sind sie bekannt als Vektor für *Salmonella* (*S.*) spp., die weltweit einen der häufigsten Auslöser für lebensmittel-assoziierte Krankheiten darstellen; vor allem in ärmeren Ländern mit unzureichenden sanitären Bedingungen. Uganda zeigt beispielhaft einen rapide ansteigenden Konsum von Schweinefleisch während sich der allgemeine Hygienestatus nur langsam verbessert. Ziel dieser Studie war eine Untersuchung zum epidemiologischen Vorkommen von *Salmonella* spp. in Schweinefleischereien, sogenannten pork joints, sowie zur Wirksamkeit von Insektizid-behandelten Netzen zur Reduzierung von Fliegen.

Von Juni bis Oktober 2014 fand eine Befragung mit 60 Fleischern und 240 Verbrauchern in 60 randomisiert ausgewählten pork joints in Kampala statt. Bestandteil dieser Interviews war die *Best-Worst* Methode, die Einblicke in die Wahrnehmung von Fleischern über Präferenzen ihrer Kunden beim Kauf von Schweinefleisch erlaubte. Darüber hinaus wurden Lebensmittelproben, sowie Proben von Ausrüstung und Umgebung der Fleischereien entnommen und auf *Salmonella* spp. nach ISO 6579:2002 untersucht. Es folgte eine Pilotstudie zur Untersuchung von Insektizid-behandelten Netzen in 18 pork joints (Intervention) und 5 pork joints als Kontrolle. Die Versuchseinheiten wurden von August bis November 2014 vor und nach Intervention wöchentlich mittels Klebefallen auf Fliegenanzahl untersucht.

Fleischer gaben im Rahmen der Interviews an, dass rohes Schweinefleisch den höchsten Absatz mit 53% verzeichnet, gefolgt von frittiertem (30%), geröstetem (15%) und gekochtem Schweinefleisch (2%). Befragungen der Kunden ergaben ein ähnliches Bild bezüglich des Kaufes von rohem Schweinefleisch (48%) bzw. des Konsums von frittiertem (38%), geröstetem (9%) sowie gekochtem Schweinefleisch (5%). Schweinefleisch stellte die am zweithäufigsten verzehrte Form von tierischen Fleischerzeugnissen nach Rindfleisch dar.

Die Mehrheit der Fleischer (87%) gab an, dass sie zubereitetes Schweinefleisch mit rohen Beilagen wie Avocado, Zwiebeln, Tomaten oder Kraut servieren. Die Hälfte der Fleischer beziehte Schweinefleisch vom lokalen Schlachthaus in Kampala; der verbleibende Teil aus anderen Ressourcen.

Fliegen wurden in 80% der pork joints beobachtet, wobei in 43% darüber hinaus Schadnager, Vögel oder andere Insekten vorzufinden waren. Die Mehrzahl der Fleischer (92%) gab an, dass Fliegen Krankheiten übertragen können. 97% der Kunden und 85% der Fleischer bestätigten, dass sie Fliegen *ablehnen* und mit Gesundheits- und Hygiene- Problemen in Verbindung bringen.

60 Fleischer wurden gefragt, was ihrer Meinung das wichtigste bzw. unwichtigste Attribut für Kunden beim Einkauf von Schweinefleisch ist. Die Ergebnisse ergaben, dass „Fleisch vom selben Tag" und „Sauberkeit in der Fleischerei" nach Meinung der Fleischer am wichtigsten für ihre Kunden sind, während „Fettgehalt des Fleisches", „Schädlinge in der Fleischerei" sowie „Präsenz von Fliegen in der Fleischerei" am unwichtigsten angesehen wurden.

Von 693 Proben zeigten sich 8,8% positiv für *Salmonella* spp. Dabei waren 7,9% positiv für *S.* Enteritidis. Die Ergebnisse reichen von 31,2% in rohem Schweinefleisch (24/77) über 22,1% in Fliegen und 9,1% in Wasser, 5,2% in Tomaten sowie 3.9% in Kraut. Weiterhin wurden *S.* Gallinarum in 0,9% der Proben gefunden, was sich aus 2,6% in Tomaten, 2,6% in Zwiebeln, 1,3% in Kraut und 1,3% in geröstetem Scheinfleisch zusammensetzt. Alle 154 Proben von Handoberflächen der Fleischer, sowie deren Ausrüstung, zeigten sich negativ für *Salmonella* spp.

Die Auszählung der Fliegen während der Untersuchungsphase ergab 7953 Individuen, zusammengesetzt aus 85% *Musca domestica,* 14% Calliphoridae und 0,4% Sarcophagidae. Mediane der Fliegenfänge für die Behandlungsgruppe vor und nach Intervention zeigten eine Reduktion der Fliegen um 48% (p=0,002), während ein leichter Anstieg in der Kontrollgruppe beobachtet werden konnte.

Das hohe Fliegenaufkommen sowie die bestehende Prävalenz für *S.* Enteritidis verdeutlichen die Notwendigkeit, die Lebensmittelhygiene innerhalb der pork joints in Kampala zu verbessern. Das trifft insbesondere für Beilagen zu, da die Befragungen zeigten, dass selbige meist roh verzehrt werden. Entsprechend der Meinung der Fleischer, ist die Gegenwart von Fliegen beim Kauf von Schweinefleisch weniger wichtig für Kunden. Sowohl Kunden als auch Fleischer gaben allerdings an, dass sie Fliegen ablehnen und mit Krankheiten assoziieren.

Die beschriebenen Kenntnisse, Einstellungen und Praktiken von Fleischern, sowie deren Kunden sollten berücksichtigt werden bei einer effektiven und kostengünstigen Intervention zur Verbesserung der Lebensmittelhygiene. Insektizid-behandelte Netze bieten hierbei eine praktikable, preisgünstige und nachhaltige Lösung zur Kontrolle von Fliegen an und können als ergänzende Komponente der Lebensmittelhygiene und als Beitrag zur öffentlichen Gesundheit empfohlen werden.

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Annex 1: Questionnaire for butchers

International Livestock Research Institute

Consultative Group on International Agricultural Research

Food safety near Pork Outlets in Kampala

Introduction

As part of a research project, we are currently carrying a pilot study on pork purchase habits and preferences among Ugandan consumers as well as the retailers' practice near butcheries on markets in Kampala. Parts of this cooperation are research institutes, namely the:

- International Livestock Research Institute (Katalima Road, P.O. Box 24384, Kampala, +256775859334, Uganda)
- Institute for Parasitology and Tropical Veterinary Medicine of Freie Universität Berlin (Oertzenweg 19b, Berlin, +49 30 83862424, Germany)
- Central Diagnostic Laboratory of the College of Veterinary Medicine Animal Resources and Biosecurity (Makarere University, Kampala, Uganda)

We kindly ask you to give us a few minutes to respond to our questions below. Furthermore we intend to take samples of the pork, environment and hands' surface from retailers as well as collecting some flies. The collected samples are supposed to be used for research reasons only.

Consent form

There is no right or wrong answer. We are only interested in your opinions. Therefore we would be glad about your participation, which is entirely voluntarily. The collected information is strictly confidential and will be treated anonymously.

I agree to join the study: *Signature:* __________ *Date:* ____________________

THANK YOU

Contact information: If you require further information about the study, please contact:

Martin Heilmann, ILRI/Bioversity International, Tel: 0 77 5859 334

Dickson Ndoboli, Central Diagnostic Laboratory, Tel: 0 78 3407 616

Time: ________________ ID: ______________ Coordinates: ____________________ / ____________________

1. Retailer identification

1.1 Gender: [ ] Male [ ] Female

1.2. Age ___________________

1.3. Educational Level __________ [1= illiterate; 2=literate; 3=primary; 4=middle school; 5=Secondary; 6=university]

1.4. Year when you started working as pork retailer __________

1.5. Have followed a professional training as butcher: [ ] Yes [ ] No (go to 1.6)

1.5.1. If yes, specify the course: _____________________________________ and when:_________

1.6. Indicate your position in this shop: [ ] Owner [ ] Manager [ ] Worker [ ] Other:___________

1.7. Is pork retailing your main business activity? [ ] Yes [ ] No

1.8. Are you involved in other business activities? [ ] Yes [ ] No (go to 1.9)

1.8.1. If yes, specify:____

2. Pork and other products retail

2.1. How long do you store your pork generally? (Tick as many)

[ ] Delivered and sold at the same day [ ] More than one day

[ ] More than two days [ ] More than three days [ ] Other: _____________

2.2. Please specify and rank which kind of pork do you sell in general?

(1: most frequently, 2: second most frequently, etc., 0: never)

[ ] Raw [ ] Cooked [ ] Fried [ ] Roasted [ ] Other (specify):___________________

2.3. Do you have different meat cuts prices for raw pork?

[ ] Yes

[ ] No, specify the standard price:____________________UGX/kg (go to 2.4)

2.3.1. If yes, what are the prices for the special cuts:

meat cut:___________________________Price: ____________________________UGX/kg meat cut:___________________________Price: ____________________________UGX/kg

meat cut:___________________________Price: ____________________________UGX/kg

2.4. What is/are the source/s of your pork meat?
(1: most frequently, 2: second most frequently, etc., 0: never)

[ ] Wambizzi slaughter house [ ] Non- formally recognized slaughter house

[ ] Meat wholesalers /processors [ ] Other butchers

[ ] Buy pigs from farmers and slaughter them by myself [ ] I have my own pigs

[ ] By pigs from traders and slaughter them by myself [ ] Other (specify):____________________

2.5. Please specify and rank the other types of meat that you might sell in your butchery:
(1: most frequently, 2: second most frequently, etc., 0: never)

[ ] Beef [ ] sheep/mutton [ ] Goat [ ] Chicken [ ] Other (specify):___________

2.6. Do you also sell **raw** relishes like avocado, cassava, kachumbari, cabbage, etc.?

[ ] No [ ] Yes, specify: ____

2.7. Do you also sell any other foods or items?

[ ] No [ ] Yes, specify: ____

2.8. On a scale of 1 to 5, where 1 is very poor and 5 is very wealthy, how would you rate the typical consumer that shops at your place?

[ ] 1 (very poor) [ ] 2 (poor) [ ] 3 (not poor) [ ] 4 (wealthy) [ ] 5 (very wealthy)

[ ] I don't know

3. Hygiene practices and flies awareness

3.1. Of what material is your cutting surface? (tick as many)

[ ] Dirt [ ] Leafs [ ] Grass [ ] Wood [ ] Concrete [ ] Metal

[ ] Hard plastic [ ] Tiles [ ] Other, specify: __________________________________

3.2. How often do you clean the surface where the pork is placed on? (tick only one)

[ ] Less than once a day [ ] Once a day [ ] 2-5 times a day

[ ] Over 5 times a day [ ] Never [ ] Other, specify: _____________________________

3.3. How do you clean the cutting surface? (tick all that apply)

[ ] Wipe on clothes [ ] Cold water [ ] Hot water

[ ] Wipe with wood shavings [ ] Wipe with leaf/grass

[ ] Detergent/soap, specify:____________________ [ ] Other, specify:
____

3.4. Where does the water come from that you use for washing hands, cleaning tools, containers, and surfaces? (tick all that apply)

[ ] Piped water (tap) [ ] Piped water (tank) [ ] Stream/river

[ ] Rainwater [ ] Well [ ] Bottled

[ ] Other, specify: ____

3.5. Do you generally notice the presence of flies in your butchery? [ ] Yes [ ] No (go to 3.6)

3.5.1. If yes, how do you evaluate the number of flies?

[ ] Low [ ] Acceptable [ ] High [ ] Very high

3.6. What do you think about having flies in the butchery?

[ ] Dislike strongly [ ] Dislike somewhat [ ] It's not a problem (go to 3.8) [_] No opinion (go to 3.8)

3.7. Why do you dislike flies (tick all that apply)

[ ] Health [ ] Hygiene [ ] Nuisance [ ] Other:
____

3.8. What have you done to reduce flies number/presence until now?

[ ] Nothing [ ] Flick off [ ] Traps - which kind of:
____

[ ] Chemically - what kind of: ___________________ [ ] Other:
____

3.9. Do you think that flies could carry diseases and contaminate the meat? [ ] Yes [ ] No

3.9.1. If yes, which type of diseases:
____

3.10. Are you willing to buy and install insecticidetreated nets? [ ] Yes [ ] No

4. Retailers' Assessment of consumers pork and retail attributes preferences

In the following exercise, you will be shown a series of choices on pork and on retail attributes. Please indicate the attribute you think is most important for your consumers and the attribute you think least important for them.

Card 1. Please indicate the attribute you think is most important for your customers and the attribute you think least important for them when buying pork meat? (Tick only one case as most important and one case as least important)

Most important	Attribute	Least important
☐	Pest animals in/around the butchery	☐
☐	Cleanliness of the butchery	☐
☐	Boney meat	☐
☐	Presence of flies in the butchery	☐

Card 2. Please indicate the attribute you think is most important for your customers and the attribute you think least important for them when buying pork meat? (Tick only one case as most important and one case as least important)

Most important	Attribute	Least important
☐	Price	☐
☐	Butchery close to main road	☐
☐	Colour of the meat	☐
☐	Boney meat	☐

Card 3. Please indicate the attribute you think is most important for your customers and the attribute you think least important for them when buying pork meat? (Tick only one case as most important and one case as least important)

Most important	Attribute	Least important
☐	Cleanliness of the butchery	☐
☐	Type of building structure	☐
☐	Price	☐
☐	Age of the animal	☐

<u>Card 4</u>. Please indicate the attribute you think is most important for your customers and the attribute you think least important for them when buying pork meat? (Tick only one case as most important and one case as least important)

Most important	Attribute	Least important
☐	Type of building structure	☐
☐	Pest animals in/around the butchery	☐
☐	Butchery close to main road	☐
☐	Trust in the butcher	☐

<u>Card 5</u>. Please indicate the attribute you think is most important for your customers and the attribute you think least important for them when buying pork meat? (Tick only one case as most important and one case as least important)

Most important	Attribute	Least important
☐	Trust in the butcher	☐
☐	Boney meat	☐
☐	Age of the animal	☐
☐	The butcher wears coat	☐

<u>Card 6</u>. Please indicate the attribute you think is most important for your customers and the attribute you think least important for them when buying pork meat? (Tick only one case as most important and one case as least important)

Most important	Attribute	Least important
☐	Butchery close to main road	☐
☐	Age of the animal	☐
☐	Presence of flies in the butchery	☐
☐	Meat from the same day	☐

Card 7. Please indicate the attribute you think is most important for your customers and the attribute you think least important for them when buying pork meat? (Tick only one case as most important and one case as least important)

Most important	Attribute	Least important
☐	Colour of the meat	☐
☐	Presence of flies in the butchery	☐
☐	The butcher is wearing coat	☐
☐	Type of building structure	☐

Card 8. Please indicate the attribute you think is most important for your customers and the attribute you think least important for them when buying pork meat? (Tick only one case as most important and one case as least important)

Most important	Attribute	Least important
☐	Boney meat	☐
☐	Meat from the same day	☐
☐	Type of building structure	☐
☐	Fat layer of the meat	☐

Card 9. Please indicate the attribute you think is most important for your customers and the attribute you think least important for them when buying pork meat? (Tick only one case as most important and one case as least important)

Most important	Attribute	Least important
☐	Presence of flies in the butchery	☐
☐	Trust in the butcher	☐
☐	Fat layer of the meat	☐
☐	Price	☐

Card 10. Please indicate the attribute you think is most important for your customers and the attribute you think least important for them when buying pork meat? (Tick only one case as most important and one case as least important)

Most important	Attribute	Least important
☐	The butcher is wearing coat	☐
☐	Price	☐
☐	Meat from the same day	☐
☐	Pest animals in/around the butchery	☐

Card 11. Please indicate the attribute you think is most important for your customers and the attribute you think least important for them when buying pork meat? (Tick only one case as most important and one case as least important)

Most important	Attribute	Least important
☐	Meat from the same day	☐
☐	Colour of the meat	☐
☐	Trust in the butcher	☐
☐	Cleanliness of the butchery	☐

Card12. Please indicate the attribute you think is most important for your customers and the attribute you think least important for them when buying pork meat? (Tick only one case as most important and one case as least important)

Most important	Attribute	Least important
☐	Fat layer of the meat	☐
☐	The butcher is wearing coat	☐
☐	Cleanliness of the butchery	☐
☐	Butchery close to main road	☐

Card13 Please indicate the attribute you think is most important for your customers and the attribute you think least important for them when buying pork meat? (Tick only one case as most important and one case as least important)

Most important	Attribute	Least important
☐	Age of the animal	☐
☐	Fat layer of the meat	☐
☐	Pest animals in/around the butchery	☐
☐	Colour of the meat	☐

5. Knowledge, attitudes

5.1. I will read you some statements about hygiene in the food retail shop. Please indicate whether you agree or disagree:

	Strongly disagree	Disagree	Don't know	Agree	Strongly agree
a. Being able to work fast is the most important skill of a good worker	☐	☐	☐	☐	☐
b. People working in food jobs are more likely to get sick than other people	☐	☐	☐	☐	☐
c. In this working environment, keeping clean is easy.	☐	☐	☐	☐	☐
d. A little dirt on the clothes or tools will not cause harm.	☐	☐	☐	☐	☐
e. If pork is labelled by an inspector it is always safe to eat.	☐	☐	☐	☐	☐
f. Ensuring hygiene is the responsibility of the management or the owner.	☐	☐	☐	☐	☐
g. If pork is well-cooked then it is safe to eat.	☐	☐	☐	☐	☐
h. Providing high quality products helps generate more profit	☐	☐	☐	☐	☐
i. I give suppliers advice on how to improve quality	☐	☐	☐	☐	☐

6. Checklist (interviewer observations)

	YES	NO	NOT OBSERVED
Facilities: Record the following for the sale location			
a. Is there a permanent structure (brick, cement, wood house)?			
b. Is the pork protected from the environment?			
c. Is there a source of electricity?			
d. Is there a refrigerator present?			
e. Is there a freezer present?			
f. Does the retailer have access to running water?			
g. Does the retailer have access to a hand-washing area with soap?			
h. Is the flooring of a material that is easy to clean (i.e. tiles, concrete)?			
i. Is there a facility to dispose rubbish, old pork (i.e. dustbin)?			
j. Are there mosquito nets installed?			
k. Are there windows in the selling area present?			
Worker/ retailer conditions: Record the following for people selling the pork			
l. Do workers have clothing and shoes free of visible dirt?			
m. Do the workers wear protective gloves and/or protective clothing?			
n. Do workers have uncovered wounds?			
o. Do workers have any visible signs of communicable diseases? **If yes, describe:**			
p. Are latrines present in area?			
q. Are workers eating, drinking or smoking while handling pork?			
r. Does the butcher have a certificate of health fitness?			
Storage conditions: ask to see where the pork is stored and record the following			
s. Are crates and storage equipment free of visible dirt?			
t. Is the area free from pest animals (i.e. birds, flies)?			
u. Are there flies present?			
v. Is the cutting/selling/processing area free from visible dirt?			
w. Is the storage equipment made of a material that is easy to clean (e.g. metal, plastic)?			
x. Are the tools free from cracks and damage?			

y. Is pork in contact with other food products? **If yes, describe:**			
z. Is there a strict separation between clean and dirty areas?			

Annex 2: Questionnaire for customers

ILRI
INTERNATIONAL
LIVESTOCK RESEARCH
INSTITUTE

International Livestock Research Institute
Consultative Group on International Agricultural Research

Food safety near Pork Outlets in Kampala

Introduction

As part of a research project, we are currently carrying a pilot study on pork purchase habits and preferences among Ugandan consumers near butcheries on markets in Kampala. Parts of this cooperation are research institutes, namely the:

- International Livestock Research Institute (Katalima Road, P.O. Box 24384, Kampala, +256775859334, Uganda)
- Institute for Parasitology and Tropical Veterinary Medicine of Freie Universität Berlin (Oertzenweg 19b, Berlin, +49 30 83862424, Germany)
- Central Diagnostic Laboratory of the College of Veterinary Medicine Animal Resources and Biosecurity (Makarere University, Kampala, Uganda)

We kindly ask you to give us a few minutes to respond to our questions below.

Consent form

There is no right or wrong answer. We are only interested in your opinions. Therefore we would be glad about your participation, which is entirely voluntarily. The collected information is strictly confidential and will be treated anonymously.

I agree to join the study: *Signature:* __________ *Date:* ____________________

THANK YOU

Contact information: If you require further information about the study, please contact:

Martin Heilmann, ILRI/Bioversity International, Tel: 0 77 5859 334

Dickson Ndoboli, Central Diagnostic Laboratory, Tel: 0 78 3407 616

Time: _________________ ID: ______________ Coordinates: _____________________ / ______________________

1. Pork purchase and consumption habits

1.1. Do you buy pork? [ ] Yes [ ] No (the survey will not be conducted)

1.2. Are you usually responsible for the household pork purchase? [ ] Yes [ ] No

1.3. Could you specify and rank the main type of pork do **you/your household buy** in general?

(1: most frequently, 2: second most frequently, etc., 0: never)

[ ] Raw [ ] Cooked [ ] Fried [ ] Roasted [ ] Other (specify):________________

1.4. Where do you/your household normally buy pork?

(1: most frequently, 2: second most frequently, etc., 0: never)

[ ] Butchery [ ] Pork Joint [ ] Supermarket [ ] Pig farmer [ ] Other (specify):_______

1.5. What is your most frequently consumed meat at home?

(1: most frequently, 2: second most frequently, etc., 0: never)
[ ] Beef [ ] Sheep [ ] Goat [ ] Poultry [ ] Pork [ ] Fish

1.6. How much do you trust the retailer to provide hygienically processes pork (on a scale of 1 to 4, where 1 is I fully trust and 4 is I don't trust at all)?

[ ] 1: I fully trust [ ] 2: I mostly trust [ ] 3: I don´t really trust [ ] 4: I don´t trust at all

[ ] I don´t know

1.7. How often do you/your household usually buy **raw pork**?

[ ] Everyday [ ] Few times a week [ ] Once/ week [ ] once/2 weeks [ ] Occasionally

1.8. Please state the amount and price of your recently purchased **raw pork**?

Amount:________________ Price 1 kg:___________ [ ] I don't remember

1.9. Do you eat pork? [ ] Yes [ ] No

1.10. How often do you/your household eat pork?: __________ per day / week / month / year

1.11. Which amount per meal per person?

[ ] A quarter kg [ ] Half kg [ ] 1 kg [ ] Other: _________________

1.12. Could you specify and rank the main type of pork do **you/your household eat** in general?

(1: most frequently, 2: second most frequently, etc., 0: never)

[ ] Cooked [ ] Fried [ ] Roasted [ ] Sausages [ ] Other (specify):_____________

1.13. How often do you eat in Pork Joint: _______ per day / week / month / year

[ ] Never (go to 1.15)

1.14. Do you normally eat your pork in Pork Joint with vegetables that have <u>not been cooked</u> like tomatoes, cabbages or onions? [ ] Yes [ ] No

1.15. Do you buy any other foods in Pork Joint? [ ] No [ ] Yes, specify:_______________________

1.16. When did you last suffer from vomiting, diarrhoea or stomach pains?

[ ] Never [ ] Last week [ ] Last Month [ ] Last Year

2. <u>Flies awareness</u>

2.1. Do you generally notice the presence of flies in the pork retail outlet? [ ] Yes [ ] No

2.2. What do you think about the presence of flies in the butchery?

[ ] Dislike strongly [ ] Dislike somewhat [ ] Not affected/No opinion (go to 2.4)

2.3. Why do you dislike flies (tick all that apply)?

[ ] Health [ ] Hygiene [ ] Nuisance [ ] Other: _________________________

2.4. Do you think that flies could carry diseases and contaminate the meat? [ ] Yes [ ]No (go to section 3)

2.5. If yes, which diseases: ____

3. Pork purchase decision

In the following exercise, you will be shown a series of three raw pork meat and outlet characteristics. These are described by 4 attributes as follows:

- Colour of the pork (3 levels): pale; pink; dark red
- Presence of flies in the outlet (3 levels): no flies at all; few flies; many flies
- Cleanliness of butcher (3 levels): wears clean coat; wears dirty coat; does not wear coat.
- Price per kg raw pork in UGX (3 levels): 8000; 10000; 12000.

Card 1

Please indicate the most and least preferred raw pork (Tick only one case in each line)

Pork 1	Pork 2	Pork 3
Pale meat color Few flies in the butcheries Butcher wearing dirty coat 8000 UGSH/Kg	Pink meat color No Flies in the butcheries Butcher not wearing coat 10000 UGSH/Kg	Dark red meat color Many flies in the butcheries Butcher wearing clean coat 12000 UGSH/Kg

Most preferred ☐ ☐ ☐

Least preferred ☐ ☐ ☐

Card 2

Please indicate the most and least preferred raw pork (Tick only one case in each line)

Pork 1	Pork 2	Pork 3
Pale meat color Many flies in the butcheries Butcher not wearing coat 8000 UGSH/Kg	Pink meat color Few flies in the butcheries Butcher wearing clean coat 10000 UGSH/Kg	Dark red meat color No Flies in the butcheries Butcher wearing dirty coat 12000 UGSH/Kg

Most preferred ☐ ☐ ☐

Least preferred ☐ ☐ ☐

Card 3

Please indicate the most and least preferred raw pork (Tick only one case in each line)

Pork 1	Pork 2	Pork 3
Pale meat color No Flies in the butcheries Butcher wearing clean coat 8000 UGSH/Kg	Pink meat color Many flies in the butcheries Butcher wearing dirty coat 10000 UGSH/Kg	Dark red meat color Few flies in the butcheries Butcher not wearing coat 12000 UGSH/Kg

Most preferred	☐	☐	☐
Least preferred	☐	☐	☐

Card 4

Please indicate the most and least preferred raw pork (Tick only one case in each line)

Pork 1	Pork 2	Pork 3
Pink meat color Few flies in the butcheries Butcher wearing clean coat 10000 UGSH/Kg	Pale meat color Many flies in the butcheries Butcher not wearing coat 8000 UGSH/Kg	Dark red meat color No Flies in the butcheries Butcher wearing dirty coat 12000 UGSH/Kg

Most preferred	☐	☐	☐
Least preferred	☐	☐	☐

Card 5

Please indicate the most and least preferred raw pork (Tick only one case in each line)

Pork 1	Pork 2	Pork 3
Dark red meat color Many flies in the butcheries Butcher wearing clean coat 12000 UGSH/Kg	Pink meat color No Flies in the butcheries Butcher not wearing coat 10000 UGSH/Kg	Pale meat color Few flies in the butcheries Butcher wearing dirty coat 8000 UGSH/Kg

Most preferred	☐	☐	☐
Least preferred	☐	☐	☐

Card 6

Please indicate the most and least preferred raw pork (Tick only one case in each line)

Pork 1	Pork 2	Pork 3
Pink meat color Many flies in the butcheries Butcher wearing dirty coat 10000 UGSH/Kg	Dark red meat color Few flies in the butcheries Butcher not wearing coat 12000 UGSH/Kg	Pale meat color No Flies in the butcheries Butcher wearing clean coat 8000 UGSH/Kg

Most preferred	☐	☐	☐
Least preferred	☐	☐	☐

Card 7

Please indicate the most and least preferred raw pork (Tick only one case in each line)

	Pork 1	Pork 2	Pork 3
	Pale meat color No Flies in the butcheries Butcher wearing clean coat 8000 UGSH/Kg	Pink meat color Many flies in the butcheries Butcher wearing dirty coat 10000 UGSH/Kg	Dark red meat color Few flies in the butcheries Butcher not wearing coat 12000 UGSH/Kg
Most preferred	☐	☐	☐
Least preferred	☐	☐	☐

Card 8

Please indicate the most and least preferred raw pork (Tick only one case in each line)

	Pork 1	Pork 2	Pork 3
	Dark red meat color Few flies in the butcheries Butcher not wearing coat 12000 UGSH/Kg	Pale meat color No Flies in the butcheries Butcher wearing clean coat 8000 UGSH/Kg	Pink meat color Many flies in the butcheries Butcher wearing dirty coat 10000 UGSH/Kg
Most preferred	☐	☐	☐
Least preferred	☐	☐	☐

Card 9

Please indicate the most and least preferred raw pork (Tick only one case in each line)

Pork 1	Pork 2	Pork 3
Pale meat color Few flies in the butcheries Butcher wearing dirty coat 8000 UGSH/Kg	Dark red meat color Many flies in the butcheries Butcher wearing clean coat 12000 UGSH/Kg	Pink meat color No Flies in the butcheries Butcher not wearing coat 10000 UGSH/Kg

Most preferred	☐	☐	☐
Least preferred	☐	☐	☐

Card 10

Please indicate the most and least preferred raw pork (Tick only one case in each line)

Pork 1	Pork 2	Pork 3
Dark red meat color No Flies in the butcheries Butcher wearing dirty coat 12000 UGSH/Kg	Pink meat color Few flies in the butcheries Butcher wearing clean coat 10000 UGSH/Kg	Pale meat color Many flies in the butcheries Butcher not wearing coat 8000 UGSH/Kg

Most preferred	☐	☐	☐
Least preferred	☐	☐	☐

4. Knowledge, attitudes

4.1. What is your opinion of food certificates / food safety labels?

[ ] I fully trust [ ] I mostly trust [ ] I don´t know

[ ] I don´t really trust [ ] Not at all [ ] I don´t even look

4.2. What is your main source of information to stay current?

[ ] TV [ ] Radio [ ] Newspaper [ ] Friends [ ] Internet [ ] Work colleagues [ ] Other: ______

4.3. How much do health aspects influence your purchase decisions?

[ ] A lot [ ] A bit [ ] Not at all

5. Respondent's characteristics

5.1. Gender: [ ] Male [ ] Female

5.2. Marital status: [ ] Single [ ] Relationship [ ] Married [ ] Divorced [ ] Widow

5.3. Age: __________

5.4. Number of persons in the household: ____________ persons

5.5. Number of children in the household:

[≤ 2] years ……. [3 – 6] years …….. [7 – 12] years ….. [13 – 18] years ……..

5.6. Level of education completed:

[ ] Illiterate [ ] Literate [ ] Primary [ ] Middle school

[ ] Secondary [ ] University [ ] I prefer not to answer

5.7. Are you the head of the household? [ ] Yes [ ] No

5.8. What is your **household** monthly income in UGX?

[ ] ≤ 150,000 [ ] 150,001 – 500,000 [ ] 500,001-1,000,000 [ ] >1,000,000 [ ] I prefer not to answer

Thank you

Publications

Parts of this thesis have been presented at the following conferences:

Heilmann, M., Mtimet, N., Roesel, K., & Grace, D. (2015). *Assessing Ugandan pork butchers' practices and their perception of customers' preferences: A best-worst approach*. Poster presented for the 9th European Congress on Tropical Medicine and International Health, Basel, Switzerland, 6 – 10 September 2015. https://cgspace.cgiar.org/handle/10568/68509

Heilmann, M., Ndoboli, D., Roesel, K., Grace, D., Huehn, S., Bauer, B., & Clausen, P. H. (2015b). *Occurrence of Salmonella spp. in flies and foodstuff from pork butcheries in Kampala, Uganda*. Paper presented at the Annual expert meeting on parasitology and parasitic diseases at the German Veterinary Association in Stralsund, Germany, 29 June – 1 July 2015. https://cgspace.cgiar.org/handle/10568/68283

Heilmann, M., Roesel, K., Clausen, P. H., Grace, D. (2016). Knowledge, attitudes and practices among customers at pork butcheries in Kampala, Uganda. Poster presented at the first joint conference of the Association of Institutions for Tropical Veterinary Medicine and the Society of Tropical Veterinary Medicine, Berlin, Germany, 4 – 8 Septmeber 2016. https://cgspace.cgiar.org/handle/10568/77090

Ndoboli, D., Heilmann, M., Roesel, K., Clausen, P. H., Wampande, E., Grace, D., Alter, T., Huehn, S. (2016). Antimicrobial resistance of *Salmonella* enterica in pork and vegetable servings at pork joints in Kampala, Uganda. Poster presented at the first joint conference of the Association of Institutions for Tropical Veterinary Medicine and the Society of Tropical Veterinary Medicine, Berlin, Germany, 4 – 8 Septmeber 2016. https://cgspace.cgiar.org/handle/10568/77109

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Declaration of authorship

I hereby truthfully declare that I have carried out this thesis myself and without the help of any third party and that all literal quotations and other authors' ideas have completely been accounted for.

Rome, 1/11/2016

Martin Heilmann