

Pathogens and antibiotic resistant bacteria in abattoir waste and animals

– a study involving abattoir wastewater, earthworms and
Marabou storks



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Pathogens and antibiotic resistant bacteria in abattoir waste and animals

Patogener och antibiotikaresistenta bakterier i slakteriavfall och djur

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SUMMARY

The aims of this study were to investigate abattoir wastewater for *Escherichia coli* and *Enterococcus* spp. (indicator bacteria for faecal contamination), *Salmonella* spp. and investigate if pathogens such as *Salmonella* spp. could be detected in earthworms living in soil contaminated by abattoir wastewater and in Marabou storks feeding from an abattoir drainage channel in Kampala, the capital city of Uganda. It was also to investigate the antibiotic resistance among *Escherichia coli* from the different sources. The study was conducted at Kampala City Abattoir where samples of abattoir wastewater, earthworms and Marabou stork faeces were collected. The results showed that the abattoir wastewater contained high levels of *Escherichia coli* and *Enterococcus* spp. *Salmonella* spp. was not detected but *Citrobacter freundii* and *Shigella* spp were found. From the earthworms *Escherichia coli* was isolated but not *Enterococcus* spp. or *Salmonella* spp. No *Salmonella* spp. was isolated from the Marabou storks. Antibiotic resistance profiling revealed differences in antibiotic resistance between the *Escherichia coli* from the different sources where *Escherichia coli* from the Marabous were most resistant.

To minimize the public health risks that bacterial pathogens in abattoir wastewater pose this study suggests that faeces and other abattoir waste is collected and destroyed/made non-hazardous, or that there is a continuous cleaning of wastewater. As well that the availability for scavenging animals such as the Marabou stork to feed from the drainage channel is minimized. The study also recommends that actions be taken to reduce the usage and availability of antibiotics to reduce antibiotic resistance.

SAMMANFATTNING

Syftet med denna studie var att undersöka förekomst av indikatorbakterierna, för fekal förorening, *Escherichia coli* och *Enterococcus* spp. samt *Salmonella* spp. i slaktavfallsvatten. Syftet var även att undersöka ifall patogener som *Salmonella* spp. kan isoleras från maskar i jord kontaminerad med slakteri-avfallsvatten och i Maraboustorkar som söker föda från en dräneringskanal på ett slakteri i Ugandas huvudstad Kampala. Studien undersökte även resistensmönstret hos *Escherichia coli* från de olika grupperna. Studien genomfördes på ett slakteri i Kampala där vattenprover från slakteri-avfallsvatten, maskar och faeces från Maraboustorkar insamlades. Slakteri-avfallsvattnet visades innehålla höga nivåer av *Escherichia coli* och *Enterococcus* spp. *Salmonella* spp. påträffades inte men *Citrobacter freundii* och *Shigella* spp. Från maskarna isolerades *Escherichia coli* men inte *Enterococcus* spp. eller *Salmonella* spp. *Salmonella* spp. lyckades ej heller isoleras från Maraboustorkarnas faeces. Undersökning av antibiotikaresistensmönstret påvisade skillnader i resistensmönstret mellan de olika bakterieisolaten och att *Escherichia coli* från Maraboustorkarna var de som var mest resistenta.

Denna studie föreslår följande för att minska de risker för folkhälsan som bakteriellt förorenat slakteri-avfallsvatten utgör. Att faeces och annat slakteriavfall samlas in och oskadliggörs, alternativt att en kontinuerlig rengöring av vattnet utförs samt att möjligheten för asätande djur som Maraboustorkar att söka föda vid slakteriet och dess dräneringskanal minimeras. Vidare rekommenderas att åtgärder vidtas för att minska användandet av och tillgängligheten på antibiotika för att minska resistensen mot antibiotika.

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INTRODUCTION

Water and water related disease

According to the WHO (2013a) approximately 1,1 billion people lack access to high quality water supply sources resulting in diarrhoea, which is the cause to 4% of the human deaths in the world. According to Black et al. (2010) 16 % of the deaths among children under five years in Uganda during 2008 were caused by gastro-intestinal diseases associated with diarrhoea and in the Uganda Demographic and Health Survey for 2011 it was found that 23 % of the children that were included in the study with an age under five years had had diarrhoea two weeks prior to the start of the survey. Water contaminated with faeces from animals can cause diarrhoea because animal faeces can contain diarrhoea-causing microorganisms (WHO 2013a). As an example animal faeces can contain pathogens such as *Escherichia coli* 0157 and *Salmonella* spp., which can infect humans (Berger & Oshiro 2002). It has been suggested that waterborne zoonosis can be a bigger problem in developing countries because of the lack of water treatment facilities and use of untreated wastewater (Dufour et al. 2012).

Several studies have revealed that abattoirs in developing countries have an unhygienic environment (Adeyemo 2002; Nwanta, Onunkwo & Ezenduka 2010) and detected the presence of pathogens that are known causes of diarrheal diseases and a possible hazard to human health in the abattoir waste and water contaminated by abattoir waste (Benka-Coker & Ojior 1995; Abiade-Paul et al. 2005; Nwanta, Onunkwo & Ezenduka 2010). It has also been suggested that scavengers feeding on abattoir waste can spread pathogens from the waste to new locations (Adeyemi & Adeyemo 2007).

Objectives

The main objective of this study was to investigate if there are pathogens in abattoir wastewater from an abattoir in Kampala, the capitol city of Uganda, and if the pathogens can be picked up from the wastewater by earthworms and Marabou storks. Also it was to investigate the antibiotic resistance pattern of *Escherichia coli* from the wastewater, earthworms and Marabous. This is of importance because the pathogens can be transferred to humans via the water or the storks and because antibiotic resistance can be transferred from animals to humans.

Specific objectives

- Investigate abattoir wastewater for *Escherichia coli* and *Enterococcus* spp. (indicators of faecal contamination)
- Investigate abattoir wastewater for *Salmonella* spp.
- Investigate earthworms living in a wastewater contaminated environment for *Escherichia coli*, *Enterococcus* spp. and *Salmonella* spp.
- Investigate faeces from Marabou stork' feeding from the wastewater for *Salmonella* spp and *Escherichia coli*.
- Investigate the antibiotic resistance pattern in *Escherichia coli* from abattoir wastewater, earthworms and Marabou storks

LITERATURE REVIEW

Slaughter and waste handling in developing countries

Abattoirs in developing countries are generally less developed compared with the situation for instance in Europe and US (Chukwu 2008). They can be modern or very simple but many of them disregarding type may constitute a threat to human health because of unsanitary conditions (Verheijen, Wiersema & Hulshoff Pol 1996). According to Odong et al. (2013) two of the abattoirs in the city of Kampala discharge untreated waste into Nakivubo channel. Nakivubo channel fall into Lake Victoria at Inner Murchison Bay (Odong et al. 2013). The Inner Murchison Bay is the source for drinking water for Kampala, its suburbs and nearby towns (Water and Environment Sector Performance Report 2012).

Abattoir waste

The parts of an animal that are not used for production of food are called abattoir waste and can consist of internal organs, blood, bone, tendons and ligaments (Franke-Whittle & Insam 2013). It can also include urine, faeces and carcasses (Adeyemi & Adeyemo 2007). Abattoir waste also includes wastewater originating from an abattoir (Adeyemi & Adeyemo 2007).

Pollution with waste

According to Ritter et al. (2002) discharge of industrial waste into surface water is the most common path for industrial waste to contaminate drinking water. Water downstream from locations that discharge wastewater can contain significant levels of pathogenic bacteria (Ritter et al. 2002). In a study by Nafarnda et al. (2012) untreated wastewater and the water bodies receiving the same were studied for bacteria at the locations of several abattoirs in Nigeria. It was found that the untreated wastewater contained levels of total coliform bacteria that were beyond the levels recommended for discharge into water bodies. It was also found that receiving water bodies were contaminated with faecal bacteria and that the levels of *Escherichia coli* in the water downstream of all the abattoirs were higher than in the water upstream of the abattoirs indicating that the abattoirs were the source of pollution. The water downstream of the abattoirs was for example used for drinking, washing and bathing water. The authors suggested that such contamination can be a hazard to public health (Nafarnda et al. 2012). It has also been shown in a study from Nigeria that an abattoir discharging untreated slaughter waste into a drainage channel had ground water in its vicinity contaminated with faecal bacteria (Adeyemo, Ayodeji & Aika-Raji 2002).

Potential pathogens in abattoir waste

Approximately 61 % of the known human pathogens in the world are zoonotic (Taylor, Latham & Woolhouse 2001). Pathogens in abattoir waste may originate from the digestive tracts or hides of the animals. Most of the pathogens are of enteric sources (Mittal 2004). *Salmonella* spp. and *Escherichia coli* are examples of zoonotic bacteria that can cause diseases in humans and can be present in high levels in abattoir waste (Adeyemi & Adeyemo 2007). Nwanta, Onunkwo & Ezenduka (2010) examined abattoir waste for bacteria with potential risk for human health at an abattoir in Nigeria, and it was found that several bacteria such as, *Escherichia coli* O157:H7, *Salmonella* spp. and *Campylobacter* spp. were present in

the waste. In addition, *Escherichia coli* O157:H7 was one of the most frequently isolated bacteria. Several studies in Africa have found *Salmonella* spp. in wastewater at abattoirs (Benka-Coker & Ojior 1995; Abiade-Paul et al. 2005; Nyamboya, Okemo, & Ombori 2013). *Salmonella* has also been isolated in effluent water from treating facilities at abattoirs (Barros et al. 2007). Also has the presence of *Salmonella* spp. in water been associated with dumping of abattoir waste into water bodies (Benka-Coker & Ojior 1995). Abattoir waste can also contain antibiotics (Adeyemi & Adeyemo 2007).

Escherichia coli

Escherichia coli is a gram negative, rod shaped bacteria belonging to the family *Enterobacteriaceae*. The majorities of *Escherichia coli* strains are non-pathogenic and part of the intestinal flora in many animals but some strains are pathogenic. The bacteria are divided into several different pathotypes depending on its virulence factors (VetBact 2013a).

Enterohemorrhagic Escherichia coli (EHEC) are a pathotype of *Escherichia coli*, which can cause severe diarrhoea and haemolytic uremic syndrome (HUS) in humans (Johnson (2011) pp.139-140). The serotype O157:H7 is a serotype belonging to the EHEC group that several times been associated with disease in humans. EHEC have the ability to produce shiga like toxin also known as verotoxin (Ray & Bhunia (2008) pp. 297-298; Johnson (2011) pp. 139-140). *Vero toxic Escherichia coli* (VTEC) has been found in several different animal species such as cattle, goats, sheep, pigs, chickens, dogs and cats. VTEC has been found in a greater percentage in ruminants than in non-ruminants and it has therefore been suggested that ruminants are important natural reservoir of VTEC (Beutin et al. 1993). Several studies have found that healthy cattle can be carriers of *Escherichia coli* O157:H7. (Chapman et al. 1997; Johnsen et al. 2001; Albihn & Eriksson 2003)

Salmonella

Salmonella spp. are gram-negative rod shaped bacteria, belonging to the family *Enterobacteriaceae* (Vetbact 2013b). In humans the most common effect of a salmonella-infection is gastro-enteritis but infection can lead to more serious illness such as septicemia (McElhany & Pillai (2011) p.227). Transmission of *Salmonella* spp. may occur by direct or indirect means. Wildlife infected by *Salmonella* spp. and contaminated water are possible ways of transmission. The main source of infection is infected animals that often are subclinical carriers (Whyte et al. (2011) pp.398-399). Several serovars of *Salmonella* that cause disease in humans have been found in animals held for meat production (Johnson (2011) p.146).

Indicator organisms

When investigating water for faecal contamination indicator organisms are often used. *Escherichia coli* is often used as an indicator of faecal contamination and is according to the WHO (2013c) regarded as the indicator organism most suitable for indication of faecal contamination. Water that has recently been contaminated by faecal material contains high numbers of *Escherichia coli*. *Enterococci*, a subgroup of faecal *Streptococci*, can also be used as an indicator of faecal contamination (WHO 2013c).

Transmission of pathogens from abattoir waste/abattoir wastewater

Pathogens can spread from animal to man by several different ways, for example via direct contact, consumption of food or water that is contaminated, indirect contact via objects that are contaminated, and transmission by vectors and by aerosols (Center for Food Security and Public Health 2008).

A study by Adeyemi & Adeyemo (2007) suggested that wild animals can transfer pathogens to humans and other animals from abattoir waste by feeding on the same. Water contaminated with pathogens can also cause infection in animals and humans drinking the water or eating crops or foods contaminated by the water (Mittal 2004). An example of the latter was shown by Breuer et al. (2001), where they connected a multistate outbreak of disease caused by EHEC O157:H7 to seeds of alfalfa sprouts contaminated with the pathogen.

Transmission by birds

Birds feeding from sewage outfalls, rubbish tips or shellfish that's been contaminated can pick up bacteria and then the bacteria can be distributed to other places by the birds (Wallace, Cheasty & Jones 1997). In a survey of faecal samples from birds (mostly gulls) in 1997 the results showed that a small percentage of the birds included were carriers of *Escherichia coli* O157 (Wallace, Cheasty & Jones 1997). In 2006 Ejidokun et al. found, when investigating the source of infection for an outbreak of disease caused by *Escherichia coli* O157 in three humans, that isolates from the humans were identical to a isolate found in a sample from wild rooks' faeces. Their results indicated that indirect contact with faeces from wild birds can result in infection with *Escherichia coli* O157 and that the infection thereafter can carry on by person to person transmission. It was suggested that the birds had picked up the pathogen from faeces from livestock (Ejidokun et al. 2006). Several studies have shown that *Salmonella* spp. can be found in several different species of wild birds and that they therefore can act as carriers of the bacteria (Al-Sallami 1991; Craven et al. 2000; Vlahović et al. 2004; Nyakundi & Mwangi 2011). Al-Sallami (1991) found that the *Salmonella* spp. that was most frequently isolated from humans with diarrheal disease was found in wild crows in the same area. According to Cízek et al. (1994) the main source of infection in wild birds with *Salmonella* spp. is the environment and that birds with links to such an environment can pick up an infection when drinking or eating. A study by Benton et al. (1983) showed that contamination with *Salmonella* spp. and *Escherichia coli* in a water supply reservoir could be connected to wild birds (gulls) roosting on the water.

The Marabou stork

The Marabou stork, *Leptofilus crumeniferus*, (see Figure 1) is one of the largest and most common storks in Africa. It weighs approximately five to six kilograms and has a wingspan up to four meters. Marabous are known scavengers and are reported to be omnipresent at abattoirs in some parts of Africa (Kahl 1966).

According to Moriearty et al. (1972) Marabou storks often are in close contact with humans for example at abattoirs. In the study by Moriearty et al. (1972) Marabou storks were euthanized and samples of faeces investigated for presence of *Salmonella*, however no isolates were found to contain *Salmonella*. In a more recent study by Nyakundi & Mwangi

(2011), the presence of pathogenic bacteria in droppings from Marabou storks was investigated and it was found that 13% of the droppings contained *Salmonella*, 14% *Escherichia coli* and 9 % contained *Shigella*. The study showed that Marabou storks can carry isolates of *Salmonella*, *Escherichia coli* and *Shigella*. It was suggested that the bacteria originated from the storks' food and water sources (Nyakundi & Mwangi 2011). The authors concluded that faeces from the Marabou stork can be a potential hazard to people's health.



Figure 1. (Peter Svanström 2013). A Marabou stork nesting.

Earthworms

Earthworms have according to Satchell (1983) been suspected to transmit animal and human pathogens. They are known sources of infection of parasites to poultry and wild birds (Taylor et al. 2007). Microorganisms that are present in the environment of the worm are also often present in the worm (Satchell 1983). Williams et al. (2006) found that *Escherichia coli* O157:H7 can be found on and inside the earthworm *Dendrobaena veneta* after feeding in an environment contaminated with *Escherichia coli* O157:H7. In a study by Kumar & Sekaran (2005), earthworms (*Lampito mauritii*) were placed in an environment with material from several sewage treatment facilities that were contaminated with *Salmonella*. *Salmonella* were found in the gut of the worms living in the contaminated sewage waste up to 70 days after the start of the study, though the study also showed that the levels of *Salmonella* in the gut of the worms decreased over time. It also showed that *Salmonella* couldn't be found in worms living in an environment with sewage sludge mixed with cattle faeces and rice straw after 70 days and the decrease of *Salmonella* levels in worms from this group was also faster (Kumar & Sekaran 2005). That the levels of *Salmonella* decreases when in presence of earthworms have previously been showed in other studies, using another earthworm named *Eisenia foetida* (Brown & Mitchell 1981; Eastman et al. 2001; Murry & Hinckley 1992).

Antibiotic resistance

Antibiotic resistance means that bacteria can resist the effect of one or more antibiotics (ECDC 2013). Some bacteria are resistant to antibiotics naturally but bacteria can also acquire resistance (ECDC 2013). Infections caused by bacteria that are resistant to antibiotics can lead to failure of conventional treatment, longer treatments and death. Antibiotic resistance also leads to higher medical costs and endangers the success of certain treatments (WHO 2013b). It is well known that animals can harbor antibiotic resistant and zoonotic pathogens (Bywater et al. 2004; de Jong et al. 2012; SWEDRES-SVARM 2012). Multiple drug resistance have been suggested to be defined as when a bacteria has acquired resistance to one or more antibiotics in at least three antimicrobial categories (Magiorakos et al. 2012). Pathogens that are resistant to antibiotics can be transmitted from animals to humans and vice versa (ECDC 2013). Antibiotic resistant bacteria that are non-pathogenic and part of the normal intestinal flora have been shown to be able to transfer resistance genes to pathogenic bacteria such as *Salmonella* and *EHEC* O157:H7 (Blake et al. 2003). Resistant bacteria present in animals can also transfer resistance genes to bacteria that are part of the human normal intestinal flora if they are transferred to humans (van den Bogaard & Stobberingh 2000). There are several studies that have established the presence of antibiotic resistant bacteria in abattoir waste (Abiade-Paul, Kene & Chah 2005; Nwanta, Onunkwo & Ezenduka 2010; Atieno, Owuor & Omwoyo 2013) and among these bacteria multidrug resistant *Salmonella* (Nwanta, Onunkwo & Ezenduka 2010) and *EHEC* O157:H7 (Olatoye, Amosun & Ogundipe 2012) have been found. As well *Escherichia coli* resistant to several antibiotics have previously been found, in faeces from animals taken for slaughter at abattoirs in Kampala (Byarugaba et al. 2011). Resistant *Escherichia coli* and *Enterococcus* spp. have previously been isolated from wild birds and have been suggested as a danger to human health if spread to humans via faecal contaminated water (Radhouani et al. 2012). In a study by Chang et al. (2010) that investigated the presence of antibiotics in sewage samples antibiotics were found in the effluents from an abattoir.

MATERIALS AND METHODS

Study site

The study was carried out at Kampala City Abattoir, which was built in 1935 by the British colonial government. The abattoir has a daily slaughter of approximately 700 cattle and 200 goats and sheep. At the abattoir, waste from the slaughtering process is washed out into a drainage channel without any regular processing and Marabou storks feeding from the drainage channel is a common scene, see Figure 2.



Figure 2. (Peter Svanström 2013). Marabou storks feeding from the drainage channel.

Abattoir wastewater

Sampling of abattoir wastewater

Effluent untreated wastewater from the abattoir was collected at five different occasions during the morning at the time when the slaughter was finished and the waste from the slaughter area was washed out into the drainage channel. All of the samples were collected from the same location of the drainage channel downstream of the slaughter area. Wastewater, 500 millilitre, was collected in a sterile glass bottle and then transported to the laboratory for analyses.

Analyses of abattoir wastewater

Duplicate dilution series were made by pipetting 10 millilitres of untreated water into a sterile glass bottle with 90 millilitre dilution liquid (0, 85% NaCl and 0, 1% peptone) and then 10 millilitre of this dilution was transferred to next bottle and so on until the dilution series ranged from 1:10 to 1:100 000. The first series ranged from 1:10 to 1:100 000 but for the following it was extended up to 1:10 000 000. The dilutions were then filtered through a filter with 0,45µm pore size with negative pressure acquired with help from a hand driven filtration pump.

Analyses for Escherichia coli

From the first sample four filters were used to grow *Escherichia coli* but for the following samples only three filters were used. The filters were placed on a Lactose TTC agar with Tergitol-7 (*E. coli* agar) for growth of *Escherichia coli*. The *E. coli* agar plates were incubated at 44°C for 24 ± 2 hours. After incubation the *E. coli* agar plates were examined for yellow and orange colonies and these were counted. The plate with the growth that was most easily counted but not with too few colonies was selected for further analyses. The counted colonies were converted into colony forming units/100 millilitre (CFU/100ml). Five colonies from the chosen plate were collected with a 1µl loop and placed on a Tryptic Soy agar (TSA) plate and then streaked with a 10µl loop and then incubated for 24 hours at 37°C. After the 24 hours, colonies growing on TSA plates were tested for oxidase and indol reaction and colonies that were oxidase negative and indol positive were considered as *Escherichia coli*. Such colonies were then gathered with a 10µl loop and mixed with a 1,5 millilitre mixture of Brain Heart Infusion (BHI) and glycerol (83% BHI and 17% glycerol) and preserved at -21°C for storage.

Analyses for Enterococcus spp.

From the first sample four filters were used to grow *Enterococcus* spp. but for the following samples only three filters were used. The filters were placed on a Slanetz and Bartley agar (SLABA) for growth of *Enterococcus* spp. and incubated at 44°C for 48± 4 hours. After 48 hours the plates were excubated and the filters transferred from the SLABA plates to Bile esculin agar plates and then incubated for additional two hours at 44°C. After two hours the plates were investigated for colonies that had gone dark/black. Such colonies were considered as *Enterococcus* spp. and were counted and converted into CFU/100ml.

Analyses for Salmonella

Undiluted water ranging from 10-40 millilitres was filtered and after filtration the filter was placed in a bottle with 50 millilitre of buffered peptone water and incubated at 37°C for 18± 2 hours. After 18 hours, 100µl was pipetted from the bottle and divided into three droplets on a Modified Semisolid Rappaport Vassiliadis (MSRV) agar plate. The MSRV plate was then incubated at 41,5°C for 24± 2 hours. After 24 hours the MSRV plates were investigated for a greyish swarming zone around the droplets. From plates with such a zone 1µl was gathered with a loop from the periphery of the swarming zone and was then streaked on a Xylose lysine deoxycholate agar (XLD agar). The XLD plate was then incubated at 37°C for 24 hours. After 24 hours the XLD plates were investigated for black/dark colonies or red colonies. Plates with such colonies were selected for further analyses and five colonies from each plate were streaked on a Purple agar plate. The Purple agar plates were incubated for 24± 2 hours at 37°C. After 24 hours the Purple agar plates were investigated and plates with growth without a yellow change in colour were selected for API 10S test. Ten µl of colony material was gathered and dissolved into five millilitre of distilled water and then used for API 10S test. The API 10S test was used as instructed in the test manual and the test result was interpreted after 24 hours at 37°C.

Earthworms

Sampling of earthworms

Earthworms were collected at approximately ten locations along the drainage channel from soil that were in contact with or contaminated by the untreated effluent water from the abattoir. The earthworms were then transported in a bottle with soil from the abattoir to the laboratory.

Analyses of earthworms

Preparation

The earthworms were washed in water to remove soil and then placed in a plastic jar. Twenty-five worms were then investigated for the presence of *Escherichia coli* and *Enterococcus* spp. Each worm was euthanized by being placed in a 50°C hot water tub for a few seconds after which they were placed on a board and a medial incision was made with a scalpel to access the lumen of the worms gut. A 10µl loop was then used to gather material from the gut lumen.

Analyses for Escherichia coli

The gathered 10µl was then streaked on a MacConkey plate for investigation for *Escherichia coli* and was incubated at 44°C for 24 hours and then investigated. Red colonies were counted and then five red colonies from each dish with red colonies were re-streaked on a Tryptic soy agar (TSA) plate that was incubated at 37°C for 24 hours. Colonies on the TSA were after 24 hours tested for oxidase and indol reaction. Cultures negative for oxidase and positive for indol were regarded as *Escherichia coli*. These colonies were then as previously described under analyses of water put into the freezer.

Analyses for Enterococcus spp.

Another 10µl from each worm was streaked on a Slanetz and Bartley agar (SLABA) plate and was incubated at 44°C for 48 hours and then colonies growing on it were re-streaked on a TSA dish and incubated for 24 hours.

Analyses for Salmonella

Twenty-five earthworms were investigated for *Salmonella*. Each sample was weighed and then homogenized by hand and a broth was made up of 1/10 sample and 9/10 Buffered peptone water. The broth was then incubated for 18± 2 hours at 37°C. After 18 hours, 100µl of broth was pipetted from each sample and distributed in three drops on a Modified Semisolid Rappaport Vassiliadis (MSRV) plate. The MSRV were incubated for 24 hours at 41,5°C. The following steps in the analyses were the same as has been described under *Analyses of effluent wastewater; Analyses of Salmonella*.

Control group

The same method as described above was used for the earthworm control group, which consisted of 25 samples. The control group was gathered at a swamp in Nangabo Sub county, Wakiso district. The environment that the worms were gathered from was free from abattoir waste and wastewater.

Faeces from Marabou storks

Sampling of Marabou faeces

Marabous at the abattoir

Marabous feeding from the drainage channel were observed close to the abattoir. When a Marabou was seen to defecate, two samples from the faeces were gathered from the top of the faecal material with two bacteriological swabs with Ames medium. All the samples were gathered during the same visit to the abattoir and from several places along the drainage channel. In total samples were gathered from 12 Marabou storks.

Control group

For the control group faeces were sampled from Marabous nesting at the campus of Makerere University. These birds were chosen because it was regarded as unlikely that they fed at the abattoir. The birds were observed during several nights to find out their nesting sites. Faeces were then sampled from several nesting sites after observation of birds defecating. The samples were collected as described for the Marabous at the abattoir. In total samples were gathered from 12 Marabou storks.

Analyses of Marabou faeces

Escherichia coli

For analyses of *Escherichia coli* faecal material was transferred from one of the sampling swabs on to a MacConkey agar plate and then streaked with a 10µl loop. The plate was then incubated for 24± 2 hours at 44°C. After 24 hours red colonies were counted and then five red colonies from each dish with red colonies were re-streaked on a Tryptic soy agar (TSA) plate that was incubated at 37°C for 24± 2 hours. Colonies on the TSA were after 24 hours tested for oxidase and indol reaction. Cultures negative for oxidase and positive for indol were regarded as *Escherichia coli*. These colonies were then as previously described under analyses of water put into the freezer.

Faecal material was also streaked on a MacConkey agar with an addition of the antibiotic Cefotaxime (two millilitre Cefotaxime with a concentration of 0,5mg/ml to two litres of agar). This was done to select for more resistant *Escherichia coli*.

Analyses of Salmonella in faecal material

The second bacteriological swab taken from each stork was cut off with a pair of scissors so the part with the faecal material was placed in a glass test tube with 10 millilitre of buffered peptone water. The test tube was then incubated at 37°C for 24 hours. After 24 hours 100µl of the liquid in the test tube was divided into 3 droplets on a MSR/V plate and incubated at 41,5°C for 24± 2 hours. The following steps of the analyses were the same as previously has been described under *Analyses of effluent wastewater; Analyses of Salmonella*.

Antibiotic resistance profiling for *Escherichia coli* isolates

Antibiotic resistance profiling of the *Escherichia coli* isolates were performed with the VetMIC™ GN-mo test purchased from the National Veterinary Institute, Sweden. The test was a Minimal Inhibitory Concentration (MIC) based broth micro-dilution panel for antimicrobial susceptibility testing.

All the frozen *Escherichia coli* isolates were after arrival to Sweden cultured on blood agar by transferring 1 µl of sample from the still frozen storage tubes to the plate. The plate was then incubated for 24 hours. After 24 hours the plate was controlled so that there was a pure culture and then 1 µl of colony material was re-streaked on another blood agar plate that also was incubated for 24 hours. After the additional 24 hours a 1 µl loop was used to collect material from 3 to 5 colonies from the blood agar. The collected material was then suspended in 4 ml of sterile 0,9% saline and blended. 20 µl was then transferred from this suspension to 10 ml of Mueller Hinton broth (CAMHB) and the suspension was then blended. The panel contained the following antibiotics in eight different concentrations; Ampicillin, Ciprofloxacin, Nalidixic acid, Gentamicin, Streptomycin, Tetracycline, Florfenicol, Colistin Sulfamethoxazole, Trimethoprim, Chloramphenicol, Kanamycin, Cefotaxime and Ceftazidime. The panels' wells were filled with 50 µl of the CAMHB mixture and afterwards the wells were sealed with a transparent covering tape. The panels were then incubated at 36°C for 18 hours. For each panel 10 µl of the CAMBH mixture was streaked on an agar plate for growth and purity control. One control panel with a strain of *Escherichia coli* (CCUG 17620) with known antibiotic resistance profile were also cultured for quality check of the test. After 18 hours, the panels were examined and for each antibiotic type the MIC value was decided as the lowest concentration that completely inhibited visible growth or for Sulfamethoxazole the lowest concentration inhibiting 80% of the growth. The antibiotic cut off values that were used to decide if the strains of *Escherichia coli* were resistant against tested antibiotics was fetched from the European Committee on Antimicrobial Susceptibility Testing website (2013). Isolates with values higher than the cut off value were considered as resistant.

RESULTS

Abattoir wastewater

Escherichia coli and *Enterococcus spp.*

Escherichia coli and *Enterococcus spp.* were found in all five of the water samples taken at the abattoir. The levels of *Escherichia coli* ranged from $6,6 \cdot 10^6$ to $1,0 \cdot 10^8$ colony-forming units per 100-milliliter water. The levels of *Enterococcus spp.* ranged from $2,0 \cdot 10^6$ to $2,4 \cdot 10^7$. For all results see table 1.

Table 1

Sample	<i>Escherichia coli</i> (CFU/100ml)	<i>Enterococcus spp.</i> (CFU/100ml)
1	$6,6 \cdot 10^6$	$5,2 \cdot 10^6$
2	$1,0 \cdot 10^8$	$4,4 \cdot 10^6$
3	$0,9 \cdot 10^8$	$2,0 \cdot 10^6$
4	$3,3 \cdot 10^7$	$2,6 \cdot 10^6$
5	$0,6 \cdot 10^8$	$2,4 \cdot 10^7$

Table 1: Levels of *Escherichia coli* and *Enterococcus spp.* presented in colony forming units per 100 millilitre in the untreated effluent water from the abattoir.

Salmonella

Four of the five water samples went as far as to the API 10S test and the results from the test are presented for each sample in Table 2.

Table 2

Water sample	API 10 S code	Bacteria according to test
1	6004	Shigella spp. 63,5%
2	6404	Citrobacter freundii 54,9%
3	6404	Citrobacter freundii 54,9%
4	6404	Citrobacter freundii 54,9%

Table 2; results from API 10 S test on water samples investigated for presence of *Salmonella*. The percentage shows the probability of it being the presented bacteria.

As shown in Table 2 *Salmonella* was not isolated from any of the water samples collected.

Earthworms

Earthworms collected from the abattoir

Escherichia coli and *Enterococcus spp.*

Out of the 25 analysed earthworms there was growth on 23 out of 25 MacConkey plates. Of these 23, there were only growths typical for *Escherichia coli* with red colonies and a yellow shift in agar colour on 11 plates. The rest of the plates had a growth with white colonies and a

yellow shift in colour on the agar. Out of the 11 samples that were further processed only two were negative on oxidase test and positive for indol. In total 8 % of the earthworms were carriers of *Escherichia coli*. There was no growth on any of the SLABA plates after 48 hours and thus *Enterococcus* spp. were not found in any of the earthworms.

Salmonella

A greyish swarming zone was found on all of the 25 samples taken to analyse *Salmonella*. Out of these 25, only three showed a growth of dark/black colonies on XLD agar, the rest of the samples showed a non-typical growth with light coloured colonies and a yellow shift in agar colour. Of the three samples that were further analysed one had a yellow shift in colour on Purple agar. The API 10 S test results for the two remaining worms are presented in table 3. In conclusion *Salmonella* was not found in any of the earthworms from the abattoir.

Table 3

Worm number	API 10 S code	Bacteria according to test
9	7004	<i>Escherichia vulneris</i> 45,9%
11	7404	<i>Citrobacter freundii</i> 34,1%

Table 3; API 10 S results of samples from earthworms at the abattoir investigated for presence of *Salmonella*. The percentage shows the probability of it being the presented bacteria.

Control group of earthworms

Escherichia coli and *Enterococcus* spp.

Out of the 25 earthworms in the control group there was growth on four of the MacConkey plates (16%) after 24 hours. None of these four plates had a growth typical for *Escherichia coli*, all had white to light yellow coloured colonies. None of the samples were further analysed. *Escherichia coli* were not found in the control group. There were no growths on any of the SLABA plates after 48 hours and thus *Enterococcus* spp. were not found in any of the samples.

Salmonella

Out of the 25 samples analysed for *Salmonella* a greyish swarming zone was seen in nine of the samples on the MSR agar. The rest of the samples showed no growth. Out of the nine samples that were streaked on XLD agar none had dark/black colonies but instead white colonies and a yellow shift in colour on the agar. Thus no *Salmonella* was found in any of the samples from the earthworm control group.

Marabou stork faeces

Marabou faeces from the abattoir

Escherichia coli

Out of the twelve samples taken at the abattoir there were growth typical for *Escherichia coli* on nine of the MacConkey plates. All of these nine samples were negative on oxidase test and positive on indol test and classified as *Escherichia coli*. On the MacConkey plates with antibiotic addition there was growth on eight of the twelve plates but only colonies typical for *Escherichia coli* on six. All six were classified as *Escherichia coli*.

Salmonella

All the twelve samples showed a greyish swarming zone on the MSR/V plates after 24 hours. After culture on XLD plates there were four samples with dark/black colonies the remaining samples had a growth of white to lightly yellow colonies with a yellow shift in colour of the XLD agar. The four samples that were further analysed did not show a yellow shift in colour on Purple agar. The four samples were identified by the API 10 S test as the bacteria presented in Table 4. None of the samples taken from Marabous at the abattoir contained *Salmonella*.

Table 4

Stork number	API 10 S code	Bacteria according to test
3	6404	Citrobacter freundii 54,9
5	7005	Pantoea spp. 2 47,7%
9	6404	Citrobacter freundii 54,9%
11	6404	Citrobacter freundii 54,9%

Table 4; result from API 10 S test on samples of Marabou stork faeces investigated for *Salmonella*. The percentage shows the probability of it being the presented bacteria.

Marabou faeces from the control group

Escherichia coli

Out of the twelve samples taken at Makerere University Campus there were growth typical for *Escherichia coli* on five of the MacConkey plates. All of which were negative on oxidase test and positive on indol test and classified as *Escherichia coli*. On the MacConkey plates with antibiotic addition there was growth on three of the plates all of which the growth were typical for *Escherichia coli*. All of the three were after oxidase and indol test classified as *Escherichia coli*.

Salmonella

All the twelve samples showed a greyish swarming zone on the MSR/V plates after 24 hours. After culture on XLD plates there was one sample with dark/black colonies, and the remaining samples had a growth of white to lightly yellow colonies with a yellow shift in colour of the agar. The sample that was further analysed did not show a yellow shift in colour on Purple agar. The sample was identified by the API 10 S test as *Citrobacter freundii*. Thus none of the samples taken from Marabous at Makerere University campus contained *Salmonella*.

Antibiotic resistance pattern of *Escherichia coli*

Water samples

The antibiotic resistance profile for each of the *Escherichia coli* isolated from the five water samples taken from the untreated wastewater at the abattoir are presented in Table 5. Out of the five bacterial isolates, there was only one that was found to be resistant. The isolate was resistant against Ampicillin and Ciprofloxacin.

Earthworms

The antibiotic resistance profiles for the *Escherichia coli* isolated from the two earthworms taken from the abattoir are presented in Table 6. One of the isolates was found to be resistant to 6/12 of the tested antibiotics belonging to four different antibiotic classes.

Marabou storks

Abattoir group

The antibiotic resistance profiles for the *Escherichia coli* isolated from the Marabou stork faeces taken from the abattoir are presented in Table 7. Seven out of nine isolates showed resistance against one or several of the antibiotics. Six out of nine isolates were resistant against five or more antibiotics belonging to three or more antibiotic classes.

Control group

The antibiotic resistance profiles for the *Escherichia coli* isolated from the Marabou stork faeces taken from Makerere University Campus are presented in Table 8. All five of the isolates showed to be resistant against four or more of the investigated antibiotics belonging to five different antibiotic classes.

Table 5

	Antibiotic													
	Am	Ci	Nal	Gm	Sm	Tc	Ff	Cs	Su	Tm	Cm	Km	Ctx	Caz
Water sample														
1	4	0,06	2	0,5	4	<1	<4	<0,5	16	1	4	<8	0,12	<0,25
2	4	0,06	4	1	16	<1	8	<0,5	16	1	8	<8	0,06	<0,25
3	32 [R]	0,12 [R]	4	0,5	4	<1	<4	<0,5	32	0,5	4	<8	0,06	<0,25
4	2	0,06	2	0,5	16	<1	16	<0,5	16	0,5	8	<8	0,06	<0,25
5	2	0,06	4	1	8	2	8	<0,5	<8	0,25	4	<8	0,12	<0,25
Cut-off value	>8	>0,06	>16	>2	>16	>8	>16	>2	>64	>2	>16	>8	>0,25	>0,5

Table 5: Resistance profile for *Escherichia coli* from untreated wastewater collected at the abattoir. Antibiotic concentrations are in µg/ml. Am, Ampicillin; Ci, Ciprofloxacin; Nal, Nalidixic acid; Gm, Gentamicin; Sm, Streptomycin; Tc, Tetracycline; Fl, Florfenicol; Cs, Colistin; Su, Sulphametoxazole; Tm, Trimethoprim; Cm, Chloramphenicol; Km, Kanamycin; Ctx, cefotaxime; Caz, ceftazidime. [R]=resistant against the antibiotic.

Table 6

	Antibiotic													
	Am	Ci	Nal	Gm	Sm	Tc	Ff	Cs	Su	Tm	Cm	Km	Ctx	Caz
Earthworm 4	2	1 [R]	>128 [R]	1	256 [R]	32 [R]	8	<0,5	>1024 [R]	>16 [R]	4	<8	0,06	<0,25
Earthworm 14	2	0,03	2	0,5	8	64 [R]	<4	<0,5	<8	0,5	4	<8	0,12	<0,25
Cut-off value	>8	>0,06	>16	>2	>16	>8	>16	>2	>64	>2	>16	>8	>0,25	>0,5

Table 6: Resistance profile for *Escherichia coli* from earthworms collected at the abattoir. Antibiotic concentrations are in µg/ml. Am, Ampicillin; Ci, Ciprofloxacin; Nal, Nalidixic acid; Gm, Gentamicin; Sm, Streptomycin; Tc, Tetracycline; Fl, Florfenicol; Cs, Colistin; Su, Sulphametoxazole; Tm, Trimethoprim; Cm, Chloramphenicol; Km, Kanamycin; Ctx, cefotaxime; Caz, ceftazidime. [R]=resistant against the antibiotic.

Table 7

	Antibiotic													
	Am	Ci	Nal	Gm	Sm	Tc	Ff	Cs	Su	Tm	Cm	Km	Ctx	Caz
Stork 1	>128 [R]	0,5 [R]	8	0,5	128 [R]	64 [R]	8	<0,5	>1024 [R]	>16 [R]	8	<8	>2 [R]	8 [R]
Stork 3	>128 [R]	>1 [R]	>128 [R]	1	8	128 [R]	16	<0,5	>1024 [R]	>16 [R]	>64 [R]	<8	>2 [R]	16 [R]
Stork 4	2	1 [R]	16	1	64 [R]	128 [R]	16	<0,5	>1024[R]	>16 [R]	8	<8	0,06	<0,25
Stork 5	>128 [R]	>1 [R]	>128[R]	>16 [R]	>256 [R]	128 [R]	8	<0,5	>1024 [R]	>16 [R]	>64 [R]	<8	>2 [R]	>16 [R]
Stork 6	>128 [R]	1 [R]	16	0,5	16	<1	8	<0,5	>1024 [R]	>16 [R]	4	<8	2 [R]	16 [R]
Stork 9	16 [R]	0,06	4	0,5	8	2	8	<0,5	32	1	8	<8	0,12	0,5
Stork 10	2	0,06	4	0,5	4	2	8	<0,5	<8	0,25	8	<8	0,06	<0,25
Stork 11	>128 [R]	>1 [R]	>128 [R]	>16 [R]	128 [R]	>128 [R]	16	<0,5	>1024 [R]	>16 [R]	16	16 [R]	>2 [R]	>16 [R]
Stork 12	2	0,06	4	1	8	<1	<4	<0,5	32	1	<2	<8	0,12	<0,25
Cut-off value	>8	>0,06	>16	>2	>16	>8	>16	>2	>64	>2	>16	>8	>0,25	>0,5

Table 7: Resistance profile for *Escherichia coli* from Marabou stork faeces collected at the abattoir. Antibiotic concentrations are in µg/ml. Am, Ampicillin; Ci, Ciprofloxacin; Nal, Nalidixic acid; Gm, Gentamicin; Sm, Streptomycin; Tc, Tetracycline; Ff, Florfenicol; Cs, Colistin; Su, Sulphametoxazole; Tm, Trimethoprim; Cm, Chloramphenicol; Km, Kanamycin; Ctx, cefotaxime; Caz, ceftazidime. [R]=resistant against the antibiotic.

Table 8

	Antibiotic													
	Am	Ci	Nal	Gm	Sm	Tc	Ff	Cs	Su	Tm	Cm	Km	Ctx	Caz
Stork 1	>128 [R]	0,5 [R]	8	1	32	64 [R]	16	<0,5	>1024[R]	>16 [R]	64 [R]	<8	>2 [R]	>16 [R]
Stork 5	>128 [R]	0,03	4	0,5	128 [R]	128 [R]	16	<0,5	>1024 [R]	>16 [R]	32 [R]	<8	>2 [R]	1
Stork 6	>128 [R]	0,5 [R]	16	1	128 [R]	64 [R]	<4	<0,5	>1024 [R]	>16 [R]	4	<8	0,06	<0,25
Stork 9	>128 [R]	0,5[R]	8	0,5	128 [R]	128 [R]	8	<0,5	>1024 [R]	>16 [R]	8	<8	0,06	<0,25
Stork 11	>128 [R]	0,06	2	0,5	128 [R]	128 [R]	8	<0,5	>1024 [R]	>16 [R]	>64 [R]	<8	>2 [R]	8 [R]
Cut-off value	>8	>0,06	>16	>2	>16	>8	>16	>2	>64	>2	>16	>8	>0,25	>0,5

Table 8: Resistance profile for *Escherichia coli* from Marabou stork faeces collected at Makerere University Campus. Antibiotic concentrations are in µg/ml. Am, Ampicillin; Ci, Ciprofloxacin; Nal, Nalidixic acid; Gm, Gentamicin; Sm, Streptomycin; Tc, Tetracycline; Ff, Florfenicol; Cs, Colistin; Su, Sulphametoxazole; Tm, Trimethoprim; Cm, Chloramphenicol; Km, Kanamycin; Ctx, cefotaxime; Caz, ceftazidime. [R]=resistant against the antibiotic.

DISCUSSION

Abattoir wastewater

The study of the abattoir wastewater showed that the water was contaminated with high levels of *Escherichia coli* and *Enterococcus* spp. indicating faecal contamination. The source of the contamination is most likely the abattoir waste that is being washed out into the drainage channel during and after slaughter. Such contamination conducts a hazard to public health since the water can spread to water sources and make such water unfit for human consumption and even transmit disease. This is a potential hazard in the case of the City Abattoir since the drainage channel eventually ends up in Murchison bay where drinking water for Kampala is extracted.

Salmonella was not found in the untreated wastewater; nevertheless the conclusion that the abattoir wastewater does not contain *Salmonella* cannot be drawn since the study was limited. Possible explanations to why *Salmonella* wasn't detected are that the results are based on five samples and that the amount of water filtered for its analyses was much lower than intended. The intended volume of water was 300 millilitres but the volume filtered was only about 10 to 40 millilitre. The small volume was due to the fact that the hand driven under pressure pump that was used didn't generate sufficient pressure to filtrate more than 10-40 millilitre of the untreated water. It is reasonable to suggest that a volume of 10-40 millilitres of water isn't enough to be representable for the total quantity of wastewater produced daily at the abattoir. Another reason that might explain why *Salmonella* wasn't found is that there might not have been any animals carrying the bacteria slaughtered on the days on which the samples were taken and thus no *Salmonella* in the effluent water.

The bacteria detected when analysing for *Salmonella* was *Citrobacter freundii* and *Shigella*. It is reasonable to suggest that the slaughter waste wasn't the source of the *Shigella* since *Shigella* spp. are bacteria with humans and primates as hosts (Strockbine & Maurelli 2005). More likely the source is faecal contamination from humans indicating that abattoir workers or other people resident at the abattoir area defecate into the drainage channel. The bacterium most common found when searching for *Salmonella* was *Citrobacter freundii* which is an environmental bacteria which can be opportunistic and that also can be found in many animals (VetBact 2013c).

Earthworms

Escherichia coli were only isolated from the earthworms collected at the abattoir. The control group was collected from an environment, which wasn't contaminated with abattoir wastewater. This suggests that earthworms living in a bacterial contaminated environment pick up bacteria. Another interesting difference between the two groups was that the initial growth on MacConkey agar was much higher in the abattoir group than in the control group. In the abattoir group growth was recorded on 92 % of the agar plates and in the control group on 16 %. These figures suggest that a higher level of environmental contamination generates a higher level of bacteria in the earthworms. Though the control group might be carriers of bacteria that do not grow on MacConkey agar.

Enterococcus spp. wasn't found neither in earthworms from the abattoir or in the control group. Possible explanations are that the environment wasn't as contaminated with *Enterococcus* spp., that the chosen method for isolating *Enterococcus* spp. wasn't the best or that *Enterococcus* spp. are destroyed by the earthworms or doesn't colonize them. Since *Enterococcus* spp. was found in all water samples and are as likely as *Escherichia coli* to be introduced into the surrounding environment, the reason may have been that there were none in the earthworms. *Salmonella* wasn't isolated from neither of the groups, though that fact doesn't mean that earthworms at the abattoir don't pick up or carry *Salmonella*. The bacteria isolated when searching for *Salmonella* was *Citrobacter freundii* and *Escherichia vulneris*. *Citrobacter freundii* is an environmental bacterium which can be opportunistic and that also can be found in many animals (VetBact 2013c). *Escherichia vulneris* is a gram-negative and motile rod that has been isolated from the environment, humans and animals (Brenner et al. 1982). It has been found to be the causing microorganism to several different infections in humans (Senanayake et al. 2006). The probability of it being these two bacteria was though quite low. Possible sources of error for not finding *Salmonella* are that the collected amount of earthworms was too low, that the chosen culture method was too insensitive or that *Salmonella* was eliminated from the earthworms. Even though *Salmonella* wasn't isolated, the fact that the earthworms was found carrying *Escherichia coli* and that the abattoir group seemed to carry bacteria in a higher frequency than the control group suggests that the earthworms living in a contaminated environment can pick up potential pathogens. Also earthworms have previously been found to carry *Salmonella* when living in a *Salmonella* contaminated environment (Kumar & Sekaran 2005). Earthworms that pick up *Salmonella* could be a possible source of infection to animals feeding on them. At the abattoir there are a lot of birds of which the majority are Marabou storks. A Marabou stork feeding on earthworms carrying *Salmonella* could get infected and then spread the bacteria to other places as water bodies or public areas and result in human infection.

Marabous

Salmonella could not be isolated from any of the groups. However this doesn't exclude that there are *Salmonella* spp. in the population of storks. A possible source of error is that the gathered amounts of samples were few and that the amounts of faeces gathered were low. That more faeces wasn't collected was because of the method that was chosen to gather the samples. The current method used a swab that was smeared in the faeces. This method was used because it made it easy to collect samples from the droppings and because it was considered that it minimized the risk of picking up anything else than faeces. The Marabou storks were abundant at the abattoir and were continuously seen feeding from the drainage channel and it is therefore suggested as likely that Marabous can pick up the bacteria if *Salmonella* contaminated water or abattoir waste is excreted. A possible way to investigate if the Marabous pick up bacteria from the drainage channel is to extract DNA from the *Escherichia coli* strains from the Marabous and the wastewater and compare to see if they are the same. Though if the *Escherichia coli* strains from the Marabous would originate from the wastewater it would be expected that they had the same antibiotic resistance profiles, which wasn't the case. *Pantoea* spp. was isolated when culturing for *Salmonella*, though the probability of it being *Pantoea* was quite low. *Pantoea* spp. are gram negative rods and can be

isolated from for example humans, animals, soil, water and plants (Grimont & Grimont 2005). *Pantoea* spp. can cause infection in humans, for example *Pantoea agglomerans* has been reported of causing septic arthritis (Champs et al. 2000) and sepsis (Liberto et al. 2009).

As previously presented *Shigella* was isolated from the effluent wastewater at the abattoir. *Shigella* spp. are bacteria with humans and primates as hosts (Strockbine & Maurelli 2005) but *Shigella* has been isolated from Marabous (Nyakundi & Mwangi 2011). Because of the fact that Marabous can carry *Shigella* and that it was isolated from the wastewater, it is therefore suggested that Marabous can pick up and spread *Shigella* to new areas and give cause to disease breakouts among humans.

Antibiotic resistance profiles

The antibiotic resistance profiling found that the *Escherichia coli* from the abattoir wastewater were more sensitive to the tested antibiotics than the *Escherichia coli* originating from the Marabou storks. As well, the only isolate from the wastewater that showed any antibiotic resistance did not have the same resistance pattern as the isolates from the Marabous. One of the *Escherichia coli* isolates from the earthworms showed resistance against several of the tested antibiotics and if using the definition suggested by Magiorakos & Srinivasan (2012) this strain could be regarded as multiple drug resistant. Concerning the strains isolated from the Marabous the results showed that several were resistant against many of the tested antibiotics and that five samples from both the abattoir and the control group were multiple drug resistant. There also didn't seem to be any difference between the abattoir group and the control group. Since the isolates collected from the water didn't show similar resistance as the isolates from the Marabous or the earthworms it may not be the wastewater that is the source of the antibiotic resistance. However it may be so that if a larger number of samples would have been collected from the wastewater a larger number of resistant *Escherichia coli* strains could have been found. Concerning the Marabous, they can have gained the resistance from other food sources which seems likely since storks from the control group proved to have multiple drug resistance as well. However there can be antibiotic residues in the water that may have been ingested by the storks at the abattoir and selected for resistant bacteria. It would be interesting to investigate the abattoir wastewater for antibiotic residues to see if this is a possible explanation for the resistance found in the Marabous. Antibiotics are according to Mukonzo et al. (2013) available for purchase over the counter, without a prescription, at some places in Uganda. This may lead to a misuse of antibiotics and maybe the residues levels in the environment would decrease as well as the resistance among bacteria if this would be stopped. No matter the source of the resistance the fact that Marabou storks in Kampala carry *Escherichia coli* strains that are multiple drug resistant is a potential threat to human and animal health. The Marabous are seen all over the city and they defecate everywhere. They can in this way spread the resistant bacteria to new places where it can come in contact with humans. Another risk is that they transfer the resistance to other bacteria in their intestinal tract that may be more pathogenic, such as *Salmonella*.

Conclusion

The disposal of abattoir wastewater containing *Shigella* spp. and high levels of *Escherichia coli* and *Enterococcus* spp. into the drainage channel, the uninhibited feeding of Marabous from the drainage channel and the presence of highly resistant strains of *Escherichia coli* in Marabous are considered to be a risk for human health. To reduce the risks and to minimize the possible transmission to animals in the environment of the abattoir it is suggested that the following preventive measures are introduced at the abattoir:

- Faeces and other abattoir waste be collected and destroyed or made non-hazardous instead of being excreted into the drainage channel.
- Minimize the availability for scavenging animals such as the Marabou storks to feed from the drainage channel for example by covering the same with a grid or using a closed piping system. To minimize the availability for scavengers would reduce the possibility of spread of pathogens from the drainage channel to other areas by the means of animals.
- It is also advisable to have a continuously running treatment facility that minimizes the amount of bacteria in the effluent water before discharge into the Nakivubo channel.
- Regarding the antibiotic resistance there are reasons to believe that wild animals such as the Marabous which most likely not have been treated with antibiotics but gained their resistance from feeding shows that there is a problem with antibiotic residues in the environment. This is a big problem with no easy solution but actions should be taken to reduce the usage and availability of antibiotics.

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