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Fakulteten för veterinärmedicin och husdjursvetenskap

Institutionen för biomedicin och veterinär folkhälsovetenskap

Seroprevalence and risk factors for bovine brucellosis, salmonellosis and bovine viral diarrhoea in urban and peri-urban areas of Kampala, Uganda

Ellen Jönsson

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Seroprevalens och riskfaktorer för bovin brucellos,
salmonellos och bovin virusdiarré i stadsnära områden av
Kampala, Uganda

Ellen Jönsson

*Handledare: Sofia Boqvist, Institutionen för biomedicin och veterinär folkhälsovetenskap
Karl Ståhl, Institutionen för biomedicin och veterinär folkhälsovetenskap*

Examinator: Susanna Sternberg Lewerin, Institutionen för biomedicin och veterinär folkhälsovetenskap

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SUMMARY

Africa is expected to go through a rapid urbanization over the next four decades and the demand for food is increasing in the rapidly growing urban and peri-urban (UPU) areas. Keeping livestock in urban areas is in particular associated with health hazards. This is due to close interaction between humans and animals, and it has been shown that zoonotic diseases are increasing in urban areas. The benefits of urban and peri-urban agriculture (UPA) are related to improved food security, in particular among low-income groups. Apart from the negative public health impact of zoonotic diseases, animal disease could have a devastating impact on the economy and food security of many households in these areas.

In the present study, three important endemic diseases, including brucellosis, salmonellosis and Bovine viral diarrhoea (BVD), were analyzed in regard to seroprevalence and risk factors. Brucellosis is a severe zoonotic disease with impact on both public and animal health and associated with economic losses in most low-income countries. Transmission to humans is mainly through consumption of untreated and contaminated milk products and contact with infected animals. Salmonellosis is an important zoonotic disease with a worldwide distribution. Transmission to humans is mainly through contaminated food. The disease can be severe in both humans and animals. BVD is spread worldwide and is considered to be one of the most economically important cattle diseases, due to decreased production and animal health implications.

The aim of this study was to conduct a health survey of cattle in UPU areas of Kampala, the capital of Uganda, to study the prevalence and herd level risk factors of the endemic zoonotic diseases brucellosis and salmonellosis and the endemic disease BVD. In total, 214 blood samples were analyzed using ELISA assays. For brucellosis a seroprevalence of 3% and 11% were shown on animal and herd level respectively. Keeping cattle in the Nakawa division of Kampala was found to be a statistically significant risk factor for brucellosis ($p=0.05$). For salmonellosis the seroprevalence was 24% and 57% on animal and herd level respectively. No significant risk factors for salmonellosis were found. The seroprevalence for BVD was 23 % on animal level and 39% on herd level. Using a bull for breeding instead of artificial insemination was found to be a statistically significant risk factor for BVD ($p=0.02$).

Since several seropositive cattle for *Brucella* spp. and *Salmonella* spp. were found in the UPU areas of Kampala, it is a possibility that transmission of bacteria between cattle and humans may occur. Several seropositive cattle for BVDV were also detected in the study area. A suggestion of measures towards increased bio-security and education for better basic hygiene measures, such as heat preparations of milk and hand sanitation in UPA, could potentially improve both human and animal health status in Kampala, Uganda.

SAMMANFATTNING

Afrika förväntas gå igenom en snabb urbanisering under de kommande fyra decennierna och i de snabbt växande städerna och i stadsnära områden kommer efterfrågan på livsmedel att öka. Boskapsskötsel i områden med hög befolkningstäthet är särskilt förknippad med hälsorisker på grund av nära kontakt mellan människor och djur. Det har visat sig att zoonoser ökar i Afrikas urbana områden. Fördelar med stadsnära jordbruk är förknippade med förbättrad livsmedelsförsörjning, särskilt bland låginkomsttagare. Förutom de negativa humana hälsorisker som zoonoser för med sig kan djursjukdomar även ha förödande effekter på hushållsekonomi och livsmedelsförsörjning för människor i stadsnära områden.

I den här studien analyserades de tre viktiga endemiska sjukdomarna brucellos, salmonellos och bovin virusdiarré (BVD) med avseende på seroprevalens och dess riskfaktorer. Brucellos är en allvarlig zoonos med inverkan på både människors och djurs hälsa och associeras med ekonomiska förluster i de flesta utvecklingsländer. Människor smittas främst genom konsumtion av opastöriserade mjölkprodukter eller genom direkt kontakt med infekterade djur. Salmonellos är en annan allvarlig zoonos med global distribution. Människor smittas främst genom förorenade livsmedel. Sjukdomen kan resultera i allvarliga sjukdomstillstånd hos både människor och djur. BVD återfinns i alla världsdelar och anses vara en av de ekonomiskt sett största nötsjukdomarna på grund av minskad produktion och försämrad djurhälsa.

Syftet med denna studie var att genomföra en hälsoinventering av nötkreatur i stadsnära områden av Ugandas huvudstad Kampala för att undersöka seroprevalens och riskfaktorer för de endemiska zoonotiska sjukdomarna brucellos och salmonellos samt den endemiska sjukdomen BVD. Totalt togs 214 blodprover från nötkreatur i Kampalas stadsnära områden som analyserades med ELISA teknik. Seroprevalensen för brucellos var 3% på individnivå och 11% gårdsnivå. En signifikant risk faktor för brucellos var att hålla boskap i området Nakawa ($p=0,05$). För salmonellos var seroprevalensen 24% på individnivå och 57% på gårdsnivå. Inga signifikanta riskfaktorer för salmonellos kunde påvisas. Seroprevalensen för BVD var 23% på individnivå och 39% på gårdsnivå. Användandet av naturlig betäckning istället för AI var en signifikant riskfaktor för BVD ($p = 0,02$).

Det finns en risk att människor i Kampala smittas av bakterier från nötkreatur eftersom flertalet seropositiva nötkreatur för *Brucella* spp. och *Salmonella* spp. påvisades i stadsnära områden av Kampala. Flertalet seropositiva nötkreatur för BVDV påvisades även i området. Åtgärder för ökad biosäkerhet i stadsnära jordbruk och utbildning i grundläggande hygienåtgärder så som värmebehandling av mjölk och god hand hygien kunna förbättra både människors och djurs hälsa i Kampala, Uganda.

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BACKGROUND

This project is a component of the larger project: “Urban and peri-urban farming” with the sub-project “Zoonotic infections among cattle in urban and peri urban areas in Uganda”, a collaboration between Makerere University in Uganda and the Swedish University of Agricultural Sciences.

Urban and peri-urban agriculture

The world’s population is split between cities and rural areas and more than 50% of the global population is currently living in cities. Africa is considerably less urbanized when compared to other areas of the world, and still six out of every ten persons live in rural areas. Africa is expected to go through a rapid urbanization over the next four decades. It is likely that the urban population in Africa will treble over the next four decades and by mid-century it is expected that 20% of the world’s urban population will be concentrated in Africa (United Nations, 2010).

Expansions of urban areas are driven by a number of different reasons including economic growth, unemployment, lack of educational opportunities, natural disasters and social instability. Peri-urban areas are also expanding and consist of areas surrounding cities that are in most ways incorporated to the cities (FAO, 1999). According to Bryld (2003) there are not sufficient resources available to provide secure nourishment for the poor urban population and in many countries UPA is still illegal.

In the rapidly growing urban and peri-urban (UPU) areas, the demand for food is increasing. To satisfy these needs urban and peri-urban agriculture (UPA) is in many ways essential. Urban agriculture refers to small areas within the city for small scale animal husbandry and growing crops. Peri-urban agriculture refers to small farm units situated close to the city where farmers keep livestock, grow vegetables and produce milk and eggs (FAO, 1999). The increase in UPA has resulted in new areas of the cities being cultivated; mostly these cultivated areas are situated in backyards and around buildings. However, public land areas such as roadsides, along rail lines, floodplains, water drains and parks are also used for UPA (Freeman *et al.*, 1993). Free range and tethered animals are frequently seen on these areas.

Benefits of UPA

The benefits of UPA are associated with improved food security and increased household income, in particular among low-income groups. It has been described that there is a strong and statistically significant association between improved nutritional status among children and UPA, which has resulted in higher height measures based on age, for children in Kampala (Maxwell, 1995; Maxwell *et al.*, 1998). Other benefits from UPA include a lesser need for packaging, storage and transportation of food. The availability of fresh food is enhanced and potential agricultural jobs are available through hired farm labour. Urban farmers in Kampala are overwhelmingly women and the UPA is mainly a way for women to secure food supply for the family members either by direct provision or as an economic investment (FAO, 1999;

Maxwell, 1995). Gender equality and education of women are proven to enhance food security in low-income countries (UNDP, 2012).

Risks of UPA

Keeping urban livestock is in particular associated with health hazards due to close interaction between humans and animals in densely populated areas, and the lack of appropriate health practices when slaughtering animals. As the urban population has grown and UPA has become increasingly practiced, it has been documented that formerly rural zoonotic diseases are increasing in urban areas (Flynn, 1999). Concerns regarding the zoonotic diseases range from the traditional zoonotic diseases such as brucellosis and trichinellosis, to microbial contamination of food from for example Salmonella and *E. coli*, and to emerging diseases that can affect both livestock and humans (e.g. avian flu) (Steinfeld, 2004). An increased number of cases of malaria and dysentery have been reported in households close to farming areas (Lee-Smith & Prain, 2006; Nuwagaba, 2002). The most important zoonotic diseases, transmitted in UPU areas of Kampala, Uganda, were identified as animal sourced food-borne gastroenteritis, bovine tuberculosis, brucellosis and *Taenia solium* neuro-cysticercosis (Makita *et al.*, 2011b).

Other risks include environmental and health risks from inappropriate agricultural practices as well as increased competition for land, water, energy and increased pollution (FAO, 1999). Animal manure can beneficially be used as fertilizer in UPA but can also become a major health risk due to, for example, contaminated drinking water containing fecal coliform bacteria (Flynn, 1999).

Uganda

Historically, the population of Uganda was divided into tribally based kingdoms, developed by migrants from central and western Africa in the 15th and 16th century. Britain colonized the country in the 19th century and Uganda's independence was declared in 1962. In 1967 Uganda was proclaimed a republic (UCOTA, 2012).

The population of Uganda is about 34.1 million (2012), with about 1.7 million (2012) people living in the capital Kampala (UBOS, 2012). Most Ugandans live in rural areas, and only 13% (2010) of the population is urban. Uganda has a high annual population growth rate of 3.2% and an urban population growth rate of 4.8% (2010-2015) (FAOSTAT, 2012; United Nations, 2012). In average, Ugandan women give birth to seven children in their lifetime resulting in the third highest population growth rate in the world (MoFPED, 2010).

About 74% of the population is involved in agriculture of some kind (FAOSTAT, 2012). Most farming is small scale and foodstuffs are mainly produced for personal consumption. About a quarter of the households in Uganda own cattle (MAAIF & UBOS, 2008). The labour force in the agriculture sector is almost equally divided between men and women (FAOSTAT, 2012). According to Ssembalirwa (2008), over 35% of Kampala city's population practice some form of agriculture.

Livestock population and cattle management in Uganda

Cattle are the most important livestock with significant economic contributions in Uganda (Mwebaze, 2006). Approximately 90% of the cattle herds (and most small ruminants) are owned by smallholders. Other important livestock species are cattle, sheep, goats, pigs, rabbits and poultry. The estimated total cattle population in Uganda is 11.4 million, and a typical cattle-holding household keeps on average 7 cattle (MAAIF & UBOS, 2008). As many as 93% of the farmers keep indigenous breeds (figure 1) and the remaining keep mixed or exotic bred cattle. This is a reflection of a low level of modernization of the livestock sector. Out of the indigenous cattle 70% are of Zebu/Nganda breed and 30% are of Ankole breed (MAAIF & UBOS, 2008). Exotic and mixed breeds are often Friesians, Guernseys, Jerseys and their crosses with the local zebu. Most of the small farms depend on artificial insemination (AI) for breeding, while larger farms use bulls (Mwebaze, 2006).



Figure 1. An indigenous cow is sampled in the central division of Kampala, Uganda, 2012 (personal photo).

Figure 2. Cattle of different breed outside a slaughter house in the Rubaga division of Kampala. (personal photo).

Exotic and mixed breeds are mostly kept under intensive zero-grazing management on small and medium sized farms. The indigenous breeds are kept in extensive traditional management systems with minimal inputs/outputs. The following grazing methods are mainly used (Mwebaze, 2006):

- Communal/pastoral systems are common in areas with low population. Herdsmen move the often mixed herds, as they search for fresh grazing and water.
- Enclosed ranching mainly includes more extensive systems with larger mixed herds.

- Tethering, where livestock are restrained by rope is common in UPU areas where the average herd size is small (1-5 animals).
- Fenced dairy farms are mostly close to urban areas and markets. Most are small farms (1–5 cows) and milk is the main product.
- Zero grazing, is increasing in UPU areas where land is scarce and the demand for fresh milk and meat is high. The livestock is continuously housed and the owner needs to provide green forage. Most of these farmers have 1-3 exotic or cross breed cows (Mwebaze, 2006).

According to a recent inventory performed within ongoing collaborative research between SLU and Makerere University, Kampala, Uganda, the livestock population in UPU Kampala reaches about 1.1 million birds, 73 000 small ruminants, 40 000 pigs and 32 000 cattle in the city (Vinnerås, B., personal communication, 2012).

This correlates with the figures from MAAIF & UBOS (2008) that reports that Kampala district has approximately 32 000 heads of cattle owned by 2% of all households. The total number of cattle in Uganda is increasing as a response to an increased demand for meat and milk. However, the number of cattle in the UPU areas of Kampala is decreasing (MAAIF & UBOS, 2008) probably due to an increased population in the city center. Since the national production cannot meet the increasing demands, Uganda imports large amount of dairy product (Mwebaze, 2006).

In Uganda it was illegal to carry out farming in the city until 2005 (Ssembalirwa, 2008), and the country has taken positive steps towards legalizing UPA. To assist the development of UPA in a safe and sustainable way the CGIAR (Consultative Group for International Agricultural Research) established an initiative known as the Urban Harvest to enhance food and nutrition security, increase incomes and improve environmental and health conditions among urban populations via agriculture. Kampala was made one of the anchor cities and a Health and UPA Coordinating Committee was formed, involving local researchers and policy makers as well as representatives of international organizations (King'ori, 2004).

Endemic animal diseases

“An endemic disease is a disease that occurs in a population with predictable regularity and with only minor deviations from its expected frequency of occurrence” (Putt *et al.*, 1987). Diseases included in this study are referred to as endemic in Uganda.

Human and animal health is closely linked and people depend on animals for nutrition, companionship and socio-economic development. The route of transmission of animal diseases, including zoonoses, can be direct or indirect. Examples of direct or indirect transmission are via contact with infected animals, animal products such as contaminated food, by consumption of contaminated drinking water or by vectors that carry infection between hosts. Possible vectors are ticks, flies, mosquitoes, fleas and lice. Studies indicate

that about 40% of all human infectious diseases are transmitted from animals. About 75% of newly emerged diseases the last decade have been transmitted from animals or animal products (EFSA, 2011).

This study includes two endemic zoonotic diseases (brucellosis and salmonellosis) and one non-zoonotic endemic disease (BVD).

Brucellosis

Brucella is one of the world's major zoonotic pathogen and is an important source of disease in humans and domestic animals. Brucellae is responsible for big economic losses and substantial human morbidity in endemic areas (Boschioli, 2001; International Conference on Emerging Zoonoses, 2007)

Characteristics

Brucellosis is caused by bacteria from the genus *Brucella* and is a severe zoonotic disease with human beings as end hosts. *Brucella* species are gram-negative small rods that can be divided into six species of which *Brucella melitensis*, *Brucella suis* and *Brucella abortus* have public health implications (Osterman & Moriyon, 2006; WHO, 1997) Out of these main zoonotic *Brucella* species, most of human disease are caused by *B. abortus* and *B. melitensis* (Franco *et al.*, 2007). *Brucella* species are mainly host specific but cross infection between species occur especially with *B. melitensis* (WHO, 2006).

Bovine brucellosis

Cattle are most often infected by *Brucella abortus*, although *B. melitensis* and rarely *B. suis* can also infect cattle. Outbreaks of bovine brucellosis are associated with abortion during late gestation (third trimester), weak newborn calves, retained placenta, metritis, orchitis, reduced milk production and infertility in cows and bulls. The outcome of the disease is dependent on virulence of the infective strain, age, reproductive and immunological status and route of infection (Carvalho Neta, 2010). Vaccination of cattle with *B. abortus* strain 19 or RB 51 can greatly reduce the susceptibility for infection of the homologous species (Schurig *et al.*, 2002; WHO, 1996).

Infection can occur through the skin, conjunctiva or the respiratory mucosa, but the main route of infection in cattle is through invasion of the intestinal mucosa in the gastrointestinal tract. *Brucella abortus* is capable of invading the intestinal mucosa mainly through the M-cells. From the gastrointestinal tract, the infection is spread to local lymph nodes where *Brucella* replicates intracellularly in phagocytes (Carvalho Neta, 2010). *Brucella* species are facultative intracellular pathogens that sequester within phagocytic cells of the reticuloendothelial system, such as lymph nodes, liver, spleen and bone marrow. The infection is systemic and can involve any organ or tissue (WHO, 2006). During systemic infection, colonization of the pregnant uterus, male genital organs and mammary glands are favoured. *Brucella abortus* has a strong tropism to the uterus during the third trimester leading to abortion through infiltration of inflammatory cells, necrosis, vasculitis and

ulceration of the allantochorion (Carvalho Neta, 2010). A large number of *Brucella* organisms are shed during parturition. Abortion generally only occurs once, and the cow often has normal subsequent parturitions, although reinvasion of organisms of the uterus can occur in the following pregnancies and bacteria are shed in the fluids and membranes at parturition. Persistent infection of the mammary gland is common in subsequent lactation with continuous shedding of bacteria in the milk. Calves can acquire the infection vertically or by ingestion of contaminated milk and they may remain serologically negative and do not show any clinical signs. However, heifers with latent asymptomatic infection can abort or give birth to infected calves, which is central in maintaining the disease in a herd. Venereal transmission is uncommon although localization of *Brucella* organisms in the testis, epididymis and accessory sex glands in infected bulls are common and may lead to infertility. Artificial insemination with contaminated semen is a potential source of infection (Carvalho Neta, 2010; McDermot & Arimi, 2002).

Public health aspects

Brucellosis, also known as “undulant fever”, “Mediterranean fever” or “Malta fever”, is a zoonosis with negative impact on both public health and economy in most developing countries. The occurrence of human disease is mostly dependent on the animal reservoir and subsequently the control and prevention of brucellosis in animals is essential for eradicating the disease in man (Carvalho Neta, 2010; WHO, 2006). Brucellosis is transmitted to humans through untreated and contaminated milk products and contact with infected animals, carcasses and genital discharges. In developing countries, many people are at risk due to lack of control of the infection in the animal population, poor routines for heat treatment of milk, poor hygienic conditions and consumption of raw milk (Franco *et al.*, 2007; WHO, 1997; WHO, 2006). A study in Uganda showed that consumption of raw milk was significantly associated with seropositivity in humans in Mbarara district (Ssekawojwa, 2007). A study made at Nsoba abattoir Kalerwe Kampala, demonstrated a *Brucella* seroprevalence of 15.1% in workers, indicating that humans working in close contact with infected animals are at risk for contracting the disease (Ssekabira, 2009).

The incubation period for human brucellosis is usually a few weeks but can extend to months. The illness is mild and self-limiting to severe. Brucellosis in humans is usually expressed as an acute intermittent febrile illness which may persist and progress to chronic disease with severe complications. Since *Brucella* species are intracellular organisms, treatment with more than one antimicrobial is required for several weeks (Franco *et al.*, 2007; WHO, 1997; WHO, 2006). In tropical regions, brucellosis is often misdiagnosed as drug-resistant malaria. As a consequence to this, brucellosis is not adequately treated with a combination of antibiotics (Gorvel, 2008). *Brucella* species are potential biological weapons due to their relative stability in aerosol form and low infectious dose. *Brucella* is classified in risk group III by the WHO laboratory biosafety manual (WHO, 1997; WHO, 2006).

Brucellosis in cattle in Uganda

Brucellosis is a common disease in large parts of the world, and especially in low-income countries. According to Makita *et al.*, (2011b), brucellosis is one of the most important zoonotic diseases, transmitted in UPU areas of Kampala, Uganda. A recent study of 423 bovine serum samples from the UPU areas of Kampala showed a herd prevalence of brucellosis of 6.5% and at individual animal level a prevalence of 5.0%, using the diagnostic test C-ELISA (Makita *et al.*, 2011a). In the same study, risk factors at herd level were identified as large herd sizes, free-grazing farming and history of abortion (Makita *et al.*, 2011a).

Several studies investigating brucellosis seropositivity in cattle have been reported from Uganda. A study performed at Nsoba abattoir Kalerwe in Kampala, sampled 500 cattle and reported a seroprevalence of brucellosis to 14% (Ssekabira, 2009). A retrospective study with serum samples collected over a 10 year period from different laboratories in Uganda showed a 12% seroprevalence for bovine samples (Mwebe *et al.*, 2011). A serological survey (n=388) on bovine brucellosis carried out in three districts in western Uganda and two districts in eastern Uganda showed a significantly higher mean prevalence of 22% in the western districts than in the eastern districts with a mean prevalence of 3.4% (Kashiwazaki *et al.*, 2012). A cross-sectional study (n=723) conducted in Uganda to assess the seroprevalence and risk of brucellosis in zero-grazing and pastoral dairy systems showed an animal level seroprevalence of 3.3% and 34% respectively (Magona *et al.*, 2009).

Out of 1535 sampled cattle, 4% were found to be seropositive in a study taken place in Mbarara district, Uganda (Ssekawojwa, 2007). *Brucella* seropositivity in cattle in this study was significantly associated with pastoral livestock production, age, sex, breed, parity and abortion (Ssekawojwa, 2007).

Magona *et al.*, (2009) states that information, gathered from cattle owners in Uganda, suggests that vaccination against brucellosis was not being practiced in the country and Makita *et al.*, (2011a) concluded that because brucellosis is endemic Uganda, vaccination is the most appropriate control measure in the country. Ssekawojwa (2007) emphasizes the need for the authorities to institute control and prevention measures for brucellosis.

Salmonellosis

Salmonella is a zoonotic pathogen of great significance with a worldwide distribution. Infections of livestock are an important cause of mortality and morbidity in cattle and constitute an important reservoir for human infections (Wray & Davies, 2000). Salmonellosis has been recognized in all countries and is most prevalent in areas with intensive animal husbandry (OIE, 2010). Antimicrobial resistance of non typhoidal salmonellosis (NTS), most commonly *S. typhimurium* and *S. enteritidis*, has become common in sub-Saharan Africa, which limits the value of important drugs for management of generalized infections especially common in HIV-infected human patients (Gordon *et al.*, 2008).

Characteristics

Salmonellosis is caused by a gram-negative bacterium from the genus *Enterobacteriaceae*. There are two species of Salmonella; *S. enterica* and *S. bongori*, which can be divided further into subspecies (Tindall *et al.*, 2005). Most of the animal and human pathogenic Salmonella serovars belong to the *S. enterica enterica* subspecies. Strains of Salmonella are classified into serovars according to the Kauffmann-White scheme and are based on the diversity of the lipopolysaccharide antigen (O) and the flagellar protein antigen (H). Salmonellae are primarily intestinal bacteria but are commonly found in the environment due to fecal contamination (Grimont & Weill, 2007).

Pathogenesis

The route of infection is mainly oral and the bacteria can be transmitted through direct or indirect contact with infected animals or humans. Sources of infection can be fecally contaminated water or foodstuffs. The bacterium penetrates the mucosal tissue, mainly in the lower intestines, via enterocytes and M cells-which overlie lymphoid follicles. The pathological changes include edematous and shortened villi as well as abnormal extrusion of enterocytes though the mechanism involved with onset of diarrhea is not entirely understood. Many of the systemic effects of salmonellosis leading to shock are associated with the release of endotoxins (Wray & Davies, 2000). A study by Hall & Jones (1977) showed that abortion is caused by rapid accumulation of bacteria in the connective tissue of the cotyledons which results in placental destruction and hormonal change.

Salmonella infections in cattle

Salmonella typhimurium and *S. dublin* are the most common serovars isolated from cattle and the distribution of these two serovars may differ between different countries. Introduction of the bacteria into naïve herds are by purchase of calves or adult cattle or through animal feed which is a recognized source of pathogenic microorganisms for farm livestock. (Davies & Hinton, 2000; Wray & Davies, 2000) Clinical findings in cattle are inconsistent. Some animals show signs of diarrhea, fever, depression, loss in milk yield, abortions, anorexia, pneumonia, sepsis or death while other go through a subclinical infection (Wray & Davies, 2000). Some cattle that recover from infection may become active carriers and continue to shed the bacteria continuously or intermittently for years and in some cases life. Some animals become latent carriers with a localized infection in lymph nodes or tonsils. A sudden change in resistance due to, for example, disease or stress can result in exacerbated clinical disease and excretion of the bacteria in the feces in these animals (Wray & Davies, 2000). Infection with *S. dublin* is associated with compromised animal health and reduced production and economic losses due to high calf mortality and losses in milk yield (Dahl Nielsen, 2012).

Public health aspects

Human salmonellosis is mainly a foodborne disease, but humans can be infected by direct contact with infected cattle (Wray & Davies, 2000). A study of human salmonellosis found that 30% of the cases were associated with infected cattle (Fone & Barker, 1994). Many cases of human salmonellosis have also been associated with the consumption of un-pasteurized

milk. The dominating serovars to cause infection in humans are *S. enteritidis* and *S. typhimurium* (Humphrey, 2000). Clinical findings in humans are diarrhea, abdominal pain, fever, nausea, muscle pain and sometimes vomiting. In healthy individuals, the infection can proceed without symptoms, whereas immunocompromised individuals are more at risk for severe infection. Lethal cases are uncommon but do exist (Humphrey, 2000).

Salmonellosis in Uganda

A study of clinical human *Salmonella* isolates collected over 10 years at the medical research council in Entebbe, Uganda, showed that the *S. enteritidis* and *S. typhimurium* serotypes were the most prevalent strains causing human infection. Results from the same study also concluded that there was significant antimicrobial resistance to commonly used antibiotic drugs such as Ampicillin, Chloramphenicol, Co-trimoxazole and Tetracycline (Kyakuwa, 2010). A similar pattern of antimicrobial resistance was seen in the *Salmonella* isolates, sampled from cattle, at two abattoirs in Kampala, Uganda. All of the isolates from that study were resistant to Chloramphenicol, 80% of the isolates were resistant to Sulfisoxazole, Streptomycin and Trimethoprim-Sulfamethoxazole, 60% of the isolates were resistant to Cefoxitin, Ampicillin, Kanamycin, Amoxicillin-Clavulanic acid and Tetracycline. The prevalence of *Salmonella* was 1.23% in this study (Ezama, 2010).

Bovine viral diarrhea

BVDV is one of the most widespread and economically important cattle pathogens worldwide and is not prioritized in most low-income countries due to presence of many other important diseases probably resulting in major losses. There are significant differences in prevalence between different areas and countries probably the result of differences in management and structure (Kalaycioglu, 2007).

Characteristics

BVD is a viral disease caused by a *pestivirus* in the family of *Flaviviridae* and is closely related to Classical swine fever and Border disease virus (Brownlie, 1986). Bovine viral diarrhea virus, BVDV, can be divided in two genotypes, type 1 and 2 (Vilcek *et al.*, 2001). BVDV of both genotypes can occur in cytopathogenic or non-cytopathogenic biotype and can be differentiated by cell culture (Brownlie, 1986).

Pathogenesis

The routes of infection are by direct or indirect animal contact. During acute infection, virus may be shed in nasal discharge and semen. Persistent infected (PI) animals constitute a continuous source of infective virus to other cattle and remain seronegative, direct contact with PI animals is probably the most important method of transmission of infection (Brownlie, 1990). Persistent infected bulls continuously shed virus in semen that are highly infectious (Lindberg, 2003). Infection can also be transmitted to the fetus vertically if the pregnant cow is infected in early gestation (before day 125). This results in a persistently infected calf. Since the calf was infected in early fetal development, before immunocompetence, the calf does not develop immunity against the virus and continues to

shed virus to the surrounding animals. In a persistent infected calf the non-cytopathogenic can mutate and become cytopathogenic. This can cause the serious mucosal disease (Brownlie, 1990).

Clinical findings

Acute BVD infections occur mainly in young cattle and may be subclinical or associated with diarrhea (Baker, 1995), but can also cause severe disease. Common clinical findings are fever, inappetence and mucosal lesions. In calves, the infection is often associated with diarrhea and respiratory symptoms. Since BVDV act as an immunosuppressive agent, secondary bacterial infections are common. In adult bulls, an acute infection may result in an impairment of semen quality. A wide range of reproductive failures can be seen during infection depending on the stage of gestation, for example, birth of PI calves, malformations, fetal death, abortion, mummification, malformations, stillborn calves and failure to conceive (Lindberg, 2003).

The clinical signs of persistent infected calves can vary from apparently healthy individuals to weak calves unable to suckle and standing. The latter often die within days of birth. PI calves have impaired immune functions and therefore more susceptible to other infections. Some animals survive to sexual maturity and all calves bred from persistent infected dams are persistently viraemic (Lindberg, 2003). After removal of the PI animal from a herd, cows will remain seropositive through life, and the in-herd prevalence will decline slowly mostly depending on replacement by new seronegative cows (Houe, 1999).

Mucosal disease

It is well known that persistent infected animals can develop mucosal disease (MD), although development of the disease are sporadic and rare (Brownlie, 1985). MD is a fatal disease that can cause rapid death, often in cattle aged six months to two years of age. The disease develops as a result of a mutation resulting in a change in biotype from non-cytopathogenic to cytopathogenic strain. MD can also develop as a result of recombination between a non-cytopathogenic and a cytopathogenic strain from, for example, a vaccine containing cytopathogenic strains. The course of the disease can be either acute or chronic. Clinical signs in the acute form often involve fever, anorexia, diarrhea and extensive erosions in the gastrointestinal tract, leading to progressive emaciation and death. In chronic cases, the animal show similar signs but over a longer period of time (Lindberg, 2003).

Bovine viral diarrhea in Uganda

Accessible scientific knowledge about the prevalence of BVDV in Uganda is limited but according to a recent limited field study in northern Uganda, the proportion of seropositive animals was 20% (Ståhl, K., personal communication, 2012). Nothing is known of the impact and prevalence of BVD in the UPU areas of Kampala to the author's knowledge.

OBJECTIVE

The main objective of this study was to conduct a health survey of cattle to study the prevalence and herd level risk factors of the endemic zoonotic diseases brucellosis and salmonellosis as well as the endemic disease bovine viral diarrhoea.

More specific aims were to:

- Assess the risk factors influencing Brucella, Salmonella and BVDV seropositivity in cattle in the study area.
- To gain experience of clinical, laboratory and epidemiological features of viruses and bacteria of great importance in terms of economic losses, human disease and animal suffering in Kampala.
- To gain insight in the UPA of a developing country.

MATERIALS AND METHODS

Study population

The study area was confined to the UPU areas of Kampala. Kampala city council is constituted of 5 administrative units also called divisions (KCCA, 2012). The study covered all the five divisions of Kampala city district, which include; Central Kampala, Nakawa, Rubaga, Kawempe and Makindye. The study also covered the area called Greater Kampala which is the highly urbanized subcounties of Wakiso and Mukono districts (UBOS, 2012).

The sample size was set to 270 cattle. This sample size is enough to estimate a prevalence of 50%, with a precision of 5% and a confidence interval of 90%.

The total number of animals in the respective divisions is viewed in table 1. These figures are based on information from a recent project called “Urban and peri-urban farming” with the sub-project “Manure Management” taking place in the UPU areas of Kampala that has mapped Kampala’s UPU domestic animal population. This data was provided by Björn Vinnerås, Department of Energy and Technology at SLU. However, due to recent reorganizations of cattle in urban and peri-urban areas of Kampala, the location of animals did not entirely coincide with the animal-data provided by Björn Vinnerås. Animals were sampled from each division using different local contact persons with local affiliations (Kwizera, M.H., personal communication, 2012). In order to sample animals from many different farms, a maximum of five animals on each farm were sampled. On animal level, sampled individuals were chosen from different age, sex and breed groups.

Table 1. The table is viewing the total number of cattle in each division of Kampala, according to the project “Urban and peri-urban farming” with the sub-project “Manure Management”. Data provided by Björn Vinnerås, Department of Energy and Technology at SLU. The table also includes the total number of sampled cattle in each division in the present study

Division	Number of cattle from previous mapping	Number of sampled animals (%)
Central	62	21 (34)
Nakawa	616	50 (8)
Rubaga	934	34 (4)
Kawempe	1628	43 (3)
Makindye	610	46 (8)

Data collection and sampling

Data was collected at each farm using a written questionnaire (Appendix A). The form included 22 closed and open-ended questions to assess the animal health status, use of antibiotics, given food stuffs, manure handling, bio-security, breeding method and movement of animals as well as information about type of labour on the farm (fig 2). Interviews were conducted directly in English or through a translator if necessary. GPS coordinates were registered on each farm.

Blood samples were collected using the jugular vein (vena jugularis) (fig 3) or the ventral tail vein (vena coccygea ventralis). The animals were manually restrained and not sedated. The blood was collected using 20G needles and a vacutainer adapter into sterile 6 ml serum tubes. The samples were kept in room temperature to clot and thereafter stored at 2-8°C until analyzed.



Figure 3. Interviewing a farmer in the central division of Kampala, Uganda, 2012. Livestock are kept in the outbuilding seen in the background. Note the boxes with banana peels, a commonly used animal feed (personal photo).

Figure 4. Bleeding a cow from the jugular vein. The young cross bred cow is kept in a zero-grazing production system in the Makindye division of Kampala (personal photo).

Laboratory analysis

All laboratory analyses were performed at Makerere University in Kampala, Uganda at the molecular biology laboratory, Institute of Environment and Natural Resources. The serum tubes were centrifuged within a maximum of 36 hours after sample collection, and the serum was manually separated from the blood. All serum samples were aliquoted into two 1.8cc cryo vials, one for diagnostic analysis stored at 2-8°C and one for future long term storage at -80°C. All samples were analyzed within a maximum of 3 weeks.

Enzyme Linked Immunosorbent Assay (ELISA)

Commercial ELISA kits were used for detection of antibodies in the serum samples (fig 4). The protocol for each assay was followed according to the manufacturer's instructions. All reagents used were provided in the kits, as well as positive and negative controls. All controls were run in duplicates. Samples were run in singles.

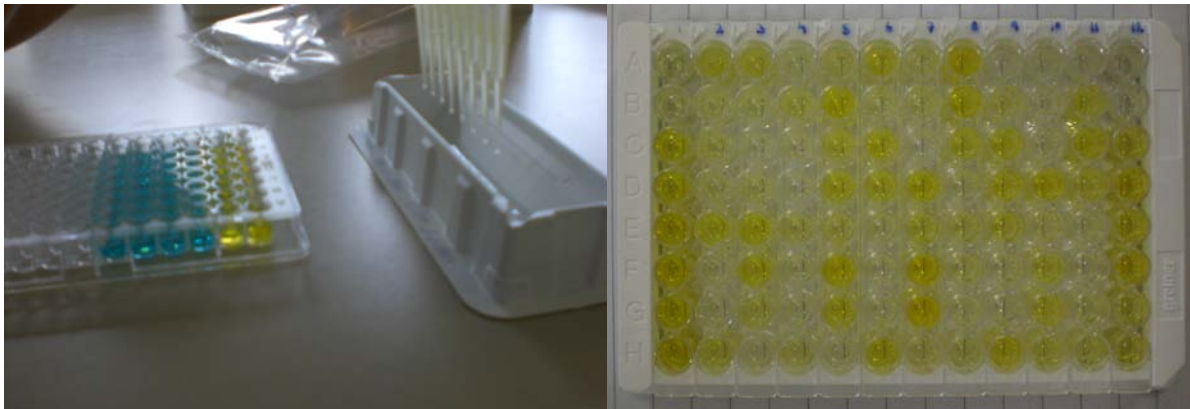


Figure 5. Competitive ELISA plate for detection of antibodies in sera against *Brucella* spp. A yellow colour change indicate a negative sample. Addition of stop solution in the last step causes a colour change in the wells from blue to yellow (personal photo).

Figure 6. Indirect ELISA plate for detection of antibodies in sera against *Salmonella* spp. Wells with a yellow colour change indicate a positive sample (personal photo).

Detection of antibodies against *Brucella*

For detection of antibodies against *Brucella* spp., a commercial competitive ELISA (C-ELISA) assay was used (SVANOVIR[®] Brucella-Ab, Svanova Biotech AB, Uppsala, Sweden). The assay is originating from the Animal Disease Research Institute, Canada. According to Nielsen *et al.* (1995) the assay has a specificity of 99.7%. The sensitivity is ranging from 98.0-99.7% for bovine samples according to an in-house study made by the manufacturer (Lindh, C., personal communication, 2012) and Nielsen *et al.* (1995).

In the *Brucella* C-ELISA procedure, 5µl of serum sample was diluted and then exposed to *B. abortus* smooth lipopolysaccharide (S-LPS) coated wells on a microtiter plate together with 50 µl mouse monoclonal antibody (mAb), directed against the M87-region on the LPS. After

incubation at room temperature for 30 minutes and washing with PBS-Tween Buffer 4 times, 100µl of goat anti-mouse antibody conjugate with horse radish peroxidase was added which binds to any mAb's bound to the S-LPS coated wells. After incubation in room temperature for 30 minutes the plate was washed with PBS-Tween Buffer to remove unbound materials before addition of 100µl substrate solution. 50µl of stop solution was then added to stop the reaction and the optical density was measured by a microplate photometer 450nm. Colour development is due to conversion of the substrate by the conjugate. In the absence of anti-Brucella antibodies (negative sample), the mAb binds to the wells and is indicated by a colour development. If the sample contains Brucella specific antibodies (positive sample) they compete with the mouse antibodies and inhibit the binding and therefore less colour development in the wells. Sera from vaccinated cattle (strain 19) do not compete with the mAb and is therefore leading to a negative reaction (Nielsen *et al.*, 1995; OIE, 2010).

Since the buffer control wells contained no serum sample they were considered to give 0% inhibition using the photometer. All data were calculated from those absorbance readings using the equation:

Per cent inhibition (%I) = $100 - (\text{absorbance} [\text{test sample}] / \text{absorbance} [\text{buffer control}] \times 100)$ (OIE 2010).

Validation of the test and interpretation of the results were done according to the instructions of the manufacturer. Samples with per cent inhibition values ≥ 30 were considered to be positive.

Detection of antibodies against Salmonella

For detection of antibodies against *Salmonella* spp., a commercial indirect (I-ELISA) kit was used (PrioCHECK® *Salmonella* bovine, Prionics AG, Zürich, Switzerland). According to the manufacturer the test detects both *S. dublin* and *S. typhimurium* strains. The test detects antibodies directed against the 1, 4, 5 and 12 O-antigens of the *S. dublin* strain and 1, 9 and 12 of the *S. typhimurium* strain.

In the *Salmonella* I-ELISA procedure, 10µl of sample serum was diluted and added to wells coated with purified LPS from *S. dublin* and *S. typhimurium* on a microtiter plate. The plate was incubated in room temperature for 1 hour. The plate was washed 6 times with diluted washing fluid. 100µl conjugate consisting of goat-anti bovine IgG coupled to horse radish peroxidase was added and the plate was incubated for 1 hour at room temperature. After washing 6 times with diluted washing fluid, 100µl of chromogen (TMB) substrate solution was added. A colour change indicated a positive sample due to the conversion of the substrate by the conjugate. The reaction was then stopped by addition of 100µl stop solution and the optical density was measured by a microplate photometer 450nm (Barrow, 1994; OIE, 2010).

All data were calculated using the manufacturers (PrioCHECK®) equations on the package insert:

1. Corrected optical density values = optical density [sample]-optical density [negative control]
2. Per cent positivity = $100 \times (\text{corrected optical density value [test sample]}/\text{corrected optical density value [positive control]})-10$

Validation of the test and interpretation of the results were done according to the instructions of the manufacturer. Samples with per cent positivity values ≥ 35 were considered to be positive.

Detection of antibodies against BVDV

For detection of antibodies against BVDV a commercial indirect ELISA (I-ELISA) assay was used (SVANOVIR[®] BVDV-Ab, Svanova Biotech AB, Uppsala, Sweden). The assay is developed to detect BVD-virus specific antibodies (IgG₁) in bovine serum, plasma and milk samples, individual and bulk tank milk. According to the manufactures in house study the assay has a sensitivity of 100% and a specificity of 98.2% using the serum neutralization test (SNT) as reference method.

In the BVDV I-ELISA procedure, 10 μ l of sample serum was diluted and added to BVDV-antigen coated wells as corresponding wells coated with control antigen on a microtiter plate. The plate was then incubated in 37°C for 1 hour to let the antibodies (if present in the serum sample) bind to the antigen in the coated wall. The plate was washed 3 times with PBS-Tween Buffer. 100 μ l conjugate was added to form a complex with the antibodies (if present in the serum sample) and the plate was incubated for 1 hour at 37°C. After washing 3 times with PBS-Tween Buffer, 100 μ l of substrate solution was added. A colour change indicated a positive sample due to the conversion of the substrate by the conjugate. The reaction was then stopped by addition of 50 μ l stop solution and the optical density was measured by a microplate photometer 450nm (Howard *et al.*, 1987; OIE, 2008).

All data were calculated using the following equations:

1. Corrected optical density values = optical density [BVDV antigen coated wells]-optical density [control antigen coated wells]
2. Per cent positivity = $100 \times (\text{corrected optical density value [test sample]}/\text{corrected optical density value [positive control]})$ (OIE 2008)

Validation of the test and interpretation of the results were done using the values in the manual cover enclosed with the commercial kit. Samples with per cent positivity values ≥ 10 were considered to be positive.

Statistical analyses

When analyzing the risk factors on herd level the seroprevalences were regarded as true prevalences, due to practical reasons. Evaluation of the different risk factors for each disease

at herd level was made using the Fisher exact p-values. The OR, CI and p-values were attained using the Epi-Info Tool version 7, from Centers for Disease Control and Prevention (CDC). P-values < 0.05 were used as statistically significant values.

A number of parameters were tested as potential risk factors. Factors tested were geographical location, herd size, keeping goats, breeding method, usage of the bull on other farms, disease history, type of labour, and production system. Seropositivity for any of the other diseases was also tested as potential risk factors.

RESULTS

Interviews

A total number of 56 farms were visited and thereby 56 interviews were conducted, using the questionnaire. Different villages in the UPU areas of Kampala's divisions were visited (see figure 7). The field trips took place in September and October of 2012.

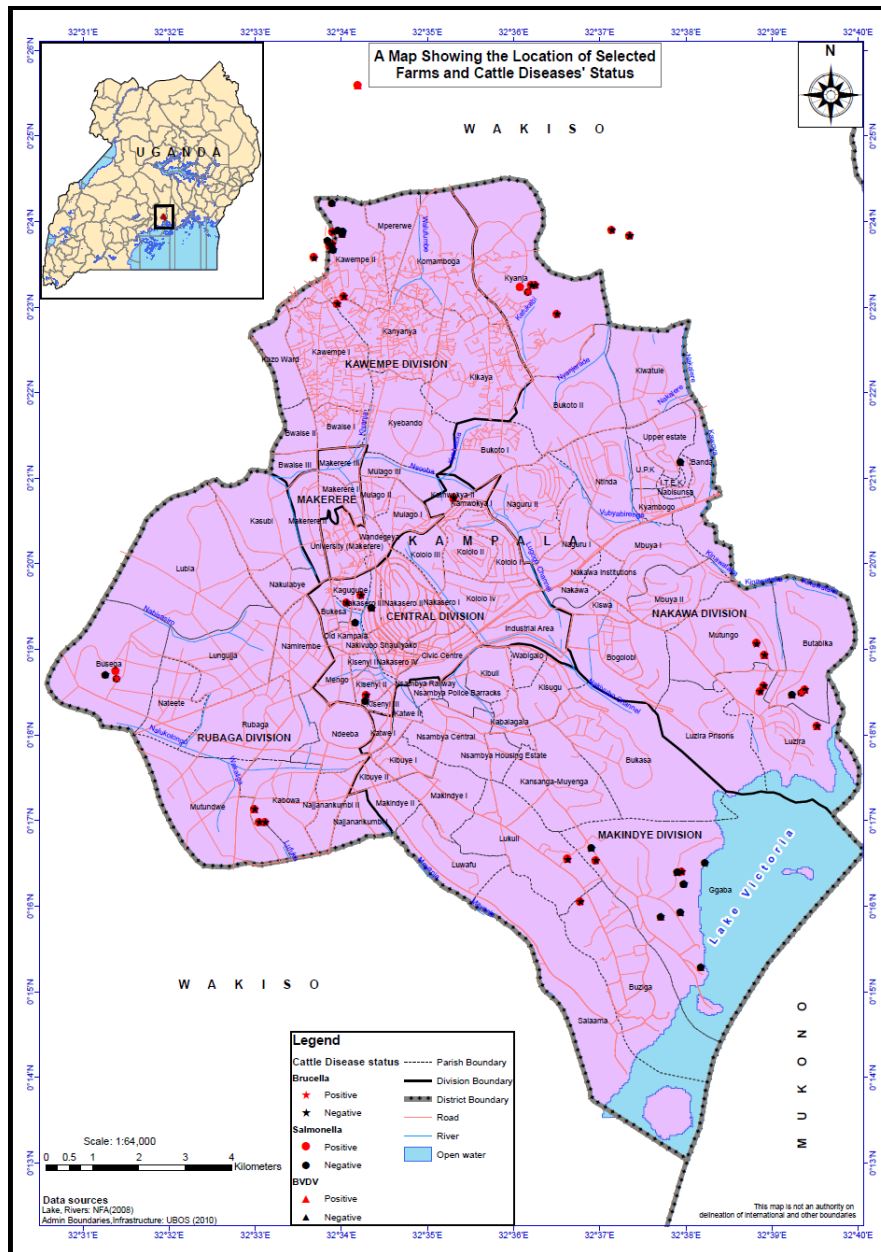


Figure 7. Map showings sampled farms in the UPU areas of Kampala. Positive herds are indicated with a red colour. Detailed maps for each disease are found in appendix 2-4.

Farm characteristics

The mean herd size was 7.9 cattle, ranging from 1 to 70 animals. In addition to cows, 22 out of the 56 farms housed goats. Most respondents were adult and male, only 18 of the respondents were female. The female respondents were to a higher degree owners to goats in addition to cattle (11/18), compared to the male respondents (11/28), (fig 8). This difference was proven to be statistically significant ($p=0.04$, OR 3.9, CI: 1.2-12.5). Out of 56 respondents, 42 were owners, 9 were herdsmen and 5 were family members. The main purpose of keeping cattle was to sell milk and 75% of respondents (42/56) kept cattle for this

purpose. In addition, many respondents also kept cattle for production of milk for own consumption (22/56). Out of 56 sampled farms, 23 were keeping cattle in a zero-grazing production system. Other common production systems were tethering (15/56), pasture (13/56) and free range (5/56) (fig 9).

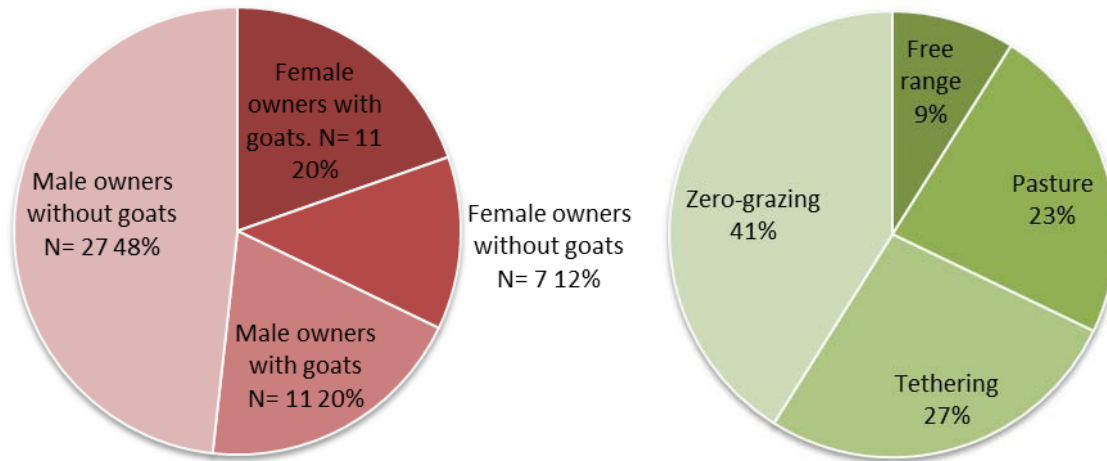


Figure 8. Gender difference in housing goats in addition to cattle. Female respondents were significantly ($p = 0.04$) more prone to keep goats in addition to cattle, compared to male respondents.

Figure 9. Common production systems in the UPU areas of Kampala. Most respondents kept cattle in a zero-grazing production system.

Feeding practices and manure handling

On all sampled farms, cattle were fed fresh grass and 82% were fed banana peels in addition to grass. Animals were to a lesser extent fed with corn, brewers mash, other peels and concentrate. Cattle were fed with products bought at a local market in 50% of all farms, 43% with crops that were grown on the farm. Other, less common feed-sources were kitchen wastes, breweries and crop grown by neighbours. Manure was, in 52% of farms, used as fertilizing for growing crops, 32% did nothing, 13% sold the manure, 7% used the manure for fuel (in most cases bio-gas) and in 4% of cases the manure was dumped. Some respondents ticked more than one option.

Livestock movements

The average number of bought and sold cattle was 0.5 (range 0-4) and 0.6 (range 0-6) respectively in the recent year. Most animals (80%) were bred on the farm. Cattle were either purchased from neighbours or the local market. In the last year, 19 farms had sold livestock and they were in most cases sold to the abattoir (10/19). Some were sold to neighbours (6/19) or the local market (3/19). Livestock were most often transported by truck when bought or sold. In some cases cattle were herded on foot.

Herd health status

Animals had been diseased on 46 out of 56 farms during the last year. The most common observed clinical sign was fever (32/56). Other common clinical signs were cough, diarrhea,

abortion and nasal discharge (table 2). Many farmers reported that their livestock had shown more than one clinical sign in the last year. Clinical signs other than fever, cough, diarrhea, abortion and nasal discharge were classified as “other” disease. Clinical signs included in this category were infertility, intestinal worm infection, mastitis, retained placenta, eye infection, drooling and skin infection. One or more animal had been treated with antibiotics in 37 out of 56 farms in the last year.

Table 2. Observed clinical signs on the 56 sampled farms

Clinical signs	Number of farms	Percentage of farms
Abortion	3	5
Coughing	17	30
Diarrhea	11	20
Fever	32	60
Nasal discharge	5	9
Other	15	27

Reproduction

For reproduction of livestock, 23 out of 56 farmers relied on natural breeding, although usage of artificial insemination (AI) was almost as common (21 out of 56). 12 respondents stated that both natural breeding and AI was used (see figure 10). The bull was used on other farms in 89% of cases (31/35) where natural breeding or both was used. Only 4 respondents had a bull that was exclusively used on that farm.

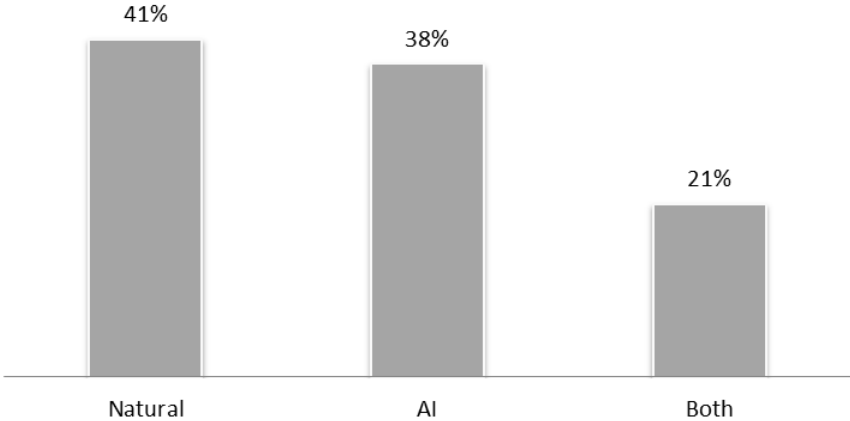


Figure 10. Distribution of breeding methods. Most respondents (23/56) relied on natural breeding.

Blood samples

In total, 214 cattle were sampled on 56 different farms to analyze the presence of antibodies against *Brucella* spp., *Salmonella* spp. and BVDV. The mean number of cattle sampled on each farm was 3.8 animals, ranging from 1-5. A maximum number of 5 animals per farm

were sampled. This was done to increase the number of sampled farms, and thereby get a better geographical distribution of sampled livestock.

Disease prevalence

The number of sampled herds and cattle, as well as seroprevalences, on herd level and animal level for the different diseases in each division, is viewed in table 3.

Table 3. Total number of sampled animals in each division of Kampala and disease prevalences for each division

Division	Number of sampled farms/cattle	Number of Brucella seropositive farms/cattle (prevalence %, herd level/animal level)	Number of Salmonella seropositive farms/cattle (prevalence %, herd level/animal level)	Number of BVD seropositive farms/cattle (prevalence %, herd level/animal level)
Central	7/21	0/0 (0/0)	4/6 (57/29)	1/1 (14/5)
Kawempe	10/43	0/0 (0/0)	6/12 (60/28)	4/13 (40/30)
Rubaga	8/34	2/2 (25/1)	5/9 (63/26)	3/5 (38/15)
Makindye	11/46	0/0 (0/0)	4/6 (36/13)	4/6 (36/13)
Nakawa	16/50	4/4 (25/8)	11/17 (69/34)	9/20 (56/40)
Greater Kampala	4/20	0/0 (0/0)	2/2 (50/10)	1/5 (25/25)

Prevalence of brucellosis

Out of 56 sampled herds, six animals in six herds were found to be seropositive, representing a prevalence of 2.8% and 10.7% on animal level and herd level, respectively. Percentage inhibition values for the six positive samples are presented in table 4.

Table 4. Percentage inhibition (%I) values calculated from optical density (OD) values for the six Brucella seropositive cattle (read at 450nm). Values ≥ 30 were considered to be positive

Animal ID	2Q1C	3Z2C	3B3C	3Y4C	3N2C	3K2C
%I-Values	30.0	30.1	31.3	31.7	35.1	38.3

Identified positive animals came from different farms, no more than one positive individual was found on each farm. The positive farms were all situated in the Nakawa or the Rubaga division of Kampala as presented in figure 11.

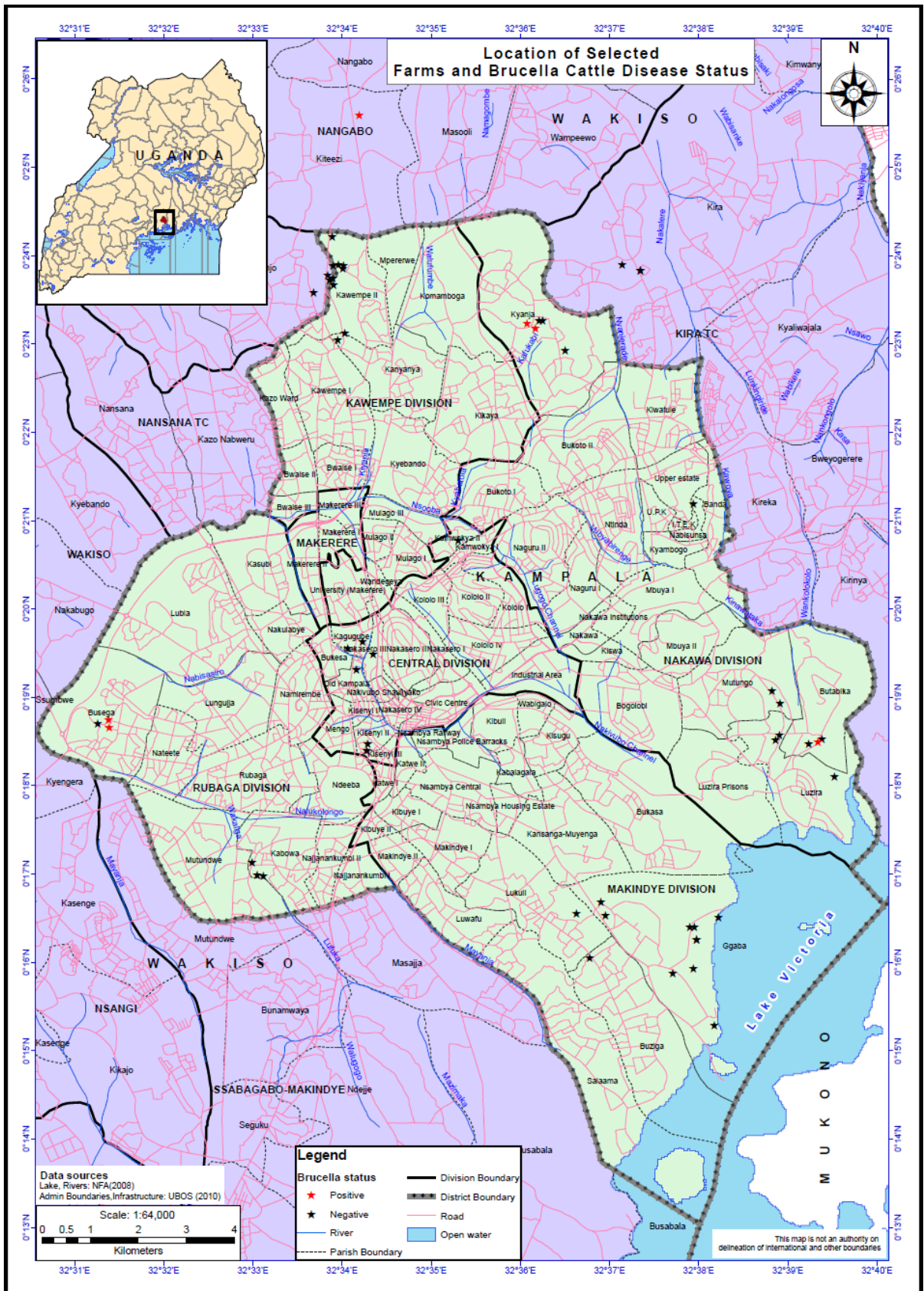


Figure 11. Location of selected farms. Red stars indicate a seropositive herd for brucellosis.

Prevalence of salmonellosis

Out of 56 sampled herds, 52 animals in 32 herds were found to be seropositive positive, representing a prevalence of 24.3% and 57.1% on animal level and herd level, respectively. The average number of seropositive animals of sampled animals in each herd was 32%. Per cent positivity values for the 52 positive samples are presented in figure 9, samples with PP values ≥ 35 were considered to be positive. Negative samples with PP values < 35 are not included in figure 12.

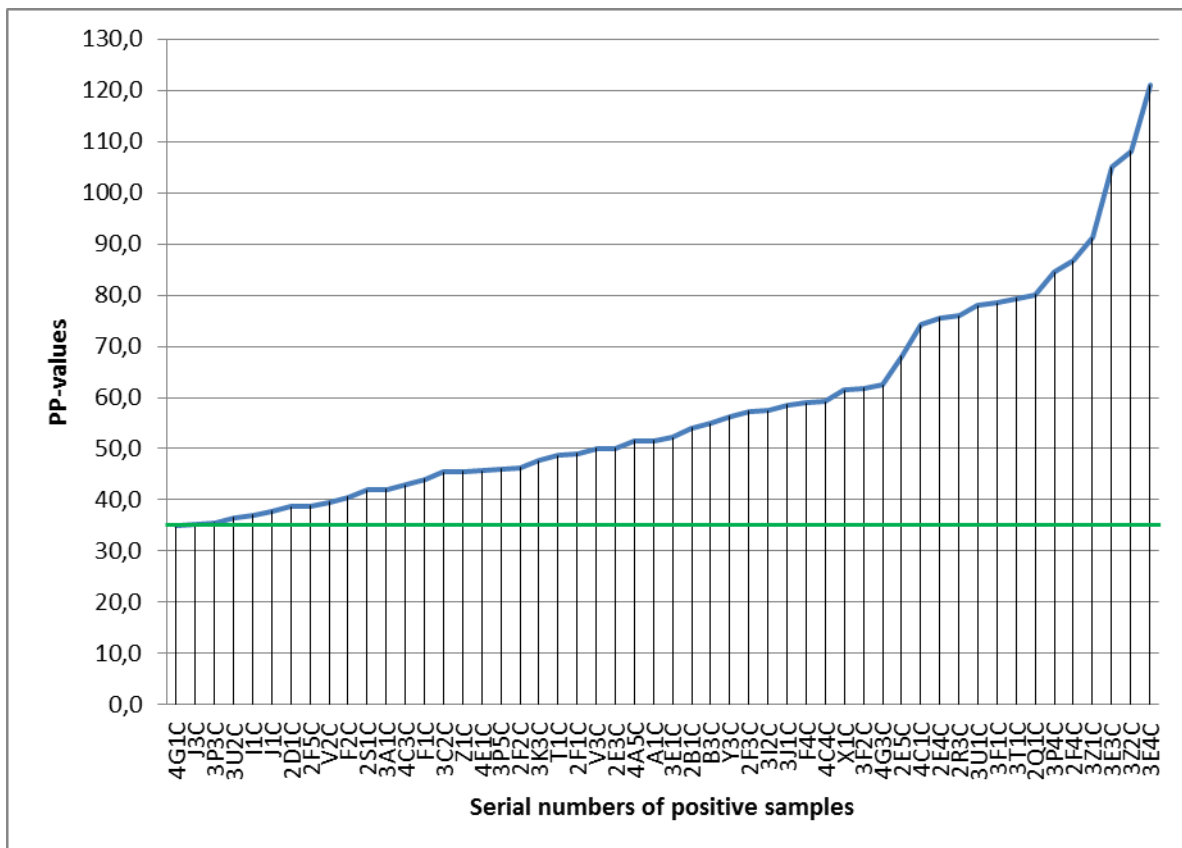


Figure 12. Per cent positivity (PP) values calculated from optical density (OD) values for the 52 *Salmonella* seropositive cattle (read at 450nm). Values ≥ 35 were considered positive. The green line represents the cut off value .

Identified positive animals came from 32 different farms. On average 1.6 seropositive animals were found on each positive farm, ranging from one to five. All divisions of Kampala were represented with positive herds as presented in figure 13.

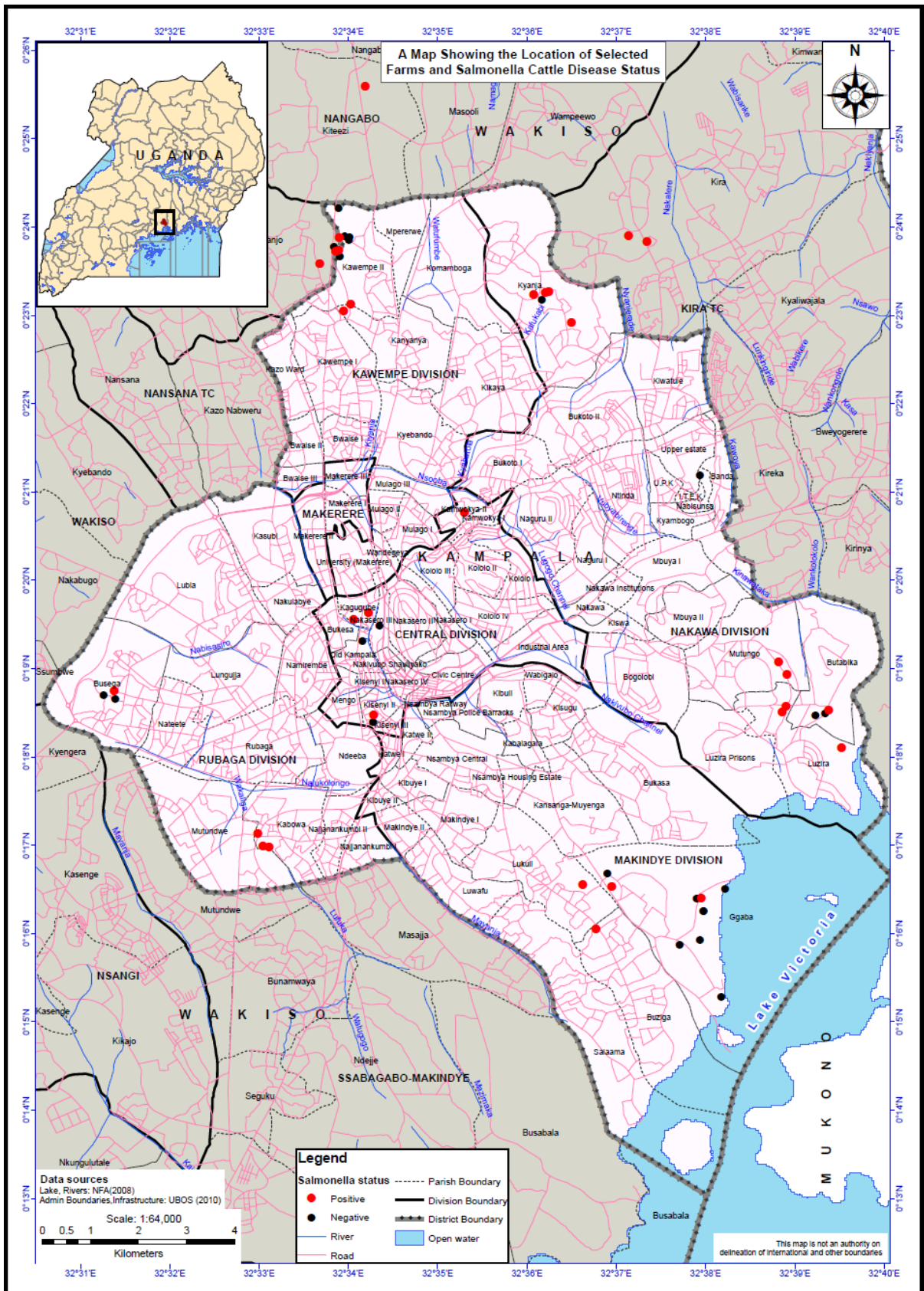


Figure 13. Location of selected farms. Red dots indicate a seropositive herd for salmonellosis.

Prevalence of Bovine Viral Diarrhea

Out of 56 sampled herds, 22 herds were found to be positive, showing a herd prevalence of BVD of 39.3%. The average number of seropositive animals of sampled animals in each herd was 26%.

Out of 56 sampled herds, 50 animals in 22 herds were found to be seropositive positive, representing a prevalence of 23.4% and 39.3% on animal level and herd level, respectively. Percentage positivity values for the 50 positive samples are presented in figure 10, samples with PP values ≥ 10 were considered to be positive. Negative samples with PP values < 10 are not included in figure 14.

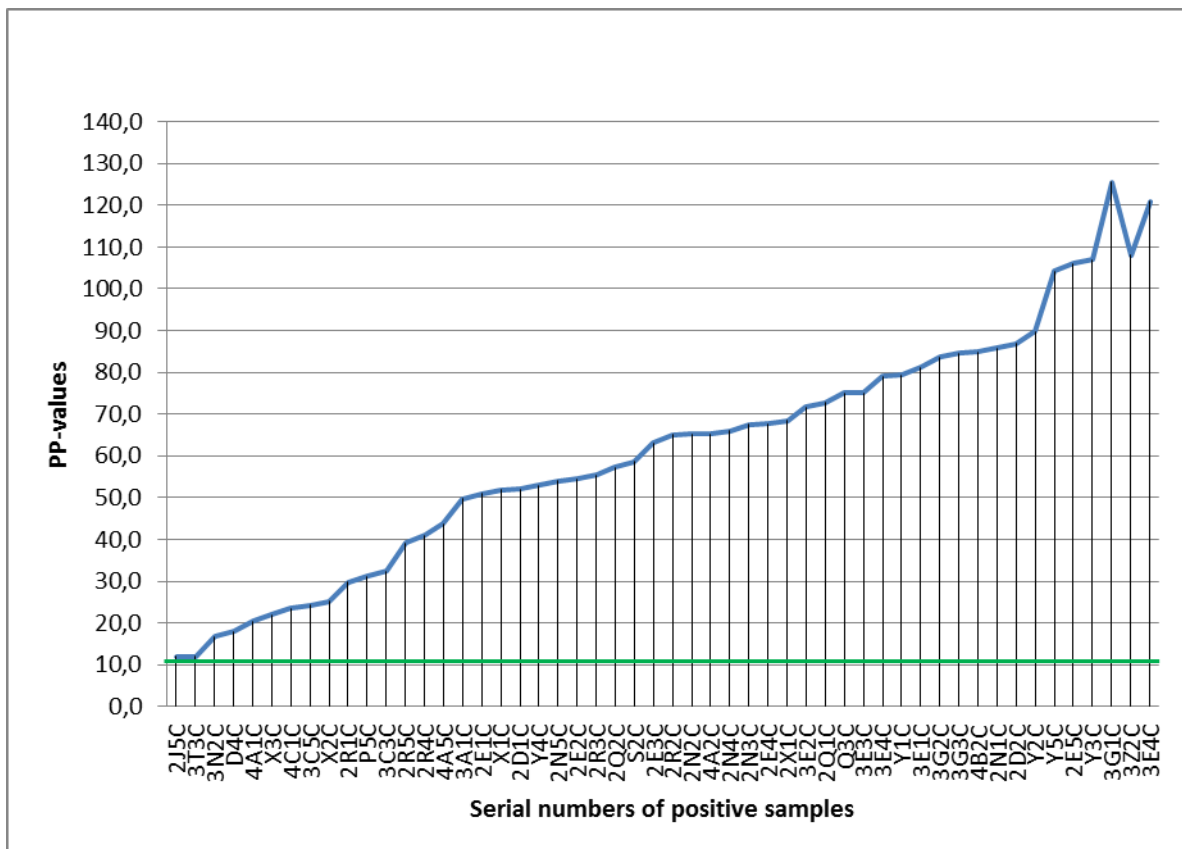


Figure 14. Percentage positivity (PP) values calculated from optical density (OD) values for the 50 BVDV seropositive cattle (read at 450nm). PP values ≥ 10 were considered positive. The green line represents the cut off value .

Identified positive animals came from 20 different farms. On average 2.5 seropositive animals were found on each positive farm, ranging from one to five. All divisions were represented in within the seropositive group of animals as presented in figure 15.

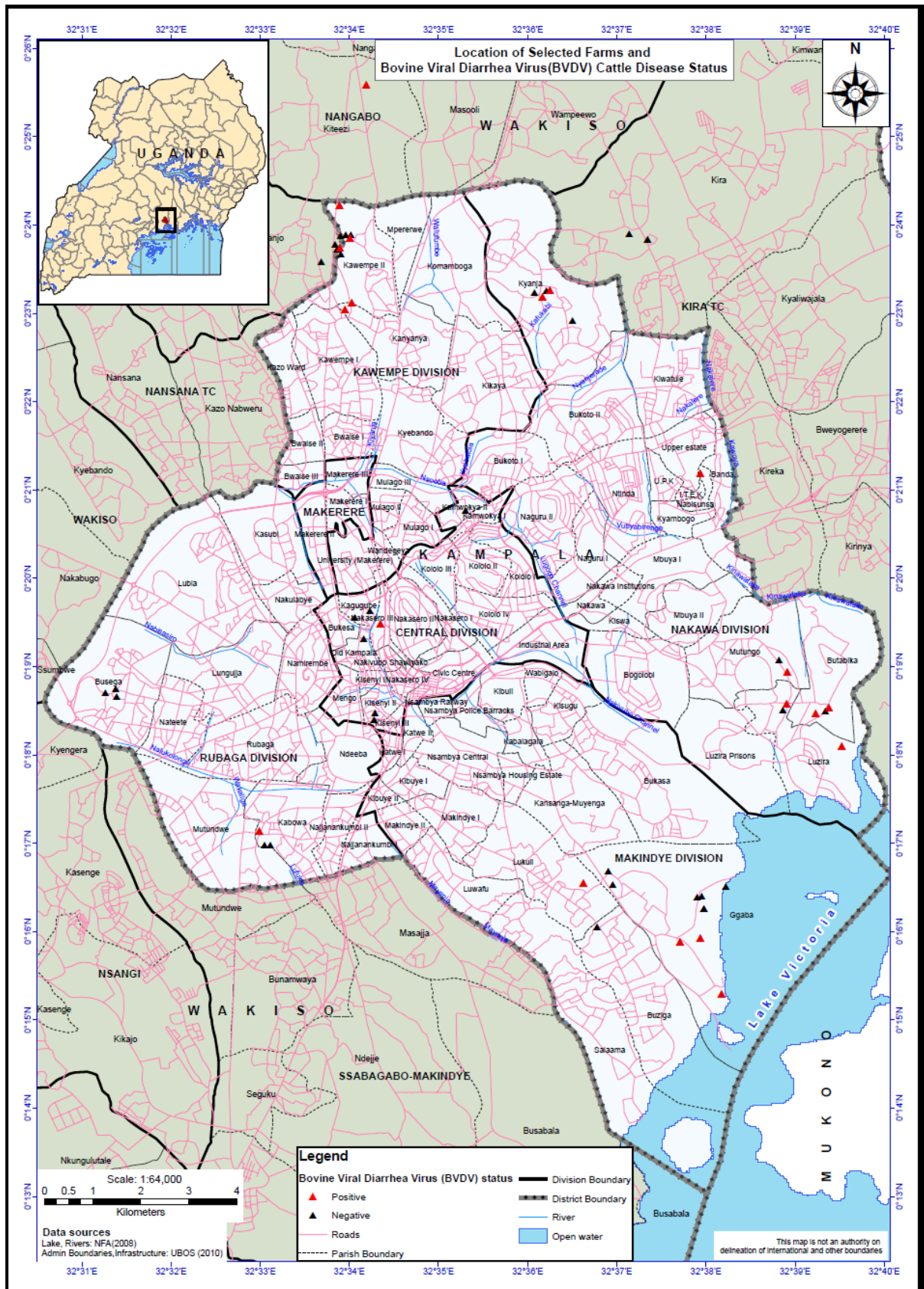


Figure 15. Location of selected farms. Red triangles indicate a seropositive herd for salmonellosis.

Risk factors

Risk factors for brucellosis

Four out of 16 farms in the Nakawa division were seropositive. The chance of finding a seropositive herd in the Nakawa division was significantly higher than finding a positive herd in the other divisions ($p= 0.05$, OR 6.3, 95% CI: 1.03-39.0). No positive herds were found in the other divisions. No other significant risk factor for brucellosis was detected (table 5).

Table 5. Univariate analysis for brucellosis at herd level

Factors	Seropositive	Seronegative	Odds ratio	95% CI	p-value
Herd size					
1-5	5	25	5.0	0.5-46.0	0.2
>5	1	25			
Breeding method					
Bull	4	42	0.4	0.1-2.4	0.29
AI	2	8			
Bull used on other farms					
Yes	4	27	1.7	0.3-10.2	0.68
No	2	23			
Production system					
Zero-grazing	3	20	1.5	0.3-8.2	0.68
Not zero-grazing	3	30			
Tethered					
Tethered	2	13	1.4	0.2-8.7	0.65
Not tethered	4	37			
Pasture					
Pasture	1	12	0.63	0.1-6.0	1.00
Not pasture	5	38			
Disease history					
Yes	5	41	1.1	0.1-10.6	1.00
No	1	9			
Type of labour					
Hired	3	36	0.4	0.1-2.2	0.35
Family	3	14			
Keeping goats					
Yes	2	20	0.8	0.1-4.5	1.00
No	4	30			
Salmonella seropositive					
Yes	3	29	0.7	0.1-3.9	1.00
No	3	21			
BVDV seropositive					
Yes	2	20	0.8	0.1-4.5	1.00
No	4	30			

Risk factors for salmonellosis

No significant risk factor or preventive factor for salmonellosis was detected on herd level (table 6).

Table 6. Univariate analysis for salmonellosis at herd level

Factors	Seropositive	Seronegative	Odds ratio	95% CI	p-value
Herd size					
1-5	18	12	1.3	0.4-3.7	0.78
>5	14	12			
Breeding method					
Bull (and both)	19	16	0.7	0.2-2.2	0.78
AI	13	8			
Bull used on other farms					
Yes	17	14	1.2	0.2-9.8	1.00
No	2	2			
Production system					
Zero-grazing	13	10	1.0	0.3-2.8	1.00
Not zero-grazing	19	14			
Tethered					
Tethered	10	5	1.7	0.5-5.9	0.54
Not tethered	22	19			
Pasture					
Pasture	6	7	0.6	0.2-2.0	0.52
Not pasture	26	17			
Free range					
Free range	3	2	1.1	0.2-7.4	1.00
Not free range	29	22			
Disease history					
Yes	26	20	0.9	0.2-3.5	1.00
No	6	4			
Type of labour					
Hired	23	16	1.3	0.4-4.0	0.77
Family	9	8			
Keeping goats					
Yes	11	11	0.6	0.2-1.8	0.42
No	21	13			
Brucella seropositive					
Yes	3	3	1.4	0.3-7.5	1.00
No	21	29			
BVDV seropositive					
Yes	10	12	1.2	0.4-3.5	0.79
No	14	20			

Risk factors for Bovine Viral Diarrhea

Using a bull for breeding, instead of AI, was found to be a statistically significant risk factor ($p=0.02$, OR 4.5, 95% CI: 1.3-16.1). A total number of 35 sampled farms used natural breeding and 18 were found seropositive. No other risk factors on herd level were found (table 7). Worth noting is that there is a difference in herd size, not significant, but numerically.

Table 7. Univariate analysis for BVD at herd level

Factors	Seropositive	Seronegative	Odds ratio	95% CI	p-value
Herd size					
1-5	9	21	0.4	0.1-1.3	0.17
>5	13	13			
Breeding method					
Bull (and both)	18	17	4.5	1.3-16.1	0.02
AI	4	17			
Bull used on other farms					
Yes	15	16	0.3	0.1-3.3	0.6
No	3	1			
Production system					
Zero-grazing					
Zero-grazing	6	17	0.4	0.1-1.2	0.11
Not zero-grazing					
Tethered	7	8	1.5	0.5-5.0	0.55
Not tethered	15	26			
Pasture					
Pasture	8	5	3.3	0.9-12.0	0.1
Not pasture	14	29			
Free range					
Free range	1	4	0.4	0.1-3.4	0.64
Not free range	21	30			
Disease history					
Yes	17	29	0.6	0.1-2.3	0.49
No	5	5			
Type of labour					
Hired	17	22	1.9	0.5-6.3	0.38
Family	5	12			
Keeping goats					
Yes	7	15	0.6	0.2-1.8	0.41
No	15	19			
Brucella seropositive					
Yes	2	4	0.8	0.1-4.5	1.00
No	20	30			
Salmonella seropositive					
Yes	12	20	0.8	0.3-2.5	0.79
No	10	14			

DISCUSSION

In the present study, several of the included farms had seropositive animals for the included diseases; brucellosis, salmonellosis and BVD, in the UPU areas of Kampala.

General aspects

In the present study the mean heard size was 7.9 cattle, and this correlates well with statistics from MAAIF & UBOS (2008) who reports that amongst cattle owning households in Uganda, a typical household owns on average seven cattle. However the MAAIF & UBOS (2008)

reports a lower herd size of 4.1 cattle for the region of Kampala. This difference could be due to the fact that the figures from MAAIF & UBOS are from 2008, and probably based on estimation.

It was found that female respondents, to a higher degree than male respondents, were owners of goats in addition to cattle. This difference was found to be statistically significant. This could be explained by the fact that women, to a higher extent than men, keep goats and that keeping livestock mainly is a way for women to secure food supply for the family members, either by direct provision, or as an economic investment as previously described by Maxwell (1995) and FAO (2008).

Manure was in most farms (52%) used as fertilizer for growing crops. Manure is considered to be a valuable product for improving soil fertility without having to buy costly artificial fertilizers. Animal manure could also be a source of transmission of pathogenic microorganisms, some of which may be of zoonotic nature, such as *Salmonella* (Bicudo & Goyal, 2002). As many as 32% of the respondents replied that the manure was not taken care of in any kind of way. This could be a potential health hazard for both animals and humans through, for example contamination of drinking water.

Most respondents (82%) stated that their cattle had been diseased in the last year. The most commonly observed clinical sign was fever which can be seen during infection of both *Salmonella* spp. and BVDV, as well as many other pathogens. Abortion, which can be seen during infection with *Brucella* spp., was only observed in 5% of sampled farms. However, these figures may not be entirely accountable since there was no definition of how to interpret the clinical signs, and the respondents only ticked the pre-made boxes in the questionnaire. For example, fever was probably not measured using a thermometer. Since housing livestock is a safety net, especially for vulnerable groups (e.g. women, poor and people with HIV), animal disease can be related to increased poverty (Perry & Grace, 2009). Apart from the increased food insecurity due to animal disease and the zoonotic aspects, the economic effects of animal disease can be directly visible (death, abortions, treatment costs) or obscured (weight loss, decreased milk yield).

Due to reorganizations of livestock in the urban areas of Kampala, included cattle could not be randomly sampled according to the list made based on the prior mapped population. Therefore cattle were sampled from each division based on different local contact persons. Visited farms were those known by the local contact person, and thereby not selected using the premade randomization list. The amount of sampled animals in each division was therefore based on logistic possibilities on that particular day. In an effort to spread the sampling as much as possible, a maximum number of 5 animals were collected on each farm, and one day of sampling was spent in each division. Many animals (34%) were sampled in the central division, related to how many animals that were found in the central division in the previous mapping. Between 3-8% of the total cattle population in the other division were sampled. Apart from the central division, the selection was representative.

During interviewing it was observed that knowledge about the different disease varied considerably, and no conclusions could be made using this question in the questionnaire, due to some noted misunderstandings. Some farmers replied that they were aware about the diseases. However while explaining about the disease, it was apparent that the farmer was not aware. Other difficulties using the questionnaire, was that discussion with some farmers had to be done using a translator. It is possible that some misunderstanding could have taken place, while translating the questions and answers, thereby leading to untrue or incorrect answers regarding some questions.

Few risk factors were found and this could be due to widespread diseases or similar conditions on the farms. The statistical analyses for risk factors, on herd level, were not optimal due to the selection of animals and the number of sampled animals on each farm. Seropositive herds could for example have been missed, when sampling of a maximum of five animals per herd.

Most sampled cattle were kept on farms with small scale husbandry. This is illustrated by the fact that 96% of sampled herds consisted of less than 20 cattle and as much as 56% housed five or less cattle. Cattle were kept very close to the home, most often in the own backyard and no advanced bio-security measures were seen, at the utmost a fence and a single entrance. On the same backyard as the cattle were kept, many other everyday activities were conducted, such as cooking, doing laundry, socialization and the same backyard also worked as a playground for children. It was clear that humans lived in close contact with their livestock and therefore a good animal health status is very important to prevent zoonotic diseases and to sustain a good economic situation, since people are very dependent on their livestock for food and income. Knowledge about basic hygiene, food security and how diseases are prevented are for these smallholders very important. Education about how taking preventive measures such as heating milk before consumption and basic hygiene barriers to livestock could constitute a major difference to human health in these areas.

Brucellosis

A previous study of 500 cattle sampled from an abattoir in Kampala, Uganda, reported a seroprevalence for brucellosis to 13.7%, using both the Rose Bengal Test (RBT) and the standard tube agglutination test (STAT) (Ssekabira, 2009). In a study of 245 bovine serum samples from the UPU areas of Kampala, 42% were seropositive, using the tube agglutination test (Mwiine, 2004). However both STAT and RBT are less specific compared to the C-ELISA (OIE, 2009), and the previous high reported seroprevalence may be due to false positive results because of strain 19 (S19) vaccination or false positive serological reactions. Sera from S19 vaccinated cattle do not compete with the mAb in the C-ELISA used in the present study and vaccinated animals are therefore leading to a negative reaction (Nielsen *et al.*, 1995; OIE, 2010). The ELISA was also proven to be the most sensitive of the three tests (Chachra *et al.*, 2009). The C-ELISA can eliminate some, but not all, false positive serological reactions caused by *Enterobacteriaceae* (Portanti *et al.*, 2006). The C-ELISA is

also capable of eliminating most false positive reactions from vaccination with S19 (OIE, 2009).

In a study of 423 cattle serum samples from the UPU areas of Kampala, a seroprevalence of brucellosis of 5.0% on animal level and 6.5% on herd level was shown, using the C-ELISA assay (Makita *et al.*, 2011a). These values coincide well with the obtained herd prevalence of the present study; 10.7%. Only one seropositive animal was found on each farm, and this could be a true prevalence since the within herd prevalence usually not is 100%, and in a study by Makita (1999) the within herd prevalence was 2%. It is a small possibility that the seropositive animals were false positive, due to cross reactions with other bacteria, especially *Yersinia enterocolitica* serotype O:19 as viewed in Chenais *et al.* (2012). However, as discussed earlier, the C-ELISA, compared to other serological tests, is capable of eliminating most FPSR caused by *Enterobacteriaceae* (Portanti *et al.*, 2006). Other possible explanations to why only one positive animal was found on each positive farm, is that a maximum of only five animals were sampled on each farm. Subsequently, not all cattle on each farm were sampled, and as a result, some seropositive animals might not have been tested. Found seropositive animals could also be newly purchased animals, and the other animals may not have seroconverted.

In the present study, the only statistically significant risk factor for brucellosis was to keep cattle in the Nakawa division of Kampala. This result indicates that animals in the same geographical area spread the disease to close by neighbours, through short distant trade (such as local markets), and through direct contact between animals and infectious discharge. It could also be due to different cattle keeping practices in that particular division. However, no such differences that stand out could be seen. Several other risk factors for brucellosis have been found in other studies such as large herd size, free grazing farming and history of abortion (Makita *et al.*, 2011a), but no such statistically significant correlations could be made in the present study.

In case of *Brucella* infection, the concentration of anti-brucellae antibodies increases and the extent and duration of the response can be affected by many factors including species, immune status, age, sex, pregnancy and virulence of the infecting strain. Following exposure to virulent *B. abortus* strains, antibodies may be detectable in 4-10 weeks or longer, depending on the route of entry, size and stage of pregnancy (WHO, 1986). The presence of anti-brucellae antibodies suggests exposure to the bacteria, but it does not necessarily mean that the animals have a current or active infection at the time of sampling. The presence of antibodies may be a result of past exposure of the bacteria resulting in a self-limiting disease, as suggested in a study by Godfroid *et al.*, (2002).

Godfroid *et al.*, (2012) concluded that, the implementation of sanitary measures such as heat treatment of milk products, as well as vaccination, could significantly lower the incidence of human cases in resource poor countries.

Salmonellosis

For salmonellosis the herd seroprevalence was found to be 57.1% in the present study. A previous study of 406 sampled cattle at the abattoir in Kampala, reported a prevalence of 1.23%, using fecal samples and culturing as method (Ezama, 2010). The difference in prevalence between the present study and the studies from Ezama (2010) and Huston *et al.* (2002), may be explained by the fact that two different analytical methods were being used. Fecal culture is considered the golden standard for defining the *Salmonella* infection status of animals but intermittent fecal shedding limits the sensitivity of this technique. Multiple cultures are required to define the true infection status of individual animals. Culture could, compared to serological tests, therefore show a lower prevalence due to that animals with subclinical infection only shed bacteria intermittently and in low numbers, and therefore results in a negative sample if cultured. A correct sampling, storage and laboratory practice is also important factors when culturing fecal samples. Incorrect techniques could result in false negative results (OIE, 2010). To differentiate recently infected cattle (increasing titers) and convalescent cattle (decreasing titers) from *Salmonella* carriers (stable titers), it would be necessary to do repeated serological testing (Smith *et al.*, 1989).

Some serologically positive animals for *Salmonella* antibodies may be false positive and thereby explain the high prevalence of salmonellosis in the present study. According to the Manual of diagnostics tests and vaccines for terrestrial animals (OIE, 2010) it is well known that some animals with a positive serological response may no longer be infected with salmonellae. It may also be the case that some animals that are actively excreting the *Salmonella* organism may be serologically negative in the early stages of the disease, before immunoglobulin production is maximal, or having an infection with less invasive serovars. Some infected animals may even never seroconvert. Young cattle that are sampled could be false negative since they are immunologically immature and can be unresponsive until 10-12 weeks of age (Hoorfar *et al.*, 1996; OIE, 2010). The I-ELISA kit used in the present study detects both *S. dublin* and *S. typhimurium* strains, although these are the most relevant serovars to infect cattle in most countries, other serovars can cause human infection that were not included in the present study. It is important to stress that the found high prevalence of salmonellosis, could be a possible hazard to human health since transmission to humans can occur through direct and indirect contact with infected animals, as described in a Dutch case study by Hendriksen *et al.*, (2004).

Bovine vial diarrhea

No published seroprevalence studies have been found regarding BVD in Kampala, however in northern Uganda, the proportion of seropositive animals in a limited field study was 20% (Ståhl, K., personal communication, 2012). The prevalence was 39.3% in the present study. Studies based on the detection of antibodies against BVDV, in either bulk milk or individual serum samples, have shown that the prevalence of seropositive herds is most often in the range of 70 to 100% (Houe, 1999), indicating that the prevalence values in the present study

is relatively low compared to studies in other countries in the 1990's probably due to small scale animal husbandry in low-income countries.

Using a bull for breeding instead of AI was found to be a statistically significant risk factor for BVD. This may be explained by the fact that virus are shed in semen during acute infection or PI infection of bulls or other related factors connected to usage of natural breeding. The virus can also be transmitted through nasal discharge during direct contact between the bull and cow. The virus could also be transmitted through indirect contact when transporting cows/bulls between farms since most farmers use the same bull for breeding. The farms that uses natural breeding could also have other related factors in common that constitutes a risk factor for BVD.

Follow up tests to identify individual PI animals in seropositive herds needs to be done since a maximum of five animals were tested on each farm.

CONCLUSIONS

In conclusion, since several of the included herds were seropositive for *Brucella* spp. and *Salmonella* spp. in the UPU areas of Kampala, it is likely that transmission of bacteria between cattle and humans may occur in the urban and peri-urban areas of Kampala. Seropositivity against BVDV was also detectable among cattle-herds in all divisions of the UPU areas of Kampala. A suggestion of measures towards increased bio-security and education for better basic hygiene measures, such as heat preparations of milk and hand sanitation in UPA, could potentially improve both human and animal health status in Kampala, Uganda

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APPENDIX 1

Date..... GPS readings.....

District.....

Sub-County.....Parish.....

Village.....

Name of Herd owner

Serial number.....

The project objectives of our Minor Field Study are to do a health survey of cattle to study the prevalence of important diseases, including zoonotic diseases, in blood and fecal samples and thereby estimate the health status of animals in Kampala and the potential risk for transmission of diseases to humans.

Gender of respondent M F

Age category (estimate) Youth Adult Elderly

Role on the farm: Herdsman Farmer/owner Family member Other (specify).....

How many livestock do you keep?

What kind of livestock do you keep? Goats Cattle

Cattle and others Goats and others If others what kind of livestock.....

For what purposes do you keep your livestock? For own consumption

To sell milk To sell meat To sell livestock Economic investment
Other.....

What kind of feeds do you give your livestock?

Where do you get feeds for your animals? I grow them by myself

From a local store Kitchen/market wastes

I buy them from a friend/neighbour Other.....

What kind of production system do you have? Zero-grazing Tethering
Paddock/pasture Other.....

How do you dispose of animal waste? Use it for growing crops Sell it

For fuel Bury it in the ground Nothing Other.....

Have your animals been diseased in the last year? Yes No

If yes, what kind of symptoms have they shown? Abortions Nasal discharge Diarrhea
 Fever Coughing Other.....

How many of your livestock have been treated with antibiotics (medicine) in the last year?

.....

How many livestock have you bought in the last year?.....

How many livestock have you sold in the last year?.....

Where did you buy your livestock from? Friends/neighbours

I only have livestock bred on my farm Local markets Other.....

How are the livestock transported to/from your farm? Herded on foot Car/truck
Motor cycle Bicycle Other (specify)

Where have you sold your livestock? To friends/neighbours I keep all of my livestock
In local markets Other.....

Type of labour on the farm : Hired labour Family labour

What bio-security measures do you have in place on your farm? Footbaths Fencing
Single entrance to farm limit visitors to farm Others(specify).....

What breeding method is used on your farm? Artificial insemination Natural breeding
 Both

If natural breeding is used, is the male used on other farms? Yes No

Are you aware about a disease called

Brucellosis Yes No

Salmonellosis Yes No

Bovine viral diarrhea virus Yes No

