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Prevalence of antibodies for Peste des petits des ruminants virus and Brucella and related risk factors in goat herds in urban and peri-urban agriculture in Kampala, Uganda

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Prevalens av antikroppar mot Peste des petits ruminants virus och Brucella samt relaterade riskfaktorer hos getter i stadsnära jordbruk i Kampala, Uganda

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SUMMARY

The world's population reached more than 6.9 billion people in July 2011. It is expected that the population growth during the next 40 years will take place mostly in low-income countries and the population increase will be 2.3 billion people during 2011-2050. A rapid urbanization is also expected to result in 57.7 per cent of Africa's population living in urban areas in 2050. The rapid growth of urban areas is driven by for example economic growth, unemployment, lack of educational opportunities, natural disasters, food insecurity and social instability. In the rapidly growing urban and peri-urban areas, the demand for food is increasing. To satisfy these needs urban and peri-urban agriculture is in many ways essential for food security. Urban and peri-urban agriculture give poor people access to food by providing for self-consumption and can thereby contribute to poverty alleviation and prevent famine. There are also risks related to urban and peri-urban agriculture such as environmental hazards and transmission of for instance zoonotic diseases.

Agriculture is the most important source of income for about 66 per cent of the population (34 million) in Uganda. Twenty-five per cent of Uganda's population is considered poor and most farming is small scale providing for self-consumption. It has been shown that urban agriculture in Kampala, the capital of Uganda, has positive effects on nutrition status and food security. Health risks have though been reported and the most important zoonotic diseases in urban and peri-urban Kampala are food-borne gastroenteritis, bovine tuberculosis, brucellosis and cysticercosis. In low-income countries, like Uganda, goats are important for poor people providing a source of income, meat, manure, milk and an insurance against emergencies. Goats are essential for poverty alleviation in developing countries.

The aim of this study was to gain knowledge about urban and peri-urban agriculture in Kampala, Uganda and to investigate the prevalence of antibodies for Peste des petits ruminants virus (PPRV) and brucella and related risk factors in goat herds. Brucellosis is considered one of the most important zoonotic diseases in urban and peri-urban Kampala. Peste des petits ruminants (PPR) is a highly infectious disease primarily affecting small ruminants. It is a disease of high socioeconomic significance in low-income countries due to the high mortality and morbidity.

Fifty-five farms in urban and peri-urban Kampala, Uganda, were visited during our study. A maximum of 5 blood samples were taken on each farm and farmers were interviewed regarding for example their animal's health, animal movements and bio-security measures. One-hundred and ninety blood samples were taken in total and analyzed using competitive ELISA. The seroprevalence at herd level was 16.4 per cent for brucellosis and 1.8 per cent for PPRV, respectively. No statistically significant risk factors associated with seropositivity for PPRV were found in our study. Seropositive herd status for brucellosis was associated with having a farm in the division of Greater Kampala. Because of the fact that seropositive goats were found in our study it may be possible that transmission of brucella bacteria between goats and humans may occur in urban and peri-urban Kampala. Furthermore, seropositivity for PPRV may indicate that the virus has to some extent spread from the northern parts of Uganda to more central parts. In order to ascertain whether this is true or not I think further studies need to be carried out.

SAMMANFATTNING

I juli 2011 var befolkningen i världen 6.9 miljarder. Befolkningstillväxten de närmaste 40 åren kommer troligen att ske i utvecklingsländer och resultera i en ökning av jordens befolkning med 2,3 miljarder under tidsperioden 2011-2050. Samtidigt kommer även en urbanisering ske vilket kommer att resultera i att 57,7 procent av befolkningen i Afrika kommer att bo i urbana områden år 2050. Denna urbana tillväxt drivs av ekonomisk tillväxt, arbetslöshet, utbildningsmöjligheter, naturkatastrofer, tillgång på mat och social instabilitet. Efterfrågan på mat ökar i takt med tillväxten av urbana och periurbana området vilket gör urbana och periurbana jordbruk otroligt viktiga för livsmedelsförsörjning. En stor fördel med urbana och periurbana jordbruk är att de ger fattiga konsumenter möjligheten till självförsörjning och kan därmed bidra till bekämpning av fattigdom och hungersnöd. Det finns dock även risker med urbana och periurbana jordbruk såsom miljöpåverkan och risk för överföring av sjukdomar t.ex zoonoser.

I Uganda är jordbruk den viktigaste inkomstkällan för 66 procent av den 34 miljoner stora befolkningen. Tjugofem procent av befolkningen räknas som fattiga och de flesta jordbruken är småskaliga och producerar för självförsörjning. Man har sett att urbana jordbruk i huvudstaden Kampala har en positiv effekt på nutritionstatus och tillgång på mat. Hälsorisker har dock också rapporterats och de viktigaste zoonotiska sjukdomarna i urbana och periurbana områden i Kampala är gastroenterit, bovin tuberkulos, brucellos och cysticerkos. Det är i Uganda vanligt att man i ett hushåll har getter som inkomstkälla, säkerhet i krissituationer samt för tillgång till kött, mjölk och gödsel. Getter är essentiella för fattigdomsbekämpningen i utvecklingsländer.

Syftet med denna studie var att samla information om urbana och periurbana jordbruk i Kampala, Uganda och även undersöka seroprevalens och relaterade riskfaktorer för Peste des petits ruminants virus (PPRV) samt brucella hos getter. Brucellos är beskriven som en av de viktigaste zoonotiska sjukdomarna i urbana och periurbana områden i Kampala och är enligt WHO av betydelse för folkhälsan. Peste des petits ruminants (PPR) är en sjukdom som framförallt drabbar små idisslare. På grund av att PPRV ofta orsakar utbrott med hög mortalitet och morbiditet så är detta en sjukdom av stor socioekonomisk betydelse i utvecklingsländer.

Femtiofem gårdar i urbana och periurbana Kampala, Uganda, besöktes under studiens gång. Vid besöken togs blodprov från max 5 getter per gård och djurägarna intervjuades med frågor om t.ex djurhälsa, djurhandel och biosäkerhet på gården. De 190 blodprover som togs analyserades sedan med kompetitiv ELISA teknik. Seroprevalensen på gårdsnivå var 16,4 procent för brucella och 1,8 procent för PPRV. Inga riskfaktorer för seropositivitet för PPRV hittades i denna studie men seropositiv status för brucella på gårdsnivå var statistiskt signifikant associerad med att ha sina getter i området Greater Kampala. Eftersom seropositiva getter hittades i denna studie så kan det innebära att det eventuellt finns risk för överföring av brucella bakterier mellan getter och människor i urbana och periurbana områden i Kampala. Dessutom kan seropositivitet för PPRV tala för att detta agens kan ha spridits från norra Uganda till mer sydliga delar av landet. För att ta reda om så är fallet tycker jag att vidare undersökningar behöver göras.

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BACKGROUND

This project, set in the urban and peri-urban parts of Kampala, Uganda, is a Minor Field Study (MFS) sponsored by the Swedish International Development Cooperation Agency (Sida) and the Swedish University of Agricultural Sciences (SLU). It is also part of a larger project “Urban and peri-urban farming” with the sub-project “Zoonotic infections among cattle in urban and peri-urban areas in Uganda”.

Urban and peri-urban agriculture

The population in the world (more than 6.9 billion in July 2011) is divided between cities and rural areas. It is expected that the population growth during the next 40 years will take place mostly in low-income countries. In 2011, 5.7 billion people lived in less developed regions accounting for 82 per cent of the world's total population. The expected population increase in these areas during 2011-2050 will be 2.3 billion people (United Nations 2011a).

Forty per cent of Africa's population is currently living in urban areas. This is considerably less compared to other areas of the world, and the continent will probably go through a rapid urbanization between the years 2011-2050. This rapid urbanization is expected to result in 57.7 per cent of the inhabitants in Africa living in urban areas by the year of 2050 and according to the Food and Agricultural Organization 40-45 per cent of the poor in Africa will presumably live in towns and cities in 2020 (FAO 2008; United Nations 2011b).

The rapid growth of urban areas is driven by for example economic growth, unemployment, lack of educational opportunities, natural disasters, food insecurity and social instability. Peri-urban areas are also expanding and consist of areas surrounding cities that are in most ways incorporated in the cities (FAO 1999). Production of livestock, in these areas, is fast-growing, representing 34 per cent of meat production and 70 per cent of egg production worldwide (FAO 2001).

In the rapidly growing urban and peri-urban areas, the demand for food is increasing. To satisfy these needs urban and peri-urban agriculture are in many ways essential. Urban agriculture refers to small areas within the city used 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.

The benefits of urban and peri-urban agriculture include less need for packaging, storage and transportation of food as well as access to food for poor consumers by providing for self-consumption. Other opportunities include availability of fresh food, more convenient recycling and disposal and potential agricultural work opportunities. It is stated that urban and peri-urban agriculture can contribute in the work of achieving the Millennium Development Goals (MDGs) such as reduction of poverty and reduction of hunger. Also reaching Goal 3 (promote gender equality and empower women) can be supported by urban and peri-urban agriculture by for example providing an income for women and the ability for women to produce food near their home (FAO 2008).

Risks of urban and peri-urban agriculture include environmental and health hazards from inappropriate agricultural practices as well as transmission of zoonotic diseases (FAO 1999). Other risks concerning urban and peri-urban agriculture include an increase in pollution and competition for land, water and energy (FAO 1999).

Uganda

Uganda is situated in Eastern Africa on the equator. The country covers an area of 241 551 km², that is approximately half the size of Sweden, and borders with South Sudan, Kenya, DRC, Tanzania and Rwanda (Utrikespolitiska institutet 2012).

In 2010 the population of Uganda reached about 34 million. Of those, 1.7 million are currently living in Kampala, the capital of Uganda. Most Ugandans live in rural areas, and about 14.7 per cent of the population is living in urban areas (Utrikespolitiska institutet 2012; UBOS 2012). Rapid urbanization in Uganda has increased the urban population by more than six times from the year of 1980 to 2012. Nearly 7.5 million Ugandans are considered poor and this constitutes 25 per cent of the population in (UBOS 2012).

The population growth in Uganda is high and the average Ugandan woman gives birth to about seven children during her lifetime. This results in the third highest population growth rate in the world (Republic of Uganda Ministry of Finance, Planning and Economic Development 2010).

Agriculture is the most important source of income for about 66 per cent of the population in Uganda attributing to 43 per cent of the national gross domestic product (GDP) (Mwebe et al. 2010; UBOS 2012).

Most farming is small scale and foodstuffs are mainly produced for own consumption (Utrikespolitiska institutet 2012). In 1995, 34.8 per cent of the households in Kampala were involved in agriculture and 9.5 per cent of these households kept livestock (Maxwell et al. 1995).

It has been shown that urban agriculture in Kampala has positive effects on food security and nutrition status. Better nutrition status of households has resulted in measurably taller children for age in Kampala (Maxwell, Levin & Csete 1998). However, health hazards are a major concern for urban and peri-urban agriculture in Kampala, including animal to human disease transmission and increased cases of malaria and dysentery in households close to farming areas (Lee-Smith & Prain 2006; Nuwagaba 2002). The most important zoonotic diseases, transmitted in urban and peri-urban areas of Kampala, Uganda, were identified as animal sourced food-borne gastroenteritis, bovine tuberculosis, brucellosis and *Taenia solium* neurocysticercosis (Makita et al. 2011a).

According to a recent inventory performed within ongoing collaborative research between SLU and Makerere University, Kampala, Uganda, the livestock population in urban and peri-

urban Kampala reaches about 1.1 million birds, 73 000 small ruminants, 40 000 pigs and 32 000 cows in the city (Vinnerås, B. SLU, personal communication., 2012).

Goats in Uganda

In developing countries, like Uganda, goats are essential for poor people providing a source of income, meat, manure, milk and an insurance against emergencies. Keeping goats is described as a possible pathway out of poverty by providing food security (Peacock 2005; Semakula et al. 2010).

In 2010 there were about 13 208 000 goats in Uganda according to the Ugandan Bureau of Statistics (UBOS) and the number of goats in Kampala district was estimated 64 072 in 2008. There are about 2.5 million households owning goats, in Uganda, and the typical household owns 2 goats. The production of goat meat was 33 619 metric tonnes in 2010 compared to 19 669 metric tonnes of pig meat and 180 300 metric tonnes of cattle meat (beef and veal) produced the same year, in Uganda (MAAIF 2011).

In Uganda the most common type of goats are the local breeds including Mubende, Small East African, Kigezi and Karamoja. Only about 4 per cent of goats are exotic breeds according to an unpublished report by the UBOS and these breeds include Boer, Galla, Anglo-Nubian, Alpine, Saanen, Toggenburg and Spanish. There are also local breeds crossbred with exotic breeds. The local breeds of goats in Uganda are resistant against diseases and used to the harsh environment (Mwebe et al. 2010; MAAIF 2011).

The most common goat management system in central Uganda is tethering but also zero-grazing systems and free range systems are frequently used (Kugonza, Bareeba & Kirembe 2001; Mwebe et al. 2010). The most profitable production system seems to be tethering. Farmers primarily keep adult female goats and they are the most valuable ones (Mwebe et al. 2010).

The major problems in goat production, in Uganda, are proposed to be lack of pasture, lack of veterinary services, lack of crossbreeds, low market demands, animal theft and conflict with neighbours (Mwebe et al. 2010). It has been stated that infection with parasites in goats is the most common health problem affecting and reducing goat production in Africa (Peacock 2008).

Zoonotic diseases

Zoonotic diseases are infectious diseases that can be transmitted between animals and humans. The route of transmission can be direct or indirect for example 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 zoonoses account for roughly 40 per cent of overall human infectious diseases. About 75 per cent of newly emerged diseases the last decade have been transmitted from animals or animal products (EFSA 2011).

When performing an evidence-based identification of the most important livestock related zoonotic diseases at the Mulago National Referral Hospital in Kampala, between 2005 and 2007, 12 potential zoonotic diagnoses were identified (gastrointestinal infections, fungal infections, tuberculosis, brucellosis, neuro-cysticercosis, cellulitis, rabies, hepatitis A and E, elephantiasis, tetanus, shistosomiasis, encephalitis). 5 841 cases suffering from potential zoonotic diseases were found accounting for 9.3 per cent out of 62 671 outpatients at the hospital. Approximately 2000 (1996-2004) cases out of 5841 were estimated infected by contact with livestock (Makita et al. 2011a).

Brucellosis

Brucellosis is caused by bacteria from the genus *brucella* and is a severe zoonotic disease. *Brucella* species are gram-negative small rods and can be divided into six species according to their main hosts. *Brucella melitensis*, *Brucella suis* and *Brucella abortus* are species with public health implications (WHO 1997).

Cattle are most often infected by *Brucella abortus* and goats by *Brucella melitensis*; these two species can also infect humans (Doganay & Aygen 2003; WHO 1986). *Brucella melitensis* is the most invasive and pathogenic species of brucella (WHO 1997) and biovars 1, 2 and 3 are the main causative agents of brucellosis in sheep and goats (OIE 2009b).

Pathogenesis and clinical signs

Infection by *Brucella* spp. in goats (and cattle) occurs via the mucous membranes in the oropharynx, upper respiratory tract and conjunctiva. *Brucella* organisms are mostly shed during parturition and the main route of transmission between animals is by direct contact with infected animals or through an environment contaminated with discharges (e.g. fetuses, placental membranes and vaginal discharge) from infected animals (Ssekabira 2009; WHO 1986). In female goats brucella organisms are vaginally shed in copious amounts and for a prolonged time compared to cows. Venereal transmission is uncommon although localization of organisms in the testis, epididymis and accessory sex glands, in the male, is common. Genital localization may result in infertility in the bull or buck (WHO 1986). Infection in pregnant goats and cows results in abortion due to invasion of the uterus by *Brucella* spp. In subsequent pregnancies brucella organisms are shed but no abortion occurs.

It is common for brucella bacteria to remain in the mammary glands and the supramammary lymph nodes causing persistent infection and shedding of organisms in the milk. This may affect the milk yield of the animal and thereby causing economic losses (WHO 1986).

Clinical findings in cattle and goats suffering from brucellosis are abortion in late gestation (third trimester), metritis, retained placenta, hygromas, arthritis and orchitis. Animals do not develop fever by brucella infection (SVA 2011; WHO 1986).

Brucellosis as a zoonosis

Brucellosis is a common, but under-diagnosed and neglected, disease in large parts of the world especially low-income countries. In more high-income countries control of the disease in animals has led to fewer human cases but in low-income countries brucellosis is still common because of lack of pasteurization, consumption of raw milk and lack of good hygienic measures in animal husbandry and food handling. In areas where the disease exists among sheep and goats most human cases are found (WHO 1997; WHO 1986). According to Makita et al. (2011a), brucellosis is one of the most important zoonotic diseases, transmitted in urban and peri-urban areas of Kampala, Uganda.

The main routes of brucella infection for humans are via small wounds, mucous membranes (e.g the conjunctival sac), and inhalation or by consumption of un-pasteurized milk (Doganay & Aygen 2003; WHO 1986). Another relevant route of transmission seems to be contact with infected goat herds and also handling of goat manure for fertilizing purposes (Wallach et al. 1997). Direct contact with infected animals of other species than goats (e.g. cattle, sheep, pigs, camels, buffaloes, wild ruminants and seals) is also a possible source of infection (WHO 1997).

Brucella bacteria present a high risk for laboratory workers and humans working in abattoirs, slaughtering cattle and goats, are also at risk (WHO 1986; Ssekabira 2009). Other frequently exposed groups are farmers and veterinarians (WHO 1997).

Clinical findings in humans are fever, flu-like symptoms, joint pain, depression and nausea (Doganay & Aygen 2003). During an outbreak of brucellosis, in Argentina, attributed to contact with goats, the clinical findings in humans were splenomegaly, adenitis, hepatomegaly, arthritis, pneumonitis, bronchitis, pharyngitis, epididymitis, retinitis, icterus, fever, asthenia, rachialgia, headache, sweats, anorexia, arthralgia, cough, dyspnea and gastrointestinal complaints. *Brucella melitensis* biovar 1 was isolated from diseased goats during this outbreak (Wallach et al. 1997).

Brucellosis in goats in Uganda

A seroprevalence study undertaken in eastern and western Uganda, in 2000, investigating seroprevalence and risk factors associated with seropositivity in goats, concluded that goats may be seropositive for both *Brucella melitensis* and *Brucella abortus* (Kabagambe et al. 2001). Further Kabagambe et al. (2001) reported that 4 per cent of the sampled goats and 13 per cent of the sampled herds were positive for antibodies directed against *Brucella* spp. Risk factors identified in this study were: use of a hired caretaker, keeping sheep and free browsing.

Two studies performed at Kalerwe slaughter house in Kampala, Uganda, reported seroprevalences of brucellosis of 7.4 per cent in goats in 2009 and 6 per cent in goats in 2010, respectively (Ssekabira 2009; Serwanga 2010). The study performed in Kalerwe slaughter house in 2010 investigated exposure to *Brucella melitensis* and only 116 goats were tested compared to 1480 goats sampled in the study by Kabagambe et al.

Sixty goats out of 812 goats sampled (7 per cent) were found to have anti-brucella antibodies in a study taken place in Mbarara district, Uganda (Ssekawojwa 2007). The herd seroprevalence in this study was 20 per cent. Risk factors for seropositivity in goats were also investigated and a history of abortion was identified as strongly associated with brucellosis (Ssekawojwa 2007).

In a retrospective study investigating brucellosis seroprevalence in livestock in Uganda from 1998 to 2008 brucellosis seroprevalence in goats was 5 per cent (Mwebe et al. 2011).

An individual seroprevalance of anti-brucella antibodies ranging from 4 to 7.4 per cent in goats, in Uganda, can be compared to an individual seroprevalence of antibodies directed against brucella ranging from 4 to 13.7 per cent in cattle, in Uganda (Ssekawojwa 2007; Ssekabira 2009; Makita et al. 2011b; Mwebe et al. 2011).

Diagnosis in goats (and sheep)

For diagnostic purposes identification of the agent or serological tests can be performed. Culture, PCR and staining are methods used for the identification of brucella bacteria. Staining has low sensitivity because of fat globules in the milk affecting the procedure negatively and should according to OIE (2009a) be confirmed by culturing the agent.

For the culturing of *Brucella* spp. basal and selective medium can be used. Selective medium contains antibiotics suppressing other bacterial growth than *Brucella* spp. Samples chosen for cultural examination depend on clinical signs observed. Aborted fetuses, fetal membranes, vaginal secretions, milk, semen and fluids from hygromas or affected joints can all be used for culturing.

PCR methods can be used for differentiation between *Brucella* species and to some extent biovars but there have not been a lot of studies presenting validation of the PCR method for primary diagnosis of brucellosis (OIE 2009a).

Different serological test procedures are preferred during different circumstances. Serum agglutination test (SAT) is considered to inaccurate to be used when screening animals for brucellosis before international trading. Enzyme linked immunosorbent assays (ELISAs), fluorescence polarization assay (FPA) and complement fixation test (CFT) are regarded more specific than SAT and are therefore preferably used (OIE 2009a). CFT detects IgG antibodies and these are present both in the acute and chronic stages of brucellosis (Kolar 1984).

The Rose Bengal test (RBT) and the buffered plate agglutination test (BPAT) are examples of buffered brucella antigen tests (BBATs) and these, including CFT, are the serological tests most widely used in small ruminants (OIE 2009a; OIE 2009b). RBT and CFT cannot distinguish vaccinated animals from infected animals and neither discriminate between serological reactions occurring due to *Brucella melitensis* and serological reactions due to *Yersinia enterocolitica* O:9, respectively.

Competitive ELISAs (C-ELISA) and indirect ELISAs (iELISA) can be used for serological diagnosis in sheep and goats with the same sensitivity (C-ELISA) or greater sensitivity (iELISA) compared to RBT and CFT. According to Portanti et al. 2006 competitive ELISA for diagnosis of brucellosis is able to properly discriminate between serological reactions due to enterobacteriaceae bacteria and serological reactions due to brucella bacteria. Competitive ELISA is also able to distinguish between goats vaccinated and goats infected by brucella (Biancifiori et al. 2000; OIE 2009b).

The bulk milk ring test cannot be used in small ruminants for diagnosis of brucellosis because fat globuli in milk of goats and sheep do not absorb agglutinin-antigen complex as efficiently as cream of cow's milk. Therefore the typical coloured ring does not occur (Kolar 1984).

Other tests that can be used for brucellosis diagnosis in small ruminants are the brucellin skin test and the native hapten test. The latter is able to distinguish between infected and Rev.1 vaccinated animals (OIE 2009b).

Preventive measures

There are several vaccines for the immunization against brucellosis in animals. The most widely used vaccine in small ruminants is *Brucella melitensis* strain Rev.1 vaccine (WHO 1986). The Rev.1 vaccine is a live attenuated vaccine and it induces sufficient immunity for a substantial amount of time, at least 4.5-5 years in goats when administered subcutaneously at the age of 4-6 months. The vaccine is of low virulence but stimulates production of protective anti-brucella antibodies (Kolar 1984; WHO 1986).

When using Rev.1 vaccine in pregnant animals one should be aware of the risk of induction of abortion. To minimize this risk immunization of goats should be performed by the conjunctival route, instead of subcutaneously, and during late pregnancy or before mating (Blasco 1997; OIE 2009b).

Conjunctival vaccination also minimizes antibody-responses interfering with serological diagnostics of brucellosis (WHO 2009b). However, more studies need to be carried out to investigate the duration of protective immunity when immunization is performed by conjunctival administration of Rev. 1 vaccine (Blasco 1997). In a study performed by Biancifiori et al., in 2000, 1 out of 5 ewes vaccinated with Rev.1 vaccine failed to get protected by the conjunctival vaccination and aborted when challenged two and a half years post vaccination.

It has also been suggested to use reduced doses (5-10 x 10⁴ viable organisms instead of the standard dose of 0.5-2.0 x 10⁹ viable organisms) of the Rev.1 vaccine to avoid induction of abortion in pregnant animals (Blasco 1997; WHO 1986). However, this has resulted in lack of protective immunity and is therefore not recommended (Blasco 1997).

Vaccination of goats using strain Rev.1 also results in excretion of the vaccine strain in milk but according to WHO (1986) there is no known public health risk.

In Uganda, national brucellosis-vaccination programs have not included goats and vaccines used have not targeted *B.melitensis* (Kabagambe et al., 2001). This is also supported by Ssekawoja (2007) stating that there is no history of vaccination against brucellosis in goats in Uganda.

Transboundary animal diseases (TADs)

TADs are epidemic diseases that are highly contagious or transmissible and can spread fast regardless of national borders. These diseases cause a high morbidity and mortality in susceptible animal populations that can lead to severe economic, animal and possibly public health consequences (FAO 2012a).

Transboundary livestock diseases are of great concern as they cause a threat to world food security through their ability to spread rapidly and cause decreased milk production as well as decreased meat production (FAO 1996). TADs are also a cause of trade sanctions thereby causing economic losses.

Low-income countries often have difficulties coping with TADs because of their lack of resources. In these countries TADs can remain endemic which poses a threat to food security, failure of agricultural economic development and also risk for spreading the disease by animal trading (Rossiter 2009).

However, there are effective means by which transmission and outbreaks of these diseases can be prevented and controlled. These strategies consist of efficient control of import of animals and animal products, control of animal movement, surveillance for TADs, emergency plans, veterinary services, having sufficient financial means to pay for extra costs caused by an outbreak, access to vaccines for use if required and adequate laboratory support (Rossiter 2009).

Peste des petits ruminants

Peste des petits ruminants (PPR) is a transboundary animal disease and a disease of high socioeconomic significance in low-income countries due to the high mortality and high morbidity of PPR infection. PPR also has negative economic impact because of trade restrictions instituted by authorities during an outbreak (Baron, Parida & Oura 2011; FAO 2012b). Although this statement is not applicable to countries, such as Uganda, where PPR is endemic.

PPR appears to be an emerging disease since the eradication of the Rinderpest virus (RPV) although it is not clear whether this is a true spreading of the disease in the world or whether it is easier to recognize PPR since RPV has been eliminated (Baron, Parida & Oura 2011).

PPR is a viral disease caused by a *Morbillivirus* in the family of *Paramyxoviridae*. Similar viruses are rinderpest, canine distemper virus and measles viruses. Members of this family have six structural proteins: the nucleocapsid protein (N), the phosphoprotein (P), the matrix protein (M), the fusion protein (F) and the haemagglutinin protein (H). The last two ones mentioned are responsible for the attachment and the penetration of the virus into the host's cell (Diallo 1990).

The main hosts for Peste des petits ruminants virus (PPRV) are goats and sheep but cattle and other ruminants can be infected and in poor conditions cattle can show signs of illness similar to rinderpest when infected (Diallo et al. 2007; Kwiatek et al. 2011). Even though other species than small ruminants might get infected there is at present no proof that for example cattle, buffaloes or camels have any significant role in the transmission of the PPR virus (OIE 2011; OIE 2012c).

The PPRV was first described in the Ivory Coast in 1942 and the disease has since then spread over the world (FAO 2012b). Nowadays the disease occurs in a band that goes through the Arabian Peninsula, the Middle East, south-west Asia, India and Africa, mainly in the area between the Sahara desert and the equator but PPR is also considered an emerging disease in the northern part of Africa (OIE 2012b).

To this day the PPRV isolated have been classified into four lineages. When investigating geographic distribution of PPR, viruses from Africa were found to belong to three lineages (Shaila et al. 1996). In this study PPRV isolated from Senegal, Nigeria and Sudan formed one lineage (group 1), viruses from the Ivory Coast and Guinea formed group 2 and viruses from Sudan, Oman and southern India formed group 3. Viruses isolated from diseased animals in Asia and the Middle East were found to belong to another lineage (group 4) but recently this lineage has also been found during outbreaks of PPR in Africa (Shaila et al. 1996; OIE 2011).

Pathogenesis and clinical signs

Infection by PPRV in goats occurs via the mucous membranes in the retropharyngeal area. The virus penetrates the mucosa and thereby enters the bloodstream causing viremia. The PPRV specifically targets cells in alimentary, respiratory and lymphoid tissues. Cells infected by the virus either undergo necrosis or proliferate (respiratory tract) (Mulindwa 2009; Radostits et al. 2007).

The major route of infection is through inhalation of virus-particles that are secreted in nasal discharge, tears and secretions from coughing during the acute phase of the disease. Clinical findings can range from a mild or subclinical infection to severe clinical signs including sudden death in the peracute form, fever, depression, anorexia, abortion, eye infection and

clear nasal discharge that becomes thicker during the disease sequel. The profuse nasal discharge can cause respiratory distress. Ulcers form in the mouth and on the tongue and infected animals can also develop a severe diarrhea that causes dehydration. Pneumonia often develops in later stages of infection. Because of these symptoms PPR can be confused with pasteurellosis and contagious caprine pleuropneumonia although these two diseases normally do not cause diarrhea and oral lesions (Baron, Parida & Oura 2011). Secondary infections in affected goats are common because of the immunosuppression induced by the PPRV. In a study performed in 2003 unvaccinated animals infected by PPRV showed severe leukopenia caused by the virus' great affinity for white blood cells (Behre et al. 2003; Baron, Parida & Oura 2011). PPRV does not infect humans (OIE 2012b).

PPR in goats in Uganda

The first outbreak of PPR in Uganda was confirmed in July, 2007, in the region of Karamoja (MAAIF 2010). During this outbreak spanning the years of 2007-2008, over 500 000 animals are believed to have died. In a seroprevalence study in goats taken place because of the outbreak in 2007, the overall prevalence of antibodies against PPRV, in the Karamoja sub-region in Uganda, was found to be 57.6 per cent (Mulindwa et al. 2011).

It was suspected that the virus had spread to other districts nearby since 2007 (MAAIF 2010) and this was confirmed by a study taking place in the districts surrounding Karamoja region in 2009. This study reported a seropositivity of 9.4 per cent for antibodies against PPRV (Ruhweza 2009; MAAIF 2010).

Vaccination against PPRV has so far taken place in 23 districts in northeastern Uganda as a part of the Vaccination against neglected animal diseases (VACNADA) project (MAAIF 2010).

In November 2009 the seroprevalence of PPRV antibodies in sheep and goats in Karamoja after vaccination was investigated. In this study 66 per cent out of the 85 vaccinated goats sampled were positive for antibodies against PPRV. 13 per cent of the 15 unvaccinated goats sampled were positive for antibodies against PPRV and 57 per cent of the 110 goats with unknown vaccination status were positive for antibodies against PPRV as well. This study also concluded 55 per cent of the vaccinated sheep and goats to be protected by immunization against PPRV (Luka et al. 2011).

A study performed to investigate the molecular characteristics of the PPRV from Karamoja showed that there are three lineages (group 1, 2 and 4) circulating in Uganda (Luka et al. 2012). This study also reported the prevalence of PPRV in small ruminants to be 38.1 per cent in 2007 and 13.0 per cent in 2008, respectively, when using the PCR method for analysis during the ongoing outbreak of PPR. According to Mulindwa et al. (2011) more studies should be undertaken to study the epidemiology of PPR in Uganda.

Diagnosis in small ruminants

For the diagnosis of PPRV infection identification of the agent can be performed using agar gel immunodiffusion (AGID), counter immunoelectrophoresis (CIEP), immunocapture enzyme-linked immunosorbent assay, PCR and by culture and isolation methods (OIE 2012c).

Positive aspects of using AGID are its simplicity, low cost and that it can be carried out in any laboratory and in the field. The advantage of CIEP is that it is the most rapid method to identify the PPRV (OIE 2012c). Immunocapture ELISA is also a rapid test method and there are commercial kits available for diagnostic purposes. A study by Libeau et al. (1994) investigating immunocapture ELISA for differential diagnosis of PPR and rinderpest reported a high sensitivity and no cross reactions between the two viruses. The advantage of PCR is that this method is more sensitive and less time consuming than virus isolation (Couacy-Hymann et al. 2002).

Positive culturing of the PPRV results in cell syncytia formation because of the cytopathogenic effect of the virus and the cell cultures should be checked for syncytia daily according to OIE (2012c).

There are also serological tests that can be used for diagnosis of PPR. The golden standard according to the OIE (2012c) is the virus neutralization test (VNT) and this is the prescribed test for international trade. The VNT has both high sensitivity and high specificity but the procedure is time-consuming. The H- or N-based competitive ELISA has a good correlation to the VNT and is therefore an alternative for serological diagnosis of PPR (Singh et al. 2004; OIE 2011). The C-ELISA is a rapid test and it does not require the same sterility when handling samples as the VNT and is therefore more suitable for analysis in the field (Singh et al. 2004).

Preventive measures

Because of similarities between the PPRV and the rinderpest virus (RPV), vaccine against the latter has been used for immunization against both diseases. The cross protection against PPRV induced by RPV immunization is probably due to homology of the fusion protein between RP and PPR viruses (Diallo et al. 2007).

Since 1998 OIE recommends using only the live attenuated PPRV vaccines for immunization of small ruminants against PPR, thereby reducing the risk for confusion when performing serosurveys for the control of rinderpest (Diallo et al. 2007; OIE 2012c).

Nigeria 75/1 is a strain of the PPRV commonly used for immunization. This vaccine, even though belonging to group 1, has been shown to protect against different strains of PPRV thereby providing effective control of PPR (Diallo et al. 2007; OIE 2011). The immune response induced by the Nigeria 75/1 vaccine strain and the Sungri 96 strain vaccine have been shown to be long-lasting (at least 3 years) after one single dose (OIE 2011).

One disadvantage of the conventional attenuated PPRV vaccine is lack of thermostability. This causes problems in tropical areas because of difficulties maintaining a cold chain. In 2001 a new method for preservation of the vaccine was reported by Worrall et al. (2001) making it possible to store the vaccine at 45°C for a period of 14 days without noticeable loss of potency.

Another disadvantage of the traditional live attenuated vaccine is trouble with differentiating infected from vaccinated animals (Diallo et al. 2007). To try to overcome this obstacle various recombinant vaccines have been developed. Junling et al. (2012) invented a recombinant Peste des Petits ruminants-Canine Adenovirus Vaccine. This vaccine was able to induce both humoral and cell-mediated immune responses in vaccinated goats (Junling et al. 2012). A study performed by Behre et al. (2003) indicated that a capripoxvirus recombinant can be used to protect goats against both PPRV and capripoxvirus. It is not yet concluded for how long the immunity induced by this capripoxvirus recombinant lasts (Behre et al. 2003).

THE IMPORTANCE OF ANIMAL HEALTHCARE

To keep urban livestock is critical for the food security of poor people but it is also seen as a potential risk for transmission of zoonoses, such as brucellosis, and is thereby a potential public health risk (Flynn 1999; UNDP 2012). It has been stated that the association between zoonoses and urban agriculture have not been studied enough and it has also been suggested that there is an emergence of zoonotic diseases in urban areas (Flynn 1999). Makita et al. (2008) declared living in urban areas in Uganda to be a risk factor for brucellosis in humans.

The importance of good animal health for food security has been described by Perry et al. (2002) and the poor are at particularly high risk because of less ability to cope with diseases in their animals. Sheep and goats are essential for poor farmers often being their main assets. PPR has been stated 'the main killer disease of small ruminants' (FAO 2009) and is thereby a disease severely affecting food security by causing loss of animals. Surveillance, prevention and good control of PPR is according to FAO (2009) of public health interest. A major part of Uganda's small ruminants are at risk of PPR and a southward spreading of the disease in East Africa could be devastating.

Therefore it seemed prudent to gain further knowledge regarding agricultural conditions and disease seroprevalence in urban and peri-urban Uganda. This knowledge could prove important in alleviating poverty and promoting both human and animal health.

AIM

The aims of my study were:

- To gain insight in the urban and peri-urban agriculture in Kampala, Uganda.
- To estimate the seroprevalence of a selection of zoonotic (brucellosis) and transboundary (Peste des petits ruminants) animal diseases in goats and thereby

investigate the potential risk for transmission of diseases to humans (brucellosis) and livestock (PPR).

- To gain knowledge about possible risk factors for seropositivity against brucellosis and PPRV in goats.

MATERIAL AND METHODS

Study area and time period

This study was carried out in urban and peri-urban areas in Kampala district, Uganda. Seven sub-counties/divisions, out of seven possible, were included in the study: Makindye, Rubaga, Nakawa, Kawempe, Wakiso, Greater Kampala and Central Kampala (see figure 1 and 2). The study took place in the month of September and October in the year of 2012.

Selection of animals

To carry out the selection of farms to be included in this study, data from a recent inventory performed within ongoing collaborative research between SLU and Makerere University, Kampala, Uganda, was used. This data, supplied by B. Vinnerås PhD, Associate Professor Environmental Engineering, Swedish University of Agricultural Sciences, contained information about the location (GPS coordinates) and numbers of livestock on farms to be found in the district of Kampala. From this data a randomized selection of farms holding goats (and cattle) was done using Microsoft Office Excel.

It was calculated that a sample size of 270 goats would be enough to estimate a prevalence of 50 per cent, with a precision of 5 per cent and a confidence interval of 90 per cent.

However, due to practical reasons this randomized selection of farms could not be used. The city of Kampala is currently going through changes, as it is growing, including movements of livestock out of the city (Kwizera, M.H. KCCA, personal communication., 2012). Because of these movements of livestock, the farms selected were not to be found.

Instead 55 farms holding goats were selected by accessibility. The farms were selected with help from local guides having knowledge of each sub-county included in the study. A maximum of 5 goats were sampled on each of the 55 farms.

Blood sampling

Six ml of blood was taken from each sampled goat, using vacutainer technique, from either left or right jugular vein under the supervision of the assisting local veterinarian. Needles used for bleeding were size 20 G.

The blood was collected in sterile serum tubes marked with a specific serial number to guarantee identification. The serum tubes were kept standing in upright position allowing the

blood to clot during the day out in the field. The serum tubes were thereafter stored overnight in room temperature (18-25°C) at the molecular biology laboratory at Makerere University, Institute of Environment and Natural Resources (MUIENR), Kampala, Uganda.

All samples were centrifuged within 36 hours and serum was thereafter divided into two sterile cryo tubes. One cryo tube, containing approximately 1.8 ml serum, was kept in 2-8°C and used for laboratory analysis. The second tube was kept in -20°C for 4 weeks and thereafter kept in -80°C for longtime storing. Laboratory analysis was performed within 3 weeks from sampling.



Figure 1. Blood sampling an adult goat (personal photograph).

Interviewing

All farmers were interviewed regarding their animals' history of disease (e.g. abortions, coughing, nasal discharge or diarrhea), feeding, waste product handling, breeding, management system, medical treatments, transportation, labour on the farm and animal movements (bought and/or sold animals). For this purpose a questionnaire (shown in appendix 1.) containing 23 questions was used.

The questionnaire was read through and approved by our local supervisor Dr. Charles Masembe, Department of Biological Sciences, Makerere University, Kampala, before interviewing began. Question number 23 was omitted because of problems with interpreting the answers given by the farmers.

GPS readings were done on each farm and noted on each questionnaire.

Interviews were performed in English whenever possible. When not possible the questions were translated to Luganda (local language spoken in the kingdom of Buganda, Uganda) by the local guide or the assisting local veterinarian. Answers were also translated to English when needed.

Data from interviews was handled in Microsoft Office Excel.



Figure 2. Interviewing animal owners in Central Kampala (personal photograph).

Laboratory Analysis

Laboratory analyzes were performed at the molecular biology laboratory at Makerere University, Institute of Environment and Natural Resources (MUIENR), Kampala, Uganda. For both diseases (Brucellosis and PPR) investigated competitive Enzyme Linked Immunosorbent Assays (C-ELISA) were used.

Competitive Enzyme Linked Immunosorbent Assay (C-ELISA)

C-ELISA is a diagnostic test procedure using microtiter plates containing 96 wells. The wells are coated with antigen. Serum samples (test samples), which are suspected of containing antibodies (primary antibodies) directed against the coated antigen, are added during the procedure. Secondary labeled antibodies are also added to the wells. These secondary antibodies are also specific to the coated antigens. The primary and secondary antibodies will therefore compete, both trying to adhere to active sites. To avoid unspecific binding by antibodies the wells are washed repeatedly. A solution containing chromogen is used and if enzyme labeled antibodies are bound to coated antigen a chemical reaction will start and

colour will appear. Optical density is measured using a photometer and the intensity of the produced color is directly proportional to the optical density. Low optical density equals a positive reaction. This means primary antibodies are occupying active sites leaving less room for secondary labeled antibodies to adhere (Biancifiori et al. 2000; Mulindwa 2009; Mythili et al. 2011).

Brucellosis

To investigate the seroprevalance of anti-brucella antibodies in the 190 goats sampled, a commercial C-ELISA was used (SVANOVIR[®] Brucella-Ab C-ELISA, Svanova Biotech AB, Uppsala, Sweden). According to an in-house study performed by the manufacturer the sensitivity and specificity of the test is 100 per cent and 100 per cent, respectively. A study performed by Biancifiori et al. (2000) reported a diagnostic sensitivity of 99.4 per cent and diagnostic specificity of 98.9 per cent.

Brucella-Ab C-ELISA is able to detect antibodies against *Brucella abortus* and *Brucella melitensis* in both cattle and goats. In this assay samples are exposed to smooth lipopolysaccharide (antigen) and the test is based on a monoclonal antibody which competes for antigen epitopes with possible antibodies in test serum (Biancifiori et al. 2000). ELISA kits for diagnosis of brucellosis containing lipopolysaccharide are the most accurate ones according to OIE (2009).

According to Portanti et al. (2006) competitive ELISA is a suitable tool for detecting antibodies against brucella in caprine, ovine and bovine serum. In this study it was also stated that C-ELISA is equal in performance to the complement fixation test (CFT).

Brucella-Ab C-ELISA procedure

The C-ELISA procedure in this study was carried out as described in the protocol written by the manufacturer. All reagents were taken out of the refrigerator in time to guarantee reagents to equilibrate to room temperature. Buffer was added into each of the 96 wells on the microtiter plate and positive, weak positive and negative controls were put into appropriate wells for validation. Samples were added to chosen wells and run in singles because of financial constraints. Monoclonal antibody solution was added to the wells. Incubation was performed and then washing of the plate to eliminate unspecific binding of antibodies. HRP (goat anti-mouse IgG horse-radish peroxidase) conjugate was added and then incubation and rinsing was repeated. Substrate was put into each well and if samples were negative a chemical reaction started resulting in the contents of the well changing colour. Stop solution was added to stop the reaction. Optical density (OD) was measured at 450 nm and calculations were done using the following formula:

$$PI = 100 - ((\text{meanOD}_{\text{samples/control}} \times 100) / \text{MeanOD}_{\text{conjugate control}})$$

The cut off value for seropositivity was $PI \geq 30$ per cent according to the manufacturer.

Peste des petits ruminants

To investigate the seroprevalance of anti-PPRV antibodies in the 190 goats sampled, a C-ELISA was used (ID Screen[®] PPR Competition, ID vet, Montpellier, France). This diagnostic kit detects antibodies against the nucleoprotein of the PPRV. Sensitivity of the test is 94.5 per cent and specificity of the test is 99.4 per cent (reference method: virus neutralization test) (Libeau et al. 1995).

According to OIE (2012c) the recommended diagnostic serological test for international trade is the virus neutralization test (VNT) but this test is time-consuming.

PPR Competition ELISA procedure

The C-ELISA procedure in this study was carried out as described in the protocol written by the manufacturer. All reagents were taken out of the refrigerator in time to guarantee reagents to equilibrate to room temperature. Buffer was added into each of the 96 wells on the microtiter plate and positive and negative controls were put into appropriate wells for validation. Samples were added to chosen wells and run in singles because of financial constraints. Incubation was performed and washing of the plate to eliminate unspecific binding of antibodies. Anti-NP-HRP conjugate was added and then incubation and rinsing was repeated. Substrate was put into each well and if samples were negative a chemical reaction started resulting in the contents of the well changing colour. Stop solution was added to stop the reaction. Optical density (OD) was measured at 450 nm and calculations were done using the following formula:

$$\text{Competition \%} = \text{OD}_{\text{sample}} / \text{OD}_{\text{negative control}} \times 100$$

The cut off for seropositivity used was ≤ 35 per cent as recommended by the manufacturer.

Test samples having competition values between 35 and 45 per cent were considered doubtful (as recommended by the manufacturer) and these samples were run in duplicates for confirmative purposes.

Statistical Analysis

Risk factors for Brucellosis and PPR

To investigate the presence of risk factors, associated with seropositivity for brucellosis and PPR, data from the questionnaires was analyzed using Epi Info[™] 7.1.0.6 (from Centers for Disease Control and Prevention). Proportions were compared in a 2x2 contingency table using the Fisher's Exact test because the frequency in one or more of the cells was less than 5 making the Chi-squared test invalid to use. Odds Ratio was also calculated when significance was found.

Risk factors at herd level were investigated. Risk factors investigated were: keeping cattle in addition to goats, large herd size, using a buck that was also used on other farms, keeping other livestock on farm in addition to goats, labour used on farm, keeping goats that had

abortions in the last year, keeping goats that had a history of coughing in the last year, keeping animals that had been diseased in the last year, production system (tethering, zero-grazing, pasture), bought/sold animals, sero-positivity for brucellosis/PPRV and sub-county.

The risk factor ‘keeping goats that had a history of coughing in the last year’ was only investigated for PPR and the risk factor ‘keeping goats that had a history of abortions in the last year’ was only investigated for brucellosis.

RESULTS

Data collection

The numbers of goats sampled in each sub-county were: 12 in Central Kampala, 51 in Greater Kampala, 15 in Kawempe, 10 in Rubaga, 27 in Makindye, 72 in Nakawa and 3 in Wakiso. In total 190 goats were sampled. Mainly adult goats were bled but a few kids (<6 months of age) were also included. In table 1 the number of goats from the recent inventory of animals in Kampala district is compared to the number of goats sampled in each sub-county in our study.

Table 1 comparing numbers of goats sampled in each sub-county in Kampala district

Sub-county	Number of animals sampled	Percentage	Number of animals in inventory	Percentage
Central Kampala	12	6	53	2
Kawempe	15	8	961	31
Makindye	27	14	497	16
Nakawa	72	38	475	15
Rubaga	10	5	1090	35
Wakiso	3	2	Data not available	
Greater Kampala	51	27	Data not available	
Total	190	100	3076	100

Characterization of farms visited

Interviews were performed on 55 different goat farms in urban and peri-urban Kampala (see figure 1 and 2). Twenty-one villages in 7 sub-counties/divisions were visited. The average herd size was 3.5 goats per farm (range 1 to 25 goats). Twenty-five farms out of 55 farms visited held cattle in addition to goats. Only 13 farms out of 55 kept other livestock than cattle in addition to goats. It was more common for women to keep goats than for men, and mainly adults responded to the questions (see figure 3). Out of 55 respondents 48 were owners, 4 were herdsmen and 3 were family members.



Figure1 showing geographic distribution of farms visited and brucella serostatus of the herds sampled.

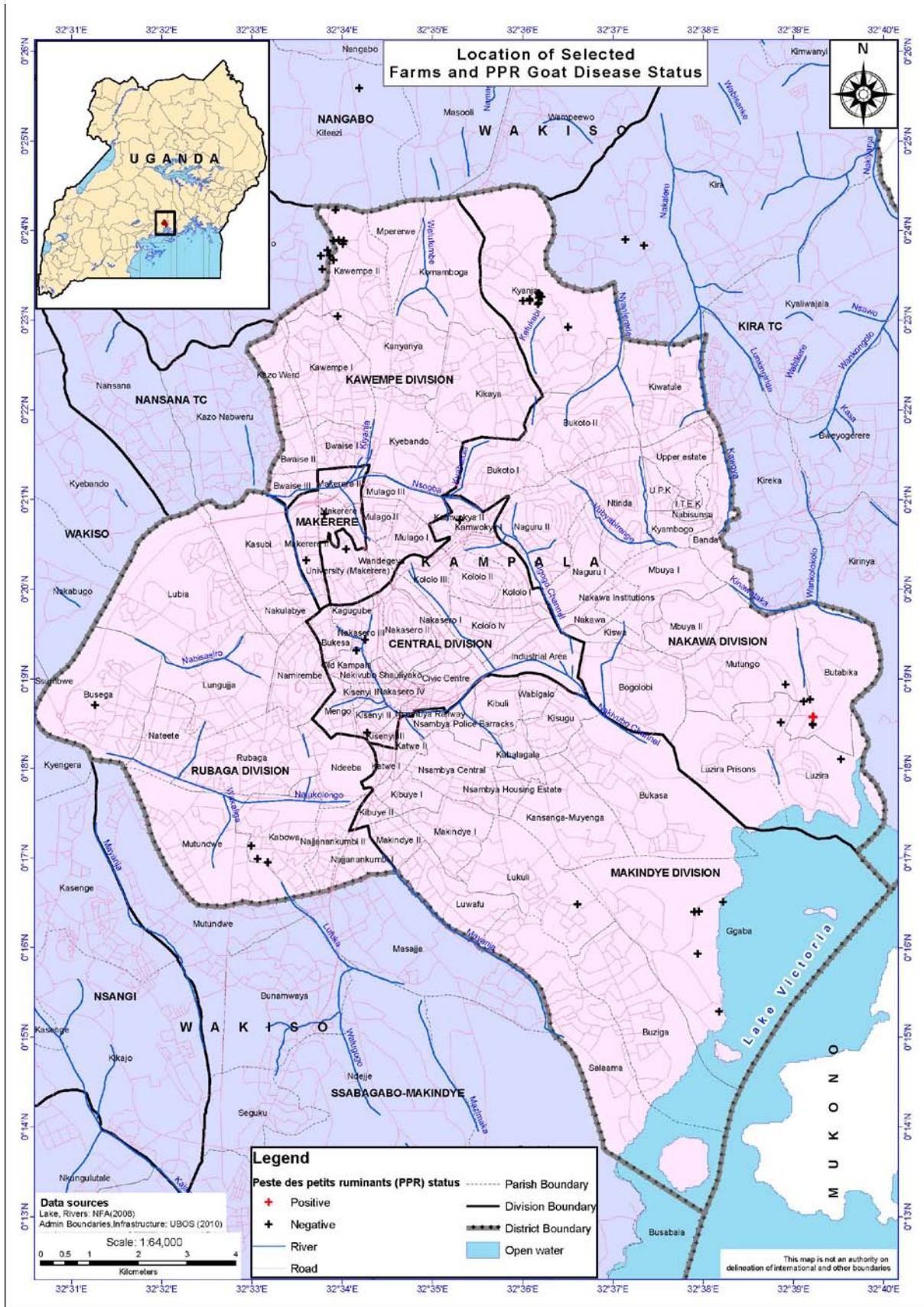


Figure 2 showing geographic distribution of farms visited and PPRV serostatus of herds sampled

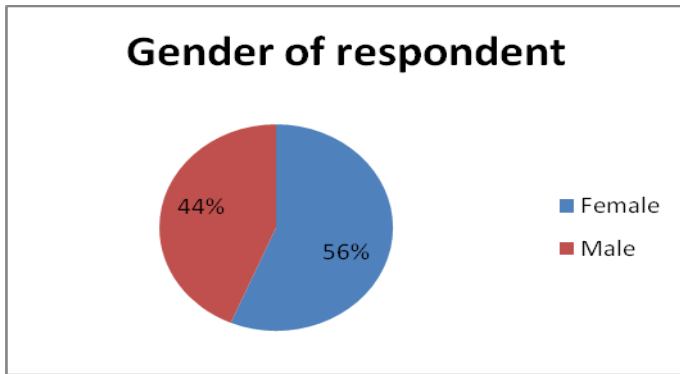


Figure 3 showing the gender distribution of the respondents.

The most common production system on the farms in this study was tethering (see figure 4).

Farmers mostly fed their goats with banana peels and grass grown by themselves. Although other peels, corn, brewer's mash and concentrate were also used for feeding purposes. Fifty-five per cent of the farmers grew their own feeds for their animals, 33 per cent bought feed stuffs in the local market, 25 per cent gave their animals kitchen wastes, 5 per cent bought/got feed stuffs from their neighbours and 7 per cent bought brewer's mash from the local brewery.

The purpose for keeping livestock is shown in table 2. Very few farmers kept their goats as pets and more than every other farmer considered their goats an economic investment.

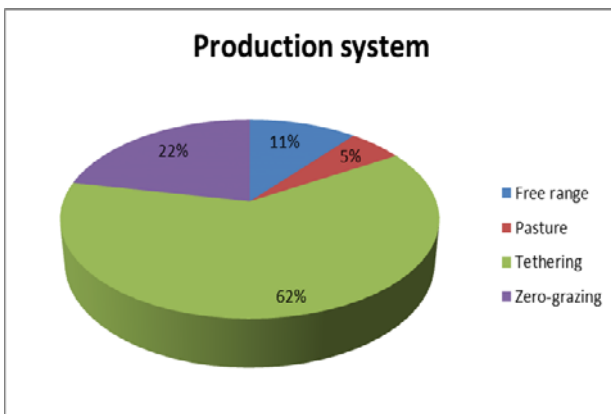


Figure 4 showing the percentage of production systems used.

Table 2 showing the purpose of livestock kept on the farms sampled

Purpose of livestock kept on farm	Number of farms	Percentage
Own consumption	19	35
Sell milk	1	2
Sell meat	5	9
Sell livestock	11	20
Economic investment	29	53
Education	1	2
Pets	1	2

The most common way to handle manure was to use it as a fertilizer (51 per cent) when growing crops on the farm. Eleven per cent sold manure, 2 per cent used it as fuel and 2 per cent dumped the manure, respectively. More than a third (35 per cent) of farmers interviewed declared they did not use animal manure for anything.

History of animal disease on farms

Sixty per cent (33 out of 55) of the respondents stated that their animals had been diseased during the last year. The most common clinical sign occurring in individual goats was diarrhea followed by coughing (see table 3). A number of goats had shown more than one clinical sign in the year of 2012 according to the respondents.

Other clinical signs than abortion, coughing, diarrhea, nasal discharge and fever were classified as 'Other'. Clinical signs in this category were mastitis (2 goats), eye disease (2 goats), weight loss (1 goat), vaginal discharge (1 goat), infertility (3 goats) and worms in feces (4 goats). On average 1,6 goats on each farm had been treated with antibiotics in the last year (range 0-15).

Table 3. This year's reported health issues in the 190 sampled goats

Clinical signs	Number of goats	Percentage
Abortion	11	6
Nasal discharge	33	17
Coughing	55	29
Diarrhea	72	38
Fever	25	13
Other	37	19

Animal movements

It was common for farmers to have goats bred on their farm and for farmers to keep all of their goats. Forty-one respondents declared that they had not bought any goats in the last year and 36 that they had not sold any goats in the last year, respectively. Farmers who had bought goats in the last year generally had bought goats from their neighbours. Nine farms stated selling their goats to the abattoir and 8 farms reported selling their goats to neighbours.

The most common way to transport goats was to herd them on foot. Transportation by truck was the second most common method of transportation. For breeding, most of the farms visited declared using a buck that was used also on other farms (see figure 5).

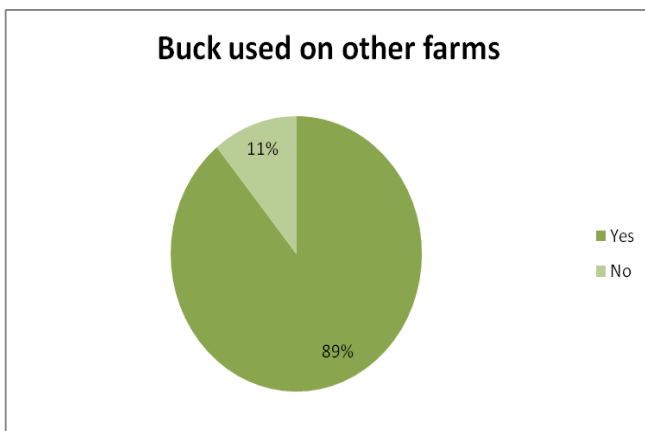


Figure 5. Percentage of farms using a buck that was also used on other farms.

Most farmers keeping goats took care of their animals by themselves although some farms used hired labour and a few farms used both family and hired labour for their goats (figure 6). Only 9 farms out of 55 responded positive when inquired about bio-security measures such as fencing, single entrance to farms, footbaths and limiting visitors to farm.

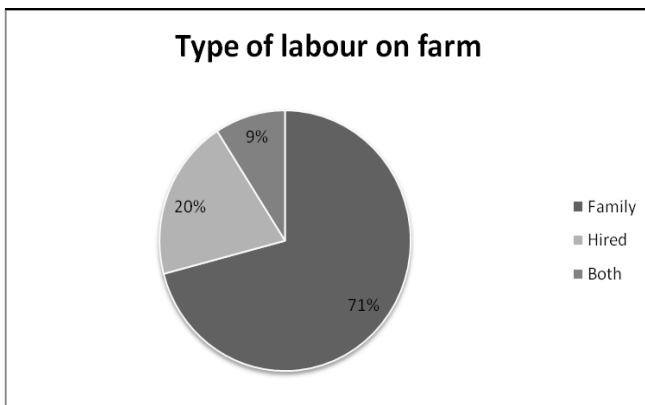


Figure 6 showing labour used on farms visited.

Laboratory results

Seroprevalence of brucellosis

One-hundred and ninety blood samples were taken and analyzed for presence of antibodies against *Brucella* spp. and PPRV. The numbers of goats sampled in each sub-county were: 12 in Central Kampala, 51 in Greater Kampala, 15 in Kawempe, 10 in Rubaga, 27 in Makindye, 72 in Nakawa and 3 in Wakiso. Mainly adult goats were bled but a few kids (<6 months of age) were also included.

Nine goats out of 190 sampled were positive (OD-value \geq 30 per cent) for anti-brucella antibodies according to the C-ELISA used in our study (see table 4). This equals a seroprevalence of 4.7 per cent (see table 5). One of these 9 brucella-positive goats was also positive for anti-PPRV antibodies.

Table 4. Optical density (OD) values for goats positive for anti-Brucella antibodies in our study

Animal id	Division	Village	OD-value (%)
WWW2G	Nakawa	Kyanja	34
VV2G	Nakawa	Bbina	50.7
RRR2G	Nakawa	Kyanja	40.6
4I2G	Kawempe	Kikoni	44.7
JJ1G	Greater Kampala	Namere	63.8
Y2G	Greater Kampala	Tula	57.5
DD2G	Greater Kampala	Tula	80.7
KK3G	Greater Kampala	Namere	34.9
CC2G	Greater Kampala	Tula	65.3

Five brucella-positive goats were from the sub-county of Greater Kampala, 3 were from the sub-county of Nakawa and 1 was from the sub-county of Kawempe, respectively (see table 4). There was only one positive goat on each of the 9 brucella-positive farms (see figure 1). Nine seropositive goat herds out of 55 sampled equals a herd prevalence of 16.4 per cent.

Seroprevalence of PPR

Three goats out of 190 sampled had anti-PPRV antibodies when samples were analyzed using C-ELISA. OD-values for the 3 positive goats were 34.7, 17.4 and 5.6 per cent, respectively (see table 5). The cut off for seropositivity was OD-values \leq 35 per cent.

Table 5. OD-values for goats positive for anti-PPRV antibodies in our study

Animal id	Division	Village	OD-value (%)
VV1G	Nakawa	Bbina	34.7
VV2G	Nakawa	Bbina	5.6
VV4G	Nakawa	Bbina	17.4

All 3 seropositive goats in this study were found on the same farm in the sub-county of Nakawa situated in the east and north-east part of Kampala district (see table 5 and figure 2). One of the 3 sero-positive goats sampled on this farm was also positive for brucella antibodies. The fourth goat sampled on the same farm was negative for both brucella and PPRV antibodies.

The seroprevalence of PPRV antibodies in our study was 1.6 per cent and the herd prevalence was 1.8 per cent, respectively (see table 6).

Table 6. Prevalence of antibodies against brucella and PPRV on farms

	Brucellosis	PPR
<i>Animal level</i>		
Number of positive animals	9	3
Seroprevalence (%)	4.7	1.6
<i>Herd level</i>		
Number of positive herds	9	1
Herd prevalence (%)	16.4	1.8

Statistical results

Risk factors for brucellosis

In our study one statistically significant risk factor was recognized at the herd level (see table 7). Keeping goats in the sub-county of Greater Kampala was associated with seropositive status of herds ($p = 0.03$; OR = 5.94 (95%CI 1.30-27.1). There was also a tendency for association between keeping goats zero-grazing and seropositivity for brucellosis ($p = 0.09$).

Risk factors for PPR

No statistically significant risk factors for PPR were recognized at the herd level in our study (see table 8).

Table 7. Risk factors investigated at herd level (brucellosis)

Factors	Seropositive herds	Seronegative herds	2tailed p-value
Keeping cattle			
Yes	4	21	1
No	5	25	
Herd size			
≤5	4	21	1
>5	5	25	
Buck used on other farms			
Yes	9	40	0.57
No	0	6	
Other livestock kept on farm			
Yes	3	10	0.43
No	6	36	
Labour			
Hired	4	12	0.42
Family	5	34	
Abortion			
Aborted	1	2	0.42
Not aborted	8	44	
Diseased animals on farm			
Yes	6	27	0.72
No	3	19	
Production system			
Tethered	4	30	0.28
Not tethered	5	16	
Zero-grazing	4	8	0.09
Not zero-grazing	5	38	
Pasture	1	2	0.42
Not pasture	8	44	
Sub-county			
Greater Kampala	5	8	0.03
Not Gr.Kampala	4	38	
Bought/sold animals			
Yes	6	22	0.47
No	3	24	
Bought animals			
Yes	4	10	0.21
No	5	36	
Sold animals			
Yes	2	17	0.47
No	7	29	

Table 8. Risk factors investigated at herd level (PPR)

Factors	Seropositive herds	Seronegative herds	2tailed p-value
Keeping cattle			
Yes	0	25	1
No	1	29	
Herd size			
≤4	1	28	1
>4	0	26	
Buck used on other farm			
Yes	1	48	1
No	0	6	
Other livestock kept on farm			
Yes	0	13	1
No	1	41	
Labour			
Hired	1	38	1
Family	0	16	
Coughing			
Coughed	1	13	0.25
Not coughed	0	41	
Diseased animals			
Yes	1	32	1
No	0	22	
Production system			
Tethering	1	33	0.28
Not tethering	0	21	
Sub-county			
Nakawa	1	22	0.42
Not Nakawa	0	32	
Brucella seropositivity			
Yes	1	8	0.16
No	0	46	
Bought/sold animals			
Yes	0	31	0.44
No	1	23	
Bought animals			
Yes	0	17	1
No	1	37	
Sold animals			
Yes	0	19	1
No	1	35	

DISCUSSION

Urban and peri-urban agriculture

In our study most farms visited were small scale keeping 3,5 goats on average and this is in agreement with statistics from UBOS (2010) reporting goat herd sizes in Uganda to be small. The most common goat management system in Uganda was tethering when investigated by Kugonza, Bareeba & Kirembe (2001) and Mwebe et al. (2010) and 62 per cent of the farms in our study used tethering.

Goats were mostly kept for own consumption and as an economic investment thereby providing food security and income, if sold in times of need, as described by Peacock (2005). The ability to keep livestock for self-consumption is one of the benefits of urban and peri-urban agriculture (FAO 2008).

More women than men owned goats in our study and it has been reported that women often have the responsibility for management of livestock (especially goats and chicken) in societies (Perry et al. 2002; Peacock 2005). Women therefore will benefit highly from improved animal health. Ensuring women income and making it possible for women to work near their homes are other benefits of urban and peri-urban agriculture (FAO 2008).

The most common way to use manure in our study was using it for growing crops (51 per cent). Using manure for fertilizing purposes is a way for poor people to be able to increase soil fertility without being able to buy expensive chemicals (Perry et al. 2002). Although it should be remembered that contact with goat manure is a possible route of transmission of diseases (Wallach et al. 1997). A significant part of the respondents (35 per cent) answered they did not use manure for anything, leaving it to pile on the ground where animals on the farm defecate. This could allow transmission of infectious agents to humans and animals.

Many farmers in this study answered that their goats had been diseased in the last year but very few farmers kept records of animal disease. The two most common clinical signs were, according to the respondents, diarrhea and coughing. These clinical signs are compatible with a lot of diagnoses including PPR. Few farmers stated that their goats had abortions, a common clinical sign of brucellosis, in the last year. It should be taken into consideration that it might be hard for farmers to notice fever in their animals without measuring rectal temperature and abortions can be missed when animals are roaming free during the day. Therefore there might be some uncertainties in our data regarding animal disease. However, animal disease reduce farm yield on many levels. Meat, milk and manure production drops. Animal trading also diminishes during outbreaks due to movement restrictions imposed by the government. Treatment costs also contribute to the overall financial loss. Although more difficult to quantify, human health and welfare are also affected by animal disease due to zoonotic properties and food insecurity (Perry et al. 2002). It is therefore essential to try to reduce animal disease in urban and peri-urban agriculture to provide food security and improve human and animal health.

Brucellosis

The herd prevalence of brucella antibodies in goats in peri-urban and urban Kampala, Uganda, in our study was 16.4 per cent. Other studies performed in Uganda recently have found seroprevalences of brucellosis ranging from 13 to 20 per cent in goats (Ssekawojwa 2007; Ssekabira 2009; Makita et al. 2011b; Mwebe et al. 2011). These studies appear to support our findings but one should remember that we only sampled 5 goats per farm. On farms keeping a large number of goats seropositive animals could have been missed because of this sampling restriction and thereby making herd seroprevalence in our study lower than it actually might be.

In our study we were not able to do a randomized selection of farms because of ongoing animal movements in Kampala. This might have affected our results considering geographical distribution of brucellosis seropositivity (and PPRV seropositivity) in goats in Kampala district. Sampling sizes from some of the sub-counties seem to be representative. According to data (shown in table 1) 16 per cent of our goat samples were taken in the sub-county of Makindye and according to the recent inventory of animals in Kampala district 16 per cent of all goats are held in Makindye. On the other hand we were only able to sample 10 goats (5 per cent) in Rubaga and according to available data 35 per cent of the goats in Kampala district are held in Rubaga sub-county. It should also be remembered that recent animal movements, due to practical reasons, in Kampala district may affect data from the mentioned inventory.

When choosing the 5 goats to sample on each farm we tried to choose randomly. On a few farms we were not able to choose randomly because these farm owners wanted us to sample specific animals and we agreed to this demand for cooperation. Animals chosen by farm owners were animals recently bought and animals that had been diseased in the last year. Test results from these goats may have caused bias and overestimation of the prevalence of antibodies against brucella and PPRV in goats in urban and peri-urban Kampala.

The commercial C-ELISA kit (SVANOVIR[®] Brucella-Ab C-ELISA, Svanova Biotech AB, Uppsala, Sweden) used was supposed to be stored in a refrigerator at temperatures between 2°C and 8°C according to the manufacturer. Because of lack of storage room and lack of communication the kit was stored in a freezer at approximately -10°C for 1.5 weeks time in Uganda. When discovering this improper storing the manufacturer was contacted to investigate if the kit had to be excluded from our study. Svanova Biotech AB, Uppsala, Sweden, was not able to tell us whether our test kit had to be excluded or not. We were encouraged to test run the kit. Dr. Charles Masembe, Department of Biological Sciences, Makerere University, Kampala, provided us with 10 known brucella-positive and 10 known brucella-negative serum samples for this purpose. When test running our ELISA kit it was able to correctly identify brucella-positive and brucella-negative serum samples and we decided to use the kit in our study. When performing the testing of our samples we used 2 known brucella-positive and 2 known brucella-negative samples on each test plate to ensure credibility of the test kit. Even though these precautions were taken it is possible that the improper storing might have altered our results.

In our study we only found one goat to be positive for anti-brucella antibodies in each positive herd (9 herds in total). OD-values for the 9 brucella-positive goats ranged from 34-80.7 per cent (positive > 30 per cent) making it possible that some of the goats with values close to the cut off value might be false-positive. According to OIE serological tests for the diagnosis of brucellosis might have difficulties differentiating between serological reactions due to *Brucella* spp and serological reactions due to cross-reacting bacteria such as *Yersinia enterocolitica* O:9. This does not seem to be a problem in our study because it has been stated that C-ELISA, for diagnosis of brucellosis, is a diagnostic tool able to solve the problem of false-positive serological reactions due to *Enterobacteriaceae* spp. (Biancifiori et al. 2000; Portanti et al. 2006). However, a specific kind of monoclonal antibody needs to be used for this to be true and also there seems to be disagreements between studies. Godfroid et al. (2002) reported that none of the serological tests used in their study, including C-ELISA, were able to differentiate brucellosis from yersinia reactions.

False-positive serological reactions due to vaccination against brucellosis in our study are unlikely because of lack of preventive measures against brucellosis in small ruminants in Uganda. Furthermore the C-ELISA is able to differentiate between infected and Rev.1 vaccinated goats with an accuracy of 90 per cent according to Biancifiori et al. (2000) thereby decreasing chances of having detected vaccinated goats, if any exist, as false-positives in our study.

In previous studies in Uganda investigating risk factors associated with seropositivity for brucellosis in goats significant risk factors were: history of abortion, keeping sheep in addition to goats, having a hired caretaker and free browsing. In our study these risk factors showed no significant association with seropositivity for brucellosis at herd level. When interviewing farmers in our study it was sometimes difficult to interpret answers given and this can have affected the outcome of the investigation of risk factors. Statistical analyzes performed were based on the answers in our questionnaires thereby relying on trustworthy answers and proper interpretation of them. We only took samples of 5 goats per farm in this study. On farms keeping a large number of goats seropositive animals could have been missed because of this sampling restriction. Thereby the number of seropositive herds might be higher and this could have affected the outcome of the risk factor analyzes.

In our study a geographical risk factor was found. Seropositive herd status was associated with having a farm in the sub-county of Greater Kampala. Fifty-one goats were sampled in this sub-county and this was the second largest sample size taken from one single sub-county thereby possibly affecting the outcome. It is also possible that seropositive goats have been bought from the same source in this area. Another explanation for the association between seropositivity for brucellosis and the area of Greater Kampala could be that goats in this area roam free during the day possibly spreading brucella bacteria between animals. The most common production system in Greater Kampala was zero-grazing (54 per cent). Only 4 farms in Nakawa and 1 farm in Kawempe (12 per cent) used zero-grazing out of the 42 farms not situated in the sub-county of Greater Kampala. This could also have an impact on the results.

The tendency of association between seropositivity for brucellosis in goats and zero-grazing is rather unexpected because the production system of zero-grazing minimizes contact between herds thereby reducing the risk for transmission of infectious agents. A possible explanation could be that farm owners feed their zero-grazing animals with feed-stuff, such as grass, contaminated by for example brucella bacteria shed by animals roaming free (Karimburo et al. 2007).

The existence of brucella seropositive goat herds in urban and peri-urban Kampala in our study suggests that there could be a risk for transmission of the zoonotic pathogen between goats and humans in these areas. Seropositivity for brucellosis means that goats in urban and peri-urban Kampala have been exposed to brucella bacteria and there can be animals that harbor bacteria making them possible transmitters of infection. Consumption of goat milk in Uganda is uncommon because most goat owners keep goats for meat production and not for milk production. Only 1 farm owner out of 55 in our study kept goats for milk production. Even though this decreases risk of transmission via infected milk contact with infected goats and goat manure may still cause infection as stated by Wallach et al. 1997. I think it would be advisable to inform goat owners that there is a potential risk of transmission of brucellosis from their goats to themselves and to other humans. I experienced that there seemed to be little knowledge about zoonotic pathogens when talking to the owners.

When trying out our questionnaire it was clear that one question (No.23) was particularly hard to ask and interpret the answers from. We tried to ask goat owners if they were aware of PPR and brucellosis but most of the ones asked did not recognize the names of the diseases even if they recognized the diseases when explained by the clinical signs they cause. Another common misunderstanding was that the owners thought we asked them if they had had animals suffering from any of these diseases. The question was cut out after one day in the field because it was predicted that the answers would not be valid.

PPR

There have been confirmed cases of PPR in goats in the northern part of Uganda and it has also been shown that the virus has spread to districts surrounding Karamoja (Ruhweza 2009). The results in our study might support that the virus have spread even further because 3 goats, out of 190, in Kampala district tested positive for antibodies against PPRV. This equals a seroprevalence of PPRV antibodies in goats of 1.6 per cent in urban and peri-urban Kampala. However, it must be taken into consideration that these goats might have been included in the VACNADA project and thereby have been vaccinated against PPRV. In our study we did not inquire about vaccination history of the goats sampled and we did not use a serological test able to differentiate between vaccinated and infected goats. On the other hand there are no records of vaccinating goats against PPRV in Kampala district.

According to answers when inquiring about animal movements on the farm with PPRV antibody-positive goats no goats had been neither bought nor sold. However, we only asked about animal movements during the last year and these seropositive goats might have been

bought further back from a district where vaccination against PPRV has been performed or where PPRV infection exists/has existed. The fact that the goats, according to the owner, had a history of coughing is compatible with a diagnosis of PPR although coughing can also derive from other aetiologies.

PPRV infection can be associated with high morbidity and high mortality but less virulent strains exist and in milder outbreaks the disease may be overlooked (Baron, Parida & Oura 2011). It is thereby possible that the 3 PPR-seropositive goats in our study had suffered from PPR while on this particular farm in Kampala district without the owner and local veterinarians noticing.

In Uganda, it is common to let goats roam free during the day and then keep them inside during the night. The 3 PPR-positive goats in our study were kept tethered and this production system permits contact with other goats that are free roaming enabling possible transmission of infectious agents.

I think that it would be advisable to perform further studies investigating the prevalence of PPR in Uganda to understand the spread of PPRV since the introduction in 2007. This is in agreement with what Mulindwa et al. (2011) concluded when performing a serological survey of PPR in Karamoja sub-region. A few seroprevalence studies have been carried out in the districts in the northern part of the country but the prevalence in districts in interior and southern parts is still unknown. Ruhweza's findings in 2009 also highlight the suspicion that the disease might be spreading. Additional outbreaks of PPR could be devastating since keeping goats is essential for a lot of people in Uganda, especially the poor. To be able to prevent outbreaks there is need of knowledge about the existence of the disease. If performing prevalence studies it would be wise to use a test method able to differentiate between vaccinated goats and goats that have been in contact with PPRV because of recent vaccination programs against PPRV taking place in the northern parts of the country. To this day a method able to do this differentiation is not available but a lot of research in this area is being conducted.

One of the 3 PPRV-seropositive goats had brucella antibodies thereby causing the only PPRV-seropositive herd in this study to be seropositive also for brucellosis. PPRV is known to induce severe immunosuppression predisposing for secondary infections and this might have made the brucella-positive goat more susceptible for brucellosis. PPRV causes leucopenia weakening the defense against pathogens (Behre et al. 2003; Rajak et al. 2005).

In our study we included a few kids (<6 months of age) but we did not inquire the owners about the kids' actual age and neither did we label these samples making it impossible to know whether any of the positive samples in our study were from young goats. When including kids in a study it must be taken into consideration that kids up to the age of 5-6 months might have maternal antibodies making them seropositive for PPRV while tested (Balamurugan et al. 2012). This should not affect the results on herd level because only seropositive does have antibodies against PPRV in their colostrum.

The problems mentioned above in the section about brucellosis (i.e. problems with the randomized selection, problems with farmers choosing what goats to sample on some farms and problems with question 23 in the questionnaire used) are also applicable to PPR.

CONCLUSION

- The results from this study demonstrate seropositivity for brucellosis among goat herds in urban and peri-urban Kampala which could indicate a possible risk for transmission of brucella bacteria between goats and humans unless people become aware and take necessary precautions.
- The existence of PPRV-antibodies among goats in urban and peri-urban Kampala in our study suggests possible spreading of the disease outside the northern part of the country and I therefore think that serosurveillance might be advisable to study the epidemiology of PPRV in 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 and goats 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.

1. Gender of respondent M F
2. Age category (estimate) Youth Adult Elderly
3. Role on the farm: Herdsman Farmer/owner Family member Other (specify).....
4. How many livestock do you keep?
5. What kind of livestock do you keep? Goats Cattle
Cattle and others Goats and others If others what kind of livestock.....
6. **For what purposes do you keep your livestock?** For own consumption
To sell milk To sell meat To sell livestock Economic investment
Other.....
7. What kind of feeds do you give your livestock?

8. 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.....
9. What kind of production system do you have? Zero-grazing Tethering
- Paddock/pasture Other.....
10. How do you dispose of animal waste? Use it for growing crops Sell it
- For fuel Bury it in the ground Nothing Other.....
11. Have your animals been diseased in the last year? Yes No
12. If yes, what kind of symptoms have they shown? Abortions Nasal discharge
- Diarrhea Fever Coughing Other.....
13. **How many of your livestock have been treated with antibiotics (medicine) in the last year?**
-
14. How many livestock have you bought in the last year?.....
15. How many livestock have you sold in the last year?.....
16. Where did you buy your livestock from? Friends/neighbours
- I only have livestock bred on my farm Local markets
- Other.....
17. How are the livestock transported to/from your farm? Herded on foot
- Car/truck Motor cycle Bicycle Other (specify)
18. **Where have you sold your livestock?** To friends/neighbours I keep all of my livestock
- In local markets Other.....
19. Type of labour on the farm : Hired labour Family labour

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

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

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

23. Are you aware about a disease called

Brucellosis Yes No

Salmonellosis Yes No

Peste des petits ruminants Yes No

Bovine viral diarrhoea virus Yes No

