Urban Livestock Production in Cambodia

Socio-Economic Benefits and Public Health Hazards

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Urban livestock production in Cambodia – Socio-economic benefits and public health hazards

Abstract

Keeping livestock can make an important contribution to the livelihoods of poor urban dwellers. There are concerns, however, that livestock keeping in and around urban areas may lead to environmental problems and increased incidence of disease transfer between animals and humans (zoonoses). This thesis examined different socio-economic benefits and public health hazards associated with pig keeping in urban and peri-urban areas in a lower middle-income country, using Phnom Penh, the capital of Cambodia, as a case study.

Interviews were carried out with householders in Phnom Penh province and faecal samples were collected from their pigs for detection of zoonotic pathogens and for antimicrobial susceptibility analysis of commensal bacteria. All households reported keeping pigs primarily for commercial purposes and the majority (60%) considered pigs to be an important income source for the household. Diseases among the pigs and low revenues were considered main constraints. None of the respondents mentioned any concerns about potential health hazards. The householders reported that pig manure was commonly dumped in the environment (46%) whereas cattle manure was used as a fertiliser (66%) ($P < 0.001$). Dumping of pig manure was more common in households with lower socio-economic position ($P < 0.001$) and in households that did not have access to agricultural land ($P < 0.001$).

Antimicrobial use was mainly based on farmers' own judgement, with 66% of respondents frequently self-adjusting treatment duration and dose. Around 45% had not heard about antimicrobial resistance. Commensal *Escherichia coli* exhibited high prevalence of resistance to several antimicrobials considered important for human health, and multidrug-resistance was found in 79% of the bacteria isolates. Higher prevalence of resistance was observed on farms that administered prophylactic antimicrobials and on farms that treated the entire group of pigs in the event of disease.

In conclusion, although pig keeping was considered an important income source by the households studied, many employed practices that may contribute to pollution and increased health hazards to urban dwellers. For pig keeping continuing to exist in proximity to urban areas in countries like Cambodia, disease prevention interventions and improvements in manure management are needed.

Keywords: pigs, pig production, livestock, socio-economic, manure management, public health, antimicrobial use, antimicrobial resistance, Cambodia, urban/peri-urban

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Stadsnära djurhållning i Kambodja – Socioekonomiska fördelar och folkhälsorisker

Abstract

Djurhållning kan utgöra en viktig inkomstkälla som bidrar till förbättrad levnadsstandard och en väg ut ur fattigdom för många invånare i städer. Att hålla djur inom och runt omkring städer kan dock medföra ökade föroreningar och en ökad förekomst av sjukdomar som sprids mellan djur och människor (zoonoser). Syftet med den här avhandlingen var att tillhandahålla vetenskaplig information om olika socioekonomiska fördelar och hälsorisker associerade med hållning av grisar i städer och stadsnära områden i ett lägre medelinkomstland som Kambodja.

Intervjuer genomfördes i hushåll i Phnom Penh och gödselprover togs från deras grisar för detektering av zoonotiska patogener och för antibiotikaresistensanalys. Majoriteten av hushållen (60 %) ansåg att inkomsten från grisarna var en av hushållets viktigaste inkomstkällor. Sjukdomar och låga intäkter från slakt ansågs vara de största begränsningarna för hushållets djurhållning. Ingen av respondenterna nämnde dock några farhågor om potentiella hälsorisker. Det fanns en tydlig skillnad mellan djurarter med avseende på gödselhanteringen, där grisgödsel vanligen dumpades i miljön (46 %) medan kogödsel användes som gödningsmedel (66 %) ($P < 0,001$). Dumpning av grisgödsel var vanligare bland hushåll med lägre socioekonomisk ställning ($P < 0,001$) och bland hushåll som inte hade tillgång till jordbruksmark ($P < 0,001$).

Användningen av antibiotika baserades till stor del på djurhållarnas egna omdömen och 66 % justerade vanligtvis själva både dosering och behandlingstid. Runt 45 % hade inte hört talas om antibiotikaresistens. Höga resistensnivåer påvisades hos tarmbakterien *Escherichia coli*, varav mot flera antibiotika som anses vara viktiga för humansjukvården, och multiresistens återfanns i 79 % av proverna. Antibiotika-resistens var vanligare på gårdar som behandlade grisarna i förebyggande syfte och på gårdar som behandlade hela gruppen eller besättningen vid tecken på sjukdom.

Den här avhandlingen visar att, även om grisproduktion ansågs vara en viktig inkomstkälla bland djurhållarna, så kunde den bidra till både miljöföroreningar och ökade hälsorisker för andra invånare. Investeringar i sjukdomsförebyggande åtgärder och förbättringar inom gödselhanteringen är nödvändigt för att stadsnära djurhållning ska kunna bedrivas på ett säkert sätt i länder som Kambodja.

Nyckelord: grisar, grisproduktion, boskap, gödselhantering, folkhälsa, socioekonomisk, antibiotikaanvändning, antibiotikaresistens, Kambodja, urban/peri-urban

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Dedication

To my son, I hope you are watching down upon us as the prettiest little angel ever ♡

However difficult life may seem, there is always something you can do and succeed at.

Stephen Hawking

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Ström G, Andersson Djurfeldt A, Boqvist S, Albihn A, Sokerya S, San S, Davun H, Magnusson U* (2017). Urban and peri-urban family-based pig-keeping in Cambodia: Characteristics, management and perceived benefits and constraints. *PLoS ONE*, 12(8): e0182247.

II Ström G, Albihn A, Jinnerot T, Boqvist S, Andersson Djurfeldt A, Sokerya S, Osbjer K, San S, Davun H, Magnusson U* (2018). Manure management and public health: Sanitary and socio-economic aspects among urban livestock-keepers in Cambodia. *Science of the Total Environment*, 62:193-200.

III Ström G, Boqvist S, Albihn A, Fernström L-L, Andersson Djurfeldt A, Sokerya S, Sothyra T, Magnusson U* (2018). Antimicrobials in small-scale urban pig farming in a lower middle-income country – arbitrary use and high resistance levels. *Antimicrobial Resistance & Infection Control,* 7:35.

Papers I-III are reproduced with the permission of the publishers.

* Corresponding author.

The contribution of Gunilla Ström Hallenberg to the papers included in this thesis was as follows:

I Was involved in planning the study. Developed the questionnaire with input from the co-authors and performed the survey with the assistance of Cambodian colleagues. Analysed the results in collaboration with the supervisors and wrote the manuscript with regular input from the co-authors.

II Was involved in planning the study. Developed the questionnaire with input from the co-authors and performed the survey with the assistance of Cambodian colleagues. Performed some of the laboratory work and was responsible for the statistical analyses with input from the supervisors. Wrote the manuscript with regular input from the co-authors.

III Was involved in the research idea and planning of the study. Developed the questionnaire with input from the co-authors and performed the survey with the assistance of Cambodian colleagues. Performed the majority of the laboratory work and analysed the results under supervision. Wrote the manuscript with regular input from the co-authors.

Abbreviations

A. suum	*Ascaris suum*
AMR	Antimicrobial resistance
CI	Confidence interval
E. coli	*Escherichia coli*
ECOFF	Epidemiological cut-off
FAO	Food and Agriculture Organization of the United Nations
FTA	Flinders Technology Associates
GDP	Gross domestic product
GHG	Greenhouse gas
GNI	Gross national income
HGT	Horizontal Gene Transfer
MALDI-TOF MS	Matrix-assisted laser desorption ionization-time of flight mass spectrometry
MDR	Multidrug-resistance
MIC	Minimum Inhibitory Concentration
NAHPRI	National Animal Health and Production Research Institute, Cambodia
NaVRI	National Veterinary Research Institute, Cambodia
OIE	World Organisation for Animal Health
OR	Odds ratio
PCA	Principal component analysis
PCR	Polymerase chain reaction
S. enterica	*Salmonella enterica*
SLU	Swedish University of Agricultural Sciences
SVA	National Veterinary Institute, Sweden
T. suis	*Trichuris suis*
WHO	World Health Organization

1 Introduction

Urban and peri-urban livestock farming has emerged as a response to the surge in demand for animal-source foods, particularly by the urban population. Keeping livestock can make an important contribution to the livelihoods of poor urban dwellers and can offer pathways out of poverty. There are concerns, however, that livestock keeping in and around urban areas may add to environmental problems and an increased incidence of infectious microorganisms transferring between animals and humans (zoonoses). Although livestock keeping in urban and peri-urban areas is not a new phenomenon, there are still uncertainties about the socio-economic, public health-related and environmental consequences that these livestock systems may have in different ecological and cultural contexts.

1.1 Demographic changes and urbanisation

During the past century, the global population grew from 2.6 billion in 1950 to 7.6 billion in 2017 (UNDESA, 2017). This growth in population is expected to continue in coming decades, albeit more slowly, and recent projections have estimated that the global population will increase to 9.8 billion by 2050. Concomitantly, the world has experienced very rapid urbanisation of its population and in 2008 we passed the milestone where more than half the global population was estimated to reside in urban areas (Figure 1) (UNDESA, 2014; UNFPA, 2007). The urbanisation occurring in the past was largely limited to high-income countries, but today urbanisation is primarily taking place in low- and middle-income countries, especially in Africa and Asia, where the urban population is expected to double between 2000 and 2030 (UNFPA, 2007). This second wave of urbanisation is happening much faster than in the past, posing serious challenges for cities in poorer countries regarding construction of new infrastructure, including power, water and

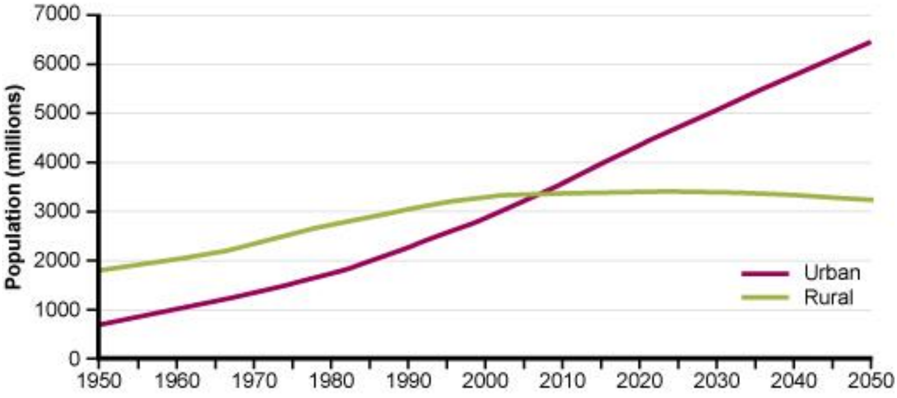

Figure 1. Urban and rural population of the world, 1950-2050 (UNDESA, 2014).

sanitation. Consequently, there is often a concentration of poverty in slum areas in the periphery of cities, where poor living conditions and limited access to basic sanitation facilities make the urban poor vulnerable to diseases and may reduce the livelihoods and well-being of this population (UNFPA, 2014; UNFPA, 2007). Although people in poorer countries often migrate to cities in search of better employment and education opportunities and improved access to healthcare and social services, poverty in these countries is growing faster in urban than in rural areas, a trend that has to some extent been neglected in the past (Satterthwaite, 2003).

1.2 The livestock revolution

Population growth, increasing income and urbanisation have fuelled a large global increase in the demand for animal-source foods, particularly by the urban population (Delgado, 2003; Delgado *et al.*, 2001; Popkin, 1999; De Haan *et al.*, 1997). This demand is expected to increase by 70% in the coming 30 years (GASL, 2015), and is projected to be particularly high in Southeast Asia, where the annual growth in consumption of animal-source foods is estimated to be 3.3% for the period 1997 to 2020 (Delgado *et al.*, 2001). As a response to this surging demand, the livestock sector has expanded considerably in recent decades, a response commonly referred to as the ‘livestock revolution’ (Delgado *et al.*, 1999). Along with this expansion, the livestock sector is also undergoing some drastic transformations, with livestock operations intensifying towards more large-scale and specialised systems, and with a shift from ruminants to monogastric animals (mainly pigs and poultry) (Gerber *et al.*, 2005), largely as a result of the better feed conversion efficiency in monogastrics (De Haan *et al.*, 1997). These livestock operations tend to be concentrated in areas with good market opportunities and with better access to cheap input supplies, conditions commonly found in the vicinity of urban areas. Small-scale producers located in these areas thereby face competition from more intensified livestock enterprises that may benefit from cheaper inputs, which may have negative effects on the livelihoods of smallholders (FAO, 2001a).

1.3 Benefits and opportunities of urban livestock

Keeping livestock has been an important part of urban agriculture since the start of civilisation. Although livestock keeping has been banned in some modern cities, it continues to emerge in others. Its purpose and the diversity of production forms that exist, however, are almost infinite, ranging from small-scale backyard subsistence farming to large commercial enterprises, mainly located in outer peri-urban areas (FAO, 2001a). This diversity complicates any attempts to make an overall assessment of urban and peri-urban livestock production and can easily lead to misunderstandings, since each of the different production forms has its own challenges and opportunities.

Keeping livestock provides many socio-economic benefits for urban and peri-urban producers. The increased demand for animal-source foods by the urban population offers great market opportunities for local producers and may constitute an important source of income for poor farmers (Poulsen *et al.*, 2015; FAO, 2001a). As the urban poor are often the most vulnerable to food insecurity, engagement in urban agriculture, including livestock production, may increase their resilience to food price fluctuations and might also enable access to more nutritious foods, such as meat and milk (Poulsen *et al.*, 2015; Warren *et al.*, 2015). Access to nutritious food is especially important for children and women of reproductive age (Black *et al.*, 2008) and several studies have identified consumption of animal-source foods as a protective factor against malnutrition (Darapheak *et al.*, 2013; Murphy & Allen, 2003; Neumann *et al.*, 2002a; Neumann *et al.*, 2002b). However, as products of animal origin are often expensive and may deteriorate in quality if not stored in appropriate conditions, it is often not economically feasible to slaughter large animals for household consumption. In this respect, the direct contribution of urban livestock production to the nutrition status of household members has been debated (Zezza & Tasciotti, 2010). An alternative situation might be that urban and peri-urban livestock production makes an indirect contribution to food security and nutrition by providing extra income that may enhance the household’s ability to purchase food (Poulsen *et al.*, 2015).

Besides providing an extra source of income and a potential source of nutritious foods, livestock production also offers efficient utilisation of resources that would otherwise go unexploited, such as the use of organic waste from markets and restaurants, or the use of by-products from urban industries (FAO, 2001a). These resources are often available in large quantities in urban areas and can be combined into nutritional feed ratios for animals. As poorer farmers may lack the resources to purchase commercial feedstuffs, such as concentrates, organic waste and by-products may provide a more economically feasible alternative.

The role and benefits of urban and peri-urban livestock production are not confined to production of food, but also comprise various social and cultural aspects. Livestock may constitute an important economic reserve and are often kept as a form of informal saving (Sansoucy, 1995). Keeping livestock can also be a means to empower urban poor, especially women, as it can often easily be combined with other household work. Studies have found that monogastric animals, such as pigs and poultry, are often found in high concentrations around urban centres and in densely populated areas in Asia (Gerber *et al.*, 2005). Monogastric animals are in many aspects more suitable to keep in these areas than ruminants and can easily be adapted to the family level, as they require little space and can be kept in backyard production systems. Feeding ruminants in urban areas can be difficult, as these species require a large proportion of roughage in their diet in order to ensure a functioning digestive system (Mertens, 1997). These kinds of feed are generally expensive in urban areas, since they are rarely produced inside cities and have to be transported from rural areas.

1.4 Public health hazards with urban livestock

Although livestock production may make an important contribution to the livelihoods of urban dwellers, keeping livestock in densely populated areas is controversial. There are rising concerns that livestock keeping can exacerbate environmental and public health problems in urban and peri-urban areas, including concerns about the transmission of zoonoses (Makita *et al.*, 2011; Bonfoh *et al.*, 2010; Kang'ethe *et al.*, 2007) and about sanitary and environmental hazards caused by the presence of manure (Bonfoh *et al.*, 2010; FAO, 2001a) (see Figure 2).

Southeast Asia has been identified as a global hotspot for emerging infectious diseases, particularly those of zoonotic origin (Coker *et al.*, 2011; Jones *et al.*, 2008). The risk of disease emergence may be largely influenced by socio-economic, environmental and ecological factors (Jones *et al.*, 2008), and transmission may be enhanced as a consequence of various livestock system characteristics and management practices. These include the close proximity and often unsanitary conditions in which human and animal populations operate (Carrique-Mas & Bryant, 2013; Randolph *et al.*, 2007), and poor risk perception and limited awareness about disease transmission among livestock keepers (Bonfoh *et al.*, 2010). Livestock production in Southeast Asia is predominantly practised in smallholder systems (Ahuja, 2013), where biosecurity measures are limited and economic resources may be scarce, conditions that favour the emergence and spread of infectious diseases (Grace *et al.*, 2012). As a consequence of their often close contact with livestock and limited availability to healthcare systems, the poor generally bear a disproportionally high share of the burden of these diseases (WHO *et al.*, 2006).

In many aspects, urban areas are particularly favourable for the emergence and spread of zoonotic diseases, as certain vectors (*e.g.* flies, mosquitos and rodents) thrive in areas with poor sanitation, standing water and access to food and other waste products (Bonfoh *et al.*, 2010). High concentrations of animals and humans facilitate disease transmission. Moreover, anthropogenic changes due to urbanisation can create new human-animal interfaces which may facilitate emergence and spread of infections (Hassell *et al.*, 2017; Bonfoh *et*

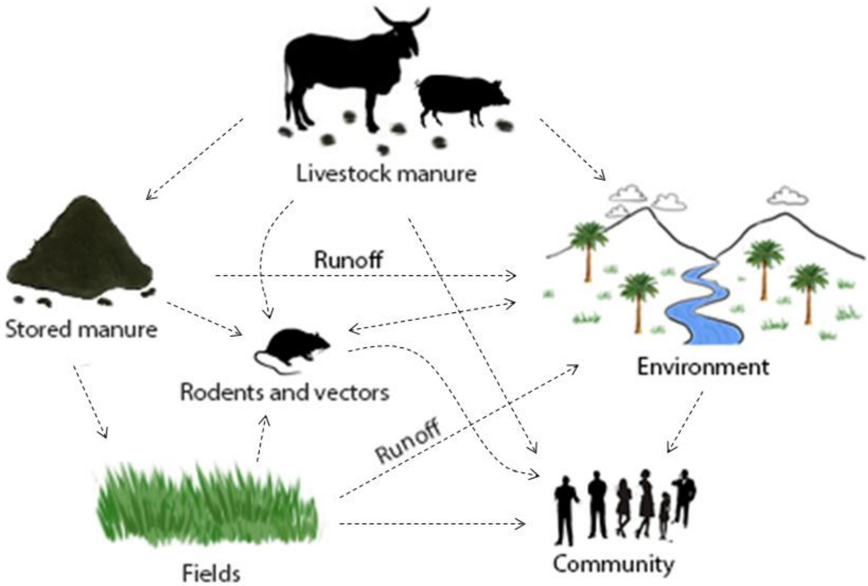

Figure 2. Schematic diagram illustrating some possible transmission pathways of pathogens in livestock manure. Transmission of pathogens via food and water, and via other animals and insects are not included (Paper II).

al., 2010). Furthermore, due to constraints on access land, there may be limited ways to dispose of livestock manure, which can worsen environmental conditions in these areas (FAO, 2001a). As improperly handled livestock manure has been identified as a risk factor for diarrhoeal diseases in humans (Pham-Duc *et al.*, 2014), and as a potential contaminator of food products (Ha *et al.*, 2008; Yajima & Kurokura, 2008) in Asian countries, improving manure management routines in both urban and rural areas is of great importance.

In addition to direct transmission of zoonotic diseases and introduction of zoonotic pathogens into the environment, there are also concerns that livestock manure may contain antimicrobial-resistant bacteria and resistance genes, thus contributing to the emergence and spread of antimicrobial resistance (AMR) (Xie *et al.*, 2018; Milinovich & Klieve, 2011; Venglovsky *et al.*, 2009). If these end up in the environment, antimicrobial-resistant bacteria may be a source of human infection and can serve as a reservoir of resistance genes for both pathogenic and non-pathogenic bacteria. Furthermore, antimicrobial residues in manure may have toxic effects on soil microorganisms and may also contribute to increased resistance (Venglovsky *et al.*, 2009).

1.5 Environmental impacts of urban livestock production

Keeping livestock in areas with little or no agricultural land may lead to negative impacts on the environment. These impacts include pollution of surface water, groundwater and soil, and are mainly associated with mismanagement of livestock manure and waste water, including improper disposal of livestock manure, over-fertilisation of crops and over-feeding of fish ponds (Gerber *et al.*, 2005).

A large proportion of the feed consumed by animals is excreted in the form of faeces and urine. These waste products contain nutrients and organic matter that may be valuable as fertiliser for crops but that could also have detrimental effects on the environment (Martinez *et al.*, 2009; Jongbloed & Lenis, 1998). Manure and waste water that are not handled or stored properly can reach the environment, lakes and rivers, either through leakage during storage, after manure application on land, or through direct discharge of manure to the environment. The excess nutrients may result in eutrophication with adverse effects on aquatic ecosystems and drinking water quality (Gerber *et al.*, 2005), while nitrate leaching from fertilised fields or manure storage units can be a source of pollution of ground water (Pastén-Zapata *et al.*, 2014). Furthermore, livestock keeping in urban areas, particularly involving cattle and other ruminants, requires a significant part of the feed to be imported from other regions, resulting in an imbalance in the crop-livestock system and exacerbating the problem of nutrient surpluses in urban areas (De Haan *et al.*, 1997).

In addition to the environmental impacts of eutrophication and nitrate leakage from the livestock sector, livestock keeping is also an important contributor to emissions of anthropogenic greenhouse gases (GHG), including carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), that may have a warming effect on the climate (Hristov *et al.*, 2013). At the global level, emissions from the livestock sector are estimated to account for around 14.5% of total anthropogenic greenhouse gas emissions (Gerber *et al.*, 2013), of which emissions from manure and manure management represent around 5-10% (Steinfeld *et al.*, 2006). Methane emissions from pig manure are estimated to account for almost half of all livestock manure emissions. During storage of manure, large amounts of methane and nitrous oxide may be emitted, especially from liquid manure (Steinfeld *et al.*, 2006). Through proper storage and application of manure, however, these emissions can be mitigated (Hristov *et al.*, 2013), although the economic feasibility of such methods is questionable and they may be difficult to implement, especially for resource-poor farmers (Teenstra *et al.*, 2014).

Due to ongoing climate change, including increasing temperatures and more extreme weather events like heavy rains and flooding, the negative impacts of livestock production are expected to increase, as a consequence of increased leakage of manure from fields and storage facilities, and of increased emissions of greenhouse gases (Thornton, 2010; Thornton *et al.*, 2009).

1.6 Manure management

Although inadequately handled livestock manure may contribute to pollution and constitute a source of zoonotic pathogens, properly managed manure represents a valuable source of nutrients for crop production and can reduce the need for inorganic fertilisers (Menzi *et al.*, 2010; De Haan *et al.*, 1997). However, if manure management is poor, nutrients and organic matter may be lost, thus decreasing the potential of manure as a fertiliser.

Today, technologies and knowledge on appropriate management of manure are available, although their implementation is often a challenge for various reasons, especially in low- and middle-income countries. A recent assessment of global manure management practices by Teenstra *et al.* (2014) identified four key barriers that could limit proper use and handling of livestock manure:

- Limited awareness and recognition of the importance of proper management of manure in order to reduce its negative effects on public health and the environment, and its potential contribution to food security through improved soil fertility and increased crop yields.
- Poor knowledge among farmers, together with inadequate infrastructure and limited knowledge support from the government or non-governmental organisations may impede the implementation of improved practices.
- Lack of incentives to improve manure management practices. This may be of particular importance for small-scale farmers that may lack the resources to undertake investments required for improvements.
- Ineffective policies or regulations that do often not support good manure management, but rather focus on the negative impacts of manure.

Proper manure management plays an important role in reducing the pathogen content in manure and in mitigating greenhouse gas emissions. Management of manure encompasses everything from excretion by the animal to collection, housing, storage, treatment, transport and finally application to land. It may also involve using manure as a building material and as a fuel for cooking. How manure is managed varies considerably throughout the world and within different livestock systems, *e.g.* animal species, production scale and intensity and land availability (Teenstra *et al.*, 2014). In low- and middle-income countries, livestock manure is either collected and stored in liquid form, together with urine and waste water, or in solid form, where faeces are collected and stored separately, although some farms discard all manure directly in the environment (Roubík *et al.*, 2018; Huong *et al.*, 2014b; Komakech *et al.*, 2014; Teenstra *et al.*, 2014; Cu *et al.*, 2012; Vu *et al.*, 2007). Discarding manure is often a result of poor knowledge among farmers or of limited means for proper storage and transportation of the manure (Teenstra *et al.*, 2014). Storage facilities commonly encompass storage in piles with or without a cover or in ponds or lagoons, with the latter usually being used for storage of liquid manure. According to studies in countries in Southeast Asia, farmers commonly place higher value on solid manure in general, and cattle manure in particular, potentially as a result of the greater difficulties in managing liquid manure compared with solid manure (Teenstra *et al.*, 2014).

1.6.1 Zoonotic pathogens in livestock manure

The term manure generally refers to animal excreta (*i.e.* urine and faeces), possibly mixed with bedding materials (Milinovich & Klieve, 2011). In addition, manure may contain water, excretions from the throat, skin, vagina and mammary glands, blood and pathogens (Pell, 1997). In this thesis, however, the term 'manure' primarily refers to faeces.

Livestock manure may harbour a large diversity of pathogens with zoonotic potential, including bacteria (*e.g. Escherichia coli, Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica*), parasites (*e.g. Ascaris suum*, *Cryptosporidium* spp., *Giardia* spp.), and viruses (*e.g.* Hepatitis E virus, rotavirus) (Christou & Kosmidou, 2013; Milinovich & Klieve, 2011; Sobsey *et al.*, 2006; Hunter & Thompson, 2005; Burton & Turner, 2003). These pathogens may pose a public health threat if they reach the environment or water bodies, where humans may be exposed through consumption of contaminated food and water (Venglovsky *et al.*, 2009; Tauxe, 1997). In order to reduce the hazards posed by pathogens in manure to public health, it is essential to reduce the pathogen burden within the animal, to decrease the amount of pathogens in the manure and to eliminate possible transmission routes of these pathogens, in the environment, in the community and within the household (Milinovich & Klieve, 2011).

Many of the zoonotic pathogens found in livestock manure are transmitted by the faecal-oral route (Milinovich & Klieve, 2011). Some of these transmission routes are illustrated in Figure 3. Transmission to humans may be facilitated by poor hygiene (Cairncross *et al.*, 2010) and through direct contact with infected animals (Klous *et al.*, 2016). In addition, contaminated food products are often a source of human infection (Tauxe, 1997). Another source of contamination of food products is use of contaminated water and manure to irrigate and fertilise fields (Guan & Holley, 2003; Tauxe, 1997). Contamination of water can occur through direct animal defecation, through manure discharge, through surface run-off from fertilised fields or through leakage from manure storage units (Williams *et al.*, 2008; Sobsey *et al.*, 2006).

The survival times of pathogens in manure vary considerably with respect to the microorganism monitored and its susceptibility to environmental conditions, including physical and chemical factors (Burton & Turner, 2003). Physical factors include the manure source, temperature, dry matter content, humidity and radiation, while chemical factors include pH, oxygen level and the presence of ammonia and other bactericidal compounds. Although there are various treatment methods designed to reduce the content of pathogens in manure, simply storing manure may lead to significant reductions

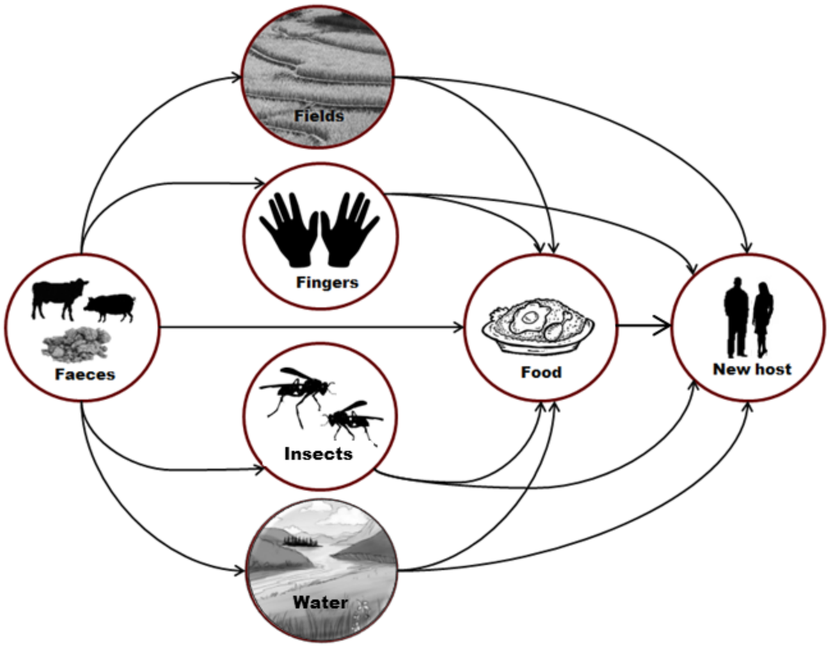

Figure 3. Illustration showing different faecal-oral transmission routes of faecal pathogens (modified from Wagner & Lanoix, 1958).

(Martens & Bohm, 2009; Martinez *et al.*, 2009; Burton & Turner, 2003). The main factors that influence inactivation of pathogens are temperature, exposure time and water activity, where higher temperature shortens pathogen survival (Martens & Bohm, 2009; Nicholson *et al.*, 2005). Elevated temperature during storage has been shown to be strongly influenced by storage design and management factors, such as size and depth of the storage facility and use of a cover (Rennie *et al.*, 2018).

1.6.2 Treatment of manure

The public health hazards posed by livestock manure have driven the development of various methods to reduce and eradicate potential pathogens in the manure. These treatment methods can be divided into chemical, physical and biological processes, which are sometimes used in combination (Milinovich & Klieve, 2011; Martens & Bohm, 2009). Chemical treatment includes treatment with lime, caustic soda or formalin, while physical treatment involves thermal treatment and drying, and biological treatment involves aerobic and anaerobic treatment. Some treatment methods that could

Box 1. Brief description of some methods that may be used for treating manure

Lime treatment. Addition of lime to manure will simplistically result in increased temperature and pH which may have detrimental effects on the viability of microorganisms. For the treatment to be effective, thorough mixing with the manure is essential (Burton & Turner, 2003). Using lime has been argued to be the cheapest chemical treatment method available (Martens & Bohm, 2009). However, for inactivation of more resistant microorganisms, including *Ascaris*, it is important to achieve high pH in the manure. In experimental studies, *Ascaris suum* eggs were inactivated in lime-treated slurry when exposed to pH > 12 for more than 3 months (Eriksen *et al.*, 1996).

Anaerobic digestion. Anaerobic digestion, for example in a biodigester, can substantially reduce the amount of pathogens in manure and the effluent is a highly valuable fertiliser (Holm-Nielsen *et al.*, 2009). The digestion process reduces the odours from manure and the gas produced can be used as fuel for cooking. Biodigestion is also a good mitigation strategy for methane emissions. In warmer climates, where methane emissions are higher, biodigestion can reduce methane emissions from manure by 75% (Hristov *et al.*, 2013). However, installation and management of biodigesters in low- and middle-income countries can be challenging and often requires financial subsidies and continuous knowledge support for proper digester operation and maintenance (Buysman & Mol, 2013)

Composting. Composting is an effective method to eliminate pathogens in manure, provided that the temperature in the whole material reaches above 55°C for more than two weeks (Martens & Bohm, 2009). It is essential that the manure is turned regularly, in order to ensure that all material is exposed to the higher temperatures. This method may not be suitable for treatment of liquid manure, as the limited amount of bedding material in liquid manure may not result in high enough temperatures due to lack of pores that enable air transport through the manure (Burton & Turner, 2003). When composting liquid manure, aeration may be used to increase the oxygen flow through the manure (Vinnerås, 2013), although the process is energy-consuming and expensive (Martinez *et al.*, 2009)

Vermicomposting. Using earthworms to compost manure and organic waste can be an efficient way of reducing and treating manure, as the worms can be used as animal feed (Lalander *et al.*, 2015b). Vermicomposting eliminates several zoonotic pathogens, including *Salmonella* spp., although additional treatment may still be required in order to achieve enough reductions in pathogen content.

Fly larvae composting. Like vermicomposting, fly larvae (*e.g*. black soldier fly) can be used to convert manure into valuable protein for use as animal feed (Lalander *et al.*, 2015a). The process can lead to drastic reductions in bacteria within the *Enterobacteriaceae* family, such as *E. coli* O157:H7 and *Salmonella* spp., and in viruses (Lalander *et al.*, 2015a; Erickson *et al.*, 2004), although reductions in other bacteria (*e.g. Enterococcus* spp.) and *Ascaris suum* eggs are limited or insignificant (Lalander *et al.*, 2015a).

be of interest for small-scale urban and peri-urban livestock keepers are described briefly in Box 1. These methods, however, may only have a marginal effect on spore-forming bacteria (Manyi-Loh *et al.*, 2016).

The most appropriate system for eradicating pathogens in manure depends to a large extent on the physical properties of the manure (*i.e.* solid or slurry), on the pathogens to be eliminated, and on whether the manure is to be applied on land or not (Milinovich & Klieve, 2011). Economic factors also need to be considered, as treatment of manure is generally not associated with any financial return to the farmer. Furthermore, many of the technological solutions developed in high-income countries are not implementable in small-scale urban livestock systems in low- and middle-income countries, partly due to high initial investment costs and to their suitability for more intensive farming systems.

1.7 Antimicrobial use and resistance

The increasing emergence and spread of antimicrobial resistance is a growing threat to human and animal health, and has largely been attributed to medically irrational use of antimicrobials[1] in humans and food-producing animals (Ventola, 2015; WHO, 2015). Irrational use of antimicrobials includes overuse, misuse and underuse, and is often a consequence of poor knowledge among physicians, veterinarians and farmers, or of economic incentives (WHO, 2012). Any usage of antimicrobials has the potential to stimulate resistance (Levy & Marshall, 2004). Paradoxically, both overuse and underuse of antimicrobials play an important role in the emergence of AMR, underuse commonly characterised by inappropriate choice of drug, substandard drugs and poor adherence to dose and treatment duration (WHO, 2012).

Antimicrobial resistance (here referring to resistance in bacteria, *i.e.* antibacterial resistance) arises when bacteria can survive and multiply in the presence of antimicrobials (Levy & Marshall, 2004). Resistance can be either intrinsic in bacteria (*i.e.* naturally occurring and independent of previous antimicrobial exposure) or acquired by previously susceptible bacteria (Zhang & Feng, 2016; Alekshun & Levy, 2007). Bacteria can acquire resistance through different mechanisms, including uptake of genes or plasmids encoding resistance, so called horizontal gene transfer (HGT), or through spontaneous

[1] An antimicrobial is a substance of natural or synthetic origin that kills or inhibits the growth of microorganisms (bacteria, virus, parasites or other) whereas an antibiotic is technically a substance that is naturally produced by a microorganism. Moreover, an antibacterial is a substance used to treat bacterial infections but the term antibiotic is commonly used instead. In this thesis the term ‘antimicrobial’ is used although generally referring to ‘antibacterial’.

mutations (Levy & Marshall, 2004; Schwarz *et al.*, 2001). Furthermore, there are various mechanisms of resistance, some targeted at the antimicrobial itself and others targeted at how the antimicrobial is transported within the bacterial cell, or altering the intracellular target of the antimicrobial. Resistance in bacteria can emerge and spread through natural selection of resistant bacteria under antimicrobial pressure, when susceptible strains are killed or inhibited, or through dissemination of resistant bacteria or resistance genes (Schwarz *et al.*, 2001).

Antimicrobial resistance will not only have severe impacts on human health, therapeutic failure will also have serious repercussions within veterinary medicine, where diseases will lead to suffering for animals and problems with animal welfare (WHO, 2015; Bengtsson & Greko, 2014). Treatment failure will also lead to substantial consequences for the global livestock sector, with a direct impact on the livelihoods of the fraction of the world's population that is entirely dependent on livestock (Laxminarayan *et al.*, 2016; FAO, 2011; Sansoucy, 1995). Estimates indicate that livestock production in low-income countries may decline by up to 11% as a result of AMR (World Bank, 2017a).

The consequences of AMR are felt worldwide, although the problems are often more severe in low- and middle-income countries, which generally suffer from a greater burden of infectious diseases (World Bank, 2017a; Laxminarayan *et al.*, 2016; WHO, 2012). Many of these countries are also characterised by poor infrastructure, poor governance and inadequate sanitation, factors that have been shown to be associated with higher levels of AMR (Collignon *et al.* 2018). Furthermore, in many low- and middle-income countries, limited access to antimicrobials and effective treatment are often major problems, particularly in rural areas, and more people die from limited access to antimicrobials than from AMR (Laxminarayan *et al.*, 2016). However, the reported increase in antimicrobial consumption in recent decades has predominantly occurred in low- and middle-income countries, a development which is thought to be driven by rising incomes and urbanisation (Klein *et al.*, 2018). There is some evidence that antimicrobial use is primarily increasing in urban areas (Laxminarayan *et al.*, 2013), potentially as a consequence of better availability of antimicrobials than in rural areas, where deploying antimicrobials might be difficult. Although this increase mainly refers to antimicrobial use within human medicine, there is reason to believe that the availability and use of antimicrobials for animals reared in the proximity of urban areas has also increased, partly as a result of the agglomeration of livestock production in these areas (FAO, 2001a).

1.7.1 Antimicrobial use in livestock

The emergence of AMR is believed to be partly caused by inappropriate use within the livestock sector (Chantziaras *et al.*, 2014; Marshall & Levy, 2011; Aarestrup, 2005). In the livestock sector, antimicrobials are widely administered to food-producing animals not only for treatment of diseases but also, more controversially, for disease prevention and growth promotion purposes (FAO, 2016; Bengtsson & Greko, 2014; WHO, 2012; Marshall & Levy, 2011; Aarestrup, 2005). Globally, estimates suggest that total antimicrobial consumption in animals is twice that in humans, although with large geographical differences (Van Boeckel *et al.*, 2015; Aarestrup, 2012). An estimate of global antimicrobial consumption in food-producing animals is presented in Figure 4. Consumption of antimicrobials in food-producing animals is expected to increase by 67% between 2010 and 2030, with the increase predominantly occurring in low- and middle-income countries and within the pig and poultry sectors (Van Boeckel *et al.*, 2015). Two-thirds of this increase can be attributed to increased numbers of food-producing animals, as a result of growing demand for animal-source foods, and one-third to a shift in farming practices, with a transition towards more intensified, large-scale farming systems. In large-scale farming systems, animals are generally kept in larger groups and at higher densities, conditions that may favour emergence and spread of infectious diseases (Tilman *et al.*, 2002). In these settings, antimicrobials might thus be used to a higher extent to mitigate the impact of diseases.

Within the livestock sector, besides being used for treatment of existing diseases, antimicrobials are often used as a prophylaxis to prevent diseases (FAO, 2016). This is common practise during periods when the risk of infection is higher, such as when animals of different origin are assembled, or

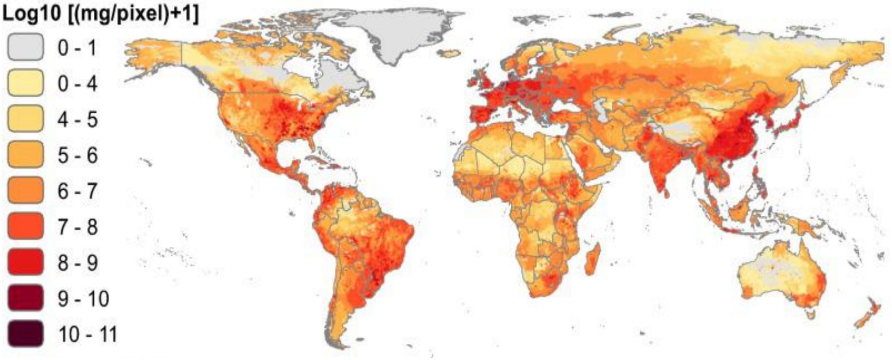

Figure 4. Estimated global antimicrobial consumption in food-producing animals (mg per 10 km^2 pixel) (Van Boeckel *et al.*, 2015).

at weaning or moving of younger animals (WHO, 2012; Wegener, 2003; Schwarz *et al.*, 2001). In addition to therapeutic and prophylactic use, antimicrobials are sometimes administered to food-producing animals in low doses in feed, for growth promotion purposes (FAO, 2016; Wegener, 2003; Schwarz *et al.*, 2001). Such sub-therapeutic use may promote the development of AMR by supporting changes in gene expression, horizontal gene transfer, and mutagenesis (Viswanathan, 2014; Laureti *et al.*, 2013). In addition, the use of substandard and falsified antimicrobial drugs may further increase the risk of AMR emergence (WHO, 2017b). These drugs may be of poor quality, either originally or as a result of poor handling, and may contain less or none of the active substances.

The quantities and classes of antimicrobials used in food-producing animals are generally insufficiently controlled globally and only a limited number of countries have monitoring programmes in place (Werner *et al.*, 2018; OIE, 2016; WHO, 2012). The data available for the EU show large differences between member countries in the amount of antimicrobials sold per animal unit (EMA & ESVAC, 2017). However, corresponding data for low- and middle-income countries are often lacking (WHO, 2012), which makes it difficult to perform risk analyses and to assess the potential impacts of interventions aimed at promoting medically rational use of antimicrobials within the livestock sector in these countries (Werner *et al.*, 2018).

1.7.2 Emergence and spread of antimicrobial resistance from livestock and the environment

The emergence and spread of AMR is a concern in veterinary medicine, as antimicrobials are necessary for treatment of infectious diseases in pets and food-producing animals, to ensure good animal welfare and secure global food production (FAO, 2016; OIE, 2016; WHO, 2012). Emergence of resistance is largely caused by the selection pressure imposed by the use of antimicrobials, where the presence of antimicrobials creates ideal conditions for resistance to emerge (Wellington *et al.*, 2013; Schwarz *et al.*, 2001).

Resistant bacteria of animal origin, such as antimicrobial-susceptible bacteria, can be transmitted to humans through direct contact and via consumption or handling of contaminated food products (WHO, 2015; Venglovsky *et al.*, 2009; Schwarz *et al.*, 2001). Other transmission routes include exposure through the environment if contaminated manure is used to fertilise crops and if manure or resistant bacteria reach the groundwater through leakage or run-off (Wellington *et al.*, 2013; Marshall *et al.*, 2009). Furthermore, several studies have found increasing levels of AMR in the environment, on fields and in fish and fish ponds fertilised with manure (Dang *et al.*, 2011a; Sapkota *et al.*, 2007; Petersen & Dalsgaard, 2003; Sengelov *et al.*, 2003; Petersen *et al.*, 2002). The presence of antimicrobial residues in manure and in the environment can also contribute to the emergence of resistance by imposing selection pressure on the bacterial population (Wellington *et al.*, 2013) and increased concentrations of antimicrobial residues have been detected on land that has received livestock manure (Guo *et al.*, 2018). These residues may eventually be degraded, but their persistence largely depends on their polarity and water solubility and the characteristics of the surrounding environment (Zhang *et al.*, 2019; Wellington *et al.*, 2013; Sarmah *et al.*, 2006; Kolz *et al.*, 2005). Moreover, globalisation, including human travel and increased international trade in animals and food products, may have a considerable impact on the dissemination of AMR (Robinson *et al.*, 2016; Laxminarayan *et al.*, 2013; MacPherson *et al.*, 2009; Aarestrup *et al.*, 2008).

In order to reduce the emergence and spread of AMR, it is essential to reduce the use of antimicrobials within both human and veterinary medicine. The most effective means to achieve a reduction in the use of antimicrobials is to ban the use of antimicrobials as growth promoters and to reduce the need for antimicrobial treatment, generally through improvements in animal health (OIE, 2016; WHO, 2012). This can be achieved through better disease prevention measures and through immunisation against prevalent infections (Clift & Salisbury, 2017).

Interventions that restrict the use of antimicrobials in food-producing animals have been shown to result in reductions in the prevalence of resistance in bacteria isolated from animals (Tang *et al.*, 2017). Such interventions have been suggested to have most effect in reducing use when targeted at pig and poultry farms, which generally administer higher levels of antimicrobials than cattle farms, as a result of high animal densities and treatment on herd level. However, restricting the use of antimicrobials in the livestock sector will be a challenge, particularly in low- and middle-income countries where regulations can be weak and compliance with existing guidelines is often difficult to monitor (FAO, 2016). In many of these countries, antimicrobials are easily accessible over-the-counter and no prescription from a qualified physician or veterinarian is required, factors that may contribute to the overall excessive use of antimicrobials (WHO, 2012). Furthermore, drug promotion by the pharmaceutical industry may contribute to irrational use of antimicrobials and may also influence prescribing behaviour, particularly as many physicians and veterinarians receive part of their income from the sale of pharmaceuticals and may therefore be reluctant to reduce their prescribing of antimicrobials (Wegener, 2012; WHO, 2012).

In 2015, the World Health Organization (WHO) issued the Global Action Plan on Antimicrobial Resistance (WHO, 2015), developed in close collaboration with the Food and Agriculture Organization of the United Nations (FAO) and the World Organisation for Animal Health (OIE). Together, these organisations have formed a tripartite collaboration to tackle the rising threat of AMR. The Global Action Plan strongly emphasises that the global AMR crisis calls for an effective multisectoral 'One Health' approach, involving human and veterinary medicine, environmental organisations and consumers, and encourages countries to develop their own National Action Plans to combat antimicrobial resistance. To support implementation of the Global Action Plan for the food and agriculture sector, the FAO developed the FAO Action Plan on Antimicrobial Resistance 2016-2020 (FAO, 2016), and the OIE launched the OIE Strategy on Antimicrobial Resistance and the Prudent Use of Antimicrobials (OIE, 2016), addressing animal health.

1.8 The case of Cambodia

Cambodia is a tropical country located in Southeast Asia, with a population of approximately 16 million inhabitants (World Bank, 2016). During the past two decades, Cambodia's economy has been among the fastest growing in the world, with more than a six-fold increase in national gross domestic product (GDP) in the period. The country has recently passed the threshold of gross national income (GNI) set by the World Bank Group and is now categorised as a lower middle-income country (World Bank, 2016). Despite this development, Cambodia is still one of the poorest countries in Southeast Asia and, although the poverty rate declined from 47.8% in 2007 to 13.5% in 2014, the majority of the population barely moved above the poverty line and around 4.5 million people in Cambodia remain near-poor (World Bank, 2018).

Cambodia is currently undergoing rapid change. Though being a predominantly rural, agrarian country, Cambodia is now urbanising rapidly as a result of natural population growth and rural-urban migration, with an estimated annual urban growth rate of 2.6% (World Bank, 2016). Around 21% of the population now reside in urban areas, mainly the capital Phnom Penh, which has a population of approximately 1.8 million inhabitants (Figure 5). Urbanisation generally offers numerous socio-economic benefits, including better employment and education opportunities (Satterthwaite *et al.*, 2010; Moore *et al.*, 2003). However, the unplanned and unregulated urbanisation seen in Cambodia has led to neglect of the environment and has resulted in

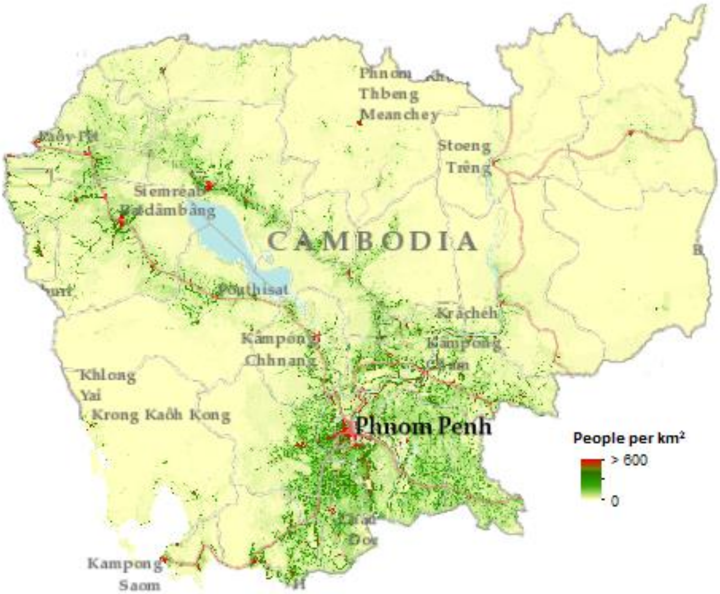

Figure 5. Population density in Cambodia displayed as people per km^2. Open Development Cambodia (www.opendevelopmentcambodia.net).

growing areas of slums and informal settlements, with an increasing economic and spatial disparity among the urban population (UNFPA, 2014). Although the country has numerous development projects in progress, most of these interventions target the population in rural areas, limiting the benefits for the urban poor.

People in Cambodia have a long tradition of keeping livestock in their backyards and livestock are deeply embedded in customs and society in the country. Livestock keepers are predominantly smallholders, although the production systems that co-exist range from extensive subsistence farming to semi-intensive commercial production and large-scale commercial production (Burgos *et al.*, 2008). There are some descriptions in the literature regarding livestock production systems and characteristics in Cambodia (Osbjer *et al.*, 2015; Sovann & San, 2010; Burgos *et al.*, 2008; Samkol *et al.*, 2006), but there is no accessible information about how and under what premises livestock are kept in the urban areas.

In 2016, Cambodia's first veterinary legal framework was endorsed, including regulations and guidelines on veterinary practices to ensure animal health, rules governing the import and export of live animals and animal products, and regulations for enforcement of sanitation standards at slaughterhouses. This legal framework, however, did not include any specific regulations concerning keeping livestock in urban areas. Furthermore, in 2018 the Multi-Sectoral Action Plan on Antimicrobial Resistance in Cambodia 2018-2022 was formulated, with the aim of guiding the government, partners and donors to identify priority areas for work and collaboration to promote rational use of antimicrobials in order to contain AMR in Cambodia. At the time of writing this thesis, however, the Action Plan had not yet been launched.

2 Aim of this thesis

The overall aim of this thesis was to obtain science-based information on different public health and socio-economic aspects of keeping livestock in urban and peri-urban areas in low- and middle-income countries, using the capital of Cambodia as a case. The main emphasis was on pig keeping, although keeping other livestock species and interaction between different species were also considered.

Specific objectives were to:

- Describe family-based urban and peri-urban pig keeping in Phnom Penh, Cambodia and identify different benefits and constraints perceived by the farmers.

- Survey practices related to management and handling of livestock manure, specifically with regard to potential public health hazards.

- Determine the occurrence in pig manure of indicator pathogens of importance for human health, using methods easily implemented in field conditions.

- Identify routines related to the use of antimicrobials in pigs and evaluate farmers' knowledge and attitudes with regard to antimicrobial treatment and resistance.

- Determine the prevalence of antimicrobial resistance in commensal *Escherichia coli* isolated from pigs and identify potential associations between antimicrobial treatment regimes and resistance.

3 Considerations on materials and methods

This section provides an overview of the materials and methods used in Papers I-III, with comments and considerations on why the methods were chosen. Detailed descriptions are presented in the individual papers.

3.1 Study area and study design

The studies included in this thesis [Papers I-III] were all conducted in urban and peri-urban areas of Phnom Penh (Figure 6). As there is no uniform definition of what constitutes an urban or peri-urban area, the entire city province was used as the study unit. In general, the ambient characteristics gradually change from urban to peri-urban with increasing distance from the city centre. Furthermore, urban areas are more densely populated and have more developed infrastructure than peri-urban areas, but have limited availability of agricultural land and other natural resources (FAO, 2001b). With increased migration and urbanisation, the city usually expands, with areas shifting from peri-urban towards urban. The continual transition in these areas makes it difficult to draw a clear distinction between urban and peri-urban. Therefore, given the current rapid development and expansion in Phnom Penh, it was decided to include both urban and peri-urban areas in the studies reported in Papers I-III and to not distinguish between the two.

Papers I and II are based on data collected in November and December 2014 and in February 2015, while Paper III is based on data collected in January and February 2017 (Figure 7). In all three studies, a cross-sectional study design was used and the aim was to include all households keeping pigs under family-farm conditions in Phnom Penh, defined as being owned and operated by the family members (FAO, 2014). In order to find all households, a census using the non-random sampling technique of snowball sampling

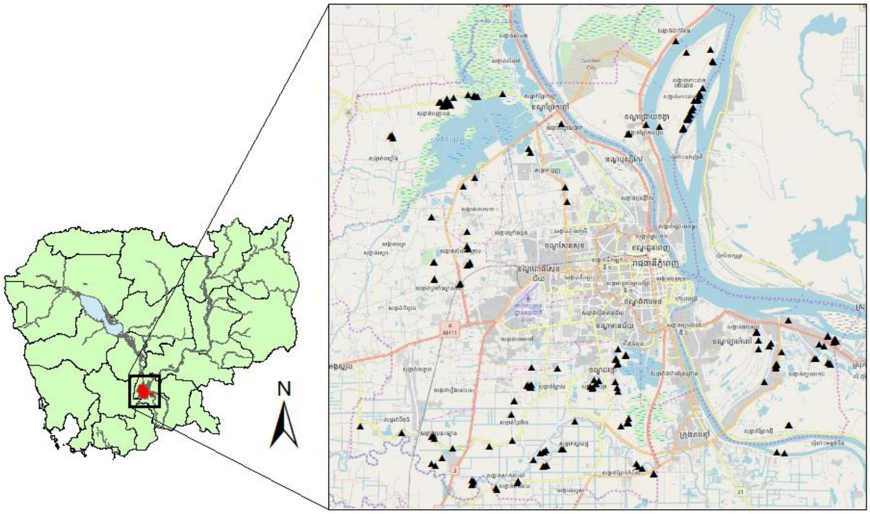

Figure 6. Map of Cambodia, showing the study area. Households included in Papers I and II are plotted. Open Development Cambodia (www.opendevelopmentcambodia.net) and © OpenStreetMap contributors (openstreetmap.org).

(Faugier & Sargeant, 1997) was performed prior to the first field collection period [Papers I and II]. Although this sampling procedure is efficient in finding participants when no information on their whereabouts exists, it has some disadvantages. The main disadvantage for the studies included in this thesis was that the method could not ensure that all households matching the inclusion criteria (*i.e.* households that kept pigs under family-farm conditions) were identified and approached. However, given that no information regarding farming households in Phnom Penh existed, this was considered the best approach in order to find the households.

Initially, 267 households were located through the census. All these households were visited and were included in the studies presented in Papers I and II based on whether the family members were at home when the household was visited and on their willingness to participate, resulting in a total of 204 households. For Paper III, the same 267 households were visited. Due to falling profitability, however, many households had stopped raising pigs and only 81 of the 267 households could be included in the third study. In addition, 10 farms that had started pig production since the census was performed were found during the fieldwork and were included in Paper III, resulting in a total of 91 households. Before each interview [Papers I-III], participating households were informed about the purpose of the study and assured that their participation was voluntary and anonymous, and consent was obtained from all

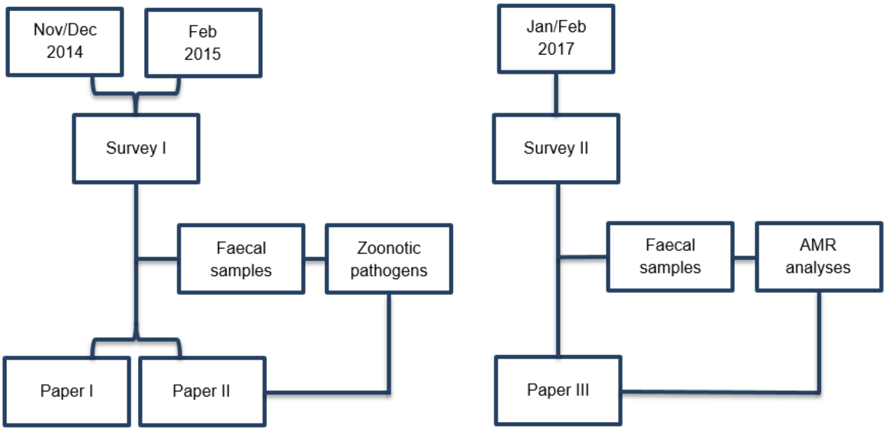

Figure 7. Schematic diagram showing how, and at what time-points, the different studies presented in Papers I-III were conducted.

respondents. After the interviews were conducted, all participants were given a towel and a bar of soap as a gift for their involvement.

3.2 Questionnaires

In all studies included in this thesis, information obtained in face-to-face interviews was used, which were all performed by the author with the assistance of one interpreter, Mr. Vor Sina. All interviews were carried out in the Khmer language and the respondents' answers were immediately translated into English. Papers I and II are based on information from the same questionnaire, whereas a separate questionnaire was used for Paper III. The questionnaire used in Papers I and II included questions on household demographics, socio-economic characteristics, practices related to animal husbandry and manure management, and the respondents' experiences and perceptions about their pig production. The aim was to interview the person responsible for the daily management of the pigs, such as feeding and cleaning. The questionnaire used in Paper III focused on routines for antimicrobial (*i.e.* antibacterial) use in the pigs and was targeted at the person in the household who was responsible for treating sick pigs.

Although the questionnaires were pre-tested prior to the studies, and the questions were thoroughly discussed together with two Khmer-speaking colleagues in order avoid any mistranslations or misinterpretations, there is always a risk of information being lost across the language barrier. It is also possible that respondents may want to provide what they believe to be desired or more socially accepted answers, even though this may not correspond with actual practices, so-called social desirability bias (Fisher, 1993). In order to avoid this, the aim was to not include any leading questions in the questionnaires. Another important aspect that needs to be considered when performing interviews is the problem of recall bias, which has been shown to be influenced to some extent by the design of the questionnaire and the motivation of the respondent (Coughlin, 1990).

3.3 Calculation of socio-economic position [Papers I and II]

In order to define the socio-economic position of households, an asset-based wealth index was constructed for each household. The calculations were based on information obtained through the questionnaire regarding construction of dwelling, availability of agricultural land and ownership of consumer durables and other household belongings. Principal component analysis (PCA) was used to assign individual weights to the different variables, representing each variable's contribution to the household's socio-economic index, as described in Vyas and Kumaranayake (2006). The calculations were performed in IBM SPSS Statistics for Windows, version 22.0 (Armonk, NY: IBM Corp.). This kind of asset-based approach is often preferred when measuring household wealth, rather than using monetary measures, as the latter often vary between seasons and the household's income is generally more variable than its consumption (Howe *et al.*, 2008). Collection of asset data may also be more reliable as it suffers from less recall bias (Sahn & Stifel, 2003). One limitation with the method that needs to be considered, however, is that information about ownership does not capture the quantity or quality of the asset (Falkingham & Namazie, 2002). Therefore, when constructing the index, assets that were broken (*i.e.* considered unrepairable by the respondents) were not included, though no adjustments could be made regarding the quantity of the assets, as the number of a specific item could potentially depend on the number of people living in the household.

Principal component analysis is one of the approaches that can be used when attempting to present socio-economic data. Other methods involve adding up the number of assets in the household, but this assumes that all assets are weighted equally in the index (Howe *et al.*, 2012). Although there are limitations of PCA, the alternative methods also have disadvantages, and the choice of data included in the calculations has been shown to have a greater influence than the specific method used (Howe *et al.*, 2008).

3.4 Collection and handling of faecal samples [Papers II and III]

Faecal samples were obtained from pigs for analysis of zoonotic pathogens [Paper II] and for antimicrobial susceptibility testing [Paper III]. The intention was to make a random selection of pens and pigs although, due to the prevailing circumstances, the sampling procedure could not be viewed as random in its true definition (Dohoo & Waltner-Toews, 1985). This sampling procedure was decided upon for practical reasons, and there is no reason to believe that it did not generate a representative sample of the population. In Paper II, fresh manure from up to four pig pens in each household was collected and samples were also taken from the manure storage unit, if available. From the pens, a pooled sample of about 200 g manure from up to five different fresh droppings was collected. From the manure storage unit, a total sample of 200 g was collected from a depth of approximately 30 cm. Subsamples of about 20 g were then transferred to sterile plastic containers and transported on ice to the National Veterinary Animal Health and Production Research Institute (NAHPRI) in Phnom Penh, formerly known as the National Veterinary Research Institute (NaVRI), for processing and analysis.

In Paper III, sterile cotton swabs were used to collect fresh faeces from three healthy pigs in each household. The swabs were placed in sterile plastic tubes containing Amies medium (Amies PS Viscose, Sarstedt) and transported on ice to NAHPRI, where they were stored at 2-8°C until analysis was performed within 48 hours.

All samples obtained for Papers II and III were kept refrigerated during transportation and storage, in order to avoid impairing the survival of the pathogens. For the same reason, analysis was performed within a day or two after sampling.

The total sample size for the respective studies was determined based on logistical and financial constraints. As no prior information was available regarding the number of pigs or pig pens kept by the households, an upper limit of samples obtained per household had to be decided in advance. In Paper II, however, very few households had more than four pig pens to be sampled. The sampling strategy of taking a pooled sample from the pen was chosen as it is an efficient method for detecting infection within a group of pigs, rather than sampling individual animals (Arnold & Cook, 2009). When investigating the prevalence of AMR in *E. coli* (as done in Paper III), simulation studies have shown that when the total number of samples is limited or pre-specified, the number of farms included should be prioritised over the number of samples obtained per farm, as this will result in increased precision of the analyses on population level (Yamamoto *et al.*, 2014).

3.5 Detection and analysis of zoonotic pathogens [Paper II]

In Paper II, faecal samples were analysed for zoonotic indicator pathogens. The bacterial species *Salmonella enterica* subsp. *enterica* and the helminths species *Ascaris suum* and *Trichuris suis* were selected for this study, as they are pathogens with zoonotic potential that commonly colonise or cause infection in pigs, with the ability to survive for months in favourable environmental conditions (McCarthy *et al.*, 2015; Katakam *et al.*, 2014; Pittman *et al.*, 2010). These organisms can thus be used as a proxy for the presence of similar pathogens in manure.

3.5.1 *Salmonella enterica*

Selective culture is a widely used standardised technique for detection of *Salmonella* in food, animal and environmental samples (ISO, 2017). This method may not always be feasible, however, as it requires laboratory materials and resources that may not be available in all settings. In Paper II, culture on-site was not possible for logistical reasons, and therefore inactivation of the live bacteria on Flinders Technology Associates (FTA) cards (Whatman®, Clifton, NJ) was chosen as an alternative method. This approach enabled transport to Sweden where polymerase chain reaction (PCR) was used to detect DNA fragments of the inactivated bacteria.

All faecal samples were initially prepared at NAHPRI by transferring 1 g portions from each sample to 5 mL plastic tubes and vortexing with 4 mL of water for 30 seconds. From the supernatant, 100 µL were pipetted onto FTA® cards (Figure 8). The cards were then transported at room temperature to the National Veterinary Institute (SVA) in Sweden.

At SVA, one punch of sample material was removed from the centre of the sample spot on each FTA® card and rinsed with 50 µL elution buffer. Detection of *S. enterica* was performed with real-time PCR, according to Hoorfar *et al.* (2000). Samples with a threshold-crossing value (C_T) ≤ 38 were considered positive. The sensitivity of the PCR method was evaluated by preparing a 1:10 dilution series of *S. enterica*, where each sample was added to FTA® cards and analysed with PCR. In addition, 100 µL of prepared *Salmonella* suspension were spread on blood agar plates and the number of colonies was counted after incubation.

3.5.2 *Ascaris suum* and *Trichuris suis*

For detection of the intestinal parasites *A. suum* and *T. suis*, a modification of the McMaster flotation technique was used, as described by Urquhart *et al.* (1996) (Figure 9). In flotation techniques, a solution with a higher specific gravity than the organism to be detected is used, which allows the organism to rise to the surface while the faecal debris sinks to the bottom. The analyses were performed at NAHPRI, where the standard procedure is not to include a centrifugation step in the analysis, due to limited human resources and logistical constraints. However, according to Pereckiene *et al.* (2007), who evaluated different modifications of the McMaster technique for detection of *A. suum* eggs in pig faeces, incorporation of a centrifugation step would lead to increased sensitivity of the analysis. Hence there is a risk that some samples were categorised as false-negative in the study.

Samples were kept refrigerated until analysis, to minimise the risk of embryonation of the parasite eggs, which would make them undetectable with the flotation technique. According to previous studies, there is very limited embryonation of *A. suum* eggs at temperatures below 11°C (Seamster, 1950) and 5°C (Kim *et al.*, 2012).

3.6 Antimicrobial susceptibility analysis [Paper III]

For antimicrobial susceptibility analysis, *Escherichia coli* was chosen as the indicator bacterial species. *Escherichia coli* is a commensal bacterial species commonly present in the intestinal tract of humans and animals. During antimicrobial treatment, the bacteria are exposed to the selective pressure of antimicrobials and may therefore constitute a reservoir of resistance genes for pathogenic bacteria (van den Bogaard & Stobberingh, 2000). Consequently, the level of resistance in *E. coli* is considered to be a good indicator of the general level of resistance in a population and a predictor of the emergence of resistance in pathogenic bacteria. Thus, *E. coli* is often used in resistance monitoring programmes as an indicator for resistance in Gram-negative bacteria (EFSA, 2018; Swedres-Svarm, 2017).

After arrival at the laboratory at NAHPRI, the samples were refrigerated before culturing, which was performed within two days. Faecal samples were cultured on MacConkey agar, from which presumptive *E. coli* isolates were sub-cultured on Tryptone Soya Agar (TSA) (Figure 10) and tested for production of tryptophanase (indole). One indole-positive isolate per sample was selected for further analysis. Because the laboratory at NAHPRI did not have the capacity to perform antimicrobial susceptibility analysis, bacteria

Figure 8. FTA® cards prepared with faecal samples for analysis of *Salmonella enterica*.

Figure 9. Sample preparation for detection of intestinal parasites, using the McMaster flotation technique.

Figure 10. Culture material for isolation of *Escherichia coli* bacteria.

isolates were frozen and transported to the Swedish University of Agricultural Sciences (SLU) at -70°C. Prior to analysis at SLU, all bacteria samples were confirmed as *E. coli* by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). For susceptibility analysis, broth microdilution was performed (CLSI, 2014), using microdilution susceptibility panels (Sensititre™ EUVSEC, Thermo Scientific). The minimum inhibitory concentration (MIC) for 14 antimicrobials (ampicillin, azithromycin, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, colistin, gentamicin, meropenem, nalidixid acid, sulfamethoxazole, tetracycline, tigecycline, trimethoprim) was visually determined and epidemiological cut-off values (ECOFFs) (EUCAST, 2017) were used to differentiate between wild-type and non-wild-type strains of the bacteria isolates (referred to as susceptible and resistant in the text).

3.7 Spatial cluster analyses [Paper II]

The geographical position of each participating household was recorded using a handheld global positioning device (GPS-Garmin eTrex H). To investigate whether there were any spatial variations in the management of pig manure, spatial scan statistics were performed using SaTScan™ software, version 9.3 (www.satscan.org). The Bernoulli purely spatial model (Kulldorff, 1997) was used to detect clusters with a higher likelihood of pig manure being discarded to the environment (*i.e.* dumped). Households that did not dump pig manure were used as controls in the analysis. The maximum spatial cluster size was set as 20% of the population, and distribution and statistical significance of the clusters were investigated through Monto Carlo simulation and Gumbel approximation, with 999 replications. However, although two significant clusters were detected, it should be remembered that the spatial scan statistics provided through SaTScan™ are sensitive to user parameter choices (Chen *et al.*, 2008), which means that the results obtained in this analysis should be interpreted with caution.

ArcMap™ 10.3.1 software was used to monitor the spatial distribution of the clusters and to produce maps of the distribution of the participating households. Open source base maps were obtained from © OpenStreetMap contributors (openstreetmap.org) and Open Development Cambodia (opendevelopmentcambodia.net).

3.8 Statistical analyses

The statistical analyses reported in Papers I-III were performed using SAS for Windows 9.4 (SAS Institute Inc., Cary, NC). Descriptive statistics were calculated to present demographic and farm characteristics, manure management practices, routines related to antimicrobial use in the pigs, and respondents' knowledge and attitudes regarding antimicrobial use and AMR. To test for differences in size of production (*i.e.* number of pigs) with type of production system and with gender differences in responsibility for the daily management of the pigs [Paper I], Kruskal-Wallis test and pairwise two-sample Wilcoxon test were performed. An independent two-sample t-test was used to test whether the socio-economic index differed between male- and female-headed households. Univariable regression analyses were used to test potential associations between socio-economic index and categorical variables, such as reported disease occurrence, manure management, household practices and respondents' awareness of zoonotic disease transmission [Papers I and II]. Pearson's chi-square (or Fisher's exact test when there were less than five observations per group) was used to test associations between different categorical variables, including household practices, education level, manure management and routines for antimicrobial treatment. In Paper II, variables that were associated ($P < 0.2$) with the categorical variable 'dumping pig manure' were included in a multivariable regression model, and manual step-down elimination was applied at a significance level of $P < 0.05$. In Paper III, generalised linear mixed models were used to investigate associations between farm characteristics, management factors, age group of pigs and the prevalence of resistance. To account for clustering, 'farm' was included in the model as a random effect, and the confounding variable 'age group of pigs' was included in all models as a fixed effect.

4 Main results and discussion

4.1 Household and farm demographics

As Paper III is based on a separate field study than Papers I and II (see Figure 7), the households in these studies are described separately.

In Papers I and II, the median household size in the 204 households was 5.0 persons (5th and 95th percentiles: 3.0 and 7.0). The households most commonly (65%) consisted of an adult couple, younger than 60 years, with young or grown-up children living in the house. In the majority of the households (96%), a man was designated as the household head by the family members. In the nine households that were female-headed, these women were widows (89%) or did not have a husband (11%). Higher education (defined as upper secondary school or above) had been completed by 40% of the men and 21% of the women ($P < 0.01$) (Table 1). The socio-economic index calculated for each household did not differ between male- and female-headed households and was not correlated with the education level.

All 204 households included in Papers I and II kept pigs, as this was an inclusion criterion. Furthermore, 68 (33%) kept cattle, 157 (77%) kept chickens and 66 (32%) kept ducks (Table 1). The number of pigs kept by the households at the time of the visit ranged from 1 to 200, with a median of 12 pigs (5th and 95th percentiles: 2 and 57). As the number of pigs kept by a household at a certain time-point may vary considerably during the year, the respondents were also asked to estimate the annual 'inflow of pigs', *i.e.* the number of pigs that had been born within, or purchased by, the household during the past 12 months, in order to get a better estimate of the size of each household's pig production. The median annual inflow of pigs was 30 (5th and 95th percentiles: 8 and 100), and most households (81%) had an inflow of 50 pigs or less. Based on the results presented in Paper I, it appears that urban and peri-urban pig keepers in Cambodia in average keep more pigs than those in rural areas, where most households keep less than 5 pigs (Osbjer *et al.*, 2015; Saroeun *et al.*, 2007). This difference might be a result of households closer to urban areas being more market oriented, which might also be reflected by the fact that all households in the present study kept pigs mainly for sale, rather than for subsistence.

The reported main occupations of the household heads are listed in Table 1. Employment outside the household was reported as the main occupation for 32% of the household heads, while pig keeping, sometimes in combination with production of rice wine, was reported as the main occupation for 41% of the household heads. As the majority of the households would be considered smallholders, pig production would perhaps not be considered a fulltime job and most households reported being engaged in several income-generating activities.

In Paper III, based on interviews with 91 households, the median number of pigs present at the time of visit was 20 (5^{th} and 95^{th} percentiles: 7 and 81 pigs). Among these participants, higher education, defined as commencing but not necessarily completing studies at upper secondary school, was reported by 31% of the men and 9% of the women. An explanation for the apparent lower education level in this study is that in Papers I and II the education level of all household members who had finished their education (*i.e.* including the education of children) was included in the calculations, while only the education of the male and female household heads was included in Paper III. As the education system in Cambodia has undergone substantial improvements in recent decades, higher education is more common among the younger generation (World Bank, 2017b), which might explain the difference between the studies. These different approaches to assessing education level were chosen because in Papers I and II the purpose was to investigate potential differences in education level influenced by socio-economic position, while in Paper III the purpose was to investigate differences in practices related to antimicrobial use that might be explained by the education level of the farmers.

According to the respondents in Papers I and II, women were most commonly responsible for the daily management of the pigs, such as feeding and cleaning (see Table 1), which is consistent with findings reported in other studies in the region (Osbjer *et al.*, 2015; Chittavong *et al.*, 2013). In Paper III, however, men were more often involved in the treatment of sick animals in the households (66%). Studies on urban and peri-urban pig farming in Cameroon (Fualefac *et al.*, 2014) and on pig farming in Indonesia (Nugroho *et al.*, 2015) have found that men are often responsible for more strenuous tasks associated with pig production, while women are involved in daily operations. This was to some extent reflected in Papers I-III.

Table 1. *Household and farm characteristics of the pig keepers participating in Papers I and II (n = 204)*

Variable	Category	*n*	%
Main occupation of household head[*]	Employment outside household	65	32
	Combined pig and rice wine production	49	24
	Pig keeping	34	17
	Mixed crop and livestock	26	13
	Fishing	14	7
	Crop production	5	2
	Cattle production	2	1
	Poultry production	2	1
	None	7	3
Completed higher education[**]	Males[a] (*n* = 337)	135	40
	Females[b] (*n* = 302)	63	21
Responsibility for daily management of the pigs	Male	57	28
	Female	88	43
	Shared	59	29
Responsibility for antimicrobial use	Male household head	60	66
	Female household head	15	16
	Other household member	7	8
	Veterinarian/Animal health worker	9	10
Access to agricultural land	Yes	98	48
	No	106	52
Keep cattle	Yes	68	33
	No	136	67
Keep chickens	Yes	157	77
	No	47	23
Keep ducks	Yes	66	32
	No	138	68
Housing pigs	Confined in pens or metal crates	199	98
	Tied up	5	2
Housing cattle (n = 68)	Tied up	64	94
	Combination tied up and free roaming	4	6
Housing chickens (n = 157)	Confined in pens	2	1
	Free roaming	56	36
	Combination confined and free roaming	99	63
Housing ducks (n = 66)	Confined in pens	4	6
	Free roaming	31	47
	Combination confined and free roaming	31	47

[*]Household head defined by the household members.

[**]Higher education defined as upper secondary school and above.

[a-b] Means within columns with different superscripts differ ($P < 0.01$).

To summarise, urban and peri-urban households in Cambodia mainly keep pigs for commercial purposes and households generally combine pig production with various other occupations. Women are most commonly responsible for the daily management, such as feeding and cleaning, whereas men are responsible for treatment of sick pigs.

4.1.1 Household practices and awareness of zoonotic diseases [Paper II]

Almost half of the households included in Paper II (46%) responded that they did not wash their hands with soap and water after handling livestock or livestock manure. There was a tendency (P = 0.09) for hand washing to be more commonly reported in households with a higher socio-economic position. Positive associations between improved precautionary practices, such as hand washing, and better socio-economic conditions have been reported by others, *e.g.* Rabbi and Dey (2013) found that the practice of hand washing was associated with access to improved facilities and hand washing materials, such as soap. They also discovered a positive association with the education level of the household head, although there seemed to be a knowledge-to-action gap among the participants. In Paper II, there were no significant associations between household practices and level of education.

More than 45% of the respondents stated that they did not think diseases could be transmitted between animals and humans. However, there were no significant associations between awareness of zoonotic diseases and hand washing, or between awareness of zoonotic diseases and the socio-economic position of the household.

In Paper I, the survey showed that pigs were kept confined by all participating households, which is an important preventive measure for disease transmission (FAO, 2010). This is a practice that seems to be more common in urban areas of Cambodia than has been reported for rural areas (Osbjer *et al.*, 2015; Saroeun *et al.*, 2007). According to the respondents in Paper I, however, small piglets were often allowed to roam freely in the household, as were poultry and companion animals, which may facilitate disease transmission (FAO, 2013).

In conclusion, there seemed to be limited awareness among the urban and peri-urban pig-keeping households surveyed about the potential health risks associated with livestock and about important measures for disease prevention. Thus there is clearly a need for more awareness-raising campaigns targeting livestock-keeping populations or the population in general.

4.2 Manure management [Paper II]

The results obtained in Paper II revealed a difference in the management of pig and cattle manure. Around 46% of the households in the study reported dumping pig manure, while 31% used it as a fertiliser for rice or vegetable production and 18% sold it or gave it away (Figure 11). In contrast, of the 68 households that also kept cattle, 7% reported dumping cattle manure, while 66% used it as a fertiliser and 21% sold it or gave it away. Notably, 27% of the households that reported using, selling or giving away cattle manure still reported dumping pig manure, indicating that respondents placed higher value on cattle manure. This has previously been suggested by Teenstra *et al.* (2014). A few respondents explained that pig manure was more difficult to collect and handle, possibly due to the fact that pig manure is often handled as slurry, where wastewater, urine and faeces are mixed and stored together, which generates large volumes of manure. The difficulty for farmers in managing liquid manure has been raised previously by Cu *et al.* (2012), who suggest that transportation is the main barrier to proper recycling. In Paper II, statistical analyses revealed that households with a lower socio-economic position were more likely to dump pig manure ($P < 0.001$; odds ratio (OR) 1.7; 95% confidence interval (CI) 1.3-2.4). Moreover, the multi-variable regression model identified lack of carts for transportation of manure and lack of

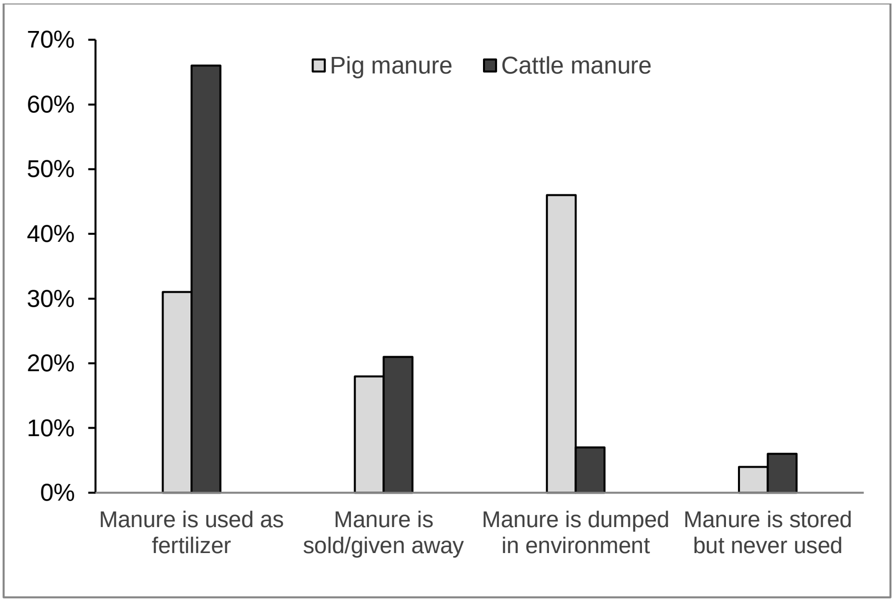

Figure 11. Management (%) of pig and cattle manure among urban and peri-urban livestock keepers in Phnom Penh (pig-keeping households n = 204; cattle-keeping households n = 68) (Paper II).

Table 2. *Results of the multivariable logistic regression analysis investigating potential explanatory factors for the practice of dumping pig manure (Paper II)*

Explanatory factor	β	OR (95% CI)	*P* value
No land	0.7	4.0 (1.9-8.5)	< 0.001
No cart	0.6	3.2 (1.5-6.9)	< 0.01
Not aware of zoonotic disease transmission	0.4	2.4 (1.1-5.3)	< 0.05
Do not collect manure manually	1.4	16.1 (7.3-35)	< 0.0001

OR = odds ratio; CI = 95% confidence interval.

agricultural land on which to apply the manure as potential explanatory factors for poor handling (see Table 2). These findings support the suggestion that resource-poor farmers may be more likely to adopt poor practices, as they have limited means to invest in the equipment necessary for proper handling and disposal of manure (Wei *et al.*, 2016; Teenstra *et al.*, 2014). It is also possible that limited awareness of the potential health threats that arise from livestock manure may contribute to hazardous practices among resource-poor farmers, as it was found in Paper II that farmers who were not aware of zoonotic disease transmission were more likely to dump pig manure ($P < 0.05$; OR 2.4; CI 1.1-5.3). In contrast, Vu *et al.* (2007) found that even though pig farmers in Vietnam were often aware of the negative consequences of poorly handled and discarded manure, many still adopted these hazardous practices. Those authors suggested that, as these farmers receive a considerable part of their income from pig production, they might be reluctant to complain to the interviewer or consider the possible adverse effects their production may have on the surrounding environment.

The spatial scan statistics detected two significant clusters in the Phnom Penh area with an increased likelihood of pig manure being dumped; one in an area where access to agricultural land was limited and the other around the island Koh Dach (Figure 12). These findings support the suggestion that lack of agricultural land is a major constraint for proper manure management and may also indicate that households located close to open water sources are more likely to dump manure. As the availability of agricultural land is often limited in more urbanised areas (FAO, 2001b), it is possible that peri-urban farmers will experience increasing problems with land access and manure disposal as a result of urban growth.

Inadequately stored livestock manure may pose a potential public health hazard if pathogens spread from the storage unit. This risk may be enhanced if manure is stored in piles or ponds with no covering, as animals and insects could potentially access the manure and manure might be washed away following heavy rains and flooding. In Paper II, around 68% of the households

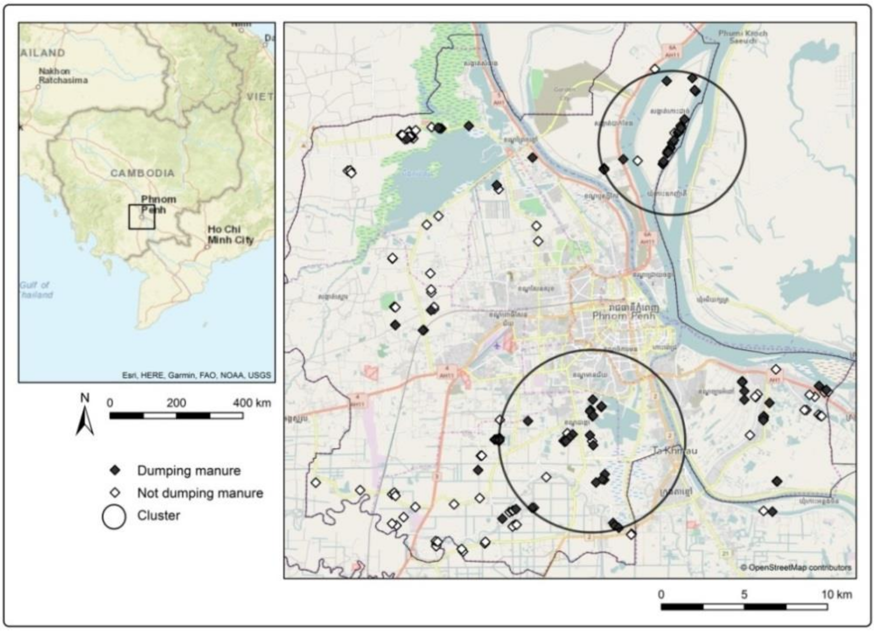

Figure 12. Spatial clusters of households in Phnom Penh, Cambodia, with a higher likelihood ($P < 0.001$) of dumping pig manure, as determined by spatial scan statistics (SaTScan software) (Paper II).

reported storing livestock manure, commonly in a pond or pile with no fences (Figure 13), but none of the households used any kind of coverage of the storage unit. Farmers in Vietnam have a tradition of using clay to cover stored manure (Dang *et al.*, 2011b), a practice that may also reduce the smell of the stored manure (Burton & Turner, 2003). Figures 14-16 show some different alternatives for storage and disposal of the manure used by the households surveyed in Paper II.

Most households that used the manure for crop or vegetable production stored it during the dry season and applied it to their fields at the beginning of the rainy season. This is a concern, as heavy rains enhance the risk of surface runoff (IAEA, 2008). Around 5% of the households, however, reported that they stored the manure during the dry season and then dumped it when the rainy season began, and several respondents explained that the stored manure would 'disappear' from the manure storage unit during the rainy season. These findings imply that pathogens from livestock manure may be spread from these households, either through leakage from the manure storage unit or from the manured fields. The way in which manure was stored and managed by the participants may not have contributed substantially to a reduction in faecal pathogens that can be harmful to humans. Although most households stored the

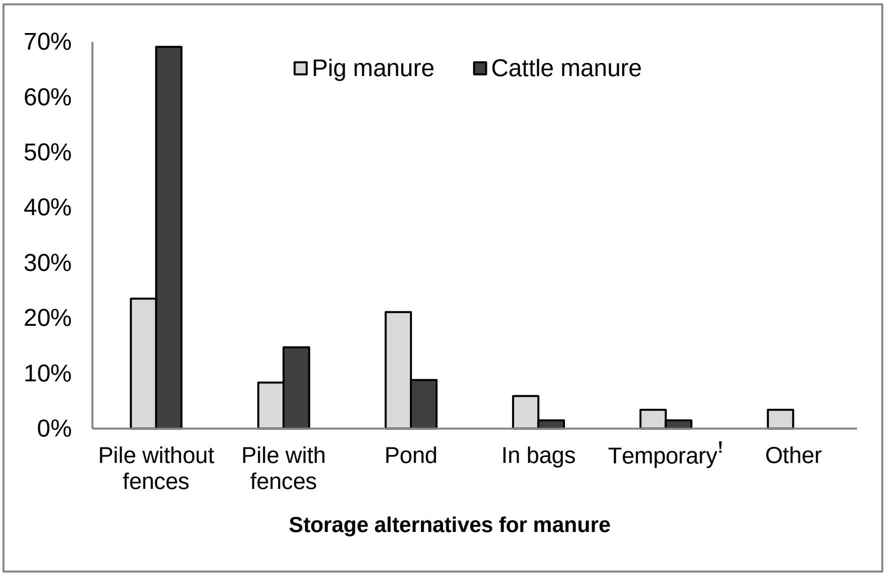

Figure 13. Storage alternatives for pig manure (*n* = 204) and cattle manure (*n* = 68) used by urban and peri-urban livestock keepers in Phnom Penh, displayed as percentage of all households. Some households used several storage alternatives (Paper II).
!Manure was taken to the fields a couple of times a week.

manure before it was applied as a fertiliser, they continuously added fresh manure to the storage unit, a practice that may cause regrowth of pathogens in the stored manure (Vinnerås, 2013). Furthermore, few farmers reported treating the manure (17%), a practice that was not associated with the socio-economic position of the household. According to the respondents, treatment was mainly applied to reduce the smell and number of flies. Treatment methods mentioned by the respondents included spraying the manure with calcium carbonate (6%), drying the manure (5%), mixing with ash (4%), using a biodigester (3%), spraying the manure with disinfectant (2%), and burning the manure (1%). Although Paper II did not evaluate these different storage and treatment methods, the efficiency of the treatment methods used by the households to eliminate pathogens may be queried. For example, in order for chemical treatment to be effective, thorough mixing of the manure is essential (Vinnerås, 2013; Burton & Turner, 2003). Furthermore, inactivation of pathogens by biodigestion is largely dependent on the retention time, which might be too short if manure is added continuously. It is also important that the manure which goes into the biodigester does not contain high levels of toxins, such as antimicrobials that could kill bacteria, as there is some evidence that this might impede the digestion process (Poels *et al.*, 1984). It was found in

Figure 14. Household kitchen area from where pig manure is directly washed out into the fields.

Figure 15. Manure from the pig pen is directly discarded into the lake.

Figure 16. Pig manure is dried and stored in bags during the dry season.

Paper III that antimicrobials were used by the majority of the households, implying that there is some risk of impaired functionality of the biodigestion progress, although the concentrations normally found in manure may not be high enough to make a substantial impact. Although anaerobic digestion in a biodigester may be a good way of managing livestock manure on small-scale farms in low- and middle-income countries, there is a limited reduction in faecal zoonotic pathogens in the effluent from these systems (Huong *et al.*, 2014a; Huong *et al.*, 2014b), implying that further investigations and optimisations of such systems are needed.

In Paper II, the management and disposal of animal faeces were mainly investigated. In retrospect, the management of urine and wastewater should also have been included since, if poorly managed, these waste fractions may constitute environmental and public health hazards (WWAP, 2017). Furthermore, in many lower-income countries, management of human wastewater is challenging, particularly in peri-urban areas where scarcity of land makes proper treatment difficult (Parkinson & Tayler, 2003). This might call for a joint approach for the management of human and animal wastewater in these areas.

In conclusion, current manure management practices undertaken by urban and peri-urban households in Cambodia may have detrimental consequences for public health. The results obtained in this thesis indicate that resource-poor households are more likely to adopt poor practices and that a large proportion of these households lack the means for proper storage and transport of manure.

4.3 Zoonotic pathogens in pig manure [Paper II]

4.3.1 *Salmonella enterica*

Only the samples obtained during the first data collection period (November and December 2014) were analysed for *S. enterica*. In total, 155 samples from 95 of 107 households were obtained; 133 from pig pens and 22 from manure storage units. The results from the PCR analyses are presented in Table 3. The occurrence of *S. enterica* in this study was lower than expected, as other studies on pig farms in Southeast Asia have found the occurrence in faecal, slurry and environmental samples to be in the range of 63 to 95% (Tu *et al.*, 2015; Huong *et al.*, 2014a; Dorn-In *et al.*, 2009). One explanation could be the relatively low sensitivity of the methodology used in the present study, as the PCR method was estimated to have a detection limit of between 1.5×10^4 to 1.5×10^5 bacterial cells per mL manure. According to Tanaka *et al.* (2014),

Table 3. *Occurrence of* Salmonella enterica, Ascaris suum *and* Trichuris suis *in faecal samples obtained from pig pens and manure storage units*

	Salmonella enterica		*Ascaris suum*		*Trichuris suis*	
	n/N*	%	n/N	%	n/N	%
Household	13/95	13.7	3/176	1.7	6/176	3.4
Pig pen	13/133	9.8	4/251	1.6	6/251	2.4
Manure storage	2/22	9.1	1/36	2.8	0/36	0
All samples	15/155	9.7	5/287	1.7	6/287	2.1

*N = total number of samples in each subgroup.

manure from *Salmonella*-infected pigs often contains as little as 7.1×10^1 colony-forming units (CFU) per gram of manure. It is therefore possible that Paper II included several false-negative samples. Ideally, the detection method should have involved an enrichment step of the samples before being transferred to the FTA® cards. However, enrichment on-site could not be arranged at the time of the study and the approach with direct inactivation of the bacteria on FTA® cards was chosen to enable transport to Sweden.

In conclusion, *S. enterica* was present among pigs in urban and peri-urban areas of Cambodia, although the occurrence was lower than expected. Despite the low occurrence, these bacteria may constitute a public health hazard through direct contact or through contamination of the environment and food products. Culture of faecal samples is needed to confirm these results.

4.3.2 *Ascaris suum* and *Trichuris suis*

For analysis of *A. suum* and *T. suis,* 287 samples were obtained from both data collection periods in Papers I and II, generating samples from 176 of the 204 households. Of these, 251 were taken from pig pens and 36 from manure storage units. The occurrence of parasites was low (Table 3) compared with that reported in other studies in Cambodia. These other studies were conducted in rural areas and detected *A. suum* in 13% (Inpankaew *et al.*, 2015) and 26% (Schär *et al.*, 2014) of pigs and *T. suis* in 20% of pigs (Schär *et al.*, 2014). This difference might be a consequence of all households surveyed in Paper II keeping their pigs confined, a practice that is known to result in reduced prevalence of parasitic infection in pigs (Agustina *et al.*, 2017). Confinement of pigs is less common among rural households. It is also possible that the frequency with which manure was removed from the pens had an effect on the occurrence of parasitic infection. We found that the majority (98%) of the farmers surveyed cleaned their pig pens more than twice a day, while only 30% of rural households report removing manure daily (Schär *et al.*, 2014).

The low occurrence of parasites found in Paper II may also be explained by the fact that 71% of the households reported deworming all their pigs. A further 19% reported deworming some of their pigs, whereas 10% did not deworm any pigs. However, due to the low number of positive samples, no associations between parasitic infection and deworming practices could be determined.

To summarise, there was a lower occurrence of intestinal parasites in the pigs included in Paper II than in studies conducted in rural areas of Cambodia. These differences might be a consequence of different management routines between urban and rural areas.

4.4 Antimicrobial use [Paper III]

In at least 78 of the 91 households (86%) included in Paper III, antimicrobials were routinely administered to the pigs. Only four households responded that they did not use any antimicrobials, mostly because their pigs were rarely sick. In nine households, the respondent did not remember the names of the pharmaceuticals used and no drug packages were present in the household, so it was not possible to determine with certainty that antimicrobials were used by these farmers. However, based on their responses to follow-up questions on disease symptoms and treatment regimes, it was concluded that at least some of the pharmaceuticals they used most likely contained antimicrobials.

In total, at least 70 different brands of antimicrobials were used by the farmers in Paper III. The antimicrobial substances most commonly mentioned or kept by the farmers are presented in Table 4, while Figures 17 and 18 show some antimicrobials used on two of the participating farms. Farmers most commonly received antimicrobials from a veterinarian or an animal health worker and many farmers reported unrestricted access to antimicrobials. Antimicrobial use was mainly based on farmers' previous experiences and on drug sellers' advice based on symptoms described by the farmer, rather than being based on veterinary diagnostics. Other studies conducted in the region report similar findings, with antimicrobials being used arbitrarily in pig and poultry production (Om & McLaws, 2016; Dang *et al.*, 2013). Although most farmers in Paper III reported discussing antimicrobial treatment with a veterinarian, many also said that they could often decide on treatment themselves, based on previous experience. In fact, 66% reported frequently deviating from the instructions provided by adjusting both drug dose and duration of treatment, based on the severity of the disease.

Table 4. *Antimicrobials most commonly mentioned or kept by the farmers surveyed*[*] *(Paper III)*

Antimicrobial	Number of households
Amoxicillin[†]	56
Ampicillin[†]	21
Colistin[†]	27
Enrofloxacin[†]	19
Gentamicin[†]	29
Lincomycin	14
Oxytetracycline	17
Penicillin G[†]	15
Spectinomycin	6
Streptomycin[†]	9
Sulfonamides	16
Thiamphenicol	6
Trimethoprim	9
Tylosin[†]	38

[*] This list is not complete, as most farmers could only name a few of the antimicrobials that were used, and it does not include potential antimicrobials in the feed concentrate.
[†] Antimicrobial considered critically important for human medicine according to WHO (2017a).

Table 5 presents self-reported practices related to antimicrobial use in the pigs. Antimicrobials were used for treatment of sick pigs by all households that presumably used antimicrobials, and at least 14 households reported administering antimicrobials as a prophylactic, most commonly to sows and piglets after farrowing. In 37% of the households, a concentrate that contained antimicrobials was used, mainly for pigs younger than three months. However, the type and concentration of antimicrobials were only specified on the concentrates used on three farms, whereas the other concentrate packages used were only labelled that they contained 'Antibiotics', which is obviously a concern.

Besides overuse and misuse of antimicrobials, the sale and use of substandard and falsified drugs are common problems, particularly in low- and middle-income countries that lack proper legislation on manufacturing and distribution of pharmaceutical products (WHO, 2012). Some respondents in Paper III reported that they had found that local antimicrobials (*i.e.* produced in Cambodia or imported from neighbouring countries) were less effective than antimicrobials imported from Europe. The respondents also mentioned that they commonly adjusted the dose depending on the origin of the drug, *e.g.* they used a lower dose or shorter treatment duration for European antimicrobials. These results might thus indicate that the local antimicrobials referred to were less effective, although this is only based on the opinions and reflections of the interviewees.

Almost half of the farmers (47%) commonly sold pigs during or directly after antimicrobial treatment and did not adhere to the recommended withdrawal periods. This is a public health concern, as antimicrobial residues might remain in the meat at slaughter if withdrawal periods are not respected (Lee *et al.*, 2001).

Figure 17. Pig farmer (left) showing the antimicrobials he uses to the interpreter Mr. Vor Sina (right).

Figure 18. Antimicrobials used at one of the participating farms in Paper III.

Table 5. *Practices related to antimicrobial use by the farmers that presumably used antimicrobials (n = 87) (Paper III)*

	Category	n	%
How do you administer antimicrobials to the pigs?	Injections when sick	87	100
	In feed/orally when sick	18	21
	In water when sick	1	1
	To sows after farrowing	5	9
	In feed routinely	8	9
If only some pigs are sick, to which pigs do you administer antimicrobials? (n = 86)	Only the sick pigs	65	76
	All pigs[b]	21	24
Do you administer antimicrobials as prophylaxis?	Yes	14	16
	No	72	83
	Unsure[c]	1	1
Does the feed concentrate contain antimicrobials? (n = 91[d])	Yes	34	37
	No	8	9
	Don't know	42	46
	Don't use concentrates	7	8
Do you sometimes give human medicines that contain antimicrobials to the animals?	Yes	9	10
	No	77	89
	Don't know	1	1
Do you sometimes end treatment prematurely if the animal gets better?	Yes	57	66
	No	30	34
What do you do with antimicrobials that are left (and have expired)? (n=84)	Throw away to	36	43
	Bury	20	24
	Burn	3	4
	Take back to	8	10
	Throw to the person	11	13
	Keep at home	4	5
	Don't know	2	2
Do you have a withdrawal period (according to instructions) between antimicrobial treatment and slaughter/trader collecting animals? (n=81)	Yes	8	10
	No	38	47
	Don't know	1	1
	Never been sick around time of slaughter	32	40
	Other	2	2

[a]Calculated based on number of farms that kept sows.

[b]This category does not necessarily includes sows, as some farmers only used traditional medicines to treat sows.

[c]Some substance was added to the feed routinely but the respondent did not remember the name.

[d]Calculated based on all 91 farms in the study.

In many low- and middle-income countries, such as Cambodia, there are often no authorities that monitor the compliance with withdrawal times and animal products are not routinely analysed for residues. According to two Vietnamese studies investigating the prevalence of antimicrobial residues in pork from local markets, 5.5% of the meat samples contained tetracycline (Duong *et al.*, 2006) and 8.8% contained sulfamethazine (Yamaguchi *et al.*, 2015). Furthermore, Duong *et al.* (2006) reported that pork meat containing residuals was more commonly obtained from markets in low-income areas, suggesting that meat products from sick animals often end up in these areas.

According to the respondents in Paper III, 43% dumped the remaining pharmaceuticals in the environment once the expiry date had passed, whereas 24% buried them and 13% threw them to the person collecting waste. This implies that discarded antimicrobials may be present locally at high concentrations in the environment, where they may contribute to resistance development (Wellington *et al.*, 2013).

To summarise, antimicrobials were used arbitrarily in pig production by the majority of the households surveyed. Besides therapeutic treatment, many farmers administered antimicrobials through the feed or as a prophylactic. Furthermore, other practices that may increase the risk of resistance development, such as group or herd treatment and the practice of discarding expired antimicrobials to the environment, were commonly occurring.

4.5 Knowledge and attitudes related to antimicrobial use and resistance [Paper III]

Although most respondents had very limited knowledge about antimicrobials and their mode of action, the majority (99%) were of the opinion that antimicrobials were necessary in order to keep the animals healthy (Table 6). However, almost half of the respondents (45%) had never heard of antimicrobial resistance. Respondents that had attained a higher level of education (defined as commencing but not necessarily completing studies at upper secondary school) were more likely to have heard about AMR (78% vs. 49%; $P = 0.036$; OR 3.6; CI 1.1-12.0). This was also more often reported by male respondents (74% vs. 39%; $P < 0.001$; OR 4.4; CI 1.8-10.9), and by households where the respondent was responsible for treating sick pigs (65% vs. 41%; $P = 0.021$; OR 2.7; CI 1.6-6.4). This could perhaps be a consequence of men generally attaining a higher level of education than women in Paper III, or of men being more often responsible for treating sick pigs and therefore probably having more discussions with veterinarians. However, when the above-mentioned variables were put in a multivariable model with knowledge of AMR as the dependent variable, it was only the variable 'sex of respondent' that was (almost) significant (P = 0.051). The model would most likely have been improved if it had been possible to interview the person responsible for treatment in all households. Unfortunately, this was not possible.

More than half of the respondents (54%) were of the opinion that the use of antimicrobials could result in negative consequences. The majority of these respondents expressed concerns about possible adverse effects on the animals when they received too much antimicrobials, or that antimicrobial residues might be present in the meat. However, none of the respondents mentioned the problem of AMR. Similarly, Om and McLaws (2016) found that farmers in

Table 6. *Knowledge and attitudes about antimicrobials and antimicrobial use among respondents (n = 91) (Paper III)*

	Category	n	%
Is it important to give antimicrobials to animals?	Yes	82	90
	No	4	4
	Don't know	5	5
Are antimicrobials needed to keep animals healthy?	Yes	90	99
	No	0	0
	Don't know	1	1
Will the use of antimicrobials result in better growth of animals? (n = 90)	Yes	30	33
	No	58	64
	Don't know	2	2
Do you think it is easy to get access to antimicrobials?	Yes	89	98
	No	2	2
Do you consider antimicrobials to be cheap?	Yes	5	5
	No	55	60
	It's acceptable	29	32
	Don't know	2	2
Do you think giving antimicrobials to animals may result in any negative consequences?	Yes	49	54
	No	3	3
	Don't know	39	43
Have you ever heard of 'antimicrobial resistance'?	Yes	50	55
	No	41	45
Do you feel you have received enough information on how antimicrobials should be used in animals?	Yes	37	41
	No	38	42
	Don't know	16	18

Cambodia did not make the connection with adverse effects of antimicrobials and AMR. These results imply that there is a need for more awareness-raising campaigns about the risks and consequences associated with antimicrobial use and resistance in Cambodia.

4.6 Antimicrobial resistance in porcine *Escherichia coli* [Paper III]

Of the 261 faecal samples obtained, 110 were from growers (1-3 months old), 122 were from fatteners (over 3 months old), and 29 were from sows. Figure 19 illustrates the results from the susceptibility analyses. Because no ECOFF were available for azithromycin, the prevalence of resistance could not be determined for that antimicrobial. Overall, bacteria isolates were classified as resistant (*i.e.* non-wild-type) to a median of five antimicrobials, whereas 21 isolates (8%) were susceptible to all antimicrobials tested and 31 isolates (12%) showed resistance to eight or more antimicrobials. Multidrug-resistance (MDR), *i.e.* resistant to three or more antimicrobial classes (Magiorakos *et al*., 2012), was found in 206 isolates (79%).

The analyses in Paper III revealed a high prevalence of resistance to the antimicrobials ampicillin, tetracycline and chloramphenicol, which is in agreement with other studies in Southeast Asia (Ström *et al.*, 2017b; Changkaew *et al.*, 2015; Nhung *et al.*, 2015; Lay *et al.*, 2012), and hence probably reflects a long tradition of use of these drugs in livestock farming in the region. A high prevalence of resistance to the last-resort antimicrobial colistin, especially among growers (34%), was also detected. This is most likely a result of extensive use of colistin, since at least 30% of the farmers reported administering colistin to their pigs, either to treat existing diseases or as a prophylactic for piglets to prevent diarrhoea.

4.7 Factors associated with antimicrobial resistance [Paper III]

In Paper III, it was found that the prevalence of resistance to several antimicrobials was significantly higher in bacteria isolates from growers (1-3 months old), compared with isolates from fatteners (over 3 months old). Similar results, where bacteria from younger pigs show higher prevalence of AMR, have been reported in other studies (Gibbons *et al.*, 2016; Mathew *et al.*, 1999). In a longitudinal study, Nguyen *et al.* (2016) found that the prevalence of resistance to the antimicrobials ciprofloxacin and gentamicin in *E. coli* from

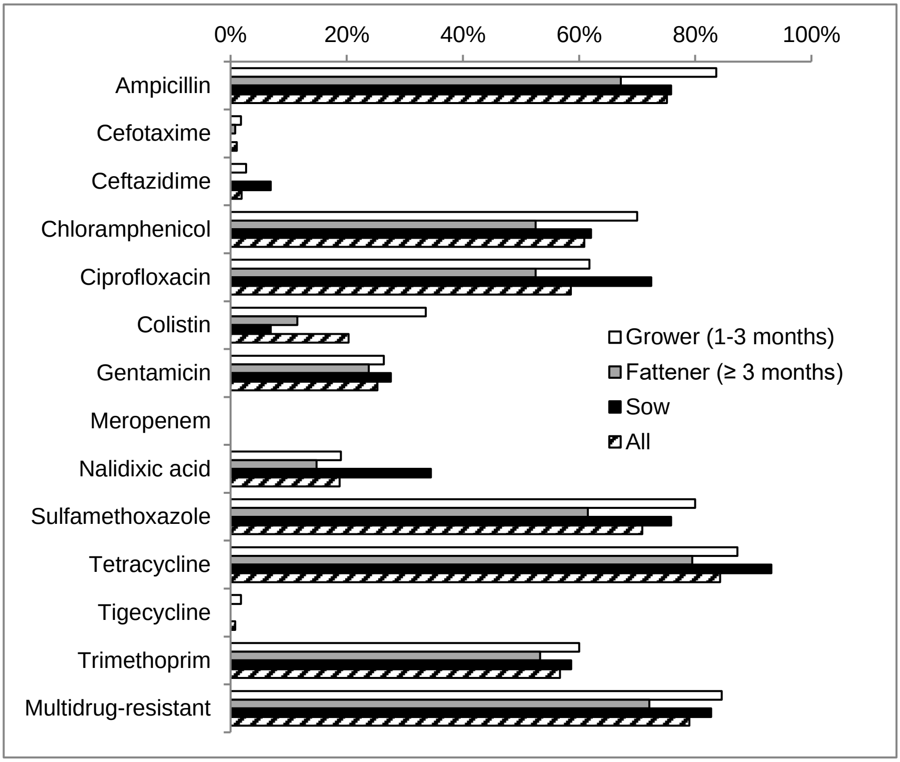

Figure 19. Prevalence of resistance to 13 antimicrobials and multidrug-resistance in commensal *Escherichia coli* isolated from growers (n = 110), fatteners (n = 122) and sows (n = 29) (Paper III).

pigs declined during the rearing process, which might be a consequence of the often reduced use of antimicrobials in the finishing phase of pig production, or of the potential fitness cost in bacteria as a result of resistance (Marchant & Moreno, 2013). In Paper III, there was also a higher prevalence of resistance in isolates from sows than in isolates from fatteners, which might be explained by the fact that sows (and also younger pigs) received oral antimicrobials to a higher extent. Oral administration of antimicrobials has been suggested to result in increased AMR in commensal *E. coli* (Burow *et al.*, 2014). Most concentrate feeds that contained antimicrobials in Paper III were fed to younger pigs and around 21% of the households where sows were sampled reported administering antimicrobials to sows on a regular basis. However, there is insufficient information on the specific antimicrobial agents used to draw any conclusions on whether the higher resistance seen in bacteria from sows and growers was due to oral or regular administration of antimicrobials to these age groups. Based on the results reported in Paper III it is evident that animal age is an important factor to consider when investigating prevalence of AMR in pigs.

Bacteria isolates obtained from households that administered antimicrobials preventatively tended to show higher prevalence of resistance to ampicillin (P = 0.081), ciprofloxacin (P = 0.072), chloramphenicol (P = 0.056), sulfamethoxazole (P = 0.028) and trimethoprim (P = 0.031), compared with households that did not report this practice. The prevalence of resistance was also higher in households that treated the entire group or herd of pigs in the event of disease, instead of just the sick individuals (ciprofloxacin P = 0.021; colistin P = 0.048; nalidixic acid P = 0.012). These results hence support the claim that medically non-rational use of antimicrobials contributes to increased prevalence of resistance (Marshall & Levy, 2011).

Age of the person responsible for antimicrobial treatment was found to be positively associated with lower prevalence of resistance to ciprofloxacin (P = 0.043) and sulfamethoxazole (P =0.021), and to MDR (P = 0.029). This could be a consequence of older farmers being more experienced and having better disease control practices than younger farmers, as suggested by Nhung *et al.* (2015).

Due to the lack of treatment records and the fact that most farmers only remembered the names of some of the antimicrobials they used, it was unfortunately not possible to determine whether higher prevalence of resistance was a consequence of higher use of certain antimicrobials. Due to the risk of recall bias, such studies are perhaps better implemented in experimental settings. Other aspects that are important to consider are whether the AMR results might be influenced by different factors related to the age group of the pigs (*i.e.* age effect) and/or by management at the farm level (*i.e.* farm effect). In Paper III, the age effect overlapped and potentially concealed the farm effect, as most farms only had pigs from one or two age groups and it might thus be difficult to separate the two. Ideally, samples from all three age groups should have been obtained from all farms, to get a better distribution. However, this was not possible as there was no prior information on the age range of the pigs on the farms studied.

In conclusion, there was evidence of high prevalence of AMR to several antimicrobials, including antimicrobials considered to be important for human medicine (WHO, 2017a). Higher prevalence of resistance was detected on farms that administered antimicrobials preventatively and on farms that treated the entire group or herd in the event of disease and not just the sick pigs.

4.8 Perceived benefits and constraints of urban pig production [Paper I]

All 204 farmers surveyed in Paper I stated that increased income was their most important reason for keeping pigs, and for more than 60% the income from pig production was defined as the main income source for the household. Many respondents also explained that the pigs were considered an economic reserve in that they could be sold in time of need, and that pig production was an easily managed complement to other occupations. In fact, only 4% of the households were solely dependent on the income from their pig production. This finding is consistent with those obtained in a study performed in Kampala, the capital of Uganda, where none of the participating households was entirely economically dependent on pigs (Katongole *et al.*, 2011). Many farmers also mentioned the benefit of using residues from other types of agricultural production as pig feed, such as rice wine residues and residues from breweries. Using agricultural residues appears to be more common in the proximity of urban areas. According to a study in three rural provinces in Cambodia, only 3-8.5% of households used rice wine residues as pig feed (Saroeun *et al.*, 2007), compared with 45% in Paper I. Furthermore, 13% of the households in Paper I used residues from breweries, whereas this was not reported by any of the households in the rural study by Saroeun *et al.* (2007). This difference between urban and rural areas might be a consequence of better market opportunities for certain products in urban areas.

The most commonly mentioned constraints to pig production are listed in Table 7. Low revenue from the slaughtered pigs was considered a major constraint by more than half of the respondents (54%), along with high cost of feed (53%). It was mainly the price of concentrate feeds that was considered to

Table 7. *Perceived constraints to pig production as reported by the farmers (n = 102)* (Paper I)*

Constraint	Number of households	%
Low prices for slaughter pigs	55	54
High cost of feed	54	53
Diseases among the pigs	51	50
Expensive to buy piglets	9	9
Smell of manure	4	4
Low fertility of sows	2	2
Lack of capital	2	2
Dependent on market prices	2	2
No constraints	14	14

*Multiple answers were possible.

be high, limiting the farmers from purchasing feed of sufficient quality. Half of the respondents also considered diseases to be a major constraint to their production, which is a problem commonly mentioned by pig keepers in Cambodia (Saroeun *et al.*, 2007) and Lao PDR (Phengsavanh *et al.*, 2011). In Paper I, more than 82% of respondents reported they had experienced diseases among their pigs in the past three years. This was more common in households with a lower socio-economic position (P = 0.025; OR 1.55; CI 1.06-2.27), possibly as a consequence of poor households having limited capital to invest in disease prevention measures or being forced to use feeds of poor quality, which are more often contaminated with pathogenic microorganisms.

To summarise, pig keeping was considered a major contributor to livelihoods by the majority of the households surveyed, while outbreaks of diseases and low revenues were the main perceived constraints. Notably, none of the respondents mentioned any concerns about food safety or the risk of disease transmission from pigs to humans, which is a concern that is often raised by international stakeholders (FAO, 2001a). This might reflect limited knowledge about biosecurity and food safety, or that food safety is not a primary concern among the pig keepers, considering their livelihoods. Limited knowledge and perception of risks and health hazards are important aspects that need to be considered, as this may affect how livestock keepers and health systems deal with health hazards of animal origin. This is a major challenge when dealing with risk mitigation and risk reduction strategies (Bonfoh *et al.*, 2010).

5 Concluding remarks

This thesis presents new knowledge on different aspects of livestock keeping in urban and peri-urban areas of Phnom Penh, Cambodia, with particular focus on potential public health hazards and socio-economic benefits and determinants. The main conclusions that can be drawn from the results presented in this thesis are as follows:

- Urban and peri-urban livestock systems pose problems, but they also offer income opportunities for poor urban dwellers and may contribute to improved livelihoods. Main constraints perceived by the pig keepers surveyed here were diseases among the pigs and low revenues from the production.

- Many surveyed households applied poor management practices for livestock manure in general and pig manure in particular. Poor practices, such as dumping of manure, were more common in households with a lower socio-economic position. Improvements in manure management are crucial in order to mitigate possible public health hazards from urban and peri-urban livestock-keeping households.

- Low occurrence of potentially zoonotic pathogens was detected in the pig manure compared with studies in rural areas in Cambodia, perhaps because of management differences in hygiene measures and deworming routines.

- Antimicrobials were administered to the pigs in the majority of the households surveyed. The use of antimicrobials was often based on the farmers' own judgements and almost half of the respondents had not heard about antimicrobial resistance.

- High prevalence of resistance was detected in commensal faecal bacteria from the pigs and 79% of the isolates were categorised as multidrug-resistant.

- Higher resistance was found in households that administered antimicrobials as a preventive measure, and in households that practised group or herd treatment if some pigs showed symptoms of disease. These findings support the hypothesis that non-rational use of antimicrobials results in higher prevalence of AMR.

The findings presented in this thesis can be of help when developing new guidelines and interventions targeting urban and peri-urban livestock farming and antimicrobial use in livestock in countries similar to Cambodia.

6 Future considerations

Cambodia is experiencing rapid and rather unregulated urbanisation. Although the majority of the poor still reside in rural areas, poverty is becoming more urban in Cambodia, as the poor are urbanising faster than the population as a whole. Urban livestock production has emerged following increasing migration to urban areas and as a response to the growing demand for animal products by the urban population. It may have an important role to play for Cambodian society, not only for the production of food, but also for social aspects and in terms of waste disposal. However, the potential for urban livestock production to play a substantial role in urban poverty and food insecurity reduction should not be overemphasised and it is important to consider the possible adverse effects such livestock systems may have on public health and the environment.

Based on the work presented in this thesis and elsewhere, inadequately handled and dumped livestock manure is problematic in urban and peri-urban areas. If livestock production in the proximity of urban areas is to continue, improved manure handling and storage are key requirements in order to mitigate its potential negative impacts. One simple solution would be to enforce legislation against livestock production within cities. However, such legislation would have detrimental effects on the income and livelihoods of poor urban farmers and their families. Another solution would be to recognise the existence of livestock production in at least peri-urban areas and work to achieve improvements in these production systems, *e.g.* through the development of recycling and discharge standards regarding management of livestock manure. Such standards could either be based on ‘the polluter pays’ principle or on incentives targeted at farmers with good management practices.

Strategies for improving manure management should focus on increasing awareness among farmers and stakeholders on the potential value of manure as a fertiliser and the hazards associated with poor management. This, together with development of best management practices (BMPs) and implementation of customised solutions for simple manure storage and application routines, might lead to improvements. However, it is important to consider the socio-economic aspects of proposed measures, as these will have an impact on compliance. One strategy that has proven successful is implementation of biodigester programmes, although this approach requires high initial investments and adoption might only be possible when financial incentives are offered. Cambodia established the National Biodigester Programme (NBP) in 2006 and since then more than 26 000 biodigesters have been installed in the country (Hyman & Bailis, 2018). However, the programme did not include the Phnom Penh area. Although the NBP in Cambodia has generally been successful, with high satisfaction rates among users, it still faces many challenges, including maintaining financial subsidies in order to make the biodigester more affordable, and problems with reduced access to credit for farmers.

The growing crisis with increasing resistance to antimicrobials is evidently a consequence of their widespread use within human and veterinary medicine. The easier access to such drugs in urban areas might to some extent contribute to the increasing emergence of AMR, especially if antimicrobials are used arbitrarily and with no supervision by trained animal health professionals. In order to prevent and contain the emergence of AMR, restricting the overuse and misuse of antimicrobials is essential, while access to antimicrobials for those who need them has to be ensured. There are many options available to reduce unnecessary use of antimicrobials, but putting them into practice has proven to be problematic and requires commitment and political leadership, not only within countries but also globally. Good governance and infrastructure also play major roles in limiting further spread of resistant bacteria and resistance genes, which are important factors for containment of AMR. Moreover, interventions and strategies need to be adapted and implemented at the national level, as this requires commitment and support from government and policy makers. An initial step towards a national action plan on AMR would be to perform a country-focused situation analysis, which is what Cambodia is currently doing (2018). Furthermore, surveillance data on country level are essential in order to monitor trends over time and to support and evaluate efforts that aim to reduce AMR.

Prevention of infections through good sanitation and hygiene is an important initial step in reducing the use of antimicrobials and the emergence and spread of AMR. Improved diagnostic services and treatment guidelines are also essential tools to limit unnecessary and medically irrational usage within human and veterinary medicine, including prohibiting the use of antimicrobials for growth promotion and as a disease prevention measure in livestock farming. Restricting access to antimicrobials and encouraging restrictions on prescriptions are also needed, but require a legal and regulatory framework that is often inadequate in many countries. Involvement of the community, physicians, animal health professionals, patients and livestock keepers in a bottom-up process, encompassing education and awareness-raising campaigns, might be a useful strategy. However, education about antimicrobial use should not be provided by the pharmaceutical or feed industry, as they might be self-serving.

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Popular science summary

Livestock production is common among households in many low- and middle-income countries. In line with increasing urbanisation, with people migrating from rural to urban areas in search of better employment opportunities and livelihoods, livestock production within cities is increasing. This increase is largely a consequence of incomers bringing their livestock with them. Urban livestock farming can be an important source of extra income for the family and may contribute to the food supply, but also involves animals and humans living closely together in high densities. Under such conditions, there is a risk of diseases transmitting between animals and humans, so called zoonoses. If there is no good system in place for the handling and disposal of livestock manure, there is also a risk of this type of livestock farming having a negative impact on the environment in the city and its surroundings.

The thesis examined the socio-economic benefits and potential public health hazards from livestock farming in the proximity of urban areas. The focus was mainly on pig farming, as it is common among Cambodian households. When investigating negative effects of livestock farming, manure management and the use of antibiotics for pigs were the main focus, because improper use of antibiotics may contribute to the increasing problem of antibiotic resistance.

The studies included in this thesis were all conducted in pig-keeping households located in and around Phnom Penh, the capital of Cambodia. For the first two studies, 204 households were interviewed and faecal samples were taken from their pigs for analysis of bacteria and parasites that may spread between animals and humans and which can be used as indicators of the presence of other pathogenic organisms. A large proportion of the households (60%) reported that pig keeping is one of the main income sources for their family. Many also reported that diseases among the pigs were a problem and that low revenues from slaughtered pigs and high feed prices were challenging. However, none expressed any concerns about potential human health hazards

caused by pig production. Almost half of households (46%) reported that they usually dump pig manure in the environment. This was more commonly stated by households with a lower socio-economic position and by respondents who were not aware that diseases could spread between animals and humans. Few households performed any form of treatment of the manure to reduce the risk of disease transmission.

In the third study, 91 households were interviewed and faecal samples were taken from their pigs for analysis of antibiotic resistance in the intestinal bacteria *E. coli*. The farmers reported that it is easy to get access to antibiotics for pigs, with use of antibiotics largely being based on the farmers' own judgement. More than one-third of the households used a pig feed containing antibiotics. Furthermore, almost half (45%) of the households interviewed had not heard of antibiotic resistance.

Antibiotic resistance was found to be common among the pigs and 79% of *E. coli* bacteria isolates were classified as multidrug-resistant, meaning resistant to three or more antibiotic classes. Antibiotic resistance was more common on farms where antibiotics were used for prevention of diseases and where the whole group or herd of pigs was treated with antibiotics if some pigs showed disease symptoms, and not just the sick pigs.

The results presented in this thesis show that, while livestock farming can contribute to increased income for urban dwellers, there are public health hazards associated with keeping animals in cities. Poor knowledge and lack of agricultural land make manure handling difficult. Moreover, proper handling and disposal of manure may often result in large costs for households, which may be problematic for resource-poor farmers. The widespread and often unnecessary use of antibiotics is worrying, as it leads to increased antibiotic resistance. Targeted information-raising campaigns, along with investments in disease prevention measures and a regional or national manure management plan, may help achieve the improvements needed for livestock production to continue in urban and peri-urban areas in countries such as Cambodia.

Populärvetenskaplig sammanfattning

Djurhållning är vanligt förekommande bland hushåll i låg- och medelinkomstländer. I takt med den ökande urbaniseringen i världen som till största del sker i dessa länder, där människor flyttar från landsbygden till städerna i hopp om bättre arbetsmöjligheter och förbättrad levnadsstandard, sker även en ökning av animalieproduktion i städerna. Detta beror till stor del på att man ofta tar med sig sina livsmedelsproducerande djur från landsbygden. Småskalig djurhållning i städer kan vara en extra inkomst för familjen och även bidra till deras matförsörjning men innebär också att djur och människor lever tätt tillsammans. Under sådana förhållanden finns risk att sjukdomar sprids mellan djur och människor, så kallade zoonoser. Om det inte finns något bra system för hantering av djurens gödsel finns även risk att den här typen av djurhållning har en negativ påverkan på stadsmiljön.

Syftet med avhandlingen var att undersöka socioekonomiska fördelar och eventuella hälsorisker till följd av djurhållning i stadsnära områden. Vi valde att fokusera våra studier till att främst handla om grishållning då det är vanligt förekommande i Kambodja. För att undersöka negativa effekter av djurhållningen valde vi att titta närmare på djurhållarnas gödselhantering och även deras användning av antibiotika till grisarna eftersom felaktig användning av antibiotika bidrar till det ökande problemet med antibiotikaresistens.

Studierna som ingår i den här avhandlingen utfördes alla i hushåll som födde upp grisar och som var belägna inom och i utkanten av Kambodjas huvudstad Phnom Penh. För de första två studierna intervjuades 204 hushåll och gödselprover togs från grisar för analys av bakterier och parasiter som kan spridas till människor och som kan användas som indikatorer på förekomst av andra sjukdomsframkallande organismer. En stor del av hushållen (60 %) ansåg att grishållningen var en av hushållets viktigaste inkomstkällor. Många upplevde att sjukdomar bland grisarna var ett problem och att låga intäkter från slakt och höga foderpriser var problematiskt. Ingen uttryckte dock någon oro för eventuella hälsorisker som kunde orsakas av grisarna. Nästan hälften av hushållen (46 %) svarade att de vanligtvis dumpade grisgödseln i miljön, vilket var vanligare bland hushåll med lägre socioekonomisk ställning och bland djurhållare som inte visste att sjukdomar kan spridas mellan djur och människor. Endast ett fåtal hushåll genomförde någon form av behandling av gödseln för att minska risken för sjukdomsspridning.

För den tredje studien intervjuades 91 hushåll och gödselprover togs från grisarna för analys av antibiotikaresistens hos tarmbakterien *E. coli*. Vi fann att det var enkelt att få tag på antibiotika till grisarna och att antibiotika-användningen till stor del baserades på djurhållarnas egna omdömen och inte utifrån en veterinär bedömning. Mer än vad tredje hushåll använde ett grisfoder som innehöll antibiotika. Nästan hälften (45 %) av de intervjuade hushållen hade inte hört talas om antibiotikaresistens.

Vi fann att antibiotikaresistens var vanligt förekommande bland grisarna och 79 % av *E. coli*-bakterierna klassificerades som multiresistenta, dvs. resistenta mot tre eller fler antibiotikaklasser. Antibiotikaresistens var vanligare på gårdar där man behandlade grisar i förebyggande syfte och där man behandlade hela gruppen eller besättningen vid tecken på sjukdom, och inte endast de sjuka grisarna.

Resultaten som presenterats i den här avhandlingen visar på att även om djurhållning kan bidra till en ökad inkomst för människor i städer så finns det hälsorisker förenat med att hålla djur i stadsmiljöer. Bristfällig kunskap och avsaknad av jordbruksmark gör gödselhanteringen problematisk och det innebär i många fall stora kostnader för hushållen att behandla och transportera bort gödseln på ett säkert sätt. Den utbredda och ofta onödiga användningen av antibiotika är också oroande och leder till ökad förekomst av antibiotikaresistens. Riktade informationskampanjer kan, tillsammans med investeringar i sjukdomsförebyggande åtgärder och en regional eller nationell plan för gödselhanteringen, leda till de nödvändiga förbättringar som krävs för att djurhållning i stadsnära områden ska kunna bedrivas i länder som Kambodja.

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