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The influence of urban livestock-keeping on the epidemiology of mosquito-borne zoonotic flaviviruses in Hanoi city of Vietnam

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Abstract
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In rapid urbanizing cities, the high density of livestock populations in close vicinity to humans, and high mosquito vector abundance have provided suitable conditions for transmission of mosquito-borne zoonotic flaviviruses, that cause several million cases of human disease annually. This thesis explores the urban livestock-keeping in Hanoi and its potential influence on the epidemiology of flavivirus infections. Four major components were investigated: livestock development of the city, knowledge and practices of local people on mosquito-borne diseases (MBDs), mosquito vector abundance, and risk factors for flavivirus exposure in animals.

The analyses of the livestock development policy, animal data and key informant interviews showed that the livestock production system of Hanoi had increased in line with the governmental policy during 2014 to 2018. However, some major challenges were identified such as the high proportion of small-scale livestock farms, animal diseases, and a slow progress of translocation of livestock farms out of the urban areas.

A cross-sectional study was conducted in 513 households in six districts of Hanoi (two urban, two peripheral, and two peri-urban). Knowledge and practices were evaluated; mosquitoes and larvae inside and outside the houses were collected and identified; blood samples of pigs and dogs were tested by a West Nile virus competitive enzyme-linked immunosorbent assay (cELISA), a kit that allowed for detection of antibodies against several flaviviruses of different animal species. JEV-specific antibodies were confirmed by plaque reduction neutralization test (PRNT). Logistic regression models using seropositivity of households as the outcome were built to identify significant risk factors.

A low level of knowledge and preventive practices against zoonotic MBDs in community was indicated in the study.

Among 12,861 adult mosquitoes and 2,427 larvae collected, *Culex* mosquitoes were the most abundant (93.01%), followed by *Anopheles* (3.82%), *Mansonia* (1.21%), *Armigeres* (1.18%) and *Aedes* mosquitoes (0.78%). In contrast, *Aedes* genus larvae were prominent at 87.02%, followed by *Culex* spp. (12.2%). There was a positive association between pig-keeping and *Culex* mosquitoes ($p < 0.001$).

In total, 475 dogs and 636 pigs were tested by a cELISA. The overall flavivirus seroprevalences in dogs and pigs were 70.7% and 88.5%, respectively. The PRNT results for a subset of 50 dog sera (34 positive, 6 doubtful and 10 negative samples by cELISA), and 50 pig sera (26 positive, 6 doubtful and 18 negative samples by cELISA) showed a lower proportion of JEV-specific antibodies (28 positives for dog samples, and 13 positives for pig samples).

In conclusion, this thesis shows associations between livestock-keeping and risks of mosquito-borne zoonotic flavivirus infections to humans in Hanoi.

Keywords: urban livestock-keeping, mosquito borne zoonotic flaviviruses, knowledge and practices, vector abundance, risk factors, Hanoi, Vietnam

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To my lovely wife, son, daughter, and parents

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. **Pham-Thanh, L**.; Magnusson, U.; Can-Xuan, M.; Nguyen-Viet, H.; Lundkvist, Å.; Lindahl, J. *Livestock Development in Hanoi City, Vietnam—Challenges and Policies.* Front. Vet. Sci., 2020. 7, 566.

II. Nguyen-Tien, T.; **Pham-Thanh, L.**; Vu-Trong, D.; Tran-Hai, S.; Vu-Thi, L.; Bui, V.N.; Bui-Ngoc, A.; Hoang-Duc, T.; Vu-Thi, Thanh.; Nguyen-Viet, H.; Magnusson, U.; Lundkvist, Å.; Lindahl, J. *Knowledge and practice on prevention of mosquito-borne diseases in livestock-keeping and non-livestock-keeping communities in Hanoi city, Vietnam: A mixed-method study.* PLoS One, 2021. vol. 16, no. 2 February, pp. 1–21, doi: 10.1371/journal.pone.0246032

III. Nguyen-Tien, T.; Bui-Ngoc, A.; Ling, J,; Tran-Hai, S.; **Pham-Thanh, L**.; Bui-Nghia, V.; Dao-Duy,T.; Hoang-Thi, T.; Vu-Thi,L.; Tran-Vu,P.; Vu-Trong,D.; Lundkvist, Å.; Nguyen-Viet, H.; Magnusson, U.; Lindahl, J. *The Distribution and Composition of Vector Abundance in Hanoi City, Vietnam: Association with Livestock Keeping and Flavivirus Detection.* Viruses, 2021. 13, 2291. https://doi.org/10.3390/v13112291

IV. **Pham-Thanh, L.**; Nguyen-Tien, T.; Magnusson, U.; Bui-Nghia, V.; Bui-Ngoc, A.; Le-Thanh, D.; Lundkvist, Å.; Can-Xuan, M.; Nguyen-Thi Thu, T.; Vu-Thi Bich, H.; Lee, HS.; Nguyen-Viet, H.; Lindahl, J. *Dogs as Sentinels for Flavivirus Exposure in Urban, Peri-Urban and Rural Hanoi, Vietnam.* Viruses, 2021. 13, 507. https://doi.org/10.3390/v13030507

V. **Pham-Thanh, L.**; Nguyen-Tien, T.; Magnusson, U.; Bui-Nghia, V.; Bui-Ngoc, A.; Lundkvist, Å.; Vu-Trong, D.; Tran-Hai, S.; Nguyen-Thi Thu, T.; Vu-Thi Bich, H.; Can-Xuan, M.; Nguyen-Viet, H.; Lindahl, J. *Flaviviruses in peripheral and peri-urban pig keeping in Hanoi, Vietnam, and knowledge and preventive practices of pig farmers*. Manuscript.

Additional Publications

Publications not included in the thesis

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Chapot, L.; Nguyen-Tien, T.; **Pham-Thanh, L.**; Nguyen-Viet, H.; Craven, L.; Lindahl, J.F. *A Mixed-Methods Approach to Understanding Knowledge of Mosquito-Borne Infections and Barriers for Protection in Hanoi, Vietnam.* Trop. Med. Infect. Dis., 2020. 5, 66

Nga Thi Thanh Nguyen, Alice Latinne, Thuy Bich Hoang, Long Van Nguyen, Ngoc Thi Pham, Anh Thi Lan Nguyen, Thai Van Nguyen, Phuong Quang Tran, Thai Van Hoang, Hai Kim Lam, **Pham-Thanh, L.**, Phuong Thanh Nguyen, Hung Van Vo, Quang Tin Vinh Le, Lan Thi Nguyen, Hoa Thi Nguyen, Christine Kreuder Johnson, Jonna AK Mazet, Scott Ian Roberton, Chris Walzer, Sarah Helen Olson, Amanda Elizabeth Fine. *Evidence of SARS-CoV-2 related coronaviruses circulating in Sunda pangolins (Manis javanica) confiscated from the illegal wildlife trade in Viet Nam.* Frontiers in Public Health, section Infectious Diseases – Surveillance, Prevention and Treatment (Accepted for publication in 2022).

Hu Suk Lee, Krishna K. Thakur, **Pham-Thanh, L.**, Tung Duy Dao, Anh Ngoc Bui, Vuong Nghia Bui, Huy Nguyen Quang. *A stochastic network-based model to simulate farm-level transmission of African swine fever virus in Vietnam.* PLoS One, 2021. | https://doi.org/10.1371/journal.pone.0247770.

Katriina Willgert, Anne Meyer, Dinh Xuan Tung, Nhu Van Thu, **Pham-Thanh, L.**, Scott Newman, Nguyen Thi Thanh Thuy, Pawin Padungtod, Guillaume Fournié, Dirk Udo Pfeifer & Timothée Vergne. *Transmission of highly pathogenic avian influenza in the nomadic free-grazing duck production system in Viet Nam.* Scientific Report, 2020. 10:8432 | https://doi.org/10.1038/s41598-020-65413-2

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Leo Loth, **Pham-Thanh, L.**, Mark Anthony Stevenson. *Spatio temporal distribution of outbreaks of highly pathogenic avian influenza virus subtype H5N1 in Vietnam, 2015– 2018.* Transboundary and Emerging Diseases, 2019. DOI: 10.1111/tbed.13259

Hu Suk Lee, **Pham-Thanh, L.**, Tien Ngoc Nguyen, Mihye Lee, Barbara Wieland. *Seasonal patterns and space-time clustering of porcine reproductive and respiratory syndrome (PRRS) cases from 2008 to 2016 in Vietnam.* Transboundary and Emerging Diseases, 2019. DOI: 10.1111/tbed.13122

Hu Suk Lee, **Pham-Thanh, L.**, Barbara Wieland. *Temporal patterns and space time cluster analysis of foot and mouth disease (FMD) cases from 2007 to 2017 in Vietnam.* Transboundary and Emerging Diseases, 2019. DOI/10.1111/tbed.13370

Contents

Abbreviations

BHK	Baby hamster kidney
CDC	Centers for Disease Control and Prevention
cELISA	Competitive enzyme-linked immunosorbent assay
CGIAR	Consultative Group for International Agricultural Research
CI	Confidence interval
Coef.	Coefficient
DARD	Department of Agriculture and Rural Development
DENV	Dengue virus
dsRNA	Double stranded RNA
EIAs	Enzyme immunoassays
ELISA	Enzyme-linked immunosorbent assay
EMEM	Eagle's minimal essential medium
FAO	Food and Agriculture Organization of the United Nations
FBS	Fetal bovine serum
GPS	Global positioning system
HH	Household
HI	Hemagglutination inhibition
HRP	Horseradish peroxidase
IFA	Immunofluorescence assay
IgG	Immunoglobulin G
ILRI	International Livestock Research Institute
IQR	Interquartile range
JE	Japanese encephalitis
JEV	Japanese encephalitis virus
MBDs	Mosquito-borne diseases
mRNA	Messenger RNA
NIHE	National Institute of Hygiene and Epidemiology
NIVR	National Institute for Veterinary Research
NS	Nonstructural
OD	Optical density
OR	Odds ratio
PFU	Plaque forming unit
PRNT	Plaque reduction neutralization test
Ref.	Reference
RNA	Ribonucleic acid

SD	Standard deviation
SDLPAH	Sub-Department of Livestock Production and Animal Health
ssRNA	Single stranded RNA
WHO	World Health Organization
WNV	West Nile virus
YFV	Yellow fever virus
ZIKV	Zika virus

Introduction

More than half of the world's population already live in cities, and the global urban population is predicted to be 60% by 2030 [1]. While urbanization does have positive effects on the increasing consumption of animal-derived food as well as income to the growing population [2], there are a number of potential negative effects. These include an increase in food insecurity and malnutrition, increased public health risks, and environmental pollution [3–5]. Urban and peri-urban livestock-keeping can provide employment and an access to per-ishable nutritious food that may otherwise be hard to procure, particularly in countries with lacking infrastructure and cold-chains. However, it can also contribute to the transmission of zoonotic infections, including mosquito-borne diseases (MBDs). In a crowded environment with high densities of hu-mans, livestock, and mosquito vectors in tropical cities, several MBDs can be transmitted between animals and humans.

Livestock development policy

According to the International Livestock Research Institute (ILRI), livestock policy is defined as a coherent set of decisions with a common long-term ob-jective or objectives affecting or relevant to the livestock subsector. Policies for livestock development may create appropriate incentives for good practice and transparent investment frameworks that consider all potential benefits and risks. Conversely, if the policies are not well developed and applied, the live-stock sector may grow in a non-adequate manner without considering poten-tial risks [6]. Some low- and middle-income countries have produced national livestock policies [7–11], but policies at provincial levels are rarely found.

In Vietnam, the national livestock development strategy toward 2020 was decided in 2008 (Prime Minister's Decision number 10/2008/QD-TTg). Based on this central government direction, some local provinces officially issued their own livestock policies [12–14], including Hanoi city [15–17]. A plan for livestock development of the city until 2020 and toward 2030 aims to increase the productivity and quality of the animal-sourced food, ensure good food hy-giene and safety, and create large-scale commodity production that meets the demand of the consumers and export standards [17].

Urban livestock and public health concerns

In the developing world, livestock play multiple roles as providers of income and employment for livestock stakeholders, an asset and safety net for the poor and women, and a source of nourishment for rural and urban households [18].

An urban livestock system, in a report of the Food and Agriculture Organization of the United Nations (FAO), can be defined as a form of livestock-keeping concentrated in and around cities. However, urban livestock-keeping can cause problems such as pollutions by live animals (manure, noise, bad odour) or by processing of livestock products (water and soil contamination from slaughtering) [19, 20]. In addition, urban livestock-keeping may bring concerns for public health in terms of zoonoses, food-borne diseases, and MBD infections.

Domestic animals in urban areas can potentially transmit various pathogens, including viruses, bacteria, parasites and fungi to humans; but risks of the introduction and transmission of zoonotic diseases strongly rely on the hygienic practice of livestock farms [21]. In addition, the storage of animal feedstuffs can be a good place for the multiplication of rodents, the reservoirs of some zoonoses [22].

The increase of human population and urbanization have increased the consumption of animal products [23]. However, food-producing animals are the source of many foodborne pathogens such as *Streptococcus suis*, *Campylobacter*, non-typhoidal *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes* [24].

Livestock-keeping has been found associated with mosquito vectors of several diseases in humans [25]. Like humans, livestock can constitute bloodmeal sources [26], and can attract mosquitoes through odours [27]. Moreover, both water sources and wastewater at animal farms are preferable sites for mosquito breeding. A close contact of humans, livestock, and mosquitoes can increase the risk for humans becoming infected with MBDs [28], especially in a rapid urbanizing city as Hanoi.

Mosquito-borne flavivirus structure

There are 73 RNA viruses of the genus *Flavivirus* described (family *Flaviviridae*), 34 are mosquito-borne, of which 22 are associated with human disease [29].

The particles of flaviviruses are enveloped, about 50 nm in diameter, with icosahedral and spherical geometries with three structural proteins (anchored capsid (anC), membrane (M), glycoprotein (E)), seven nonstructural proteins (NSs) and a single stranded RNA (ssRNA) genome of positive polarity. The genome is approx. 10.5 kb and translated as a polyprotein of about 3,500 amino acids [30] (Fig. 1).
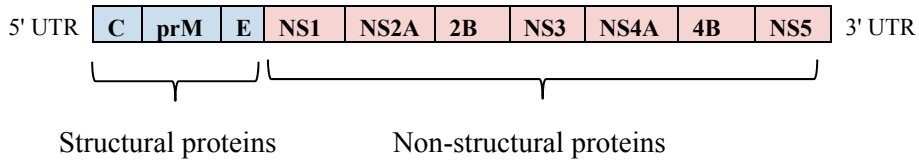
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| 5' UTR | C | prM | E | NS1 | NS2A | 2B | NS3 | NS4A | 4B | NS5 | 3' UTR |

Structural proteins   Non-structural proteins

Figure 1. The genomic structure of flavivirus

Virus replication begins with the attachment of the viral envelope protein E to host receptors, followed by fusion of the viral membrane with the host endosomal membrane. The viral RNA genome is released into the cytoplasm and translated into a polyprotein, which is cleaved into all structural and non-structural proteins. A dsRNA genome is synthesized from the genomic ssRNA(+), transcribed and replicated thereby providing viral mRNAs and new ssRNA(+) genomes. Virus assembly and virions bud at the endoplasmic reticulum before transported to the Golgi apparatus. In the final stage, new virions are released by exocytosis [31].

Mosquito-borne zoonotic flavivirus transmission

In nature, mosquito-borne zoonotic flaviviruses circulate between the vertebrate hosts and the arthropod vectors, generally *Aedes* sp. mosquitoes for dengue virus (DENV), Zika virus (ZIKV) and yellow fever virus (YFV), and *Culex* sp. mosquitoes for Japanese encephalitis virus (JEV) and West Nile virus (WNV) [32]. Mosquitoes acquire flaviviruses mainly through horizontal transmission by a bloodmeal from a viremic animal, or possibly through vertical transmission from mother to offspring [33], while vertebrate hosts become infected by the probing process of blood-feeding by an infected mosquito [34]. The *Culex* mosquitoes prefer to breed in organically polluted aquatic habitats such as rice production areas, wetlands and ponds [35]. In contrast, the *Aedes* vectors have been more adapted to human environments and have their breeding sites mainly in clean and undisturbed water [36].

Dengue fever, Japanese encephalitis (JE), and Zika are the most important MBDs in urban Vietnam [37].

The first report of dengue in Vietnam was in 1958 and it has been endemic for decades [38]. There are four antigenically distinct serotypes of DENV (DENV1 - 4) [39], of which DENV1 and DENV2 are predominant in Vietnam [40]. The DENV transmission route from humans to humans through *Aedes* mosquito biting is determined as the human cycle, while the transmission between non-human primates and *Aedes* mosquitoes is defined as the sylvatic cycle (Fig. 2). Both transmission cycles exist in the Southeast Asia region [39].
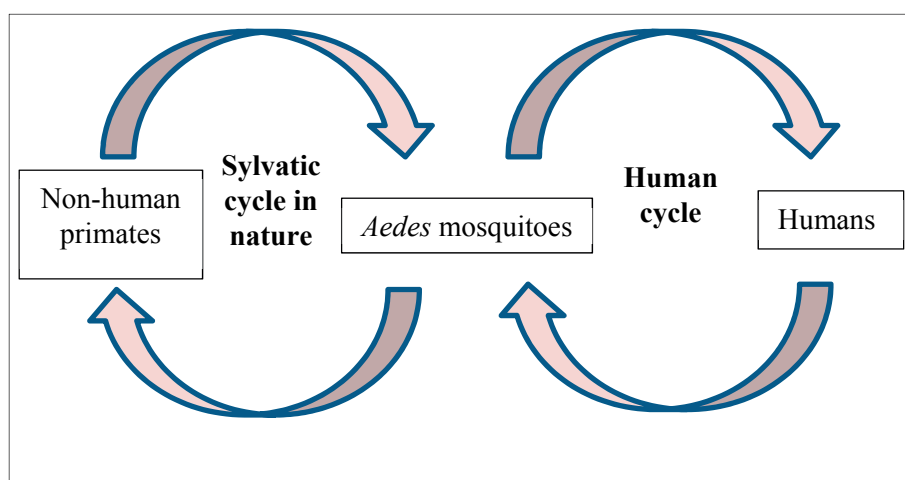
Figure 2. The transmission cycles of dengue virus

The clinical manifestations of dengue patients generally are fever with severe headache, myalgias, arthralgia, and rash that last 2 to 7 days. Most patients will recover after about a week [41]. In contrast, non-human primates rarely develop the clinical signs as seen in humans [42]. The viremia in DENV-infected patients is high from 2 days before the onset of the fever and remains for 5 to 7 days [29]. In non-human primate models, the viremia appears from 1 to 10 days after primary inoculation [42].

JE has been endemic in Vietnam since JEV initially was isolated in 1951 [43]. Ardeid birds play a role as JEV natural reservoirs, and domesticated pigs (*Sus domestica*) and wild boars (*Sus scrofa*) are the main amplifying host [33–35]. Mosquitoes from the genera *Culex, Aedes, Anopheles, Armigeres* and *Mansonia* are all suspected vectors transmitting the virus to humans who are dead-end host [36, 37]. Other livestock species, such as cattle, buffaloes, goats, poultry and dogs can be infected by JEV without clinical signs, with the exception of horses, that develop a disease similar to in humans [49] (Fig. 3). Experimentally, JEV can transmit directly through an oro-nasal route within pig herds without the presence of mosquitoes [50], however the viremia in infected pigs is normally shorter than 5 days after initial infection [51]. In humans, the viremia is usually from 3 to 7 days, but some cases have shown viremia up to 8 months post-infection [29].

The incubation period of JE is between 5 to 15 days [52]. Common symptoms of JEV-infected patients are mild with fever and headache. Gastrointestinal pain and vomiting may be observed in children. Severe cases, about 0.3% of the patients [53], are characterized by a rapid onset of high fever, headache, neck stiffness, disorientation, coma, seizures, spastic paralysis and ultimately death. The surviving patients may suffer permanent intellectual, behavioral or neurological sequelae [54].

In pigs, JEV infections can result in pyrexia, anorexia, and reproductive failure such as stillbirth, abortion, or mummified or hydrocephalic fetuses in the litters. In addition, infected horses may show pyrexia, but severe cases can lead to death in neurological disease [55].
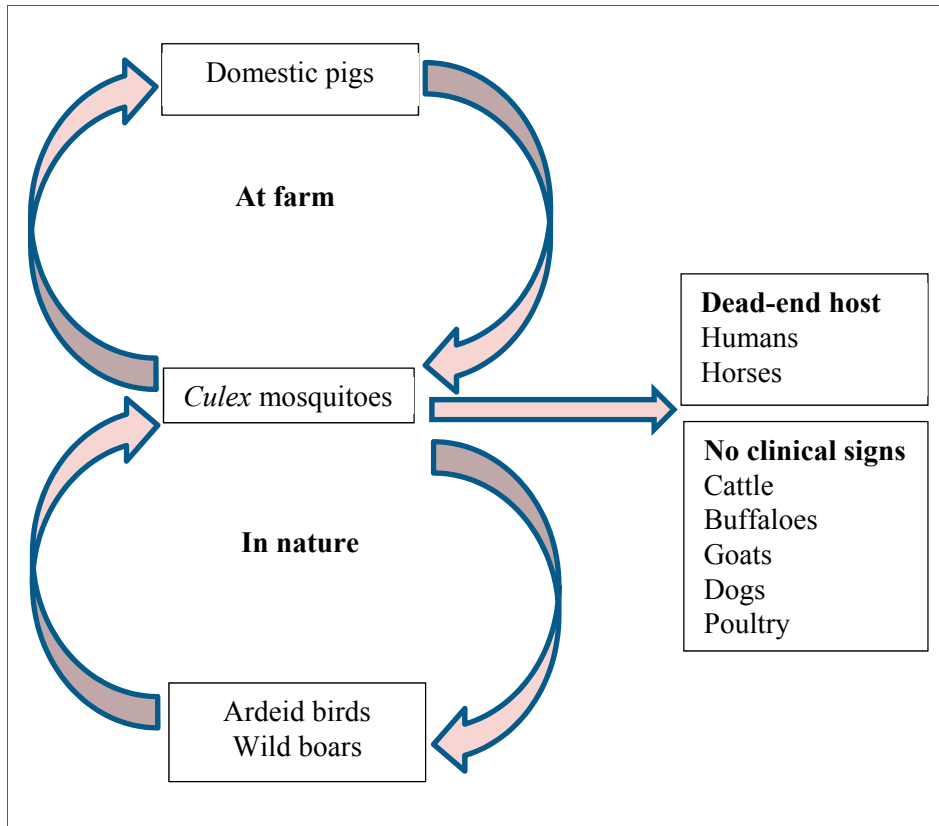
Figure 3. The transmission cycles of Japanese encephalitis virus

Seropositivity for Zika in humans was first confirmed in 1954 and since then, the first patients of ZIKV were reported in southern Vietnam in 2015 [56], however the disease has so far not been detected in the northern parts of the country [57]. There are two major transmission cycles, the human cycle and the sylvatic cycle with infected *Aedes* mosquito biting. The transmission cycles of Zika are similar to the cycles of DENV transmission. In addition, ZIKV can be infected in humans through blood transfusion, perinatal transmission from mother to infant, and by sexual contact [44–46] (Fig. 4). The viremia in patients ranges from 1 to 10 days after the onset, but the duration of viremia is normally from 3 to 5 days [61]. Infected non-human primates develop viremia that peak at 2 to 6 days after infection and typically became undetectable by day 10 [62].
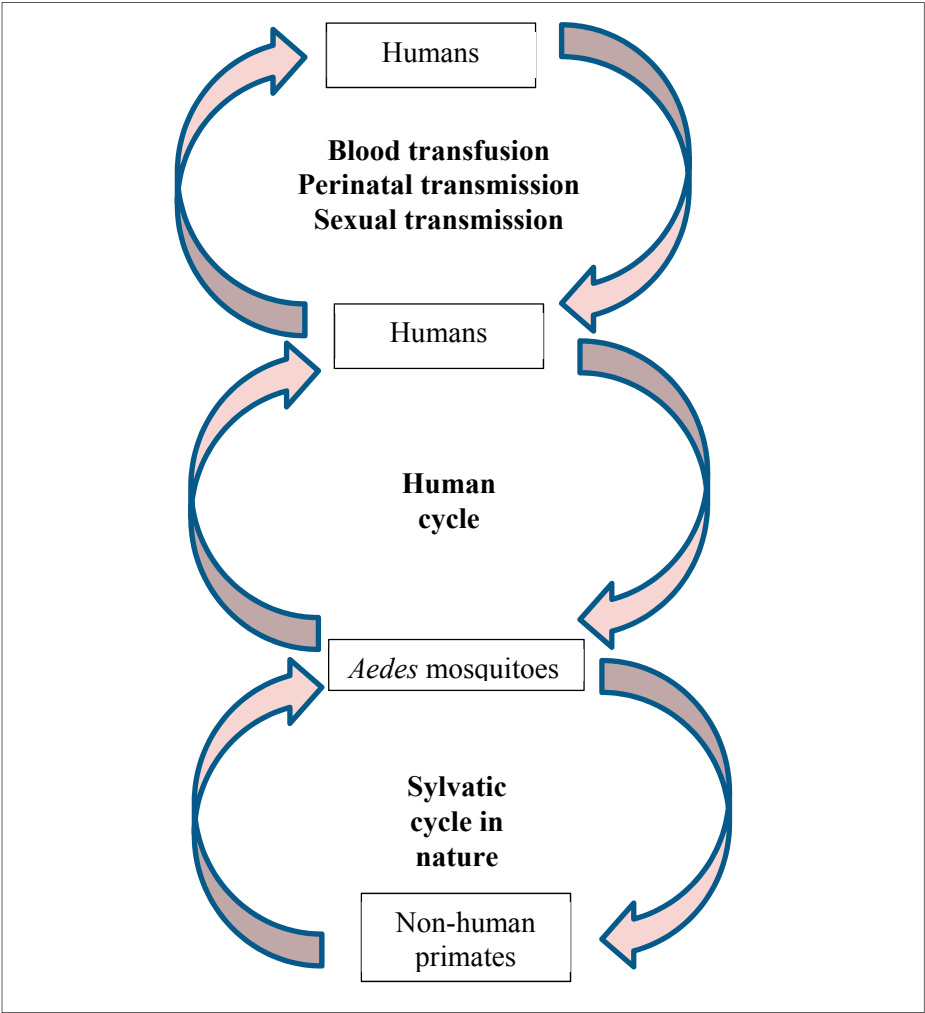
Figure 4. The transmission cycles of Zika virus

The incubation period in Zika patients is between 3 to 14 days. The most common symptoms are rash, fever, arthralgia, conjunctivitis, myalgia, and headache, and usually last for 2 to 7 days [63]. ZIKV infections in non-human primates may result in fever [62], some cases with mild weight loss and rash [64].

Laboratory diagnosis

Laboratory diagnosis is mandatory to confirm a specific flavivirus infection, which includes detection of the virus or virus-specific antibodies.

Detection of virus is usually performed during the early stages of the infection (less than 7 days post-infection) from serum, plasma, circulating blood cells and some tissues [65]. The virus can be identified by virus isolation on cell culture, by viral nucleic acid amplification tests, or by ELISA tests or rapid tests for antigen detection [29].

At the end of the acute phase of infection, serology is the preferred method. Virus-specific IgM commonly arise after 3 days from the onset and peak after about two weeks, and then decline to undetectable levels [66]. In contrast, the appearance of virus-specific IgG occurs after the first week and increases at detectable levels from 2 weeks to several months [65]. The antibodies that are usually measured target the envelope protein, precursor membrane protein, or the non-structural 1 protein (NS1) [67].

Several serological assays are available, which include enzyme immunoassays (EIAs) and hemagglutination inhibition (HI), as well as neutralization assays such as plaque reduction neutralization tests (PRNT) [67]. However, the antigenic similarities between flaviviruses result in flavivirus cross-reactive antibodies [68]. Although PRNT typically provide the greatest specificity, this assay may still suffer of some cross-reactivities [69]. Therefore, only a four-fold higher titer of neutralizing antibodies as compared to the titers to other heterologous flaviviruses can be considered positive [56, 57].

Mosquito control

There are several conventional and novel tools for mosquito control [72]. However, there is no single approach for mosquito control that makes a perfect solution [73]. Therefore, a combination of various control measures based upon analyses of the local situation will produce best results.

Elimination of sites for mosquito breeding can affect the larval habitats and interrupt the life cycle of mosquitoes [74]. This includes the empty and change standing water in containers, flower vases, buckets, vehicle tires, or drinking water tanks for livestock.

Another common measure is the installation of mosquito screens on windows, doors, and other entry points, and using mosquito nets while sleeping. These barriers can reduce the human – mosquito contact [75].

Chemical control by using larvicides or insecticides can be considered as a complementary measure if there is a difficulty of accessing vector habitats [76]. However, toxicity to humans as well as the change of the taste, odour or color of the water by the chemicals must be precautioned [75].

Biological control by some species of larvivorous fish, waterbugs and predatory copepods works effectively against the immature stages of vector mosquitoes in the larval habitats [63–65]. Using microorganisms such as the bacterium *Bacillus thuringiensis* var. *israelensis* (Bti) can release insecticidal toxins and virulence factors targeting the larval stages of insects, or entomopathogenic fungi can kill mosquitoes by toxins [80]. Since mating between *Wolbachia* bacteria-infected male mosquitoes and uninfected females yields eggs that fail to develop, this is another biological control method, which is effective for both *Aedes* and *Culex* mosquito species [81].

There have been several studies on novel genetic methods such as the sterile insect technique that releases large numbers of chemo-sterilized male mosquitoes, release of insects carrying a dominant lethal gene, or homing endonuclease genes [82].

In Vietnam, bed-nets, mosquito electric racquets, electric traps and coil burning are frequently used in many families.

Aims

The overall aim of this thesis was to explore the influence of urban livestock-keeping in Hanoi on the epidemiology of zoonotic MBDs caused by flaviviruses.

The more specific aims were:

Paper I
To understand the policy versus the actual situation of the livestock production, as well as to identify potential major challenges of the livestock sector development.

Paper II
To assess the level of knowledge and practices related to prevention and control of MBDs in livestock-keeping and non-livestock-keeping households.

Paper III
To explore how livestock-keeping in and around the city is associated with the presence of mosquito vectors and the risk of them spreading flaviviruses.

Papers IV and V
To identify risk factors of flavivirus infection in humans through serological tests in pig and dog populations.

Methods

Brief description of the study site

Hanoi city is located in the northern region of Vietnam covering an area of 3,358.6 km^2 with 7.9 million of permanent inhabitants, including 3.9 million in urban and 4 million in peri-urban areas (2018) [83] (Fig. 5). Every year, the city receives more than 3 million tourists, travelers, and workers. There are 30 districts of the city classified into 12 urban/peripheral districts and 18 peri-urban/rural districts.

As of 2018, there were 1.8 million pigs, 136,000 cattle, 23,500 buffaloes, 21.8 million chickens, and 6.2 million waterfowls in the city [84]. Additionally, large rice paddy areas of suburban Hanoi, the most preferred habitat for mosquito vectors [85], occupied 179,546 hectares [84].

Dengue and JEV are both prevalent in northern Vietnam. In 2017, there were 37,651 patients and 7 fatal cases of dengue reported in Hanoi [86], and the seroprevalence for JEV in pigs was 62.5% [87].
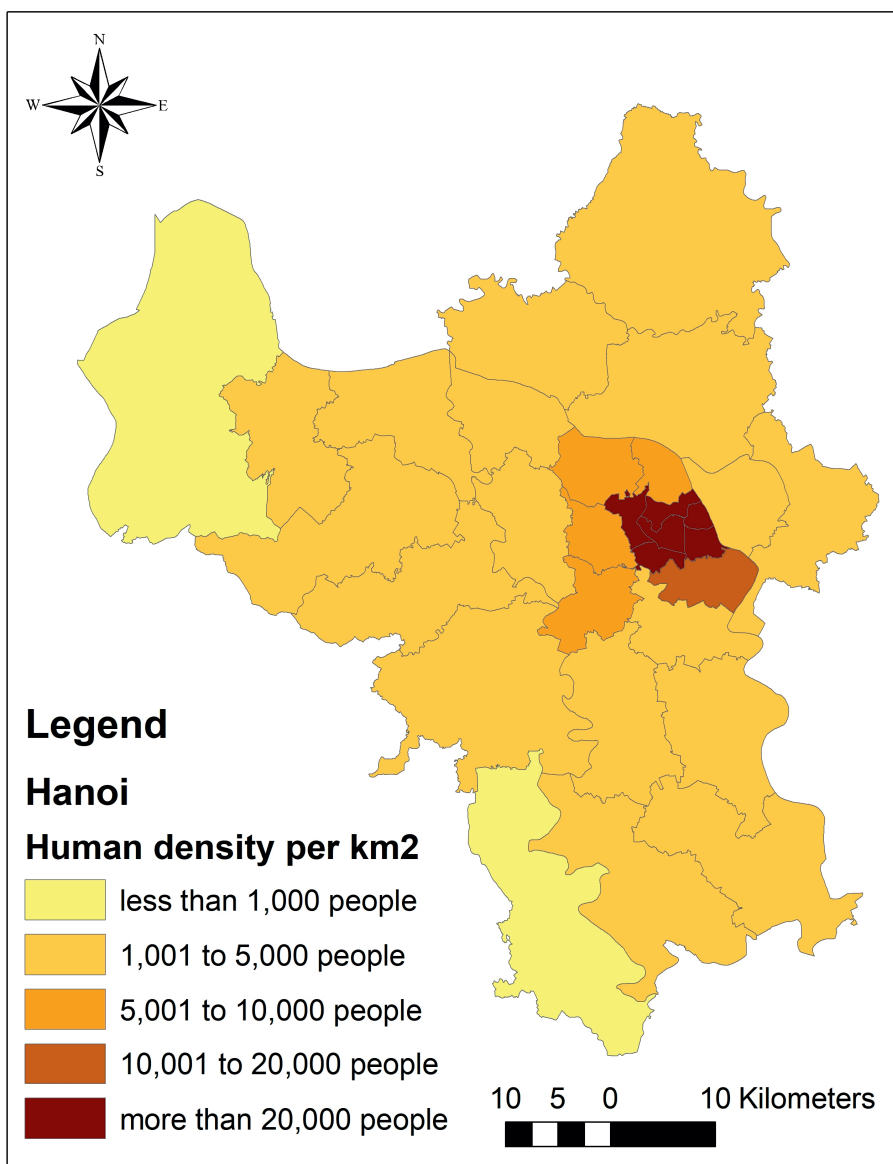
Figure 5. Map of Hanoi with human density (Source of data: Hanoi Statistics Office, 2018)

Livestock development analysis

In Paper I, a method combining interviewing key informants from provincial agencies and collecting documented data from them was applied. Six representatives from the Sub-Department of Livestock Production and Animal Health (SDLPAH) under the management of Department of Agriculture and Rural Development (DARD) and the chiefs of Livestock Production and Animal Health station from five districts of Hanoi were interviewed via telephone to understand their views on the contents of livestock development strategy, advantages versus challenges in the current livestock system, zoonotic threats caused by urban livestock-keeping, and their suggestions for policies that could maintain livestock sustainability in the future.

A recording device and taking written notes were applied during an interview and then transcribed verbatim for analyses. Key answers for current livestock development in these districts were collated in the livestock policy of the city while constraints were grouped with challenges of livestock production of Hanoi.

Legal documents on livestock development of Hanoi People's Council and Hanoi People's Committee were collected, and livestock data were referred from Hanoi Statistics Office at provincial levels and the SDLPAH office for district levels.

The key authorized papers on livestock development of Hanoi city are listed in Table 1.

Table 1. The official documents on livestock development of Hanoi issued by local authorities

Name of document	Organization that authorized	Time of issuing
Resolution number 03/2012/NQ-HDND on the agricultural development plan of Hanoi city till 2020, orientation in 2030	Hanoi People's Council	April 5, 2012
Decision number 1835/QD-UBND on approving the plan on development of Hanoi husbandry by 2020, orientation in 2030	Hanoi People's Committee	February 25, 2013
Resolution number 10/2018/NQ-HDND regarding some policies to promote production and cooperation development, linkage of production and consumption of agricultural products, building rural infrastructure of Hanoi city	Hanoi People's Council	December 5, 2018
Decision number 07/2019/QD-UBND on the issue of mechanism and policy for agricultural production assistance after natural disasters and animal epidemics in Hanoi city	Hanoi People's Committee	April 11, 2019
Document number 2933/UBND-KT on management and restructuring livestock husbandry of the city	Hanoi People's Committee	July 11, 2019

Cross-sectional survey

A total of six districts of Hanoi including two peri-urban, where large populations of livestock are kept (with more than 1,000 large ruminants, 15,000 pigs and 150,000 poultry per district) - namely, Chuong My and Dan Phuong, two peripheral districts (less than 1,000 large ruminants, 15,000 pigs and 150,000 poultry per district) - namely, Ha Dong and Bac Tu Liem and two urban districts with no livestock-keeping - namely, Ba Dinh and Cau Giay were purposively selected to represent a gradient of livestock-keeping.

Random selection of 20 global positioning system (GPS) points in each district was conducted, and within a radius of 2 km from each GPS point, about three households with livestock-keeping and other three households without livestock were visited. If the first household did not agree to participate in the study, another consenting household nearby was replaced.

The minimum number of 93 households in each category was calculated from assuming a power of 0.8, alpha 0.05, and a desire to detect a difference of 20% between households with livestock compared to households without livestock. In total, 513 households were recruited in the study (Table 2).

A house keeping at least a ruminant or a pig or five small animals such as chickens, ducks, geese, or rabbits was defined as a livestock-keeping household, meaning at least 0.05 tropical livestock units [88].

Table 2. The number of livestock-keeping households and non-livestock-keeping households in the study

Area	Households with livestock	Households without livestock
Urban districts	0	102
Peripheral districts	104	98
Peri-urban districts	129	80
Total	**233**	**280**

Questionnaires and interview

In Paper II, a questionnaire form to collect data at households was first developed in English and then translated into Vietnamese. The questionnaire consisted of four sections: (i) Demographic characteristics of the respondent (ten questions), (ii) Livestock information (eight questions); (iii) Awareness about MBDs (seven questions on knowledge with 39 items and one question on practice with 12 items), (iv) Sources of information that participants have heard about the MBDs (two questions). For section iii, every appropriate answer to either knowledge or practice questions was awarded one point, while incorrect answers received zero point, to generate scores of knowledge and practices. The maximum score for knowledge was 35, and 11 for practices. For the livestock-keeping households, additional information on the animals sampled was also collected.

The district veterinary staffs were trained to use paper-based questionnaires to interview the household owners in Vietnamese. The face-to-face interviews took about 20 minutes for each household.

Entomological studies

In Paper III, mosquito and larvae collection was conducted through trained staffs of the National Institute for Veterinary Research (NIVR), Vietnam on using Centers for Disease Control and Prevention (CDC) light traps [89]. A pair of the CDC traps were used at each household, in which one trap was placed close to livestock farm and another trap was near the animal owner's bedroom, at about 1.5-meter above the ground. In households without livestock, the outdoor collection was performed in the garden or yard.

All water-filled open containers in each household were searched for larvae using a larvae collection tool kit. Shallow containers were observed by eye and larvae, if present, were collected using a pipette. For deeper containers, a hand-net was stirred for 5 turns in the water to catch mosquito larvae.

The CDC traps were activated from 5 pm of the interviewing day until 7.30 am of the next day. The mosquitoes collected in each trap were transferred to a labelled 50mL conical centrifuge tube using a battery-operated aspirator, kept in a cool box in the field and sent to the National Institute of Hygiene and Epidemiology (NIHE), Vietnam at the same day. The mosquito samples were stored at -80°C until identification. The collected mosquitoes were counted and identified for species and gender, while larvae were also identified by genus and species at NIHE.

Animal blood sampling

In Papers IV and V, animal blood was sampled for detection of antibodies against flaviviruses.

If dogs were present, blood sampling was conducted by trained veterinarians of the Hanoi SDLPAH, using venipuncture of *Vena cephalica* or *V. saphena*. If a house had several dogs, the maximum number of dogs sampled was five.

Similarly, in a household with pig-keeping, pig blood was collected from *Vena cava* and the maximum pigs sampled in each farm was five.

The samples were stored in a cool box in the field and transferred to NIVR on the same day. Sera were centrifuged and separated immediately and kept at -20°C.

Serological analyses

In Papers IV and V, a commercial competitive enzyme-linked immunosorbent assay (cELISA) was used for screening of dog and pig sera. A subset of 50 dog and 50 pig serum samples were subsequently tested by a plaque reduction neutralization test (PRNT) for JEV.

Competitive enzyme-linked immunosorbent assay (cELISA)

A commercial cELISA kit for detection of IgG antibodies against WNV manufactured by IDvet company (No. 310, rue Louis Pasteur, Grabels, France) was employed. In principle, samples to be tested and controls were added to the pr-E protein of WNV pre-coated plate, which includes epitopes common to several flaviviruses such as WNV, JEV and DENV. Hence, this ELISA kit does not only detect antibodies against WNV but also cross-reactive antibodies induced by infection by other flaviviruses [90]. Anti-pr-E antibodies in a serum sample form an antigen-antibody complex. An anti-pr-E antibody horseradish peroxidase (HRP) conjugate binds to the remaining free pr-E epitopes, forming an antigen-conjugate-peroxidase complex. Calculation of percentage inhibition (S/N%) was equal to the mean of OD value of a sample divided by the mean OD value of a negative control, multiplied with 100. Samples presenting a S/N% less than or equal to 40% were considered positive; higher than 40% and less than or equal to 50% were considered doubtful; higher than 50% was considered negative.

Doubtful results were not considered when calculating the seroprevalence as well as analyzing risk factors.

Plaque reduction neutralization test (PRNT) for JEV

A selected subset of dog and pig sera were analyzed by a PRNT following the protocol of NIHE (code VR-5.5-01. QTXN.09, version 1.16).

Monolayers of BHK-21 cells [91] was cultured in 12-well plates with a density of 1.0×10^4 to 2.0×10^4 cells per well. Plates were incubated at 37°C with 5% CO2 from 1 to 3 days until the cell density reached 70% to 90%.

Serum specimens were inactivated at 56°C for 30 minutes in a water bath, and then diluted in Eagle's minimal essential medium (EMEM) with 10% heat-inactivated fetal bovine serum (FBS) from an initial dilution of 1:10, followed by serial two-fold dilutions of each serum up to the dilution of 1:640.

The JEV strain Nakayama [92] was prepared at 2.5×10^3 plaque forming unit (PFU)/ml. A mixture of 125 µl of diluted serum and 125 µl of virus was incubated at 37°C for 60 minutes before 50 µl was inoculated into each well of the cell culture. The adsorption period was 60 minutes at 37°C and subsequently, 2 ml of maintenance medium (EMEM with 2% FBS and 1% methylcellulose) was added in each well. The plates were incubated at 37°C for 5 to 7 days. When viral plaques were observed through a microscope, the cell monolayers were fixed in 3.7% formalin solution for 60 minutes at room temperature and stained with Methylene blue 1x for 60 minutes.

The PRNT end-point titer calculation was the reciprocal of the last serum dilution to show more than 50% reduction of the input plaque count (PRNT50).

Spatial mapping

Spatial mapping for plan areas for livestock development, livestock densities (Paper I), human density (Paper II), and household positions (Papers IV and V) was performed using ESRI® ArcMap™ 9.2.

Statistical analyses

In Papers II to V, data obtained from the questionnaires and the laboratory results were recorded in an Excel® spreadsheet and then transferred into STATA/SE 15.0 (StataCorp LLC, College Station, TX, USA) for analysis.

In Paper II, social-demographic characteristics of householders were expressed in percentages. Chi-square, Fisher exact and Mann-Whitney tests were used to compare the differences between households with livestock and without livestock. Spearman's rank correlation analysis was used to determine the relationship between knowledge and practices since the knowledge and practice scores were not distributed normally. The Mann–Whitney U test, Kruskal–Wallis test and negative binomial regression were used to identify

associations of demographic factors and knowledge and practice scores. Variables with a p-value less than 0.1 in univariable analysis were included in the multivariable negative binomial regression model.

In Paper III, since the quantity of mosquitoes and larvae did not show a normal distribution, the discrete variables related to them were described by the median and interquartile range (IQR). Spearman's rho was used to describe the correlation between the quantity of each of the types of mosquitoes and larvae with the numbers of pigs, cattle, and poultry.

In Paper IV and Paper V, descriptive statistics for the categorical variables for animals and households displayed by the cELISA results were used. A chi-square test was used to evaluate the association of the explanatory variables in the univariable analyses. At the household level, all independent variables were compared to assess the correlation among variables. A stepwise selection of variables in univariable analyses with a p-value at below 0.25 [93] and correlation measurements was applied in logistic regression models. Variables changing more than 25% from the coefficients of other variables were classified as confounding factors and they were forced into the model if the affected variables were significant. The likelihood ratio test was performed to build a parsimonious model, and the Hosmer–Lemeshow goodness-of-fit test was used for model fitness [94]. A p-value less than 0.05 was considered statistically significant.

Ethical considerations

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Ethical Committee of Hanoi University of Public Health (No 406/2018/YTCC-HD3, 6 August 2018).

In Paper I, the public veterinarians were informed that they could refuse to provide answers to the questions.

In Papers II, III, IV and V, the household owners were asked for their informed consent for the interview and the collection of mosquitoes and larvae. The research team guaranteed that the data only served research purposes. All information was handled anonymously, and the research data was only handled by the research team.

Results

Paper I

Livestock production of Hanoi

The general direction in the current policies for the development of livestock is towards industrial farms, which are the large-scale farms applying industrial techniques, concentrated outside the most highly populated areas (Resolution No.03/2012/NQ-HDND in 2012 of Hanoi People's Council). More than 70% of animal products at local markets is expected to be produced by these large-scale farms by 2020.

In the short-term, the pig population is planned to be reduced gradually, poultry stabilized, while dairy and beef cattle are to be further developed. The growth rate of animal production is planned to be maintained in the period of 2012 to 2020 at about 1.6% per year and then reduced to 1.4% per year during 2021 to 2030.

The livestock sector is planned to contribute to more than 54% and 58% of the agriculture gross output by 2020 and 2030, respectively. Livestock meat supply of the city is expected to be 420,000 tons in 2020 and increases to 492,000 tons in 2030 (Decision No. 1835/QD-UBND in 2013 of Hanoi People's Committee).

The livestock strategy of five districts where the interviews were conducted showed a consistency with the plan of livestock development of the city.

Land use for livestock production

The spatial distribution of livestock production of Hanoi is planned based on the geography of the rural districts. In brief, the keeping of beef and dairy cattle, and fattening pigs is to be concentrated in hilly districts while chickens and waterfowls are to be expanded in plain areas (Fig. 6). Translocation of most livestock farms out of residential areas in rural districts, as well as removal from urban districts, is expected to be achieved by 2030 (Decision No. 1835/QD-UBND in 2013 of Hanoi People's Committee). However, livestock populations were still present in at least 6 out of the 12 urban districts of Hanoi, as of 2018.
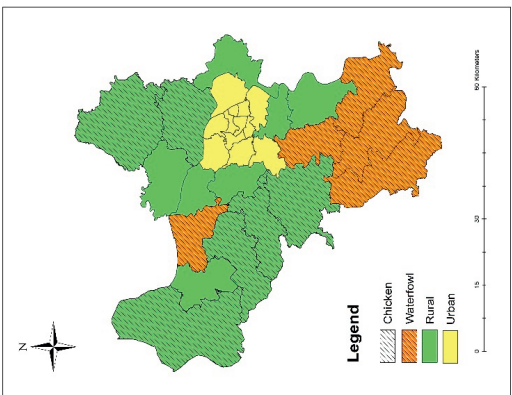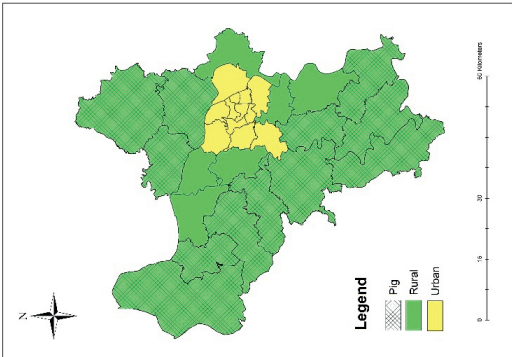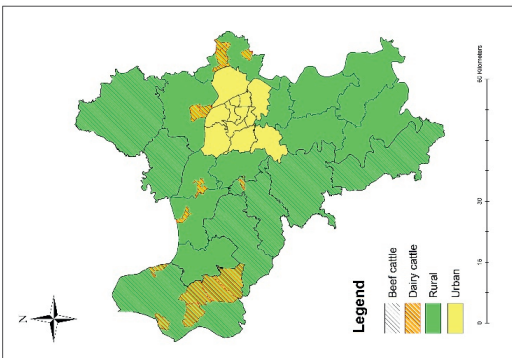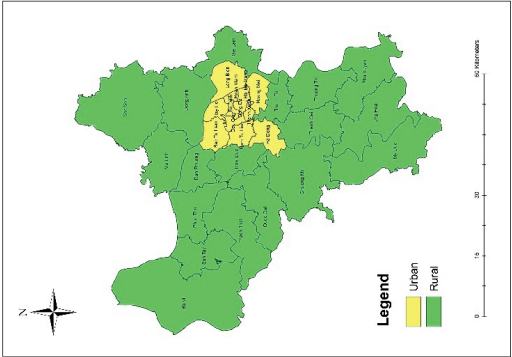
Figure 6. Planned areas for livestock development in Hanoi by 2020

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Livestock population

The livestock population of the city is planned in the Decision No. 1835/QD-UBND in 2013 of Hanoi People's Committee. By 2020, the beef cattle were projected to be from 150,000 to 155,000 heads, while dairy cattle should reach 20,000 heads. A stabilized cattle population of 145,000 to 150,000 heads is expected by 2030. The total number of pigs was planned to reach 1.4 to 1.5 million by 2020. Until 2030, the pig population is projected to decrease to 1.3 million, but the pork volume is maintained at 340,000 tons per year, reflecting an expected higher productivity. The poultry population was expected to be over 11.6 million chickens and 2.8 million waterfowls providing more than 66,000 tons of meat and 800 million eggs per year by 2020. In 2030, the poultry population is projected to be 14.3 million.

Compared to the expected livestock populations in 2020, pigs and poultry in 2018 were much higher, but in contrast, the cattle production was lower.

According to Hanoi SDLPAH, the reduction rate of small-scale farms from 2014 to 2018 in urban areas was higher, 32%, as compared to rural areas, 24% (Table 3). A small-scale farm in Vietnam is defined as a livestock farm that has less than 30 livestock units, in which one livestock unit is equal to 500 kilograms of live animal(s) [95].

Table 3. The number of small-scale farms for livestock-keeping during 2014 – 2018

Area	Year 2014	Year 2015	Year 2016	Year 2017	Year 2018
Urban	4,229	4,096	4,155	3,426	2,871
Rural	396,202	364,283	351,557	331,552	300,879
Total	**400,431**	**368,379**	**355,712**	**334,978**	**303,750**

There were five large-scale cattle farms in the Hanoi area, accounting for 12% of the total cattle population, 283 large-scale pig farms, accounting for 22% of the total pig population, and 290 poultry farms which accounted for 8% of the total poultry population, as of 2018.

The majority of small-scale farms in the city could have some challenges for livestock production such as low biosecurity practice, high risk of disease exposure, low productivity, poor quality of waste treatment, bad quality feed sources and breeds, low technology investment in livestock production, and more difficult access to the capital [96]. The constraint to livestock development of Hanoi due to small-scale farms was mentioned by the key informants in the study.

Nevertheless, given the assets and aspirations of farmers, policies toward small-scale farms are still needed [97]. For instance, shifting extensive production to semi-intensive and/or intensive production systems is a solution [96].

Veterinary services

The veterinary services play a key role in livestock production to ensure a high animal health status and food safety [98]. However, some constraints caused by animal health services such as shortage of manpower, long distances from service centres, and high costs for services have been identified [99].

Before October 2018, the veterinary service system of Hanoi was managed by the Sub-Department of Animal Health that operated from a village level up to a provincial level through an animal health worker network of more than 3,000 persons.

The People's Committee of Hanoi decided to merge the veterinary service system and livestock production agency (the Decision number 5682/QD-UBND on October 23, 2018 on Establishment of Hanoi Sub-Department of Livestock Production and Animal Health). Hence, the number of veterinarians in Hanoi has been reduced at all levels, especially the community-based animal health worker forces that were mostly affected. In some low and middle-income countries, the community-based animal health workers play an important role in early detection and reporting disease outbreaks, vaccination campaigns against the most important livestock diseases, and providing treatment to sick animals [86–87].

Compensation

According to the policy of the city, the owner of a domestic animal compulsorily culled during a disease outbreak in Hanoi is planned to be compensated with the amount of 1.6 USD per kg of pig; 1.9 USD per kg of cattle, buffalo, goat, sheep, and deer species; and 1.5 USD per number of chicken, duck, Muscovy duck, and goose species (Decision No. 07/QD-UBND in 2019 of Hanoi People's Committee).

Reported by Hanoi SDLPAH, the city compensated more than 60 million USD to pig farmers whose animals were culled due to an epidemic of African swine fever in 2019.

Other concerns to livestock development

Animal diseases

Another major challenge is diseases in the livestock. In 2018, foot and mouth disease, highly pathogenic avian influenza, porcine reproductive and respiratory syndrome, and other animal infectious diseases were reported by SDLPAH of Hanoi.

According to data published by the Department of Animal Health of Vietnam, an epizootic of African swine fever occurred during February to December 2019 in Hanoi that caused culling of more than 540 thousand pigs, which accounted for more than 25% of the pig population of the city.

The risk of zoonotic transmission associated with livestock-keeping in Hanoi was mentioned during the interviews. The participants listed highly pathogenic avian influenza and *Streptococcus suis* type 2 as potential zoonoses of the city. Many other zoonotic diseases can occur by urban livestock-keeping, including vector-borne and food-borne diseases, but were not mentioned, potentially reflecting which diseases have been more frequently mentioned in the media.

Climate change

The climate change can affect livestock production through its negative impacts to the quality of feed crop, water resource availability, and the health situation of livestock [102]. An increase in the number of outbreaks of transboundary animal diseases by climate changes has caused loss of several billion USD over the past decades and low-income countries are most affected [103]. In addition, Vietnam was on the list of the five most affected countries in the world by extreme weather events in 2016 [104].

There are studies on climate conditions and climate change scenarios for Vietnam, including effects on livestock production, proposed solutions for minimizing losses, and optimizing investment efficiency [105]. However, such overall mapping of climate change challenges specific to Hanoi has not yet been conducted.

Paper II

Socio-demographic characteristics of the households

Males accounted for 69.6%, and 95% of the respondents were married. Almost half of the respondents (46.1%) were from 46 to 60 years-old, followed by the age-group between 31 and 45 years (28.3%). Regarding educational levels, 39.1% of the respondents had graduated at high school, while 37.6% of them

had completed secondary level. There were more participants that had obtained a degree at high school or college/university from households without livestock than from households with livestock.

Half of the participants had farming (52.3%) as their main job. There were more respondents that were farmers in households with livestock than in households without livestock, whereas there were more office workers and retired people in households without livestock. The households without livestock (79.3%) were much more likely to use tap water than the households with livestock (56.7%). In contrast, the households keeping livestock were two times more likely to utilize water from wells than the households without livestock.

Knowledge of the households

Most respondents (98%) had heard about MBDs, with more households without livestock having heard about MBDs as compared to households with livestock (p<0.05). The most common MBD that the participants mentioned was dengue (96.5%), followed by malaria (72.1%).

More participants from households not keeping livestock knew about malaria than the participants from households keeping livestock (p<0.01). Fewer participants (14.4%) had heard about JE, and only 1.8% had heard about filariasis.

Many households with or without livestock recognized stagnant water containers (84.3%) and tanks (71.2%) as suitable sites for mosquito breeding. More participants of households without livestock than households with livestock could list stagnant water containers, car tires, vases, and bonsai rockery as potential breeding sites of mosquitoes (p<0.05).

Most participants (79.9%) thought that mosquitoes could lay eggs in drain or polluted water, while only 18.5% responded that mosquitoes could breed in clean water. More than 96% of the respondents could list at least one risk factor for getting MBDs. However, more households with livestock could not identify any risk factor of getting MBDs than households without livestock (p<0.05). In contrast, more households with livestock (43.6%) mentioned that livestock-keeping could increase the risk of getting MBDs than households without livestock (25.5%) (p<0.001).

About 80.5% of the respondents identified the warm and humid weather as a risk factor of MBDs. More households without livestock considered warm and humid weather as a risk factor than the households with livestock (p<0.05). Most respondents (92.2%) identified correctly that the rainy season is the period with the highest risk of getting MBDs.

Regarding symptoms of MBDs, 91.8% of the respondents thought that high fever is a prominent symptom of MBDs. However, 5.6% of the respondents could not list any symptom of MBDs. Other symptoms such as muscle pains,

nausea/vomiting, severe headache, rashes, and haemorrhages were less mentioned in both households with and without livestock, ranging from 20.4% to 59%. The respondents of households without livestock were more likely to mention high fever or muscle pains as symptoms of MBDs than the respondents of households with livestock (p<0.05).

Regarding knowledge on preventive measures of MBDs, all respondents of households without livestock could list at least one method to prevent mosquitoes, while about 1.7% of the respondents of households with livestock could not mention any (p<0.05). The preventive measures of screening of doors/windows and mosquito repellent creams/liquid were more known by the households without livestock than the households with livestock (p<0.01). In contrast, more households with livestock knew measures of elimination of mosquito breeding sites than the households without livestock (p<0.05). Most participants (96.6%) considered mosquito nets help in prevention of MBDs. Other measures such as using electric rackets, mosquito coils /incense sticks, lids on water tanks, anti-mosquito products, fish in the water containers, or wearing long sleeves were also mentioned, ranging from 39.4% to 61.8%. Using chemicals in water containers was the least mentioned (9.2%).

In summary, households without livestock had a higher knowledge score than households with livestock (p<0.05) (Table 4).

Table 4. Knowledge about MBDs

	HH with live-stock (n, %)	HH without live-stock (n, %)	p-value
Heard about MBDs			
Yes	222 (96.52%)	272 (99.26%)	0.028
No	8 (3.48%)	2 (0.74%)	
Heard about each dis-ease			
Dengue fever	216 (96.86%)	259 (96.28%)	0.72
Japanese Encephalitis	25 (11.21%)	46 (17.1%)	0.06
Zika	86 (38.57%)	99 (36.8%)	0.68
Malaria	148 (66.37%)	207 (76.95%)	0.009
Filariasis	5 (2.24%)	4 (1.49%)	0.53
Breeding sites of mos-quitoes			
Stagnant water con-tainers	183 (80.62%)	236 (87.41%)	0.038
Car tires	72 (31.72%)	124 (45.93%)	0.001
Vase	83 (36.56%)	134 (49.63%)	0.003
Bonsai rockery	81 (35.68%)	132 (48.89%)	0.003
Risk factors for get-ting MBD			
Don't know any	13 (5.73%)	6 (2.22%)	0.042
Warm and humid sea-son	173 (76.21%)	227 (84.07%)	0.028
Livestock-keeping	99 (43.61%)	69 (25.56%)	<0.001
Season that MBDs are highest			
Rainy season	211 (92.54%)	249 (91.88%)	
Dry season	1 (0.44%)	6 (2.21%)	0.23
Same	16 (7.02%)	16 (5.9%)	
Symptoms while get-ting MBDs			
Don't know any	17 (7.46%)	11 (4.04%)	0.09
High fever	203 (89.04%)	256 (94.12%)	0.04
Muscle pains	73 (32.02%)	118 (43.38%)	0.009
Nausea/vomiting	71 (31.14%)	104 (38.24%)	0.09
Severe headache	99 (43.42%)	124 (45.59%)	0.62
Rash	48 (21.05%)	54 (19.85%)	0.74
Haemorrhagic	132 (57.89%)	163 (59.93%)	0.64

	HH with live-stock (n, %)	HH without live-stock (n, %)	p-value
Ways to prevent getting MBDs			
Don't know any	4 (1.75%)	0	0.04
Screening of doors/windows	33 (14.47%)	77 (28.31%)	0.001
Mosquito repellent creams/ liquid	84 (36.84%)	132 (48.53%)	0.009
Mosquito nets	221 (96.93%)	262 (96.32%)	0.7
Electric rackets	135 (59.21%)	174 (63.97%)	0.27
Mosquito coils/ Incense sticks	91 (39.91%)	114 (41.91%)	0.65
Long sleeves	84 (36.84%)	113 (41.54%)	0.28
Keep lids on water tanks	95 (41.67%)	127 (46.69%)	0.26
Chemical in water containers	15 (6.58%)	31 (11.4%)	0.06
Anti-mosquito products (e.g: insecticides)	131(57.46%)	145 (53.31%)	0.35
Eliminate breeding sites	83 (36.4%)	74 (27.21%)	0.027
Using fish in water containers	108 (47.37%)	137 (50.37%)	0.5
Mean score ± SD	17.8 ± 6.9	18.7 ± 6.4	0.048

Abbreviations: HH, Household; SD, Standard deviation.

Practices of the households

All respondents had conducted at least one personal protection action against MBDs at home. About 90.5% of them had used mosquito bed-nets. The percentage of households with livestock that used bed-nets was significantly higher than in the households without livestock ($p<0.01$).

Half of the respondents used electric rackets (51.3%) and anti-mosquito products (49.3%) to kill mosquitoes. Other preventive measures were less used by the respondents including repellent, mosquito coils, eliminating breeding sites, keeping fish in water containers, and covering lids on water tanks.

In our study, the percentage of households with livestock applying preventive measures against MBDs such as using mosquito bed-nets, using mosquito coils, using anti-mosquito products, eliminating breeding sites and keeping fish in water containers was significantly higher than in households without

livestock ($p<0.05$). However, overall, there was no significant difference be-
tween the practice score of households with or without livestock.

Table 5. Practices for MBDs prevention

Personal protection used to prevent mosquito-borne diseases	HH with livestock (n, %)	HH without livestock (n, %)	p-value
Screen of windows/doors	14 (6.19%)	28 (10.41%)	0.09
Mosquito repellent creams/liquid	32 (14.16%)	48 (17.84%)	0.26
Mosquito bed-nets	215 (95.13%)	233 (86.62%)	0.001
Electric rackets	117 (51.77%)	137 (50.93%)	0.85
Mosquito coils/ incense sticks	53 (23.45%)	30 (11.15%)	<0.001
Long sleeves	62 (27.43%)	79 (29.37%)	0.63
Covering lids on water tanks	76 (33.63%)	80 (29.74%)	0.35
Chemical in water containers	8 (3.54%)	12 (4.46%)	0.6
Anti-mosquito products/Insecticide	124 (54.87%)	120 (44.61%)	0.02
Eliminating breeding sites	72 (31.86%)	58 (21.56%)	0.01
Using fish in water containers	77 (34.07%)	66 (24.54%)	0.02
Mean score ± SD	3.7 ± 2.4	3.3 ± 2.1	0.11

Abbreviations: HH, Household; SD, Standard deviation.

Correlation between knowledge and practices

Spearman test indicated an overall positive correlation between knowledge
and practices (Spearman's rho (r) = 0.67, $p < 0.001$). There was a strong pos-
itive correlation (r = 0.73, $p<0.001$) between knowledge and practices of the
households without livestock, with a less strong positive correlation (r = 0.58,
$p<0.001$) between knowledge and practices of households keeping livestock.

Associated factors with knowledge and practices about MBDs

Compared to households in peri-urban districts, households in central urban
districts were more likely to have better knowledge regarding MBDs (p =
0.016). Being farmers, unemployed and retired people correlated to a poorer

knowledge on MBDs as compared to office workers (p<0.05). However, farmers were more likely to have better preventive practices on MBDs as compared to office workers (p = 0.044), despite poorer knowledge.

Table 6. Associated factors with knowledge and practices about MBDs

	Knowledge score			Practice score		
	Coef.	CI 95%	p-value	Coef.	CI 95%	p-value
District *(ref. – Peri urban)*						
Peripheral	0.005	-0.06-0.07	0.89	0.036	-0.07-0.14	0.52
Central urban	0.13	0.02-0.24	0.016	-0.14	-0.32-0.02	0.09
Gender *(ref. – Male)*						
Female	0.05	-0.01-0.12	0.13	0.09	-0.01-0.19	0.09
Occupation *(ref. – Office worker)*						
Farmer	-0.17	-0.28- (-0.06)	0.003	0.18	0.004-0.35	0.044
Unemployed	-0.38	-0.55- (-0.22)	<0.001	0.01	-0.27-0.29	0.94
Retired	-0.16	-0.28- (-0.04)	0.011	-0.007	-0.2-0.19	0.94
Others	-0.1	-0.23-0.01	0.09	0.03	-0.16-0.2	0.76
Livestock-keeping *(ref. - Yes)*						
No keeping livestock	-0.04	-0.1-0.04	0.32	-0.03	-0.15-0.08	0.53
Knowledge score	-	-	-	0.06	0.05-0.07	<0.001

Abbreviations: Coef., Coefficients; CI, confidence interval; Ref., Reference.

People with a higher score of knowledge were more likely to have higher score of preventive practices (p<0.001). There was no significant difference of knowledge and practice scores of households with or without livestock-keeping.

Sources of information

Television was the most common source of information that the respondents heard about MBDs (92.9%), following by loudspeakers (70.6%), and health staff (54.9%) (Table 9).

Schools were the least widespread source of information on MBDs that the participants mentioned (1.61%). There were no differences between households with livestock or without livestock regarding the sources of information that they had heard about MBDs, except from the friends. The percentage of

households with livestock heard about MBDs from their friends was significantly higher than the percentage of households without livestock (p<0.01).

Table 7. Sources of the information participants have heard about MBDs in households with and without livestock

	HH with livestock (n, %)	HH without livestock (n, %)	p-value
Never heard	1 (0.44%)	3 (1.11%)	0.63
Television	209 (92.07%)	253 (93.7%)	0.4
Broadcast	89 (39.21%)	115 (42.59%)	0.44
Loudspeaker	164 (72.25%)	187 (69.26%)	0.46
Internet	61 (26.87%)	88 (32.59%)	0.16
Communication materials	54 (23.79%)	58 (21.48%)	0.54
Health staff	135 (59.47%)	138 (51.11%)	0.06
Friends	33 (14.54%)	19 (7.04%)	0.007
School	4 (1.76%)	4 (1.48%)	1.00

Abbreviations: HH, Household.

Paper III

A total of 12,861 mosquitoes and 2,427 larva samples were collected from 513 households. Notably, no mosquitoes at all were collected in 103 households. *Culex* was the most dominant genus, comprising more than 93% of the total collected adult mosquitoes. Among these, *Cx. tritaeniorhynchus* made up the majority, with 67.18%, followed by *Cx. gelidus*, *Cx. quinquefasciatus*, and *Cx vishnui* with approximately equal proportions of 8%. *Aedes* constituted the least abundant of the adult mosquitoes, with less than 1% amongst the five genera.

More *Ae. albopictus* were collected than *Ae. aegypti*. Other genera - including *Mansonia*, *Armigeres* and *Anopheles* - ranged from 1.21% to 3.82%.

Most mosquitoes were collected in the peripheral districts, while fewer mosquitoes were found in the central urban districts - especially in Ba Dinh. The peripheral district of Ha Dong had the highest number of mosquitoes collected, followed by the peri-urban district of Dan Phuong. *Cx. tritaeniorhynchus* accounted for the highest proportion in the peri-urban and peripheral districts, while *Cx. quinquefasciatus* was the dominant species in the central urban districts. *Ae. aegypti* were found mainly in the central urban districts, whereas they were not found in the peripheral districts. *Ae. albopictus* appeared in the peri-urban and peripheral districts much more often than in the central urban districts. In the urban districts, *Armigeres* and *Anopheles* were not found, whereas only one *Mansonia* mosquito was found there (Table 8).

Of the total number of larvae collected, *Ae. albopictus* were the most common larvae (>80%), while the percentage of *Ae. aegypti* larvae was 2.84%. Most of the *Ae. albopictus* larvae were collected in the peri-urban districts, followed by the peripheral districts. *Ae. aegypti* larvae were mainly collected in the central urban districts; however, their quantity was still lower than that of *Ae. albopictus* larvae. A total of 12.2% of the larvae collected belonged to the *Culex* species and were mostly found in the peripheral district of Ha Dong and in the peri-urban district of Chuong My. Only one *Culex* larva was collected in Dan Phuong district, and no *Culex* larvae were detected in Bac Tu Liem district. There were 18 *Armigeres* larvae found in total and all from the peripheral district of Bac Tu Liem. Only one *Anopheles* larva was collected in Chuong My district (Table 9).

Table 8. Distribution of adult mosquitoes by collection sites

| Mosquito species | Peri-urban districts | | | | Peripheral districts | | | | Central districts | | | | All | |
| | Chuong My | | Dan Phuong | | Bac Tu Liem | | Ha Dong | | Ba Dinh | | Cau Giay | | | |
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Aedes aegypti	1	0.08	2	0.09	0	0	0	0	3	5.9	5	2.7	11	0.09
Aedes albopictus	15	1.15	30	1.38	25	1.58	12	0.16	3	5.9	1	0.5	86	0.67
Other Aedes spp.	1	0.08	0	0	0	0	0	0	1	1.9	1	0.5	3	0.02
Culex tritaeniorhynchus	798	61.0	1,578	72.2	1,020	64.5	5,237	69.4	3	5.9	5	2.7	8,641	67.2
Culex vishnui	31	2.37	231	10.6	213	13.5	535	7.09	0	0	4	2.2	1,014	7.89
Culex. pseudovishnui	0	0	4	0.18	0	0	4	0.05	0	0	0	0	8	0.06
Culex quinquefasciatus	106	8.11	88	4.03	127	8.03	589	7.8	41	80.4	170	90.4	1,121	8.72
Culex gelidus	91	6.96	71	3.25	116	7.34	878	11.6	0	0	1	0.5	1,157	8.99
Culex fuscocephala	22	1.68	0	0	0	0	0	0	0	0	0	0	22	0.17
Mansonia spp.	31	2.37	41	1.88	20	1.27	63	0.84	0	0	1	0.5	156	1.21
Armigeres spp.	9	0.68	21	0.96	7	0.44	114	1.51	0	0	0	0	151	1.18
Anopheles spp.	202	15.4	119	5.46	53	3.35	117	1.55	0	0	0	0	491	3.82
Total	**1,307**	**100**	**2,185**	**100**	**1,581**	**100**	**7,549**	**100**	**51**	**100**	**188**	**100**	**12,861**	**100**

Table 9. Distribution of larvae species by collection sites

Larval species	Peri-urban districts				Peripheral districts				Central districts				All	
	Chuong My		Dan Phuong		Bac Tu Liem		Ha Dong		Ba Dinh		Cau Giay			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Aedes aegypti	0	0	1	0.4	0	0	0	0	45	23.2	23	57.5	69	2.84
Aedes albopictus	1,100	88.36	233	99.2	156	89.7	388	72	149	76.8	17	42.5	2,043	84.18
Culex	144	11.56	1	0.4	0	0	151	28	0	0	0	0	296	12.2
Armigeres	0	0	0	0	18	10.3	0	0	0	0	0	0	18	0.74
Anopheles	1	0.08	0	0	0	0	0	0	0	0	0	0	1	0.04
Total	**1,245**	**100**	**235**	**100**	**174**	**100**	**539**	**100**	**194**	**100**	**40**	**100**	**2,427**	**100**

In general, more mosquitoes (both indoor and outdoor) were trapped in households with livestock than in households without livestock. The median of total mosquitoes; mosquitoes indoor and mosquitoes outdoor collected in households with livestock was 9 ± 30 (med $\pm$ IQR), 3 ± 11 and 3 ± 16, respectively. While in households without livestock, the median of total mosquitoes, mosquitoes indoor and mosquitoes outdoor collected was 2 ± 5, 1 ± 3 and 0 ± 2, respectively. Likewise, households keeping pigs had much more of total mosquitoes, mosquitoes indoor and mosquitoes outdoor, than households without pigs. Not much of difference between the median of quantity of total mosquitoes, mosquitoes indoor and mosquitoes outdoor collected of households with or without cattle was found in this study. The total number of mosquitoes caught in households with poultry were higher than in households without poultry, however, the median quantity of mosquitoes indoor and outdoor collected was similar in households with or without poultry.

Regarding mosquito species, as described above, *Culex* was the most collected one, of which, the number of total *Culex* mosquitoes was higher in households with livestock, pig presence, cattle presence, and poultry-keeping, as compared to households without livestock. There was no difference between the median number of total mosquitoes, mosquitoes indoor, mosquitoes outdoor of other species and total larvae species collected in households with and without livestock.

Table 10 presents a correlation of the number of mosquitoes and larvae collected with the number of livestock in the households. Spearman'rho test showed that the number of pigs kept positively correlated with the number of total mosquitoes, total mosquitoes indoor, total mosquitoes outdoor, total *Culex*, *Culex* indoor, *Culex* outdoor, total *Anopheles*, *Anopheles* indoor, *Anopheles* outdoor, total *Armigeres*, *Armigeres* outdoor, total *Mansonia*, *Mansonia* indoor, and *Mansonia* outdoor.

There was no positive correlation of the numbers of mosquitoes and larvae with the number of cattle. While the test indicated weak positive correlation between total *Anopheles*, *Anopheles* indoor and outdoor; all larval species were positively correlated with the quantity of poultry.

Table 10. Correlation between the quantity of mosquitoes/larvae and the numbers of livestock kept

	Number of pigs		Number of cattle		Number of poultry	
	Spearman'rho	p-value	Spearman'rho	p-value	Spearman'rho	p-value
Total mosquito	0.34	<0.001	0.04	0.35	0.08	0.06
Total mosquito indoor	0.23	<0.001	0.008	0.84	-0.03	0.47
Total mosquito outdoor	0.29	<0.001	0.07	0.07	0.07	0.07
Total *Aedes*	0.02	0.59	-0.07	0.09	-0.04	0.27
Aedes indoor	0.01	0.76	-0.06	0.17	-0.06	0.14
Aedes outdoor	0.02	0.52	-0.04	0.35	0.005	0.9
Total *Culex*	0.34	<0.001	0.05	0.26	0.07	0.09
Culex indoor	0.22	<0.001	0.01	0.79	-0.02	0.56
Culex outdoor	0.28	<0.001	0.08	0.054	0.07	0.1
Total *Anopheles*	0.33	<0.001	0.07	0.1	0.2	<0.001
Anopheles indoor	0.26	<0.001	0.06	0.15	0.15	<0.001
Anopheles outdoor	0.24	<0.001	0.06	0.12	0.14	0.001
Aedes larvae	0.04	0.37	0.005	0.89	0.14	0.001
Culex larvae	0.06	0.16	-0.02	0.58	0.15	<0.001
Armigeres larvae	0.07	0.09	-0.01	0.8	0.15	<0.001
Anopheles larvae	0.06	0.16	-0.007	0.86	0.12	<0.01

Papers IV and V

Identification of risk factors of flaviviruses infection was conducted through a serological survey of dogs and pigs in Hanoi

Risk factor identification in dog models

Apparent seroprevalence in dog population

A total of 486 dogs from 224 households in the six districts of Hanoi were examined for the presence of antibodies against flaviviruses. The results by the cELISA for 486 dog sera revealed 336 samples positive to the pr-E protein of WNV, 139 negative and 11 doubtful. The 11 doubtful samples were removed from the calculation of seroprevalences and risk factor analysis.

Out of the remaining 475 dogs (336 seropositive and 139 seronegative) in 221 households, the flavivirus seropositivity was 70.7% (95% CI = 66.4% - 74.8%). The seroprevalences of males and females were 73.2% (95% CI = 66.6% - 79.0%) and 75.7% (95% CI = 67.9% - 82.1%), respectively.

The seroprevalences of crossbreed (91.3%; 95% CI = 84.1% - 95.5%) and local-breed (71.6%; 95% CI = 65.6% - 77.7%) were significantly higher than exotic-breed dogs (33.3%; 95% CI = 17.3% - 54.4%). Significantly higher seropositivity was found in dogs under 12 months-old (77.5%; 95% CI = 72.1% - 82.1%) as compared to dogs above 12 months (56.1%; 95% CI = 46.5% - 65.2%). The seroprevalence was significantly higher in peri-urban districts (93.3%; 95% CI = 88.1% - 96.4%) as compared to peripheral districts (74.5%; 95% CI = 68.5% - 79.7%) and central urban districts (22.5%; 95% CI = 14.9% - 32.4%). Dogs kept outside the house showed a significantly higher seroprevalence (79.5%; 95% CI = 70.9% - 86.0%) than indoor dogs (58.2%; 95% CI = 47.8% - 68.0%).

A total of 50 dog blood samples were tested by the PRNT for JEV-specific antibodies, of which 31 samples were positive and 19 samples were negative. By the cELISA, 35 sera were positive, 5 samples were doubtful, and 10 serum samples were negative (Table 11).

Table 11. Results of PRNT and cELISA on the same dog serum samples

	PRNT						
	Negative	*1:40*	*1:80*	*1:160*	*1:320*	*1:640*	*Total*
cELISA							
Positive	7	2	8	8	9	1	35
Doubtful	3		2				5
Negative	9	1					10

Univariable analyses at animal level identified significant associations be-tween seropositive dogs and district location, age of dog, breed of dog and keeping practice of dog in the households (Table 12).

Table 12. Association between seropositivity of dogs and exposure variables

Expo-sure variable	Label	Total test	Pos-itive	Seropreva-lence (95% CI)	OR (95% CI)	p-value
Sex	Female	140	106	75.7 (67.9 - 82.1)	Ref.	0.61
	Male	198	145	73.2 (66.6 - 79.0)	0.88 (0.52 - 1.48)	
Breed	Exotic	24	8	33.3 (17.3 - 54.4)	Ref.	<0.001
	Cross-breed	104	95	91.3 (84.1 - 95.5)	21.11 (6.28 - 72.6)	
	Local	215	153	71.6 (65.6 - 77.7)	4.94 (1.87 - 13.9)	
Age group	>12 months	107	60	56.1 (46.5 - 65.2)	Ref.	<0.001
	≤ 12 months	271	210	77.5 (72.1 - 82.1)	2.70 (1.62 - 4.46)	
District	Urban	89	20	22.5 (14.9 - 32.4)	Ref.	<0.001
	Periph-eral	235	175	74.5 (68.5 - 79.7)	10.06 (5.47 - 18.9)	
	Peri ur-ban	151	141	93.3 (88.1 - 96.4)	48.6 (20.4 - 121)	
Dog keeping at house	Inside	91	53	58.2 (47.8 - 68.0)	Ref.	0.001
	Outside	112	89	79.5 (70.9 - 86.0)	2.77 (1.43 - 5.42)	

Abbreviations: OR, odds ratio; Ref., Reference; CI, confidence interval.

Univariable analysis at household level

The results obtained by univariable analyses at household level in Table 13 revealed that households getting seropositivity against flavivirus were significantly associated with district location, presence of livestock such as pigs or chickens, mosquito-borne disease history in the family, and mosquito coil burning measure.

On one hand, the risk of households being seropositive in peri-urban districts (OR = 40.6, p <0.001) and peripheral districts (OR = 12.8, p <0.001) was significantly higher than in urban districts. Likewise, the risk of seropositivity was higher (p <0.01) in households keeping livestock, pigs and/or chickens than houses without livestock. Families without reported previous human cases of MBDs had a higher risk of having seropositive dogs (OR = 5.96, p = 0.002).

There was no significant difference in seroprevalence depending on presence of cats in the houses.

On the other hand, burning coils to control mosquitoes was significantly associated with an increased proportion of positive households (OR = 3.263, p = 0.019). Other practices at households including door/window screening, use of repellents, mosquito bed-net, mosquito electric traps or rackets, lid covered on water tanks, larvicides, insecticides, eliminating breeding sites of mosquitoes, and fish keeping in water tanks did not show a significant risk associated with flavivirus exposure.

Table 13. Association between seropositivity of dog-keeping households and exposure variables

Expo-sure var-iable	Label	Total HH	HH posi-tive	OR (95% CI)	p-value
District	Urban	65	19	Ref.	-
	Peripheral	85	75	18.2 (7.77 - 42.5)	<0.001
	Peri-urban	71	67	40.6 (12.9 - 127)	<0.001
HH keep-ing live-stock in general	No	137	83	Ref.	<0.001
	Yes	81	75	8.13 (3.31 - 20.0)	
HH keep-ing pig	No	148	94	Ref.	<0.001
	Yes	70	64	6.13 (2.49 - 15.1)	
	No	180	123	Ref.	0.007

Exposure variable	Label	Total HH	HH positive	OR (95% CI)	p-value
HH keeping chicken	Yes	38	35	5.41 (1.60 - 18.3)	
Family member had experienced with mosquito disease	Yes	15	5	Ref.	0.002
	No	199	149	5.96 (1.94 - 18.3)	
Mosquito prevention practice by using					
Electric racket / portable electric trap	No	87	67	Ref.	0.16
	Yes	126	86	0.64 (0.34 - 1.20)	
Mosquito coil / Incense stick	No	173	118	Ref.	0.019
	Yes	40	35	3.26 (1.21 - 8.78)	
Lid covered on water tank	No	136	103	Ref.	0.09
	Yes	77	50	0.59 (0.32 - 1.09)	
Chemical/ larvicide in water container	No	201	146	Ref.	0.29
	Yes	12	7	0.527 (0.16 - 1.73)	
Breeding site elimination	No	162	113	Ref.	0.23
	Yes	51	40	1.58 (0.75 - 3.33)	
Fish in water container	No	144	54	Ref.	0.15
	Yes	69	54	1.64 (0.84 - 3.20)	

Abbreviations: HH, Household; OR, odds ratio; Ref., Reference; CI, confidence interval.

Multivariable analysis

The paired variables between district location and livestock-keeping (r = -0.75), between district location and pig (r = -0.71), and between livestock-keeping and pig production (r = 0.89) were strongly correlating. Of the 81 households keeping livestock, 86% (n=70) of the households kept pigs and the final models of pig-keeping and livestock-keeping variables were not different. Therefore, the variable for livestock-keeping was moved out from the modelling. Two multivariable logistic regression models were built (Table 14).

In model 1 that excluded the variables of livestock-keeping and pig-keeping, coil burning had an effect of more than 33% on the coefficient of larvicides using and changed this exposure variable to become insignificant, therefore this confounding factor was added back to the model. The final model determined that district location and use of larvicides in water tanks were significantly associated with the antibody positivity of the households (p<0.05).

The model 2 without the variables of district location and livestock-keeping identified significant risk of the antibody positivity of households as pig-keeping, mosquito electric trap using, coil burning, and mosquito-borne disease history of the family (p<0.05).

Both models showed a good fit (Hosmer-Lemeshow statistic test, p = 0.114 and 0.541, respectively).

Table 14. Multivariable analysis of risk factors for household level flavivirus sero-positivity in dogs

Exposure variable	Categories	Coef.	OR	95% CI	p-value
Model 1. Without the variables for livestock-keeping and pig-keeping					
District	Urban	Ref.	Ref.		
	Rural	3.70	40.6	12.3 - 134	<0.001
	Peri-urban	2.81	16.7	6.96 - 40.2	<0.001
Larvicides in water containers	Yes	Ref.	Ref.		
	No	1.68	5.39	1.06 - 27.3	0.042
Coil burning	No	Ref.	Ref.		
	Yes	0.78	2.18	0.58 - 8.11	0.247
Model 2. Without the variables for district location and livestock-keeping					
Pig keeping	No	Ref.	Ref.		
	Yes	1.75	5.76	2.27 - 14.6	<0.001
Not use mosquito electric racket/trap	No	Ref.	Ref.		
	Yes	0.73	2.08	1.05 - 4.14	0.036
Coil burning	No	Ref.	Ref.		
	Yes	1.13	3.09	1.04 - 9.17	0.042
Had experience with MBDs in family	Yes	Ref.	Ref.		
	No	1.60	4.94	1.50 - 16.3	0.009

Abbreviations: Coef., Coefficients; Ref., Reference; OR, odds ratio; CI, confidence interval.

Risk factor identification in pig models

Apparent seroprevalence in pigs

In the four districts that had pigs, 192 pig farms were visited, and 704 blood samples were tested by the cELISA. However, 13 pig farms missed information in the questionnaire, and 53 serum samples from these 13 farms and 15 serum samples of other farms provided doubtful results by the cELISA were excluded in the analyses. Finally, the results from a total of 179 farms and 636 pigs were analysed.

Apparent seroprevalence by the cELISA test was 88.5% (95% CI = 85.8% - 90.9%). The seroprevalence of pigs based on cELISA of smallholders with less than 10 animals (71.4%; 95% CI = 62.4% - 80.4%) was significantly lower than small-medium farms with 10 to 100 pigs (91.6%; 95% CI = 89.2% - 90.0%), and medium farms with more than 100 pigs (92.1%; 95% CI = 83.4% - 100%). The seroprevalence of pigs under 4 months varied from 90.1% for 4 months-old pigs to 98.2% for 2 months, which were significantly higher than slightly older pigs, above 6 months, at 65.9% (p <0.05).

The seropositivity found in the peri-urban district Dan Phuong (68.3%; 95% CI = 60.0% - 76.7%) was significantly lower than in the peripheral districts of Ha Dong (89.7%; 95% CI = 83.6% - 95.8%) and Bac Tu Liem (92.6%; 95% CI = 83.6% - 95.8%), and Chuong My peri-urban district (95.8%; 95% CI = 92.9% - 98.6%).

In total, 50 pig serum samples were tested by the PRNT for JEV-specific antibodies, of which 13 samples were positive and 37 samples were negative. By the cELISA, 26 samples were positive, 6 samples were doubtful, and 18 samples were negative (Table 15).

Table 15. Results of PRNT and cELISA on the same pig serum samples

| | **PRNT** | | | | | | |
	Negative	1:20	1:40	1:80	1:160	1:320	Total
cELISA							
Positive	16	6	2	1		1	26
Doubtful	6						6
Negative	15	3					18

Univariable analyses at pig level (Table 16) determined significant associations between seropositive pigs and herd size, age of pig, and district location.

Table 16. Association between seropositivity of pigs and exposure variables

Expo-sure var-iable	Label	Total test	Posi-tive	Seroprev-alence (95% CI)	OR (95% CI)	p-value
Herd size	<10 pigs	98	70	71.4 (62.4-80.4)	Ref.	-
	10-100 pigs	500	458	91.6 (89.2-90.0)	4.36 (2.54-7.49)	<0.001
	>100 pigs	38	35	92.1 (83.4-100)	4.67 (1.33-16.4)	0.016
Breed	Cross-breed	387	326	84.2 (80.6-87.9)	Ref.	-
	Exotic	84	76	90.5 (84.1-96.8)	1.78 (0.82-3.87)	0.15
	Local	3	3	100	-	-
Age group	> 6 months	44	29	65.9 (51.7-80.1)	Ref.	-
	2 months	56	55	98.2 (94.7-100)	28.4 (3.58-226)	0.002
	3 months	228	217	95.2 (92.4-98.0)	10.2 (4.28 - 24.3)	<0.001
	4 months	192	173	90.1 (85.9-94.3)	4.71 (2.15-10.3)	<0.001
	5 months	71	53	74.6 (64.4-84.9)	1.52 (0.67-3.46)	0.31
District	Dan Phuong	120	82	68.3 (60.0-76.7)	Ref.	-
	Chuong My	189	181	95.8 (92.9-98.6)	10.5 (4.68-23.5)	<0.001
	Bac Tu Liem	230	213	92.6 (89.2-96.0)	5.81 (3.10-10.9)	<0.001
	Ha Dong	97	87	89.7 (83.6-95.8)	4.03 (1.89-8.61)	<0.001

Abbreviations: OR, odds ratio; CI, confidence interval; Ref., Reference.

Univariable analysis results at farm level

A positive farm was defined as having at least one pig positive by the cELISA test. The results of univariable analyses at farm level identified significant associations between seropositive farms and herd size, some mosquito control measures of the owners consisting of using repellent, wearing long-sleeve clothes, and covering lid on water tank (Table 17).

The odds of being seropositive in small-medium farms were significantly higher than in the small size farms (OR = 15.1, p <0.001). Pig farms where the owner was not using repellent had a higher odds ratio of being seropositive (OR = 6.22, p =0.008). A similar higher risk of seropositivity at pig farms was recorded by not wearing long-sleeve clothes (OR = 6.05, p =0.009), and not using closing lids of water tanks (OR = 7.58, p =0.01).

There was no significant difference in seropositivity of farms depending on district location, or mosquito vectors. Likewise, significant associations between seropositivity and a history of mosquito-borne disease in the family, mosquito-prevention by window/door screening, electric traps or rackets, mosquito coil burning, larvicides, spraying insecticides, and keeping fish inside water tanks were not found in this study.

Table 17. Association between seropositivity of pig-keeping households and exposure variables

Exposure variable	Label	Total HH tested	HH positive	OR (95% CI)	p-value
Herd size	<10 pigs	35	26	Ref.	-
	10-100 pigs	134	131	15.1 (3.83-59.6)	<0.001
	>100 pigs	10	10	1	-
Mosquito prevention practice by using					
Window /door screen	Yes	26	22	Ref.	0.07
	No	152	144	3.27 (0.91-11.8)	
Repellent	Yes	63	54	Ref.	0.008
	No	115	112	6.22 (1.62-23.9)	
Long-sleeve clothes	Yes	64	55	Ref.	0.009
	No	114	111	6.05 (1.58-23.3)	
Lid covered on water tank	Yes	76	66	Ref.	0.01
	No	102	100	7.58 (1.16-25.7)	
Chemical/ larvicide in water container	Yes	11	9	Ref.	0.14
	No	167	157	3.49 (0.66-18.3)	
Fish in water container	Yes	77	69	Ref.	0.10
	No	101	97	2.81 (0.81-9.71)	

Abbreviations: HH, Household; OR, odds ratio; CI, confidence interval.

Multivariable analysis results

The logistic regression model identified significant associations between the antibody positivity of pig farms and farm size with 10 to 100 pigs, lid covered on water tank, and elimination of breeding site ($p<0.05$) (Table 18). The model showed a good fit (Hosmer-Lemeshow statistic test, $p = 0.621$).

Table 18. Multivariable analysis of risk factors for pig-keeping households

Exposure variable	Categories	Coef.	OR	95% CI	p-value
Farm size	<10 pigs	Ref.	Ref.		
	10-100 pigs	2.56	12.9	2.87 – 58.5	0.001
	>100 pigs	0	-	-	-
Repellent	Yes	Ref.	Ref.		0.051
	No	1.62	5.05	0.99 – 25.7	
Lid covered on water tank	Yes	Ref.	Ref.		0.046
	No	1.81	6.09	1.03 – 35.8	
Breeding site elimination	Yes	Ref.	Ref.		0.021
	No	-2.14	0.12	0.02 – 0.72	

Abbreviations: Coef., Coefficients; Ref., Reference; OR, odds ratio; CI, confidence interval.

Discussion

Urban livestock production of Hanoi has developed consistent to the local policy. Like other growing economies, urban livestock-keeping of Hanoi has contributed to improved food and nutrition security, not only for the wealthy, but also for the poor. In addition, more than three hundred-thousand small-scale farmers of the city had earned their income and had employment from livestock-keeping.

An increase of pig and poultry sectors of Hanoi is consistent with a previous report that forecasted future livestock to 2020 in developing countries [106]. In a survey in Hanoi, more than 80% of respondents have consumed pork in their daily diets [107]. Furthermore, poultry meat is the second most important meat after pork of the Vietnamese people [108].

However, urban livestock-keeping of Hanoi with a majority of small-scale livestock farms could cause several negative impacts in terms of poor animal health and environment management, shortage of veterinary workforce, and risk of zoonotic disease transmission. Especially, some mosquito-borne zoonotic diseases can be spread widely in urban areas that have densely populated inhabitants and livestock. A previous study in Vietnam demonstrated that the urban pig-keeping has increased the number of mosquitoes competent as vectors for JEV [109].

Our study indicated that mosquito species competent for JEV, DENV and some zoonotic flaviviruses were present in Hanoi city. Particularly, a total of five genera of mosquito vectors for MBDs (*Aedes*, *Culex*, *Mansonia*, *Anopheles*, and *Armigeres*) were identified. These genera were also collected in a study from other provinces in Vietnam [110]. *Culex* mosquitoes were most abundant, which was similar to previous entomological studies in Vietnam [85], [111]; Guwahati city, India [112]; Khyber Pakhtunkhwa province of Pakistan [113]; Lorestan province, Iran [114]; and Atlanta, USA [115]. The results indicate a wide dispersion of *Culex* mosquitoes in tropical and subtropical regions around the world.

In contrast, the genus *Aedes* was the least abundance compared to the other genera. This is consistent with the finding in Lao PDR [116]. *Ae. albopictus* larvae were more commonly detected than other species. An aggressive spread of *Ae. aegypti* and *Ae. albopictus* mosquitoes has been recorded across the world [117]. These *Aedes* species, that are vectors of dengue fever, were found in all six districts of Hanoi.

This study determined associations between the abundance of mosquitoes - especially *Culex* mosquitoes and the district location, pig-keeping, and preventive practices of local people. Compared to peri-urban districts, central urban districts had fewer mosquitoes, whereas the peripheral districts had more mosquitoes. This might be related to the activity of livestock-keeping. In the central urban districts of Hanoi, livestock production has recently been restricted.

Some factors related to environment could also contribute to the difference in mosquito-presence between the districts. The wastewater and sewer systems, the potential breeding sites of many mosquito species, such as *Aedes* [114, 115] and *Culex* [115], are more developed in the central urban districts as compared to the peri-urban districts. Meanwhile, peripheral districts are in a mixed situation, between central urban and peri-urban districts, with a rapid urbanization but still retaining livestock-keeping practice.

Pigs can potentially provide bloodmeals for *Culex* mosquitoes and act as amplifying hosts for JEV [120]. In our study, pig-keeping showed an increase of the number of mosquitoes - especially *Culex* mosquitoes, while cattle-keeping was not significantly correlated with the number of mosquitoes, although cattle may contribute to abundance of *Anopheles* mosquitoes [121]. Poultry-keeping was significantly correlated with the number of *Anopheles* mosquitoes and larvae. However, there was not any evidence of the relationship between poultry-keeping and mosquito abundance in this study.

Mosquito-borne flavivirus transmission in Hanoi city was determined in our study. A very high seroprevalence against flaviviruses was detected in dogs (70.7%), and in pigs (88.5%) by the cELISA kit. It noted that WNV has never been reported in both humans and animals in Vietnam [139], therefore antibodies from dog and pig blood samples detected in the study could be from infections of other flaviviruses endemically circulating in Vietnam. In addition, vaccination against MBD flaviviruses for dog or pig populations is not practical in Hanoi city, therefore serological monitoring in domestic animals could provide information on potential risks and epidemiology of mosquito-borne zoonotic flaviviruses in the community.

JEV-specific antibodies found in pigs in our study were similar to other findings in Vietnam [125, 126], but the detection of antibodies against flaviviruses and JEV-specific antibodies in the dog population of Hanoi was the first report in Vietnam.

In the dog model for identifying risk factors of flavivirus infections, the seropositivity in dogs in Hanoi was much higher (70.7%) than in China, at 5.7% [124], Romania, at 42.1% [125], and Morocco, at 62% [126].

Dog puppies under 12 months of age showed more than two times higher odds of having flavivirus-specific antibodies as compared to adult dogs. Naturally, old animals have had more chances of being exposed to infectious agents. However, maternal antibodies from infected mother dogs to their puppies through colostrum could be maintained for several months, which could

explain this higher rate. However, maternal immunity to flaviviruses in dogs is still unknown. Generally, immunity relies on flavivirus antibody persistence in vertebrate hosts, but the mechanism for persistence is still poorly understood [127].

Dogs with greater outdoor exposure obtained a higher level of flavivirus seroprevalence, which was also consistent with earlier findings [124], [128]. Generally, exotic breeds of dogs imported to Vietnam have a very high economical value and they are kept closer to their owner. In contrast, crossbreed and indigenous dogs that have lower value may be kept outdoors more; therefore, they have a greater possibility of being infected with flaviviruses through mosquito feeding. Significantly higher seroprevalences to flavivirus of local and crossbreed dogs, likely due to more frequent outdoor keeping, were also found in this study.

District location was significantly associated with seropositivity among the dogs, while sex of dogs was not a risk factor, which was similar to an earlier study [49]. Peri-urban dogs and peripheral dogs showed forty times and eighteen times higher risk of exposure as compared to urban dogs, respectively. This is likely related to the greater livestock presence in peri-urban and peripheral areas than in urban Hanoi, since more mosquitoes have been found in livestock shelters than in non-livestock-keeping households [86].

The risk of flavivirus exposure in dogs was about six times higher in families without historical infection of MBDs, as compared to households that reported earlier human cases of DENV or JEV. The reduced risk of flavivirus seropositivity in dogs in households with previous experience of MBD could also be correlated to the risk mitigation behaviour of these families.

Some mosquito prevention measures applied in households were reported in this study. In particular, door and window screens can block entry points for mosquitoes in a house [129], while repellents can influence mosquito olfaction [130]. Mosquito bed-nets are the most commonly used measure by people in Hanoi, followed by anti-mosquito products such as insecticides, elimination of breeding sites and electric rackets [131]. Water containers are the most likely breeding sites of some mosquito species such as *Ae. albopictus* and *Cx. quinquefasciatus* [132]. Previous studies in Vietnam concluded that the use of an appropriate cover on water storage containers effectively reduces pre-adult mosquito infestation levels [130, 131]. Keeping fish as predators of mosquito larvae, a biological control for MDBs, has also been studied [132, 133]. However, some methods that protect humans and pets from mosquito bites in this study such as using a window screen, repellents, mosquito bed-nets and insecticides spraying did not show any efficiency of lowering seropositivity against flavivirus for the dogs.

Burning a mosquito coil indoors generates smoke that can control mosquitoes [134, 135]. In this study, a significant increase in the seropositivity of dogs in the houses burning coils as compared to dogs from the houses without coils was identified, but dogs' behaviour in reaction to coil smoke is unclear.

If dogs are sensitive to coil smoke, they may avoid it the same way as mosquito vectors and thus still be at risk of infection. A previous study suggested that mosquito coils do not significantly affect the risk of a MBD in humans if the coils are burnt just once per week [139].

Multivariable logistic regression models identified more rural location, pig-keeping, no application of mosquito control measures such as electric rackets or larvicides in home water tanks, burning coils and family without mosquito-borne disease experience as the main risk factors for flavivirus exposure of dog-keeping households.

The flavivirus seropositivity of pigs in Hanoi city in this study was much higher (88.5%) than in a previous study in northern Vietnam (60.4%), which used the same cELISA kit [122]. We recorded a gradual declining seropositivity in univariable analysis from 98.2% of two months-old pigs, the age that maternal antibodies start to disappear [140], to 65.9% of pigs older than 6 months. In fact, most of the pigs sampled in this study were young, with only 45 animals being above 6 months including the 3 oldest ones (18 months-old).

Similar to the findings of the dog models, most of the mosquito control measures applied by pig farmers in Hanoi could not reduce seropositivity against flaviviruses in their pig herds. In fact, mosquito vector control can only work effectively in certain conditions [66], [142–144]. Multivariable logistic regression model determined that the small-medium herd of 10 to 100 pigs, and not covering lid of water tank as major risk factors for flavivirus exposure of pig farms. However, not eliminating mosquito breeding sites significantly reduced seropositivity of pig farms found in our study. This is an unusual result that could be affected by unidentified factors from the pig farms, because the elimination of mosquito breeding site can break down the life cycle of mosquito vectors.

Regarding the assessment of knowledge and practices about MBDs of local people of Hanoi, our study determined a gap in both knowledge and practices of the community. Particularly, the average score for knowledge was 18.3 out of 35, a low level that is similar to a previous study in Hanoi [144]. This finding is in agreement with other studies in Lao PDR [145], Indonesia [146], Nepal [147] and India [148].

Most participants (96%) had heard about dengue fever. In fact, in Hanoi, dengue has been recorded as the most common MBD, while malaria has almost disappeared during last decades, and Zika virus infection in humans has only been detected in the central and southern regions of Vietnam [57]. JE and filariasis rarely appear in the city, leading to few participants had recognized them. Moreover, the immunization program for children under 5 years by a domestic inactivated mouse brain derived JE vaccine started in 1997 has significantly decreased the incidence of JE in Vietnam [43]. Most respondents could mention breeding sites of mosquitoes, but many of them (80%) did not know that mosquitoes can breed in clean water, which is the preferred site of the dengue vectors.

The negative binomial model revealed that people living in central urban districts had better knowledge on MBDs than people living in peri-urban districts of Hanoi.

The average score for practices against MBDs was only 3.5 out of 11, which is lower than other studies in Lao PDR [145], Indonesia [149], and Malaysia [150]. Our study determined that households without livestock had a better knowledge ($p<0.05$), but poorer practices on MBDs as compared to households with livestock. Households without livestock were assumed to have better living conditions since they had higher average income in this study (data not shown). Thus, they might have had more chances to approach the sources of health information.

This study found that farmers had a higher score of using preventive practices against MBDs than office workers. The farmers belonged to the households with livestock that had better practices against MBDs than households without livestock. They were more aware of unhygienic conditions when they kept livestock nearby home, so they more frequently cleaned animal shelter and house, and used insecticides to prevent insects. This finding is in agreement with a study in southern Vietnam [151].

There was a positive correlation between knowledge and practices on MBDs of the community. The respondents with better knowledge on MBDs had better practices against MBDs, that is similar to previous studies [95–97]. When people perceived a problem adequately, they would tend to execute more correctly.

The most common source of information of MBDs mentioned by the respondents was television (92.9%), followed by loudspeakers (70.6%), but their effectiveness was unclear. The information on MBDs was heard least in schools. However, students are amongst the high-risk group to get dengue infection in Hanoi [152], and students and pupils have previously been reported to have poor knowledge and practices against MBDs [153], and be less likely to use repellent and bed nets to prevent MBDs [154]. Hence, it is recommended that communication campaigns in schools and universities should be organized more regularly.

Overall, our study indicated risks of mosquito-borne zoonotic flavivirus transmission in urban areas of Hanoi city. Particularly, dense populations of dwellers and urban livestock of the city were evident. In addition, the association between livestock-keeping, especially pig-keeping, and mosquito vector abundance was determined. Furthermore, seropositivity against flaviviruses of dogs and pigs was at very high level. This is because the domestic animals had exposed to flaviviruses, and some dogs and pigs were confirmed to have specific antibodies against JEV. Although, there was a gap of knowledge and preventive practices of local people against MBDs identified. Common mosquito control measures of animal owners did not reduce seropositivity of dogs and pigs kept in the households.

Conclusions

This thesis investigates the associations of livestock-keeping in a rapid urbanized city as Hanoi, the capital of Vietnam, and the various risks of mosquito-borne zoonotic flavivirus infections to humans.

- The livestock development of Hanoi had remarkably increased during the period from 2014 to 2018 in line with the provincial policy. However, the densely populated livestock, and a high number of small-scale livestock farms create a large population of susceptible animal hosts for mosquito-borne zoonotic flavivirus transmission (Paper I).
- A low level of knowledge and preventive practices against MBDs of the livestock-keeping community in Hanoi was identified in this study. The households with livestock had poorer knowledge but better practices for control of MBDs than households without livestock (Paper II).
- Mosquito vector abundance in Hanoi - mainly *Culex tritaeniorhynchus*, followed by other *Culex* species was significantly associated with livestock presence, especially pig-keeping. This indicated that urban livestock-keeping may pose a risk for zoonotic transmission of MDBs (Paper III).
- In urban Hanoi, where livestock and pets are closely kept to human living area, a high flavivirus seroprevalence in dogs (70.7%,), and even higher (88.5%) in the pig population indicated a potential risk of mosquito-borne zoonotic flavivirus infection to local people. Evidence of JEV circulation in pig and dog populations through the PRNT was determined. Furthermore, our study found that some common mosquito preventive measures of local people in Hanoi did not significantly mitigate the risk of flavivirus infections in livestock-keeping households (Paper IV and Paper V).

Summary in Vietnamese

Những khu vực đô thị hóa cao, nơi có mật độ chăn nuôi lớn xen kẽ với khu dân cư và có sự hiện diện của quần thể muỗi véc tơ, sẽ là địa điểm thuận lợi đối với sự lây truyền một số bệnh gây ra bởi flavivirus cho con người. Nghiên cứu của chúng tôi tập trung vào bốn nội dung chính gồm: (1) Phát triển chăn nuôi; (2) Kiến thức và hành vi của người dân đối với việc phòng ngừa một số bệnh lây truyền từ muỗi; (3) Quần thể muỗi véc tơ; (4) Các yếu tố nguy cơ đối với sự phơi nhiễm flavivirus của vật nuôi trên địa bàn thành phố Hà Nội.

Các phân tích về chính sách phát triển chăn nuôi, quần thể vật nuôi, phỏng vấn người cung cấp thông tin chính cho thấy mặc dù chăn nuôi trong giai đoạn từ năm 2014 đến năm 2018 phù hợp với định hướng của thành phố, tuy nhiên một số hạn chế như chăn nuôi quy mô nhỏ lẻ còn phổ biến, các dịch bệnh nguy hiểm trên đàn vật nuôi và sự tồn tại của nhiều trang trại chăn nuôi tại khu vực đô thị sẽ ảnh hưởng đến sự phát triển bền vững của ngành chăn nuôi Hà Nội.

Nghiên cứu cắt ngang được thực hiện tại 513 hộ gia đình trên địa bàn 6 quận, huyện của Hà Nội cho thấy nhận thức và hành vi của người dân trong phòng, chống bệnh lây truyền từ muỗi là chưa cao. Qua phân tích 12.861 mẫu muỗi trưởng thành và 2.427 bọ gậy thu thập được tại các hộ gia đình, muỗi *Culex* chiếm tỷ lệ cao nhất (93,01%), kế tiếp là muỗi *Anopheles* (3,82%), *Mansonia* (1,21%), *Armigeres* (1,18%) và *Aedes* (0,78%); ở chiều ngược lại thì bọ gậy của muỗi *Aedes* chiếm 87,02% và của muỗi *Culex* là 12,2%. Có sự tương quan theo tỷ lệ thuận giữa chăn nuôi lợn và quần thể muỗi *Culex* ($p<0.001$).

Tổng số 475 con chó và 636 con lợn đã được lấy mẫu máu để xét nghiệm huyết thanh học bằng phương pháp ELISA cạnh tranh (cELISA) phát hiện kháng thể kháng flavivirus, kết quả cho thấy tỷ lệ dương tính huyết thanh trên vật nuôi là rất cao, 70,7% đối với chó và 88,5% đối với lợn. Kết quả trung hòa kháng thể với virus gây bệnh Viêm não Nhật Bản trên môi trường tế bào BHK-21 (viết tắt là PRNT) của 50 mẫu huyết thanh chó (gồm 34 mẫu dương tính, 6 mẫu nghi ngờ, 10 mẫu âm tính bằng phương pháp cELISA), và 50 mẫu huyết thanh lợn (gồm 26 mẫu dương tính, 6 mẫu nghi ngờ, 18 mẫu âm tính bằng phương pháp cELISA) cho thấy 28 mẫu huyết thanh chó và 13 huyết thanh lợn có kháng thể đặc hiệu kháng virus gây bệnh Viêm não Nhật Bản.

Những kết quả nêu trên đã chứng minh có sự liên quan giữa chăn nuôi gia súc và nguy cơ lây truyền một số bệnh do flavivirus từ muỗi sang người trên địa bàn Hà Nội.

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