

Health and economic burden of human *Streptococcus suis* infection in Viet Nam and the contribution of undercooked pig product consumption practices: implications for prevention and control

Huong Vu

The Queen's College

Clinical Medicine

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Abstract

Streptococcus suis is a zoonotic bacterium capable of causing severe systemic infection in humans, including central nervous system infection (meningitis) which can lead to long-term sequelae including deafness and vestibular dysfunction. Despite a growing body of literature on the epidemiology, microbiology and molecular aspects of the disease, there has been little progress on the prevention and control of this disease in Asia. This thesis aimed to examine the long-term consequences of the disease in patients, quantify the health and economic impact, and investigate the local practice of consuming raw pig blood, one of the main risk factors for *S. suis* infection in Viet Nam.

I performed a systematic review and meta-analysis and the review results showed that hearing loss and vestibular dysfunction were the most common health sequelae in patients surviving *S. suis* infection. In a patient follow-up study at a referral hospital in Ha Noi, I found that severe hearing loss and vestibular dysfunction both occurred, and persisted over a long period in 40% of patients. Morbidity caused by these two conditions accounted for about half of the disability adjusted life years lost due to the infection. The analysis of disease impact showed that *S. suis* infection caused a considerable impact on the patients and their family. The direct cost of hospitalization was estimated to be 35 million VND per episode (~ 1600 USD), which was greater than the average annual per-capita income in Viet Nam. The total annual cost of the disease was 54-70 billion VND (2.5-3.3 million USD), more than 80% of which was attributed to productivity loss due to premature deaths and long-term disability.

A mixed-methods community-based study, consisting of both quantitative survey and focus group discussion, showed that raw pig blood consumption was common in Viet Nam (reported in 35% of rural and 8.6% of urban participants). Many misperceptions about food safety and risk of disease transmission existed in the community; most importantly was the belief that apparently healthy or free-ranging pigs did not pose health risks to consumers. Data from this thesis can be used by public health and policy makers to prioritise and justify resource allocation and to identify options for disease prevention and control.

Co-Authorship

The work presented in this thesis was primarily completed by me, under the close supervision and support from my supervisors: Professor Heiman FL Wertheim, Professor Peter Horby, and Dr Ngo Thi Hoa and with the close support from my colleagues at Oxford University Clinical Research Unit – Viet Nam (OUCRU). For most of the work, I led day-to-day management and coordination under supervision of Professor Heiman FL Wertheim - my director of studies, and Dr Rogier van Doorn - director of OUCRU Ha Noi. The systematic review and meta-analysis was carried out in collaboration with Nagasaki University, Japan. I was involved in all activities from searching, data extraction through to data synthesis and analysis under the supervision of my supervisors and Associate Professor Nguyen Tien Huy at the Nagasaki University. I designed and implemented the community assessment of raw pig blood consumption practice in collaboration with Hanoi Medical University (HMU) under the support of Associate Professor Nguyen Thi Kim Chuc and Dr Tran Khanh Toan. I facilitated all the focus group discussions, while quantitative interviews were carried out by HMU field surveyors. I designed and implemented the cohort study in collaboration with the National Hospital for Tropical Diseases (NHTD) under the support of Associate Professor Nguyen Van Kinh. A study team consisting of study doctors, nurses and coordinators of both OUCRU (including myself) and NHTD conducted patient and control recruitment, assessments and interviews. I ran all statistical analyses with the support from Vu Tien Viet Dung, OUCRU biostatistician and Le Viet Thanh, OUCRU research scientist, particularly for the burden of disease analysis. I also received advice from Dr Marc Choisy (Institute of Research for Development, France) on the modelling of disease incidence. Surveillance data on *S. suis* infection were provided by Dr Pham Quang

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Declaration

Other than the assistance outlined in the co-authorship and acknowledgements, the work described in this thesis is my own work and has not been submitted for a degree or other qualification to any other university.

Abbreviations

CI	Confidence interval
CNS	Central nervous system
CPS	Capsular polysaccharide
CRFs	Case Record Forms
CSF	Cerebrospinal fluid
DALY	Disability Adjusted Life-Year
DHI	Dizziness Handicap Inventory
dB HL	decibels Hearing Level
FG	Focus group
GBD	Global Burden of Disease
GHEs	Global Health Estimates
HCMC	Ho Chi Minh City
HES	Household economic status
HHIA	Hearing Handicap Inventory for Adults
HMU	Hanoi Medical University
IQR	Interquartile range
MAR	Missing at random
MCAR	Missing completely at random
m-CTSIB	Modified Clinical Test of Sensory Interaction and Balance
MICE	Multiple imputation by chained equations
MMSE	Mini Mental State Examination
MNAR	Missing not at random
NHTD	National Hospital for Tropical Diseases
NIHE	National Institute of Hygiene and Epidemiology

HTD	Hospital for Tropical Diseases
OR	Odds ratio
PCR	Polymerase chain reaction
PR	Prevalence ratio
PRRSV	Porcine Reproductive and Respiratory Syndrome Virus
PVLE	Present Value of Lifetime Earnings
SD	Standard deviation
TSLS	Toxic-shock like syndrome
UI	Uncertainty interval
USD	U.S. Dollar
VAS	Visual analogue scale
VND	Viet Nam Dong
VSS	Vertigo Symptoms Scale
WHO	World Health Organization
YLD	Year lived with disability
YLL	Year of life lost due to premature mortality

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Chapter I

Introduction

1. 1. *Streptococcus suis* infection in the global context

1.1.1. Global epidemiology of *Streptococcus suis* infection

Streptococcus suis is a zoonotic bacterium that is a major problem for the pig industry worldwide and a common cause of adult bacterial meningitis in human in pig-rearing areas of Southeast Asia [1]. The first human case with *S. suis* was reported in Denmark in 1968 [2]. It has been considered rare in humans, causing sporadic cases among those occupationally exposed to pig or pork-derived products until a decade ago when a large outbreak with a high case-fatality rate occured in China in 2005 [3]. Since then the number of reported cases rapidly increased. By 2009, over 700 cases of *S. suis* infections were reported worldwide, most of them from China and Viet Nam [4]. In the most recent review by 2013, this number increased to over 1,600 cases [5]. These only included cases that were identified and reported, likely representing only a small fraction of the real burden. For example, in Viet Nam alone, around a hundred cases per year are reported to the surveillance system and have not been included in the total count of published cases in the literature. More cases have been reported in Europe and Asia than in other regions. Variable levels of diagnostic capacity and awareness about the disease, and differential distribution of strains and virulence factors may all contribute to the geographical variation in the reported incidence of *S. suis* infection worldwide [6].

Cases have mainly been reported among adults, particularly men and in people with occupations that involve exposure to pigs or pig derived products [4, 7, 8]. *S. suis* infections in children are rare: an infection has been reported in a 2-year-old girl [9] and in a 2-month-old infant [10] in Thailand. It is unclear if *S. suis* infection in humans is seasonal. While sporadic cases occur without any clear seasonal patterns, outbreaks tend to occur in warmer months of the year in Asia [11-15]. In some areas with high incidence such as Thailand and in the north and central Viet Nam, cases also occurred mainly during summer or rainy periods [16-19], but this pattern was not observed in the south of Viet Nam [19, 20].

Epidemiological charateristics of cases differ between Western and Asian regions [6], with a recent recognition of the importance of high-risk eating habits (i.e. consuming raw or undercooked pig parts including pig blood, organs, and meat) in some Asian communities [21-23]. Rates of *S. suis* infection are low among the general population in Europe and North American countries, and cases concentrate among occupationally exposed groups including abattoir workers, butchers and pig breeders [24, 25]. The estimated annual risk of developing *S. suis* meningitis in the Netherlands has been estimated to be 3.0 per 100 000 population for abattoir workers and pig breeders and 1.2 cases per 100 000 population for butchers in 1968-1984. For the general Dutch population without any obvious exposure the risk was estimated at 0.002 cases per 100 000 population per year [24]. In Hong Kong, the annual rates are 32 per 100 000 in population with pig related occupations and 0.09 per 100 000 in the general population, using data in cases admitted at all public hospitals in 2003-2005 [26]. In an observational prospective study across 12 hospitals from central to northern Viet Nam in 2007-2010, Nghia estimated an incidence rate of 0.57 cases per 100 000 person-years in the adult

population. The rate varies greatly between regions: 0.39 in the Mekong river delta versus 1.68 cases per 100 000 person-years in the central region [19].

To date, a total of 35 serotypes have been described (serotypes 1-34 and serotype 1/2) and defined based on the composition of the capsular polysaccharide (CPS) of the bacteria [1]. The distribution of serotypes differs geographically, with serotype 2 being the most common causing invasive diseases in both human and pigs [5, 27].

1.1.2. Sources and transmission of *S. suis* infection

S. suis is commonly found in the upper respiratory (tonsils and nasal cavities), alimentary and reproductive tracts of both healthy carriers and diseased pigs. Transmission between herds may occur when healthy carrier pigs harboring virulent strains are introduced into a non-infected herd, triggering a disease outbreak in the herd [1]. A recent genomic analysis of human isolates associated with the Sichuan outbreak in 2005 suggested a link between this human outbreak and the distribution of infected piglets from industrialized breeding companies in China [28]. Outbreaks can also occur when piglets are infected from sows especially during the weaning and mixing period [29]. Prevalence of disease varies greatly among herds, and this tends to be affected by the hygiene conditions and concurrent disease rather than the carriage rate in each herd. Crowding, poor ventilation, and any other stress-inducing conditions can predispose pigs to *S. suis* disease [29]. Viral infection such as Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) can also increase the susceptibility of pigs to bacterial infection such as *S. suis* [30]. In pigs sampled from 103 farms affected with PRRSV, 18% and 11% of sick pigs were systemically infected with *S. suis* and *S. suis* serotype 2, respectively [31].

S. suis in humans is acquired from pigs via several hypothesized routes: through cuts or abrasions on their skin while exposed to pigs or pig-derived products, or through colonization of the nasopharynx or the gastrointestinal tract [6], although the route of infection can not be explained in a large number of cases [32]. Frequent exposure to pigs and pork can increase the risk of bacterial transmission to people working in the swine industry. Not only diseased pigs but also healthy colonized pigs can be a source of infection. In fact, *S. suis* infection is listed as an industrial disease in France and the U.K. [3]. The higher rate of infections among occupationally exposed groups in Asia compared to European countries, such as the Netherlands, might be due to a higher prevalence of *S. suis* in the local pig populations [3], and poor hygiene practices of people working in the food production and supply chain [33, 34]. Prolonged and recurrent exposure to pigs or pig-derived products might enable bacterial colonization among these workers. Nasopharyngeal carriage of *S. suis* among slaughterhouse and meat-processing workers was 5.3% in Germany [35]. Higher prevalence of sero-positivity was observed among occupational groups having extended contacts compared to those with minimal contacts with pigs or pork in a study in New Zealand. 21.4% of pig farmers (compared with 0% in those without pig contact living in the farm) and 15% of meat inspectors with regular pig contact (compared with 4% in those without such regular contact) had titres of 400 or greater, a cut-off level used to indicate sero-positivity [36]. In the U.S., swine-exposed persons also had higher prevalence of sero-positivity to *S. suis* than the non-swine-exposed group (9.6% versus 1.5%) [37]. Nonetheless, it is not possible to make definitive comparisons in seroprevalence due to differing methods and populations as well as lack of test validation.

While occupational exposure is documented as a risk factor for the majority of cases in industrialized countries, a different risk pattern is found for Asian communities,

related to backyard pig production, close contact between pigs and other animals and humans, and food preparation and consumption practices [6]. In the Asian setting, it is common practice for animals to be raised within close proximity of where the farmers live, sharing the same living environment with humans, and being slaughtered at home – favourable conditions for zoonotic disease and transmission and occurence of outbreaks. An illustrative example is the Sichuan outbreak, where diseased pigs were killed and consumed and over 200 people were infected with *S. suis* presumably after unprotected physical contact with and/ or ingesting contaminated pork products [38].

In addition to occupational exposure, the oral route has a potential role in the acquisition of *S. suis* infection for the general population in Asian countries [4, 39]. The higher rate of *S. suis* infection in the general population in Asia might be explained by higher levels of exposure to uncooked pig derived dishes in these communities compared to Western countries [3]. Significantly more housewives contracted *S. suis* infection in this region than in the other parts of the world, presumably through their daily contact with raw pork [4, 12, 22]. Skin injuries might increase the risk of infection, and these are often found among housewives, cooks and slaughterers [21]. Local delicacies such as fresh pig blood and fresh pork, or undercooked pig organs including tongue, tonsils, intestines, stomach or uterus are potential sources of infection in settings like Viet Nam and Thailand [21, 22]. In some areas, fresh pork is transfered directly from slaughterhouses to wet markets and sold to consumers usually within a short time frame. Hygiene standards are usually not maintained along this supply chain [33]. *S. suis* is found in over 6% of raw pork samples collected from three of six wet markets in Hong Kong, specifically isolated in samples from tongue, tonsil, bone and tail [40]. The carriage rate of *S. suis* was 41%, of which serotype 2 was the most dominant (14%), in tonsil specimens collected from healthy pigs at three slaughterhouses in the south of Viet

Nam [41]. The observation that peaks in human *S. suis* infection rates follow outbreaks of PRRSV in pigs in the north of Viet Nam [15] highlights the need to understand community perceptions and practices in household-scale pig farming and behaviours in responses to outbreaks in pigs.

It is interesting to note that many patients with *S. suis* infection report no exposure to pigs or pork prior to their hospitalization [16, 18, 42]. This might be due to recall bias or shortcomings of the restrospective study designs used. This also suggests that other sources or routes of infection can contribute to transmission of disease. In fact, *S. suis* can be isolated from a wide range of mammalian species and birds [43], and it can survive in the environment for certain time periods (e.g. 1-2 weeks in water at 4°C and 8 days in feces and 12 days in pig carcasses at 22-25°C for *S. suis* serotype 2) [1]. Serotypes 2 and 5 have also been recovered in samples of purulent lesions in the lungs and extra-mammary sites from cattle, sheep, and goats [44]. In the Sichuan outbreak, two probable *S. suis* cases had a history of slaughtering a dead goat a few days before the disease onset [11]. In addition, some serotypes have recently been suggested to belong to a different bacterial species [27, 45]. These reports add more complexity to the epidemiology of *S. suis* infection [39], and suggest other potential sources and routes of transmission. Interrogation of patient exposure history should not therefore be limited to certain established risk factors.

1.1.3. Clinical features of human *Streptococcus suis* infection

Important clinical characteristics of *S. suis* infection have been reviewed in several papers [4, 7], and include meningitis, sepsis, endocarditis, arthritis, sensorineural hearing loss, and skin lesions. Meningitis is the most common clinical syndrome among *S. suis* patients, and the pathogen has been identified as one of the most common causes of adult bacterial meningitis in Viet Nam, Thailand and Hong Kong [16, 20, 23, 46].

Meningitis patients often present with high protein concentration, low glucose levels and neutrophilia in the cerebrospinal fluid (CSF) [16]. A striking feature is the severe to complete sensineural hearing loss that often complicates *S. suis* meningitis, occasionally accompanied by vestibular dysfunction [12, 20, 22]. Severe sepsis syndrome has also been reported in a number of cases, presenting with extensive skin findings such as petechia, purpura, ecchymoses, hemorrhagic bullae and skin necrosis [4]. A significant feature of this severe toxic-shock like syndrome (TSLS) is the high case fatality rate, reaching over 60% among cases in the Sichuan outbreak [11] and 80% in cases in Thailand [47]. Multiple organ failure, purpura fulminans and disseminated intravascular coagulation have also been described in these cases [11, 48-51].

1.1.4. Virulence of *Streptococcus suis* and pathogenesis of infection

Pathogenic strains of *S. suis* bacterium are highly virulent and invasive. However, most studies on virulence and pathogenesis have focused on serotype 2 strains [3]. Virulence factors that have been identified include the CPS, extracellular protein factor, muramidase-released protein, suilysin, several adhesins, hyaluronate lyase, and surface antigen one [4], although the most critical factor is the CPS. *S. suis* can survive in blood after penetrating the first defense barrier of the host facilitated by the antiphagocytic properties of the CPS, and subsequently spreads into multiple organs including liver, kidney, spleen, lung and heart [39]. If the infection does not progress to acute fatal septicemia, one hypothesis is that the bacterium is able to cross the blood-brain barrier and reach the central nervous system (CNS) [43]. The immune response appears to play an important role in the pathology of *S. suis* infection, both in the early phase in which septic shock might occur as a result of exaggerated inflammatory reaction and in the later phase causing localized meningitis signs. Inflammation has been shown to be a key pathogenetic factor in both systemic and CNS infections in a number of studies [52]. *S.*

suis can induce inflammation process in the meningitis phase through various mechanisms such as the activation of different pro-inflammatory cytokines and chemokines, increased secretion of inflammatory mediators involved in the disruption of of the blood-brain barrier, or increased expression of adhesion molecules in human monocytes in endothelial cells [43].

Hearing loss with or without vertigo in *S. suis* meningitis is thought to be the result of cochlear sepsis, a condition when the organism present in the subarachnoid space traverses the cochlear aqueduct to the perilymph [53]. One alternative is that hearing damage occurs as a consequence of inflammatory cells accumulating around the vestibular-cochlear nerve [53]. However studies have demonstrated that this accumulation appears to be insufficient to cause disruption in the normal neural conduction process [3]. Hearing loss might or might not improve [4], however, robust data on the long-term progression of disease sequelae are lacking.

1.1.5. Treatment of *Streptococcus suis* infection in human

S. suis infection requires approximately two weeks of intravenous antibiotic treatment, with similar principles used for other bacterial causes of meningitis [4]. Prompt treatment is required as soon as bacterial meningitis is suspected. Ceftriaxone (with or without vancomycin to cover resistant *Streptococcus pneumoniae*) is usually a good choice for empirical treatment until the results of culture and antimicrobial susceptibility become available [19]. *S. suis* strains are generally susceptible to penicillin and this drug has been used successfully in most cases [3]. Treatment needs to be tailored as required, particularly in the event of treatment failure, drug resistance, or relapse which may occur after two weeks of treatment [4].

Outcomes of bacterial meningitis have been associated with the severity of the immune response in the subarachnoid space, and studies have shown steroids can reduce this inflammation [54]. Adjunctive treatment with steroids starting before or at the first parenteral dose of antibiotics has been recommended for treating patients with community acquired bacterial meningitis as it was shown in a meta-analysis that it could reduce mortality and possibly neurological sequelae with no excess adverse events [55]. However, a later meta-analysis indicated that adjunctive dexamethasone therapy could reduce the risk of hearing loss regardless of whether this was initiated before or after antibiotic treatment [56]. A randomized double-blind placebo – controlled trial in Viet Nam in patients with confirmed bacterial meningitis, of which *S. suis* was the main etiology, also showed that dexamethasone significantly increased survival and reduced disability including deafness at 6 months after hospital discharge [57]. Dexamethasone was given 15 minutes before antibiotic therapy, however many patients in this study had already been given antibiotic prior to hospital admission.

1. 2. Prevention and control of *Streptococcus suis* infection

The purpose of prevention and control of infectious diseases is to block or reduce disease introduction and maintain it at a tolerable level. It is also referred to as primary prevention attempting to prevent the occurence of disease in a population and secondary prevention aiming at minimizing the impact of disease after it has already occurred, while tertiary prevention refers to mitigation of the long-term health effects [58]. Generally the economic cost per unit of benefit increases as interventions move along this continuum. The basic principles for prevention and control actions involve targeting the chain of transmission, including the pathogen in the reservoir host and the transmission of pathogen to the susceptible host [58]. Multiple stakeholders are required to work together in the efforts to prevent and control zoonosis such as *S. suis* infection [59].

Transmission of *S. suis* in swine is affected by several factors including the animal health status, the virulence of the strains, and the environment [1]. A combination of control and prevention strategies should be applied at farm levels to reduce disease transmission and minimize the impact of *S. suis* infection in swine. Systemic antimicrobial treatment is recommended for infected pigs while other pigs in the same pen with the infected pigs can also be pre-emptively treated through feed or water [60]. However, metaphylaxis should be minimized and strictly monitored, and should never be used in place of good management practices in farms [61]. These includes practices such as all-in/all-out pig flow, cleaning rooms between pig groups or dividing large room into smaller rooms to reduce the incidence of the disease [1]. Immunization can be an effective solution, however, no effective vaccines for pigs curently exist [3, 39]. Eradication in pigs is not recommended since medicated early weaning does not work due to early bacterial colonization; depopulation and restocking are not cost-effective; reliable diagnostic or monitoring tool are not available; and *S. suis* is not only confined in pigs but also found in a wide range of other species [1].

Transmission of *S. suis* to humans can be prevented by adopting simple actions. Although *S. suis* can survive in the environment, disinfectants or cleansers commonly used in farms or laboratories can be used to effectively inactivate the bacteria [3]. People working in close contact with pigs or pork products are recommended to adopt personal and environmental preventive measures such as covering open wounds during contact, washing hands after contact, and proper cooking of pork [60]. Splenectomized individuals should be excluded from working in the swine industry due to high mortality and morbidity if infected with the organism [62]. Slaughtering and consumption of diseased pigs should be prohibited by law in order to prevent outbreaks occurring in humans following disease outbreaks in pigs [11, 15]. The practice of consuming raw or

undercooked pork products should also be avoided since it has been associated with a large number of cases [15, 22, 63]. However, many of these practices are deeply embedded in the traditions of local communities and the development of culturally appropriate public health interventions to minimize the risk of disease transmission requires careful exploration.

1. 3. *Streptococcus suis* infection in humans in the Viet Nam context

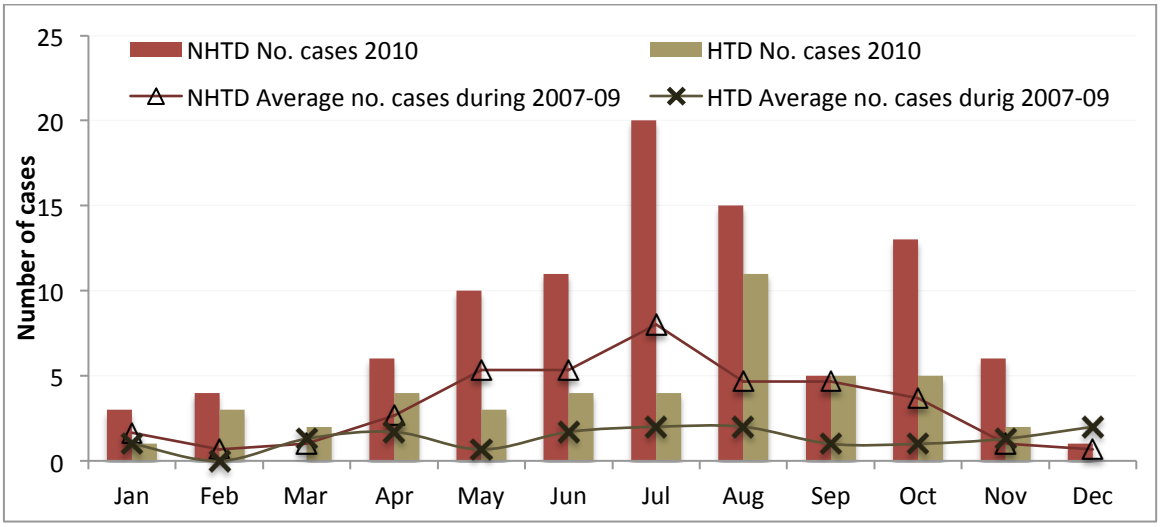
1.3.1. Epidemiology of *Streptococcus suis* infection in Viet Nam

Several reports of large numbers of *S. suis* cases from Viet Nam have been published in the international literature. Case series from the hospitals for tropical diseases in the north and the south of Viet Nam identified *S. suis* as the most common etiology for adult bacterial menigitis [16, 20]. *S. suis* was detected in specimens of 151 in 450 patients with presumed bacterial meningitis between 1996 to 2005 at Hospital for Tropical Diseases (HTD) in Ho Chi Minh City (HCMC) [20]. In 2007, the second case-series reported 50 cases with confirmed *S. suis* infection at the National Hospital for Tropical Diseases (NHTD) in Ha Noi [16]. A case-control study carried out at HTD also identified 108 cases of *S. suis* meningitis between May 2006 and June 2009 [21]. Another case-control study at NHTD reported 92 confirmed *S. suis* cases in 2010 [15]. Between August 2007 and April 2010, in a prospective surveillance study of suspected CNS infections in 13 provincial hospitals in central and southern Viet Nam, *S. suis* was confirmed in 147 cases (24%) among patients aged 15 years or older [64]. In total, over 500 Vietnamese cases have been identified in the English-language academic literature by the year 2010. This number did not include many cases reported in Vietnamese language journals. A higher number of cases were observed in 2010 (Figure I-0-1), possibly as a result of the occurrence of PRRSV outbreaks in pigs in the same year [15,

31]. Apart from the occupational exposure, food consumption including the practice of eating raw pig blood presents a major risk factor to *S. suis* infection in Vietnamese communities [21].

Shortly after the recognition of *S. suis* as the most common causes of adult bacterial meningitis in 2007, the Ministry of Health updated the guidelines on surveillance and control of communicable diseases which included a section on *S. suis* infection [65]. Advice not to slaughter and consume sick pigs, to cover wounds and wear protective clothing during slaughtering of pigs, and not to consume undercooked pork was also included in the guidelines. As of 2011 the surveillance of human *S. suis* infections was implemented and is managed by the National Institute of Hygiene and Epidemiology (NIHE). The annual total number of cases reported to the surveillance system ranged from 74 to 130 cases between 2011-2014, which appears to be lower than the total number of cases identified from the specific studies at hospitals, indicating a problem of under-reporting and/ or under- case ascertainment. Unfortunately, there have been no data on the disease incidence in pigs or the main serotypes affecting pigs in Viet Nam [19].

Figure I-0-1. Monthly distribution of human *S. suis* infections in two main referral hospitals in Viet Nam



Higher number of cases admitted at NHTD (Ha Noi) and HTD (HCMC) in 2010 (solid bar) compared to the average number of cases observed in the previous years 2007-2009 (line). Chart was adapted from Hoa et al [31].

1.3.2. Healthcare system and access to healthcare services in Viet Nam

An analysis of disease impact has to be placed into the local context of healthcare delivery system. The total Viet Nam population was 85.8 million people in April 2009 (National Population Census of 2009). The healthcare system is organized at three levels: primary (district health centres, commune health stations and village health workers), provincial (provincial health bureaus, provincial hospitals, centres for preventive medicine, medical schools), and tertiary level (Ministry of Health, research institutions, medical colleges and central hospitals). Health financing in Viet Nam used to be based on general government revenue until 1986 when major reforms were implemented that included: the introduction of user charges, the introduction of health insurance, permission for private healthcare provision, and opening-up of the pharmaceutical market. Since then, the level of funding for health care has increased and multiple sources have been utilized [66]. However, it also resulted in increasing out-of-pocket spending of

households, which still accounts for the largest share of the total health expenditure (55.5%). Health insurance is currently fragmented and coverage rates are different between membership groups. About 40% of population are not covered by health insurance and have to make out-of-pocket payments for using healthcare services, many of whom are not in the public sectors and belong to near-poor low-income groups [66]. Patients with *S. suis* in Viet Nam are often farmers or manual workers [15, 16] with low income and poor health insurance coverage. The incidence of *S. suis* infection is likely to exert a considerable impact on the patients and their family, considering its high mortality and morbidity consequences.

1. 4. Problem Statement

Despite the proliferation of new data since the Sichuan outbreak in 2005, the prevention and control of *S. suis* infection has not resulted in a reduction in the incidence of disease, particularly in Asian countries. This may be explained by the lack of awareness in the academic community and decision makers about the impact of the disease both in terms of health and economic consequences caused by premature deaths and long-term disability. In Viet Nam, it is challenging to quantify the burden of this disease due to a lack of diagnostic capacity in many hospitals as well as poor patient continuum of care making it difficult to track the patients at different levels of care from onset to recovery. Additionally, scientific evidence is generally not translated into evidence-based policies and practices [65]. Public health interventions should be tailored to specific risk factors contributing to the disease burden. To bridge this gap and support effective policies and planning for prevention and control of *S. suis* infection in Viet Nam, we need to gain more understanding on the practice of consuming undercooked pork products as an important risk factor to human *S. suis* infection, quantify the long-

term health and economic consequences of the disease, and to synthesize all data in outcome measures that comprehensively reflect the true burden of disease.

1. 5. Purpose and Research Questions

The purpose of my DPhil programme is to generate action-oriented data to support public health policy makers and professionals to reduce the burden of disease caused by *S. suis* infection in humans. I aimed to address the following research questions:

Question 1: Is the consumption of raw pig blood common in the community and can it be changed or modified to reduce the risk of disease transmission?

Question 2: Do hearing loss and vestibular dysfunction resulting from *S. suis* infection improve after hospital discharge?

Question 3: What are the health and economic consequences of *S. suis* infection and its sequelae on the patients and their family?

Question 4: What is the annual burden of disease caused by *S. suis* infection and its sequelae at a population level measured in years of life lost and in monetary value?

I have organized my DPhil programme in four parts to address the above four questions: a systematic review and meta-analysis to summarize the up-to-date data on human *S. suis* infection and derive key epidemiological parameters for burden of disease analysis; a community assessment of raw pig blood consumption practice; a cohort study to evaluate the long-term sequelae of *S. suis* infection in hospitalized patients; and a burden of disease analysis to estimate the health burden and economic impact of *S. suis* infection and its sequalae.

1. 6. Thesis Structure

The methods and findings of the research are presented in four chapters. Chapter II describes a systematic review and meta-analysis on the epidemiology, clinical manifestations and outcomes of human *S. suis* infection in the international literature. Chapter III provides details on a community assessment of raw pig blood consumption in rural and urban settings in the north of Viet Nam. The results described in chapters II and III have been published in Emerging Infectious Diseases journal in 2014 and 2015. In Chapter IV, I describe the findings from a follow-up study on patients hospitalized at NHTD due to *S. suis* infection and compare the long-term health sequelae among these patients with a group of controls. Chapter V presents an analysis of the incidence of *S. suis* infection, estimation of annual Disability Adjusted Life Years (DALYs) and quantification of economic loss caused by human *S. suis* infection and its sequelae. In Chapter VI, the findings from this DPhil programme are discussed with respect to the prevention and control of *S. suis* infection in the local context.

Chapter II

Systematic review and meta-analysis of human *Streptococcus suis* infection

2. 1. Introduction

2.1.1. Rationale

Recent years have seen the accumulation of substantial new data on the incidence, clinical and microbiological characteristics, and risk factors for *S. suis* infection. To date, there has not been any systematic review to summarize these data. Such data need to be reviewed and combined to produce global and regional epidemiological estimates for appropriate use and translation of such data into actions supporting policy making. In my thesis, I used specific epidemiological data to estimate the total impact of human *S suis* infection in Viet Nam. Some of the used data are obtained from a cohort study described in Chapter IV. Other data are extracted from the existing literature as part of this systematic review and meta-analysis.

2.1.2. Objectives

This systematic review and meta-analysis aims to:

- Provide summary estimates of important epidemiological and clinical parameters of human *S. suis* infection
- Update the evidence on human *S. suis* infection globally and identify data gaps that still exist for further research.

2. 2. Methods

2.2.1. Eligibility criteria and search process

In this review, I aimed to include all *S. suis* infection cases ever reported in humans, therefore broad eligibility criteria for inclusion of studies were used. All papers containing the phrase "streptococcus suis" found in the following searched databases, with a restriction to humans, were eligible for review.

I searched systematically and inclusively five main electronic databases (PubMed, Scopus, ISI Web of Science, ScienceDirect, and Google Scholar) for all studies published by the end of December 2012. I used the following search terms as text words in each database:

- PubMed: 'streptococcus suis' in all fields, limited to humans
- Scopus: 'streptococcus suis' in all fields, excluding veterinary papers
- ISI Web of Science: 'streptococcus suis' in topic with exclusion of veterinary science areas
- ScienceDirect: 'streptococcus suis' in all fields with exclusion of papers in veterinary journals
- Google Scholar: 'allintitle: "streptococcus suis"'

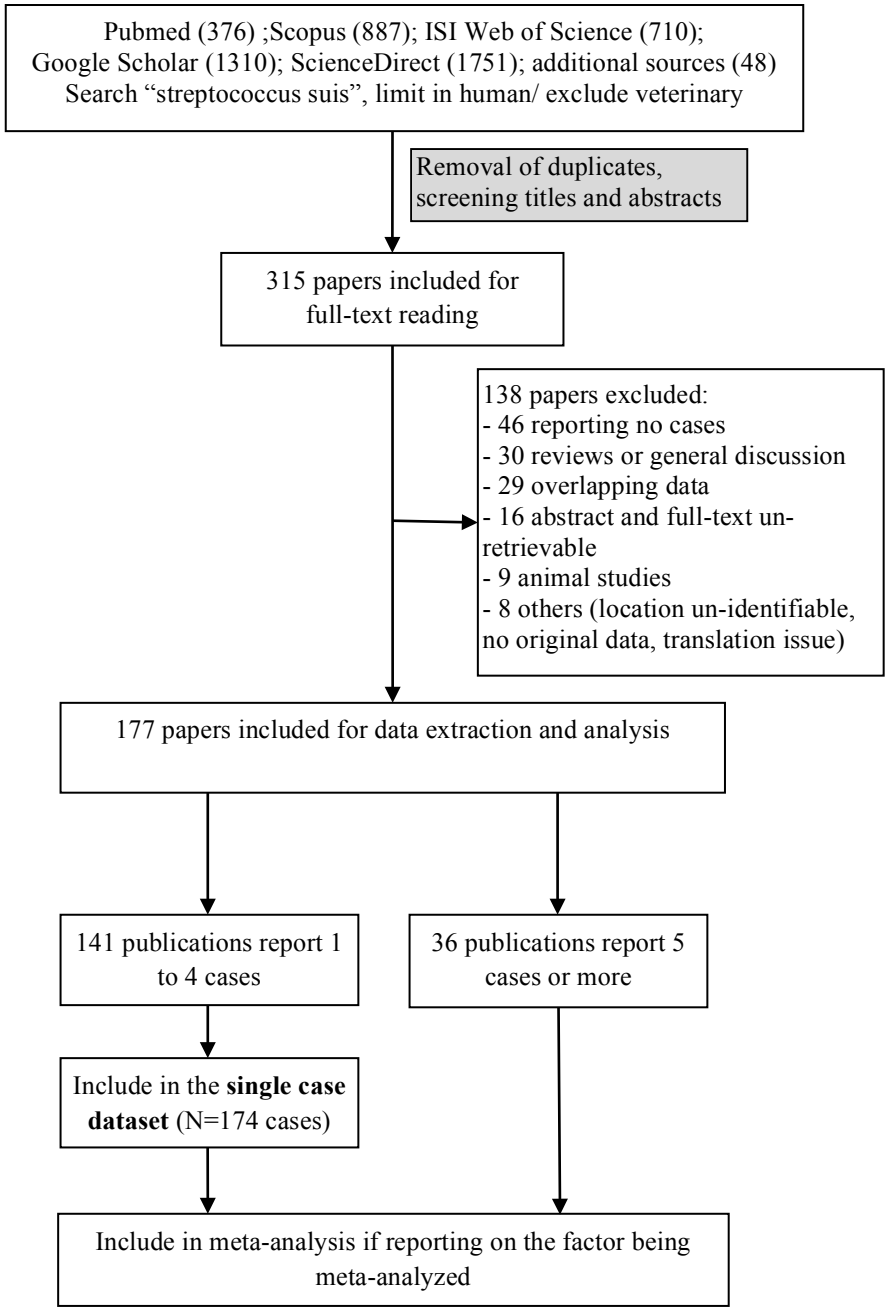
Besides these five main databases, I also made additional searches using the same search term "streptococcus suis" in other databases including Virtual Health Library, SIGLE, WHOLIS, LILACS, IMSEAR-HELLIS, China Academic Journals Full-text Database and checked the reference lists from retrieved articles. There were no restrictions on types of studies and publication languages, and non-English papers were translated for review (either by native team members or through the Google Translate service).

2.2.2. Study selection

The study selection process is described in Figure II-0-1. Publications were excluded based on the following exclusion criteria:

- No human case of *S. suis* infection was reported in the publication; or
- Publication reported data on human *S. suis* cases, but the data were considered overlapping with already included publications and providing no additional information; or
- Publication reported data on human *S. suis* cases without information indicating the location of the patients; or
- Publication reported data on human *S. suis* cases but the data could not be reliably extracted.

Figure II-0-1. Flow chart of study screening and selection process in systematic review and meta-analysis



2.2.3. Data extraction process

The data extracted included: year of publication, year of data collection, study design, data collection approach, country of origin, hospital where the patients were recruited, patient characteristic, clinical manifestations, methods of diagnosis, clinical and laboratory parameters, outcomes, and histories.

Together with another researcher working at NHTD, we independently screened the titles and abstracts, and examined the full-text publications using identical selection criteria and data abstraction forms. The results from the data extraction showed high degree of agreement between the reviewers (kappa values > 0.90 for the main variables). Any disagreements were resolved by discussion and consensus between the reviewers and with supervisors. I contacted the authors of the papers containing ambiguous data through email (one email attempt per author) for clarification, and the ambiguous data would be excluded from analyses if no responses were received from the original authors.

A human *S. suis* case was defined as a patient with *S suis* infection confirmed either by standard bacterial culture or real-time polymerase chain reaction (PCR) and whose geographical location could be identified. Probable cases identified through outbreak investigation were also included. A probable case was defined as a patient having compatible clinical illness without laboratory evidence of infection by another organism, and having an epidemiological link with the confirmed case.

Main risk factors under review included occupational exposure (working in the pig related occupations prior to illness), history of eating pork containing meals prior to illness, history of contact with pigs or pork (any recent contact with pigs or pork prior to illness other than oral consumption and usual contacts through occupational exposure), and skin injury prior to illness (any open wound). Other notable conditions prior to

illness described in the papers were also captured during the review including alcohol consumption, concurrent health conditions, and smoking, regardless of the amount or severity of the condition.

Similarly, clinical characteristics and outcomes were also captured as being present (mentioned or described in the paper) or absent (the authors described it as being absent or did not described it in the paper). For outcomes including hearing loss and vestibular dysfunction, these were ascertained either through standard audiometry or vestibular assessments, or through symptoms reported by the treating physicians as described in the papers. Due to heterogeneity and lack of information on the methods described in the papers, I decided to restrict my review and data synthesis to summarizing the number and proportion of cases with the presence of the factor/ condition under review as described in the next section.

2.2.4. Data synthesis and analysis

Non-duplicate cases were counted to calculate the total number of cases for each country which was then used to estimate the period prevalence up to 2012 (population denominator was from country population data for 2008-2012 published by World Bank [67]). All single cases from the publications that reported less than 5 cases were pooled into one dataset to produce summary outputs. This single-case dataset was then meta-analyzed with other large studies (reporting ≥ 5 cases). Meta-analysis was conducted by using Comprehensive Meta-analysis software version 2 (Biostat, Englewood, NJ, USA; http://www.Meta-Analysis.com) when >2 studies reported the reviewed factor.

Estimates from individual studies were combined using either a fixed-effects or a random-effects model depending on the level of heterogeneity between studies. I used both Q statistic and I-squared test [68] to examine heterogeneity. If heterogeneity was not

significant, the result generated from a fixed-effects model was reported. Otherwise, results from a random-effects model were reported, which incorporated both sources of variances, sampling error and between-study heterogeneity [69]. Median (range) was converted to mean (SD) using formulas proposed by Hozo et al [70], and interquartile ranges to SDs and sub-group values to total values by methods suggested in the Cochrane handbook [71]. Results of this review are presented in three sets: summary values from the single case dataset; median values (range) of the large studies; and pooled values from the meta-analysis as appropriate. Forest plots were used to visualize the estimates of individual studies and the combined estimate from meta-analysis.

Type of study design and study sample size were used to reflect study quality and incorporated in the analyses to examine the risk of bias within and across studies including the evaluation of heterogeneity as described above. I used both forest plots and funnel plots to visually examine possible publication bias for each main variable. The Egger's regression test was also used to quantify the bias when there were ≥10 studies included in the meta-analysis. If publication bias was found, the Duvall and Tweedie's trim and fill method was performed to add possible missing studies to improve the symmetry and re-estimate the combined mean and its variance [72].

I used subgroup analyses (when ≥10 studies included) and bivariate meta-regression (when ≥20 studies included) to examine the effect of study-level charateristics including year of publication (2005 and before versus after 2005 (as the Sichuan outbreak occurred in 2005)), study design (case series, outbreak investigation, cross-sectional), location (China mainland, Hong Kong, Thailand, Viet Nam, and others), data collection (retrospective versus prospective) and proportion of meningitis (<0.5, 0.5-0.9, and >0.9) on the main outcome variables. The general linear model was used for meta-regression,

with adjustment for multiple comparisons using the Bonferroni method and weighting by study sample size.

This review was designed and implemented in accordance with the recommendations of the PRISMA statement [73]. The protocol for this review has been registered at PROSPERO International prospective register of systematic reviews (No. CRD42011001742, Available from http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42011001742).

2. 3. Results

2.3.1. Characteristics of included studies

From the original list of 315 papers included for full-text review, I selected 177 publications that met inclusion and exclusion criteria for data extraction and final analyses (Figure II-0-1). A summary of the main characteristics of each paper is given in Appendix 1. The included studies were diverse in terms of design, data collection and reporting approaches. A total of 20 case series (8 from Southeast Asia, 8 Western Pacific and 4 European region) and 21 cross-sectional studies (9 from Southeast Asia, 8 Western Pacific and 4 European) were identified. Five papers on 3 outbreaks (in Sichuan and Jiangsu- China, and Phayao- Thailand) were classified as outbreak investigation reports. A retrospective methodology was the main study design in 90% of all papers. The majority of papers were published in the English language, and a large number of papers has just been published recently after the Sichuan outbreak [11] occurring in 2005 (Table II-0-1).

Table II-0-1. Characteristics of 177 papers included in the systematic review

	No. papers (%)	No. cases reported (%)*
Geographical Region †		
European	98 (55)	168 (10.5)
Western Pacific	47 (27)	836 (53)
South-East Asia	24 (14)	572 (36)
Americas	8 (4)	8 (0.5)
Type of study design		
Case report	130 (73)	151 (7)
Case series	20 (11)	511 (25)
Cross-sectional	21 (12)	761 (37)
Outbreak investigation	5 (3) ‡	532 (26)
Case-control	1 (1)	101 (5)
Data collection approach		
Retrospective	159 (90)	1299 (63)
Prospective	15 (9)	697 (34)
Mixed §	3 (1)	60 (3)
Language of publication ¶		
English	130 (74)	1947 (94.7)
Spanish	13 (7)	15 (0.7)
French	12 (7)	13 (0.6)
Others #	22 (12)	81 (3.9)
Year of publication		
1968-1980	13 (7.5)	18 (1)

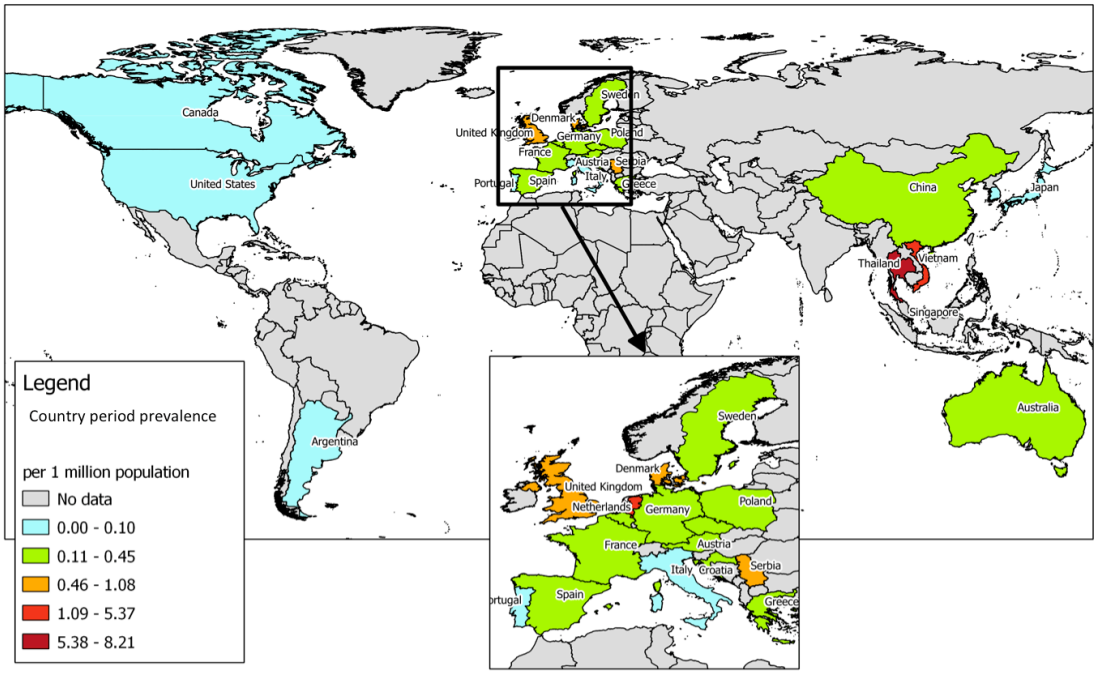
	No. papers (%)	No. cases reported (%)*
1981-1990	27 (15)	95 (4.5)
1991-2000	32 (18)	119 (6)
2001-2005	28 (16)	115 (5.5)
2006-2010	55 (31)	1052 (51)
2011-2012	22 (12.5)	659 (32)

*Case duplicates were removed in the counts for the geographical region sub-heading (adding up to a total 1584 cases, no duplicates). Duplicates were not removed in the counts for other sub-headings in the table (adding up to 2056 cases, with duplicates). †Geographical regions defined by the World Health Organization. ‡Three papers reporting about the patients in the Sichuan outbreak in China were included here, each was included for analysis of different factors. §These studies included both retrospective and prospective datasets and were included in the prospective groups in subsequent analyses. ¶Almost all large studies were published in English. Most reports in languages other than English were case reports. #German (7 papers), Dutch (4), Czech/ Italian/ Japanese (2 each), Chinese/ Polish/ Serbian/ Swedish/ Thai (1 each).

2.3.2. Epidemiology and risk factors

A total of 1 584 cases were reported in the literature by the end of the year 2012 (including 189 probable cases identified in 3 outbreaks), mainly from Thailand (36%), Viet Nam (30%), and China (22%). Over half were in the Western Pacific (53%), 36% in the South East Asia, 10.5% in the Europe, and 0.5% from the Americas. The global distribution of *S. suis* infection is presented in Figure II-0-2, with the highest prevalence rate in Thailand (8.21 per 1 million population), followed by Viet Nam (5.40) and the Netherlands (2.52) (country population data for 2008-2012 by World Bank [67]). The pooled mean age of the patients was 51.4 years and 77% were men (Table II-0-2).

Figure II-0-2. Global period prevalence of *S. suis* infection including all cases reported from 1968 up to 2012



Source of population data: The World Bank, http://data.worldbank.org/indicator/SP.POP.TOTL

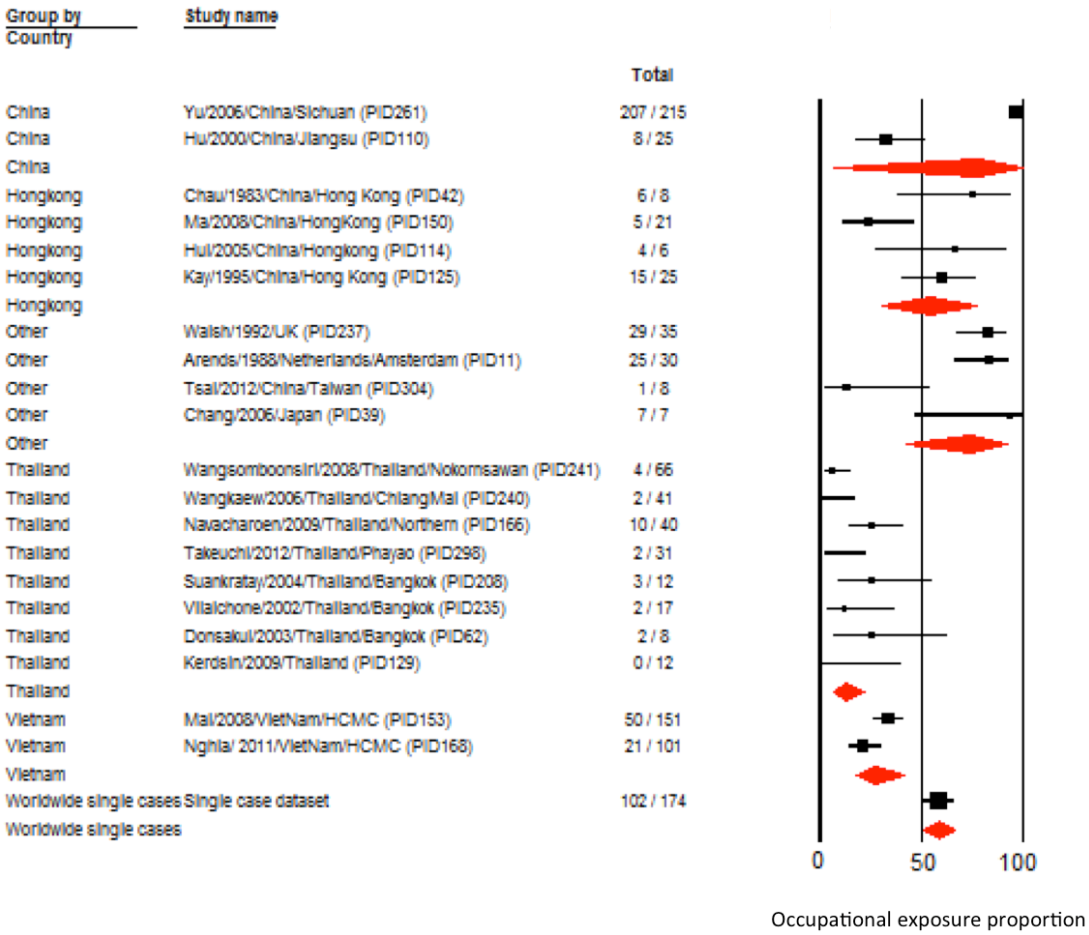
Having a pig-related occupation was the most common documented risk exposure in the single-case dataset (58.6%) (Table II-0-2). The reported proportion was generally lower in large studies, and the pooled proportion of cases with occupational exposure was 38.1% (95%CI: 24.4-53.9%) in the meta-analysis. By region, occupational exposure was more frequently reported for industrialized countries than for other countries (Figure II-0-3). The pooled proportion of occupational exposure for three countries including the United Kingdom, Netherlands, and Japan was 83.8% (95% CI 73.4%–90.7%). No publication bias was observed for the studies reporting occupational exposure.

Table II-0-2. Characteristics of the patients with *S. suis* infection included in the systematic review

Variable	Single case dataset *	Large studies (median (min-max))	Meta-analysis † (pooled mean (95%CI))	no. studies meta-analyzed ‡
Mean age (y)	49.4 (n = 156)	50.5 (37.0-61.2)	51.4 (49.5-53.2)	25
Sex (M)	83.2 (n = 155)	77.5 (37.5-100)	76.6 (72.2-80.6)	26
Pig-related occupation§	58.6	25.0 (0-100)	38.1 (24.4-53.9)	21
Contact with pigs or pork prior to illness	15.5	33.3 (2.4-100)	33.9 (21.1-49.5)	14
Eating pork meals prior to illness	4.0	53.3 (5.9-88.7)	37.3 (20.2-58.3)	9
Skin injury prior to illness	19.5	16.0 (9.5-71.4)	25.1 (15.1-38.7)	8
Drinking alcohol§	8.6	23.0 (4.8-83.9)	29.7 (17.2-46.3)	13
Concurrent diabetes¶	2.9	7.2 (3.2-25.0)	8.0 (4.6-13.7)	9

Data are presented in % or otherwise indicated. *N = 174, or otherwise specified. †Random-effects model. ‡ Include the single case dataset and the large studies. § Details on alcohol drinking history were often not described for the majority of cases, and varied greatly in the remaining cases. In the case-control study, alcoholism was defined as drinking beer 1500 ml/day or wine (spirit 30-40^{o}) 250 ml/day in at least 5 days/week. ¶ Other less common underlying conditions are in Table II-0-3.

Figure II-0-3. Forest plot of subgroup meta-analysis (random-effects) for proportions of *S. suis* patients with pig-related occupation by country reported in the 21 included studies



For each study, proportion and 95%CI are presented, with the size proportional to the study weight. The red rhombus presents the pooled proportion for each country group.

Recent contact with pigs of pork and history of eating meals containing pork also were commonly documented risk factors for the infection. Pig/pork contact history was reported for 15.5% of single cases but for 33.9% (95% CI 21.1%–49.5%) in the meta-analysis. Eating history was reported mainly in Asia (Thailand and Viet Nam); the pooled estimate was 37.3% (95% CI 20.2%–58.3%). For Thailand only, the proportion was 55.8% (95% CI 33.7%–75.9%). In other countries, only 1 patient in France was reported

eating artisanal dry sausage [74], and 1 patient in the United States ate raw pork while traveling in the Philippines [75] before the infection.

Skin injury was an important factor in the disease transmission among people with documented history of recent contact with pigs or pork. In one case-control study in Viet Nam, Ho et al showed that contact with presence of skin injury substantially increased the infection risk compared to contact with no evidence of skin injury [21]. In this systematic review, skin injury was shown in 19.5% of the patients in the single case dataset, while the pooled proportion was 25.1% (95%CI 15.1%-38.7%).

Alcohol use was evident in a very low proportion of patients in the single case dataset, but reported much more frequently in many large studies. The pooled proportion was 29.7% (95%CI 17.2%-46.3%) in the meta-analysis. Nevertheless, the Viet Nam case control study did not identify alcoholism as an independent risk factor after adjustment for other risk factors and confounders [21]. In this study, alcoholism was defined as drinking beer 1500 ml/day or wine (spirit 30-40^{o}) 250 ml/day in at least 5 days/week. The most commonly reported pre-existing condition was diabetes. Other conditions included underlying heart disease, hypertension, cirrhosis, and cancer (Table II-0-3). Smoking was mentioned in 5.2% of the cases in the single case dataset.

Table II-0-3. Pre-existing conditions (other than diabetes) of human *S. suis* cases worldwide

Pre-existing condition	Number of cases documented
Heart disease	14 (10 had infective endocarditis)
Hypertension	13
Cirrhosis	10

Pre-existing condition	Number of cases documented
Splenectomy	9
Cancer	5
Dyslipidemia/ hyperlipidemia	2
Tuberculosis	2
Psoriasis	2
Chronic liver disease	1
Aplastic anemia	1
Spinal canal stenosis	1
Rheumatoid arthritis	1
Chronic pancreatitis	1
Pelger Huet	1
History of corticoid use	2
Past *S. suis* infection	1

2.3.3. Microbiological diagnosis

Blood and/ or CSF culture were the most common reported methods for diagnosis among patients in the single case dataset (Table II-0-4). Molecular diagnosis was more common in the large studies (11 studies) than in case reports. The most prevalent strain was serotype 2 (87%), followed by 14 (2.3%) and 1 (0.6%) of all 1 156 patients with serotype information mentioned in the papers. Serotypes 4, 5, 16, 24 were also reported (one patient per serotype).

Misdiagnosis in *S. suis* infection was not uncommon, either by conventional biochemical tests or commercial identification systems. The bacterium was often reported as viridans streptococci in initial cultures. In fact, up to 70% of all viridans streptococci

cases in Thailand were confirmed to actually be *S. suis* in follow-up investigations [47]. 62.5% of *S. suis* patients in another series in Thailand [76] and 20% in the Netherlands [24] were initially reported as viridans streptococci. Misidentification with *S. bovis* (2 patients), *S. pneumoniae* (1 patient), and *Enterococcus faecalis* (1 patient) were also reported in the Dutch series. Tsai and colleagues [77] also showed large variations between the two commercial systems (Phoenix and Vitek), and misidentification with *S. acidominimus* was common using the Phoenix system.

S. suis is mostly sensitive to penicillin, resistance was reported in only two patients [10, 78]. After cessation of antibiotic treatment, a relapse occurred in a small proportion of the patients, including those with the organism tested highly sensitive to penicillin [12, 79]. The median proportion of relapse among large studies was 7.3% (range 2.9%-8.3%) and the mean proportion was 4.4% (95%CI 2.4%-7.8%) in the meta-analysis of 5 included studies (fixed-effects model). The reported relapse proportion was lower among patients in the single case dataset (2.9%).

Table II-0-4. Microbiological characteristics of patients in the single case dataset in the systematic review of *S. suis* infection

	Number of cases. (%)
Microbiological diagnosis (n = 141)	
Blood culture only	33 (23.4)
CSF culture only	34 (24.1)
Both CSF and blood culture, no PCR	53 (37.6)
PCR and culture (blood and/or CSF)	18 (12.8)
PCR only	3 (2.1)

	Number of cases. (%)
Initial misdiagnosis (n = 174) (9 viridans streptococci, 2 *E. faecalis*, 1 *S. acidominimus* and 1 *S. bovis*)	13 (7.5)
Serotype 2 (n = 174)[*]	94 (54)
Penicillin sensitivity (n = 92)[†]	90 (97.8)
Penicillin resistance (n = 92)	1 (1.1)

[*]Other serotypes among the single case dataset included serotype 1 (3 cases), 14 (3), 4 (1), 5 (1), 16 (1), and 24(1). For the remaining 64 cases (36.2%), serotype was not identified or no information on serotype was provided. †For one patient the authors reported the Minimum Inhibitory Concentration of 0.25 but did not conclude on the sensitivity status of the isolated organism.

2.3.4. Clinical manifestations

Duration from onset to hospital admission was about 3-4 days on average (Table II-0-5), but could be up to 1-3 months among cases with endocarditis presentation (e.g. [80-82]). Among the survived cases in the single case dataset, the median duration of hospitalization was 20 days (range: 5-77 days) with a mean of 25.5 (95%CI 20.4-30.6) days. The median hospitalization duration was 1 day (range 0-26 days) for 16 fatal cases with data available within this dataset. Similar durations were also reported from the large studies, ranging from 1-74 days for all cases.

Table II-0-5. Main clinical and laboratory parameters at admission of the patients with *S. suis* infection included in the systematic review

Variable	Single case dataset [*]	Large studies (median value (min-max))	Meta-analysis [†] (pooled mean value (95%CI))	No. studies [‡]
Clinical syndrome[$] (%)				
Meningitis	69.5	64.5 (30.2-100)	68.0 (58.9-75.8)	26
Sepsis [¶]	19.5	23.8 (11.8-39.4)	25.0 (20.5-30.2)	12
Arthritis	2.87	16.7 (1.5-50.0)	12.9 (6.0-25.6)	12
Endocarditis	8.6	14.3 (1.9-39.0)	12.4 (6.7-21.9)	10
Endophthamiltis	2.9	4.5(1.5-28.6)	4.6 (2.8-7.4)[#]	9
Spondylodiscitis	4.6	-	-	-
TSLS	2.9[**]	37.7 (28.9-64.0)	41.1 (25.0-59.4)	3[††]
Mean duration (d)				
Onset to admission	7.3 (n = 90)	3.5 (2.0-11.4)	4.1 (2.7-5.4)	7
Hospitalization	20.5 (n = 68)	17.4 (13.0-19.2)	17.2 (15.6-18.9) [#]	5
Symptoms (%)				
Meningeal sign[‡‡]	49.4	66.7 (12.5-95.1)	67.1 (54.9-77.4)	18
Skin rash	10.9	12.5 (0-52.0)	15.4 (8.6-25.9)	10
Shock	8.6	11.8 (1.3-64.0)	11.9 (6.3-21.5)	12
Respiratory	5.2	20.0 (8.3-35.8)	16.7 (8.6-29.9)	6

Variable	Single case dataset *	Large studies (median value (min-max))	Meta-analysis † (pooled mean value (95%CI))	No. studies ‡
failure				
Acute renal failure	5.2	8.3 (1.3-28.0)	7.1 (2.2-20.5)	5
Coagulopathy	10.3	6.0 (2.4-57.1)	10.3 (5.4-18.8)	9

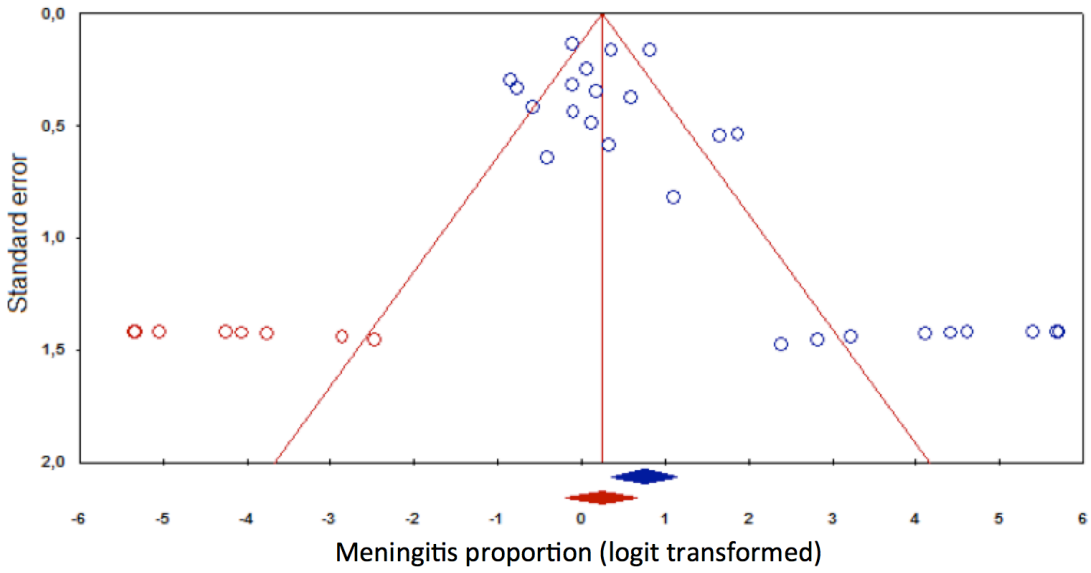
*N = 174, or otherwise specified. †Random-effects model, or otherwise specified. ‡Number of studies included in the meta-analysis, include both the single case dataset and the large studies (Appendix 1). §Other less common syndromes included peritonitis, myositis, pneumonia, sacroiliitis, abdominal aortic aneurysm, haemorrhagic labyrinthitis, gastroenteritis, vertebral osteomyelitis, lymphadenopathy, cellulitis, vertigo. ¶Cases with TSLS in China and in Thailand not included in this sepsis category. #Fixed-effects model. **Counted if the author described the case as TSLS or toxic shock syndrome. ††Three large studies reporting TSLS include 2 outbreaks in China (*2, 30*) and 1 prospective study in Thailand (*24*). ‡‡Mainly reported with a sign of neck stiffness.

Meningitis was the most common clinical syndrome (pooled proportion: 68.0%, 95%CI 58.9-75.8%), followed by sepsis (25.0%, 95%CI 20.5-30.2%), arthritis (12.9%, 95%CI 6.0-25.6%), endocarditis (12.4%, 95%CI 6.7-21.9%), and endophthalmitis (4.6%, 95%CI 2.8-7.4%). Spondylodiscitis was reported in 4.6% among the single case reports, but not in large studies. TSLS was also reported as a distinct severe clinical presentation at high rates in two outbreaks in China (64.0% and 28.9% of the patients) [13, 83] and in Thailand (37.7%) [47], but only 2.9% among the case reports.

There was evidence of publication bias found in the meta-analysis of meningitis proportions (Figure II-0-4) (significant Egger's test result). The adjusted rate based on the Duvall and Tweedie trim and fill method was 56.0% (95%CI 45.2-66.2%). Meta-regression showed that meningitis proportion was significantly associated with country of publication, study design and data collection approach (Table II-0-6), although only country of publication remained significant in a multivariable model. All patients in the

four studies conducted in Viet Nam had meningitis (Figure II-0-5). If I excluded these studies, the pooled proportion of meningitis was reduced to 59.4% (95%CI 51.1-67.1%) and the adjusted value after using the trim and fill method was 54.8% (95%CI 46.0-63.4%). In contrast, if I excluded the two outbreak investigations in China with large number of sepsis cases, the pooled proportion of meningitis increased slightly to 72.2% (95%CI 62.4-80.2%).

Figure II-0-4. Funnel plot of 26 studies included in the meta-analysis of meningitis proportions in *S. suis* infection



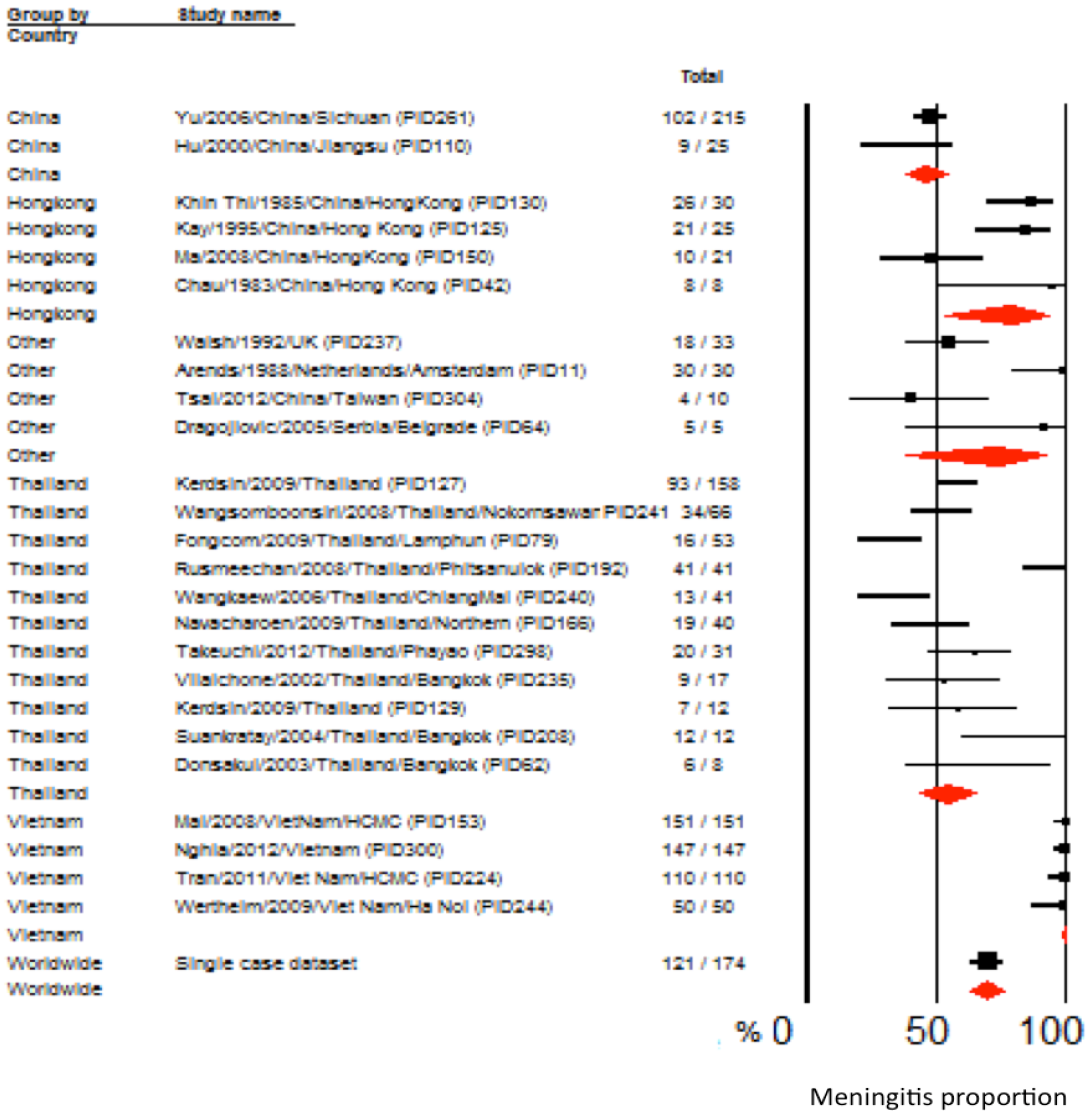
This figure plots meningitis proportion of each study against its standard error (meningitis proportion is on a logit transformed scale). Each blue circle represents each study in the meta-analysis, forming an asymmetric funnel plot with a pooled meningitis proportion (blue rhombus). Eight missing studies (red circles) added in the left side through the trim and fill method to make the plot more symmetric and gave an adjusted pooled meningitis proportion (red rhombus), which was lower than the original one.

Table II-0-6. Bivariate meta-regression for meningitis proportion among included studies in the systematic review of *S. suis* infection

Study-level factor	No. studies	Pooled proportion (95%CI)	Difference*
Country of publication			
United Kingdom, Netherlands, Serbia, Taiwan, Japan	4	0.73 (0.38-0.92)	-0.258
China mainland	2	0.46 (0.37-0.54)	-0.538†
Hong Kong	4	0.79 (0.53-0.93)	-0.226
Thailand	11	0.54 (0.43-0.65)	-0.436†
Viet Nam	4	0.99 (0.98-1.0)	reference
Year of publication			
2005 and before	10	0.77 (0.60-0.88)	0.034
After 2005	15	0.63 (0.51-0.74)	reference
Study design			
Case series	17	0.67 (0.54-0.78)	-0.234‡
Outbreak	2	0.46 (0.37-0.54)	-0.404§
Cross-sectional	6	0.92 (0.74-0.98)	reference
Data collection			
Retrospective	19	0.59 (0.50-0.68)	-0.298¶
Prospective	6	0.93 (0.70-0.99)	reference

*Meta-regression using General Linear Model, weighted by sample size with adjustment for multiple comparisons by the Bonferroni method; Difference reported is the difference in meningitis proportion between the evaluated group and the reference group as indicated in the table for corresponding study-level factor. † $p<0.001$. ‡ $p=0.05$. § $p=0.004$. ¶ $p=0.003$.

Figure II-0-5. Forest plot of subgroup meta-analysis (random-effects) for the proportions of meningitis by country reported in the 26 included studies



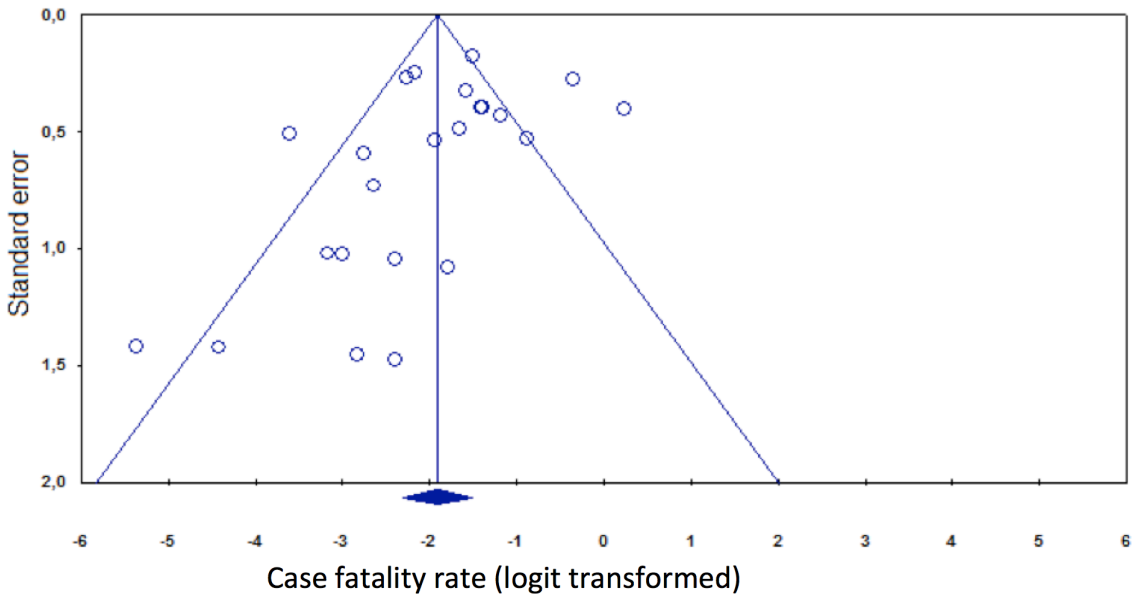
For each study, the proportion of meningitis and 95%CI is presented, with the size proportional to the study weight. The red rhombus presents the pooled proportion for each country group.

Hearing loss was the most common condition complicating *S. suis* infection (detailed description in Section 2.3.6). Other complications included skin findings ranging from petechiae to extensive purpura, ecchymoses and skin necrosis observed in 10.9% of patients in the single case dataset and up to 52% as reported in an outbreak in China [13].

2.3.5. Case fatality

Case fatality rates were variable between the single case dataset and the larger studies, ranging from 0-56% (Table II-0-7). The pooled case fatality rate was 12.8% (95%CI 9.0-18.0%). No evidence of publication bias was indicated from the funnel plot for studies included in the meta-analysis of this outcome (Figure II-0-6). Nevertheless, case fatality rates varied by country with lowest rates reported in Viet Nam (Figure II-0-7).

Figure II-0-6. Funnel plot of 25 studies included in the meta-analysis of case fatality rates among the *S. suis* cases



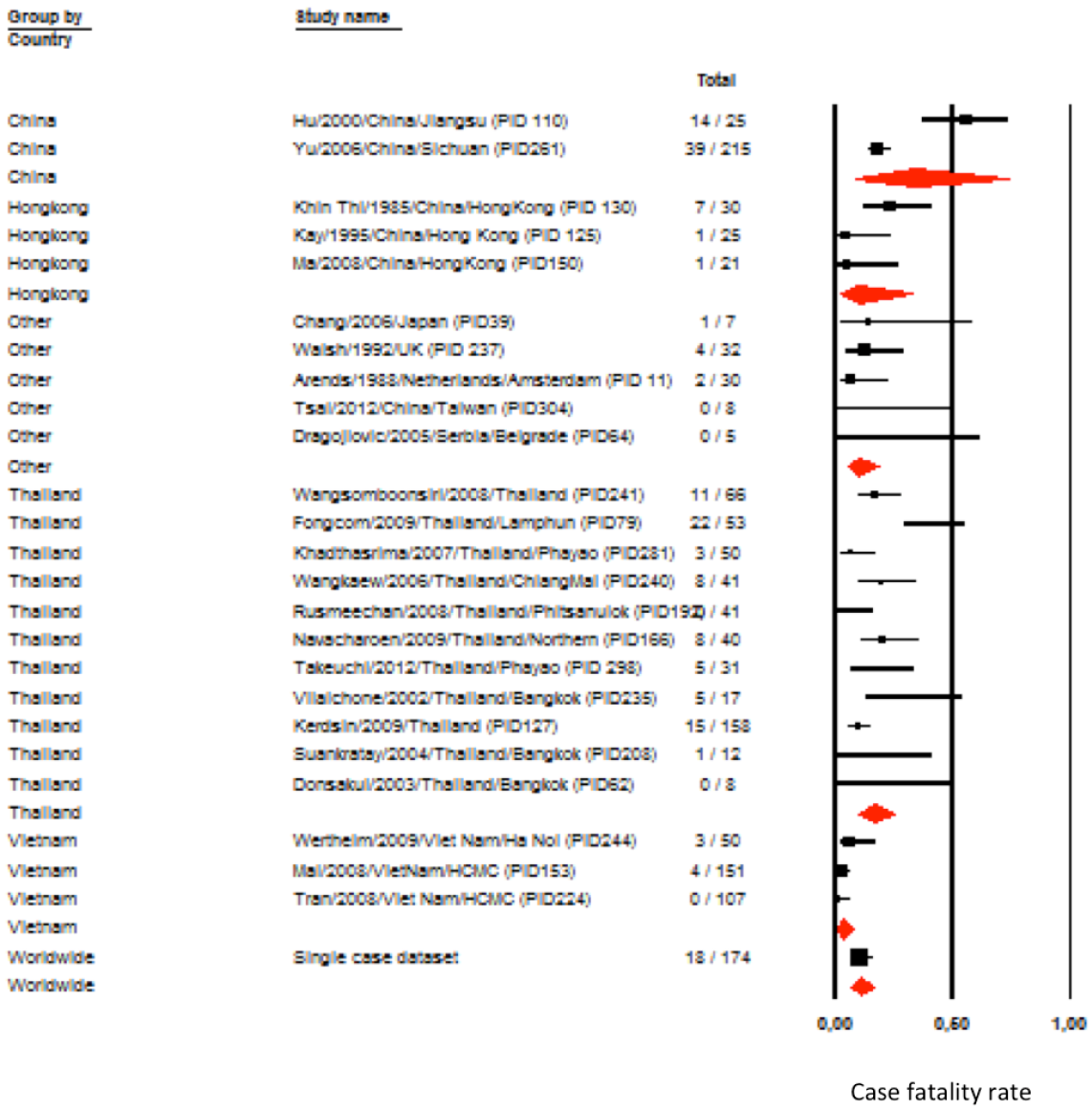
This figure plots case fatality rate of each study against its standard error (case fatality rate is on a logit transformed scale). Each blue circle represents each study in the meta-analysis. The funnel plot is relatively symmetric with a pooled case fatality rate (blue rhombus).

Table II-0-7. Proportion of the main clinical outcomes among patients with *S. suis* infection included in the systematic review

Variable	Single case dataset (%) *	Large studies (median % (min-max))	Meta-analysis † (pooled mean % (95%CI))	no. studies meta-analyzed ‡
Death	10.3	8.9 (0.0-56.0)	12.8 (9.0-18.0)	25
Hearing loss §	44.8	38.7 (6.0-100)	39.1 (31.0-47.8)	26
Recovery from hearing loss	-	5.0 (0.0-52.3)	15.4 (5.3-37.3)	8
Vestibular dysfunction ¶	16.7	25.0 (3.3-60.0)	22.7 (15.6-32.0)	13
Visual impairment	4.0	-	-	-

*N = 174. †Random-effects model. ‡Include the single case dataset and the large studies. §Studies included if cases were reported to have any degree of hearing impairment either unilateral or bilateral, temporary or permanent. ¶Studies included if cases were reported to have ataxia, vertigo, loss of balance, vestibular dysfunction.

Figure II-0-7. Forest plots of subgroup meta-analysis (random-effects) for the case fatality rates by country of origin where the *S. suis* cases were reported in the 25 included studies



For each study, case fatality rate and 95%CI are presented, with the size proportional to the study weight. The red rhombus presents the pooled case fatality rate for each country group.

However, country of publication was not significant in the bivariate meta-regression after adjusted for multiple comparisons (Table II-0-8). Instead, only meningitis proportions remained significant in explaining between-study variations in case fatality

rates. There was a negative correlation between meningitis proportions and case fatality rates among the included studies (Figure II-0-8). Studies with meningitis proportions below 50% had significantly higher case fatality rates compared with those having meningitis proportions above 90% (mean case fatality rate difference 20.3%, p = 0.001). The pooled case fatality rate was 4.0% (95%CI 2.2-7.0%) estimated for the studies in which all patients had meningitis while the pooled rate for the other studies was 17.1% (95%CI 12.3-23.4%). Cases reported in outbreaks had a higher case fatality rate compared to non-outbreak cases (21.6%, 95%CI 6.4-52.5% versus 11.5% 95%CI 7.9-16.7%).

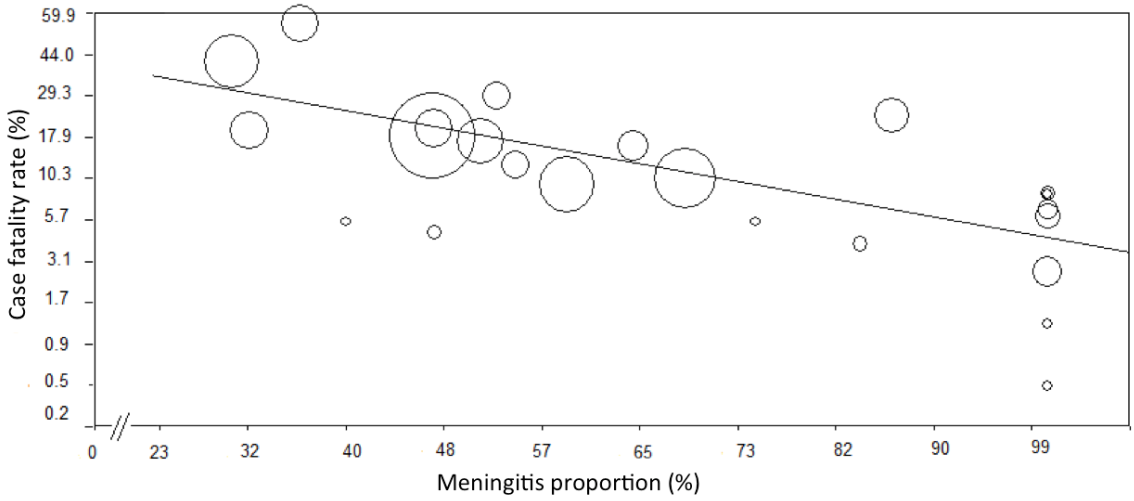
Table II-0-8. Bivariate meta-regression for case fatality rate among included studies in the systematic review of *S. suis* infection

Study-level factor	Number of studies	Pooled case fatality rate (95%CI)	Difference*	p-value
Country of publication				
United Kingdom, Netherlands, Serbia, Taiwan, Japan	5	0.10 (0.05-0.19)	0.063	1.0
China mainland	2	0.34 (0.08-0.74)	0.198†	0.06
Hong Kong	3	0.10 (0.02-0.32)	0.096	1.0
Thailand	11	0.16 (0.10-0.24)	0.129	1.0
Viet Nam	3	0.03 (0.01-0.08)	reference	-
Year of publication				
2005 and before	9	0.16 (0.08-0.31)	0.069	0.32

Study-level factor	Number of studies	Pooled case fatality rate (95%CI)	Difference*	p-value
After 2005	15	0.11 (0.07-0.17)	reference	-
Study design				
Case series	16	0.15 (0.10-0.22)	0.103	0.16
Outbreak	3	0.22 (0.06-0.52)	0.140‡	0.07
Cross-sectional	5	0.06 (0.02-0.14)	reference	-
Data collection				
Retrospective	19	0.13 (0.09-0.19)	0.035	0.52
Prospective	5	0.11 (0.03-0.32)	reference	-
Meningitis proportion				
<0.5	7	0.24 (0.14-0.38)	0.203§	0.001
0.5 - 0.9	8	0.15 (0.10-0.21)	0.106	0.11
>0.9	7	0.04 (0.02-0.07)	reference	-

*Meta-regression using General Linear Model: Difference reported is the mean difference in case fatality rate between the evaluated group and the reference group as indicated in the table for corresponding study-level factor, with the p-value calculated after weighting by sample size and adjustment for multiple comparisons by the Bonferroni method.

Figure II-0-8. Meta-regression scatter plot for the correlation between case fatality rate and meningitis proportion

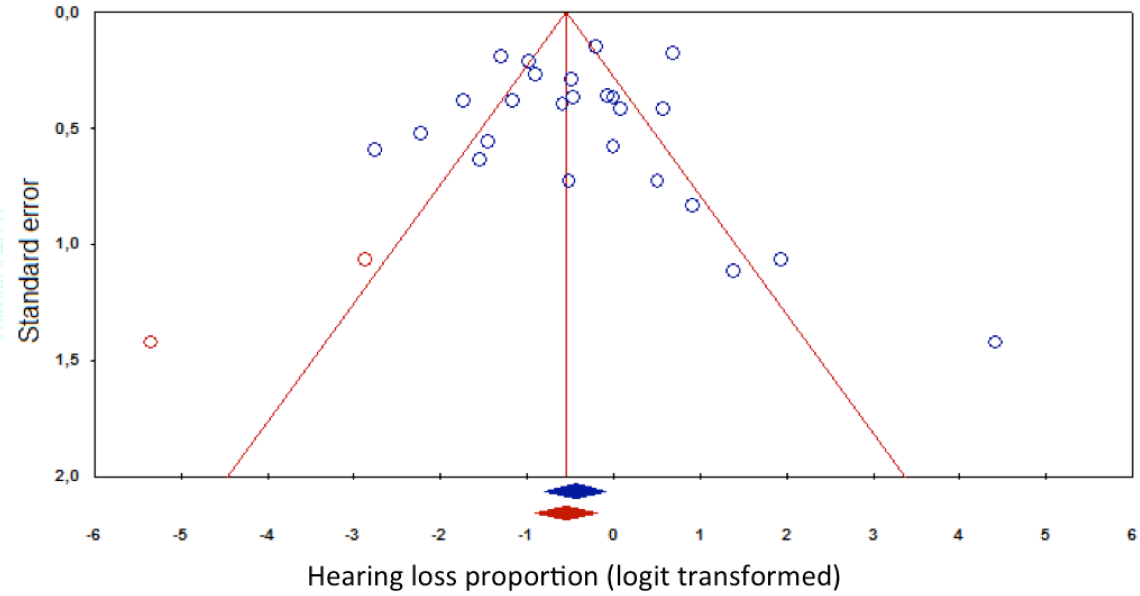


Case fatality rate was logit-transformed in the regression model. The logit rate was calculated for case fatality rate as follows: logit rate = ln[rate/(1 − rate)]. Each circle represents a study in the meta-analysis, and the size of the circle is proportional to study weighting. Studies with higher meningitis proportions tend to report lower case fatality rates.

2.3.6. Hearing loss outcome

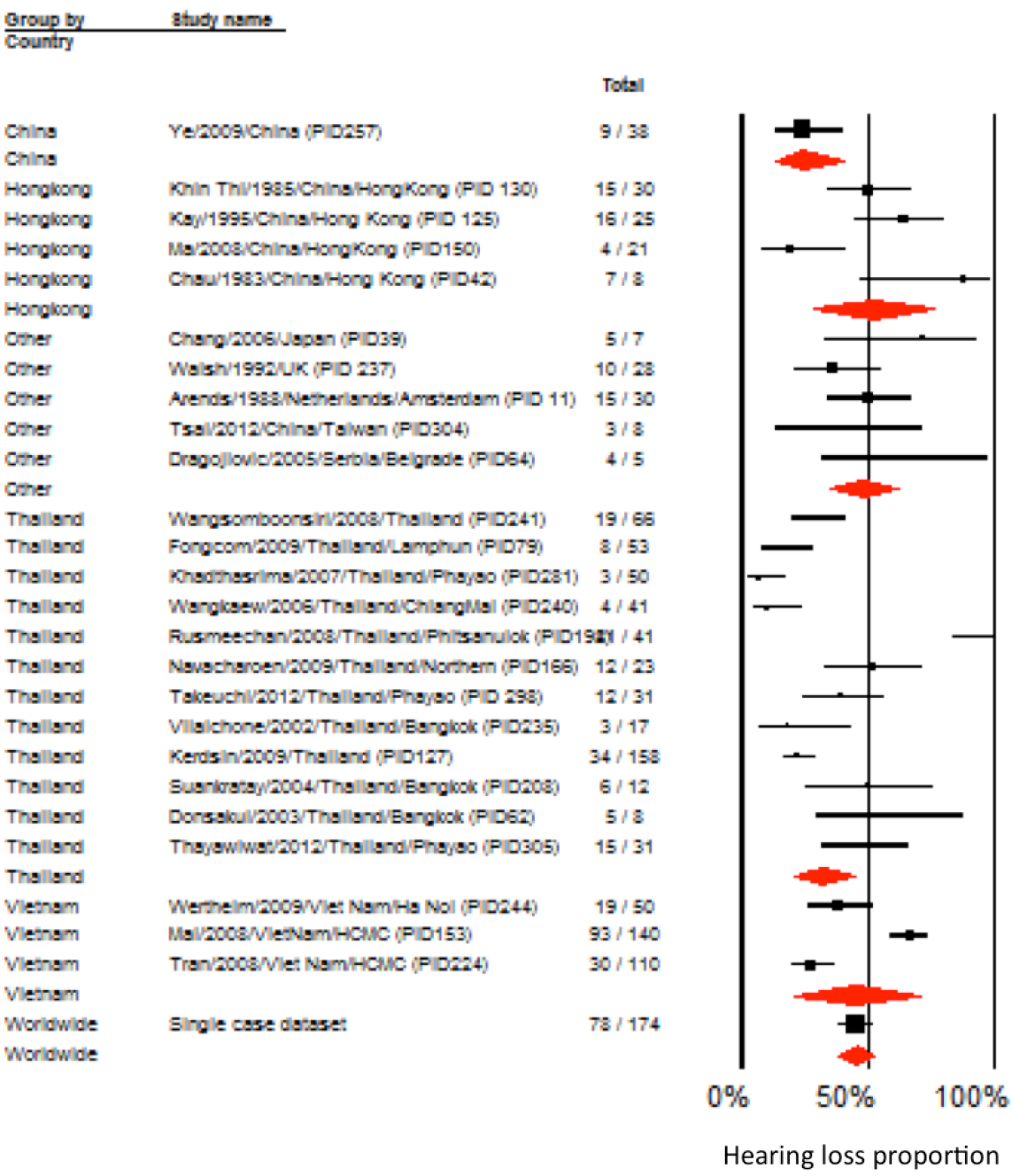
Hearing loss was the most common sequelae among patients with a pooled proportion of 39.1% (95%CI 31.0-47.8%) (Table II-0-7). Large variations are observed in the reported proportions for this outcome, and there was an evidence of some publication bias (Figure II-0-9). The reported hearing loss proportions varied greatly between studies (Figure II-0-10).

Figure II-0-9. Funnel plot of 26 studies included in the meta-analysis of hearing loss proportions among the *S. suis* cases



This figure plots hearing loss proportion in each study against its standard error (hearing loss proportion is on a logit transformed scale). Each blue circle represents each study in the meta-analysis, the funnel plot was slightly asymmetric with a pooled hearing loss proportion (blue rhombus). Using the trim and fill method, two missing studies (red circle) added in the left side and gave an adjusted proportion (red rhombus), which was slightly lower than the original one.

Figure II-0-10. Forest plot of subgroup meta-analysis (random-effects) for the proportions of hearing loss reported in the 26 included studies by country



For each study, proportion and 95%CI are presented, with the size proportional to the study weight. The red rhombus presents the pooled proportion for each country group.

There was a positive correlation between hearing loss and meningitis proportions reported in large studies ($p \approx 0.05$) (Table II-0-9). The pooled proportion of hearing loss among studies in which all patients had meningitis was 55.3% (95%CI 36.2-72.9%), compared with 34.0% (95%CI 26.0-43.1%) among the remaining studies.

Table II-0-9. Bivariate meta-regression for hearing loss proportion among included studies in the systematic review of *S. suis* infection

Study-level factor	no. studies	Pooled proportion (95%CI)	Difference*	p-value
Country of publication				
United Kingdom, Netherlands, Serbia, Taiwan, Japan	5	0.48 (0.34-0.62)	-0.014	1.0
China mainland	1	0.24 (0.13-0.40)	-0.245	1.0
Hong Kong	4	0.52 (0.28-0.75)	0.018	1.0
Thailand	12	0.31 (0.21-0.44)	-0.170	1.0
Viet Nam	3	0.44 (0.20-0.70)	reference	-
Year of publication				
2005 and before	9	0.51 (0.39-0.63)	0.128	0.33
After 2005	16	0.33 (0.23-0.45)	reference	-
Study design				
Case series	17	0.42 (0.31-0.54)	0.012	1.0
Outbreak	2	0.13 (0.03-0.41)	-0.266	0.95

Study-level factor	no. studies	Pooled proportion (95%CI)	Difference*	p-value
Cross-sectional	6	0.42 (0.25-0.62)	reference	-
Data collection				
Retrospective	19	0.38 (0.28-0.50)	-0.096	0.33
Prospective	6	0.40 (0.24-0.59)	reference	-
Meningitis proportion				
<0.5	5	0.24 (0.11-0.43)	-0.303†	0.07
0.5 - 0.9	9	0.36 (0.26-0.47)	-0.241‡	0.05
>0.9	8	0.59 (0.40-0.75)	reference	-

*Meta-regression using General Linear Model: Difference reported is the mean difference in case fatality rate between the evaluated group and the reference group as indicated in the table for corresponding study-level factor, with the p-value calculated after weighting by sample size and adjustment for multiple comparisons by the Bonferroni method.

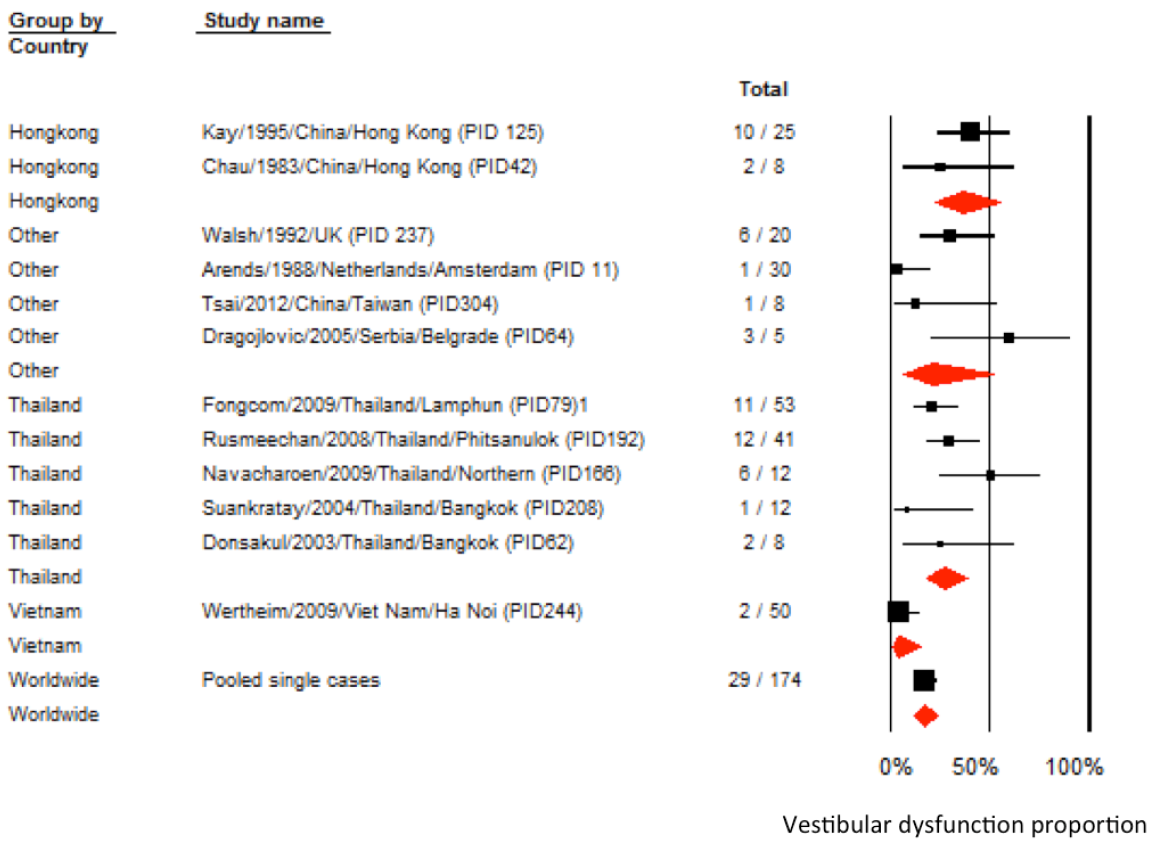
The onset of hearing loss was on average 3.8 days (range 2-7 days) after the onset of meningitis in a case series from Thailand [76]. In other case series in Hong Kong and Thailand, hearing loss was reported to start as late as 12-14 days after hospital admission [12, 23]. Limited information described how hearing loss was evaluated during and post infection among the studies reporting this clinical outcome. Only eight out of 25 large studies indicated if hearing loss was permanent after hospital discharge. Only 4 described their follow-up process, with the follow-up time ranging from 2 to 30 months [12, 20, 22, 84]. Based on these limited data, I estimated a comparatively low median proportion of recovery from hearing loss at 5.0% (range 0.0-52.3%) and the pooled proportion of 15.4% (95%CI 5.3-37.3%).

Regarding adjunctive corticosteroids treatments, a trial in Viet Nam showed that the proportion of severe hearing loss was significantly lower in the dexamethasone treatment group (15.5%, n = 71) compared with the placebo group (33.3%, n = 69) at discharge [20]. The proportions were reduced to 9.8% (n=41) and 21.7% (n=46) at 6 months follow-up, respectively. For non-severe hearing loss, the recovery process also appeared to be better in the treatment group, a reduction from 47.9% to 26.8% compared with a reduction from 36.2% to 34.8% in the placebo group. However, this study did not assess change in hearing thresholds between two time-points taking into account within and between-subject variation.

2.3.7. Vestibular outcome

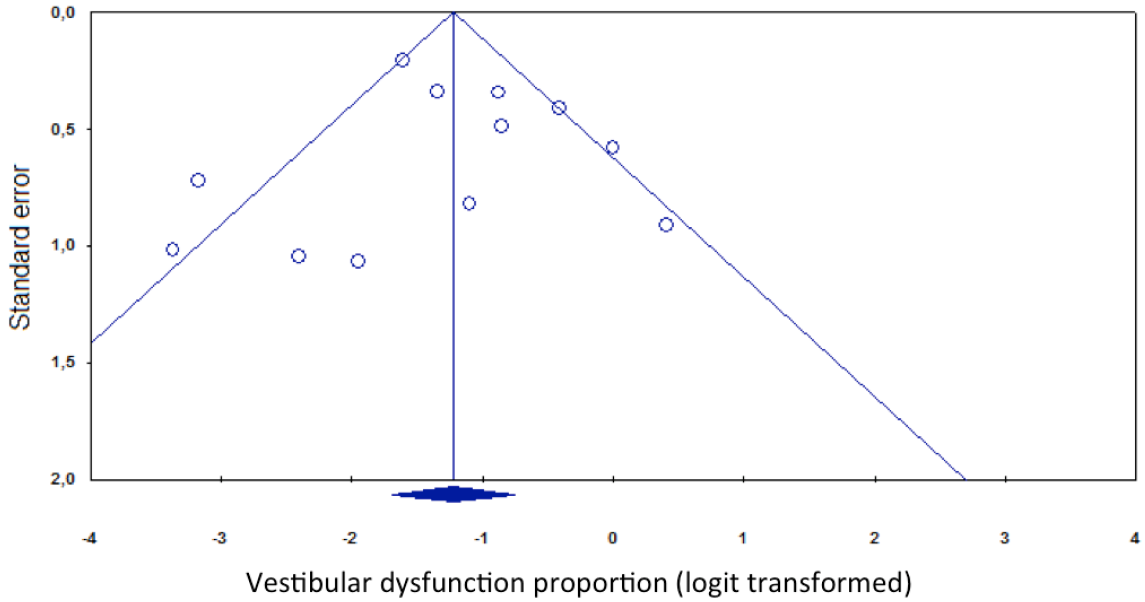
Vestibular dysfunction was the second most common neurological outcome seen in *S. suis* patients with a pooled proportion of 22.7% (95%CI 15.6-32.0%) (Table II-0-7). Large heterogeneity exists across the studies reporting this outcome (Figure II-0-11), although no evidence of publication bias was indicated from the funnel plot (Figure II-0-12).

Figure II-0-11. Forest plot of subgroup meta-analysis (random-effects) for the proportions of vestibular dysfunction by country reported in the 13 included studies



Note: For each study, proportion and 95%CI are presented, with the size proportional to the study weight. The red rhombus presents the pooled proportion for each country group.

Figure II-0-12. Funnel plot of 13 studies included in the meta-analysis of vestibular dysfunction proportions among the *S. suis* cases



This figure plots vestibular dysfunction proportion in each study against its standard error (case fatality rate is on a logit transformed scale). Each blue circle represents each study in the meta-analysis, the funnel plot was relatively symmetric with a pooled proportion (blue rhombus).

Unlike case fatality and hearing loss, I found no evidence of correlation between study-level meningitis proportions and proportions of abnormal vestibular findings. For patients with reported symptoms of vestibular dysfunction, limited information was available on the specific diagnostic and follow-up assessments. Caloric testing was used to assess the vestibular function in one large study [22] and for some patients in the single case dataset [78, 85-88]. Common symptoms described in the included studies were vertigo/ dizziness, gait unsteadiness/ ataxia/ loss of balance, and nystagmus. Vestibular findings were mostly reported among patients who also had either bilateral or unilateral hearing loss (93.1% among 29 patients with signs of vestibular dysfunction in the single case dataset; the remaining two patients had no information on their hearing performance provided). Duration of vestibular dysfunction was unclear for the majority of the patients

in both single case reports and large studies. Some authors reported progress of vestibular symptoms over short follow-up time periods [84, 88-91]. Symptom improvement was reported in some patients [84, 90, 91] and vestibular rehabilitation was considered to support this process [90].

2.3.8. Other clinical outcomes

A less common clinical outcome reported in the literature was visual impairment, found in 4% of the patients in the single case dataset. In most of these cases, patients were diagnosed with unilateral or bilateral endophthalmitis. Diplopia was also noted in some patients, possibly resulting from ocular nerve palsy [74, 92]. Some other uncommon outcomes include paralysis [16, 20], digital gangrene and amputation [93, 94], and cognitive dysfunction [95].

2.4. Discussion

2.4.1. Global epidemiology of human *Streptococcus suis* infection

After possible duplicates were removed, the total number of *S. suis* infections as of 2012 was close to 1 600 cases, doubling the figure published in 2009 [4]. Most of the increase comprised the cases from Thailand and Viet Nam, placing both countries in the highest disease prevalence stratum in the world. In contrast, only a few cases have been reported from the Americas, particularly the U.S., the second biggest pig producer worldwide [96]. This low number of cases might be because of highly industrialized pig farming systems in the region. Nevertheless, we have seen far more cases in Europe, a region with presumably similar modern farming operations, than in the Americas. Other plausible explanations include the lower virulence of North American bacterial strains [52] or different slaughtering practices.

I only counted the published cases in this review, the actual number of cases is likely to be considerably higher, particularly in the endemic areas such as Asian countries with extensive pig rearing. The problem of underestimation is further exacerbated by the fact that *S. suis* infection is not a notifiable disease in most countries. In addition, a lack of diagnostic capacities or limited disease awareness in the local laboratories can result in cases undiagnosed or misidentified.

2.4.2. Main epidemiological parameters

Meningitis was the main syndrome in approximately two in three patients with *S. suis*, although this varied by country. It is important to note that the syndromic distribution of the reported cases depends on the study design and case ascertainment methods. All major studies in Viet Nam ascertained *S. suis* cases from the population of patients with central nervous system diseases. This could lead to the under-representation

of *S. suis* cases with clinical syndromes other than meningitis. To date, only one case without meningitis (diagnosed as spontaneous bacterial peritonitis with serotype 16 infection) has been reported in this country [97]. Nevertheless, whether the existing Vietnamese human infecting strains mainly cause meningitis still remains unclear. In fact, lumbar puncture is done regularly for all *S. suis* patients including those with severe sepsis at a hospital for tropical diseases in Viet Nam, and almost all had typical CSF characteristics of bacterial meningitis. On the other hand, the diagnosis of meningitis might not be done or reported from other countries, and therefore reducing the estimated global *S. suis* meningitis proportion.

The fatality rate of *S. suis* meningitis seems to be lower than that of other common causes of adult bacterial meningitis including *Streptococcus pneumoniae* (19-37%) [98] and *Neisseria meningitidis* (10%) [99]. However, the proportions of *S. suis* patients developing sequelae appear much higher compared to the other causes [100]. Much uncertainty still exists in understanding about sequelae of *S.suis* and recovery of those conditions over time. Careful prospective assessments of these debilitating outcomes are essential for understanding and reducing the burden due to *S. suis* infection. More studies are also needed to assess the effects of adjunctive corticosteroid on hearing loss or other neurological sequelae.

In this review, I was unable to establish the pooled risk estimates for different risk factors due to a lack of studies with appropriate designs. In Viet Nam, *S. suis* cases were more likely to have consumed high-risk dishes (odds ratio 4.38), to have pig-related occupations (5.52), and to have pig exposure whilst having skin injuries (15.96) than the community controls [21]. The lower proportions of cases with occupational exposure in Thailand and Viet Nam as compared to Europe shown in our meta-analysis supports the

hypothesis that other risk factors including food consumption practices, play an important role in the epidemiology of *S. suis* in Asia.

2.4.3. Review limitations and identification of information gaps

This review is not without limitations. The included studies were highly heterogeneous in quality and in the factors that were reported, thus reducing the number of studies included in each meta-analysis. The summary values of the single case dataset should be interpreted with caution since the patients in this merged "sample" were heterogeneously "recruited" from different populations, with completely different assessment protocols and variables reported between cases. Most studies were retrospective and thus data on risk factors and clinical characteristics and outcomes could be underreported due to recall bias or by collecting from the existing data records. I was unable to assess the extent to which this misinformation could affect the overall estimates. However, the pooled proportions for death and hearing loss were not statistically different between the two data collection approaches (prospective versus retrospective) in the meta-regression analysis.

This systematic review helps to highlight areas where additional research is needed. There are obviously geographic gaps in the data on *S. suis* cases especially in the pig rearing countries in the Americas, Eastern Europe and Asia such as Mexico, Brazil, Russia, or Philippines. More research is needed to examine the medical, social, and economic sequelae of *S. suis* infection and the impact on the patients and their families. More studies are also needed to investigate risk factors including the food practices and the infection risk acquired from these practices in the Asian communities. Additional work is also necessary to assess the fraction of *S. suis* cases that can be attributed to specific risk factors (the population attributable fraction) and the proportion of *S. suis* cases of that might be preventable were specific risk factors reduced.

2.5. Conclusion

In conclusion, existing data showed that the burden of human *S. suis* infection was mainly in Asia where pig rearing and consumption is a common practice. While data on epidemiological, clinical and microbiological aspects of the infection have increased considerably in the past decade, there are still important research gaps which need to be addressed. In addition, large variation in the estimates for main disease epidemiological parameters implicates the necessity to have data specific to the local context for more accurate quantification of the disease burden and effective targeted public health prevention and control activities.

Chapter III

Community assessment of consumption of uncooked pig blood dish - a risk factor to *Streptococcus suis* infection in Viet Nam

3. 1. Introduction

3.1.1. Rationale

In Asian countries including Thailand and Viet Nam, in addition to the infection risk acquired from occupational exposure, consumption of raw or undercooked pig products has been identified as a risk factor for *Streptococcus suis* infection in many studies reporting large number of cases [14, 16, 17, 21, 22, 47, 49, 63]. In a case control study in Viet Nam, up to one in two cases with *S. suis* infection reported eating high-risk pig dishes including fresh/ undercooked pig blood, tonsils/ tongue, stomach/ intestines, or uterus in the past two weeks prior to illness [21]. Cases were much more likely to have consumed these high-risk dishes compared to their community controls (OR 4.44, 95%CI 2.15-9.15) in this study. In our study investigating the presence of *S. suis* in raw pig blood dish (*tiet canh)*, 15.4% of 188 samples collected from shops in 4 provinces across Viet Nam were found to contain viable strains of this pathogen (unpublished data, manuscript in writing). Another study showed a *S. suis* carriage rate of 41% of pigs at some slaughterhouses in southern Viet Nam, illustrating how common this bacterium is among the pig population [41]. This high carriage prevalence may contribute to the relative large proportion of *S. suis* infections in humans through consumption of raw/ undercooked pig dishes. Among the risk factors that remained independently significant in the multivariate analysis of the case-control study mentioned above, 47% of cases had consumed high-

risk pig dishes while only 27% and 20% had pig related occupations or had any exposure to pigs/ pork with skin injuries respectively [21].

Tiet canh (a raw pig blood dish) is one of very few high-risk pig dishes that are often consumed raw in Viet Nam. This dish is made of fresh pig blood (blood of other animals such as duck or goat can also be used) mixed with chopped cooked meat (e.g. pork cartilage, throat, intestine), and is left to coagulate before serving (Figure III-0-1). After some recent disease outbreaks happening in humans and animals, the Vietnamese government has issued an official order regarding the control of slaughtering, selling and consumption of animal products, which also includes a ban on selling of *tiet canh* (No. 18 BNN/CĐ, dated 21/05/2009). However, *tiet canh* continues to be among the most reported high-risk dish consumed by patients prior to the *S. suis* infection from our observation of patients at NHTD.

Figure III-0-1. A typical *tiet canh* - raw pig blood dish in Viet Nam



Better understanding of the practice of consuming raw/ undercooked pig products in the community is crucial for the development and implementation of effective interventions to control and prevent *S. suis* infections. In this study, I investigated the practice of consuming *tiet canh* dish in a northern province in Viet Nam.

3.1.2. Objectives

- To describe the frequency and socio-demographic patterns of raw pig blood consumption in the community;
- To explore community perceptions about the practice of raw pig blood consumption, and associated disease risks, and their attitudes towards control interventions.

3. 2. Methods

3.2.1. Study settings

This study was conducted in two existing health and demographic surveillance sites, FilaBavi in rural Ba Vi district and DodaLab in urban Dong Da district of Ha Noi, Viet Nam. These sites were established in 1999 and 2007 respectively by the Ha Noi Medical University and have been described elsewhere [101, 102]. Each site contains all households in a selected number of administrative areas (19.6% randomly selected clusters in Ba Vi and 3 out of 21 communes representing different socioeconomic levels in Dong Da district). These two surveillance sites share common characteristics of typical rural and urban areas in the north of Viet Nam, and provide a good contrast between the two types of community settings in a rapidly urbanizing country. Basic demographic and socioeconomic information of individuals and households are updated through biannual household survey at the sites.

3.2.2. Study design

This study employed a mixed-methods design, comprising both a quantitative behavioral questionnaire and focus group discussions. Raw pig blood consumption in the community was quantitatively assessed with a short survey form attached to the routine household survey in the two sites. This was followed by a qualitative assessment comprising ten focus group discussions (FGDs) to explore community perceptions about the food practice and associated aspects including disease risks and control interventions.

3.2.3. Data collection

3.2.3.1 Quantitative survey

I carried out the raw pig blood community survey from May through June 2012 in Ba Vi and from December 2012 through January 2013 in Dong Da. Trained interviewers visited the households as scheduled in their routine household survey and asked one household member about their practices of consuming raw pig blood using a short survey form. In both sites, respondents were asked the same questions for the practice of consuming raw pig blood in the past one year and answered by choosing one of the options offered: none, 1-3 times, 4-8 times, 9-12 times, >12 times, and unsure (Box III-0-1). Participants were required to specify the type of animal blood that they consumed, which include pig blood, duck blood and others. These questions were checked by the field interviewers (who were also local people working for the surveillance sites) to find the most appropriate way of stating the questions using the local language before the survey was started.

Box III-0-1. Raw pig blood survey questions

1. How many times each member has eaten tiết canh (raw pig blood dish) **in the past 1 year**?

□ None □ 1-3 times □ 4-8 times □ 9-12 times □ >12 times

2. What type of animal blood did you eat?

□ Pig blood □ Duck blood

□ Blood of other animal, specify the animal:

Socio-demographic data of the respondents were extracted from the latest household update survey in both sites from the database stored centrally at the Hanoi Medical University. These included sex (male versus female), age (years), highest education level, marital status, occupation, and household economic status (HES). Age was calculated based on the recorded date of birth and collapsed into six categories: <20, 20-29, 30-39, 40-49, 50-59, and ≥60 years old. Education was recorded as the highest grade or level that the person had completed, therefore the total number of years spent on formal schooling was counted and then collapsed into four categories: ≤5, 6-9, 10-12, >12 years (corresponding to primary school, secondary school, high school, post-high school training in the existing Vietnamese education system). Marital status was recoded as married versus non-married (or others) group from the existing seven categories recorded in the original database. Similarly, occupation was also recoded into six categories (office workers, manual laborers, services and sales, farmers, others, and not in the workforce) from the existing 21 categories recorded in the database. I used the existing HES classifications from the surveillance sites, which was determined based on the wealth index. The wealth index had been calculated using all household variables describing housing and assets from the routine survey. The wealth index was then grouped into

quintiles, representing five HES groups each containing one fifth of all households in the surveillance sites.

I calculated a minimum sample size using on the following formula using the expected proportion of eating raw pig blood in one year in the young population group aged <20 years who could attend the survey at the time the field interviewers made the home visit using the following formula [103]:

$$N = z^2_{\alpha/2} \quad * p * (1 - p)/e^2$$

where N is the minimum required sample size; $z_{\alpha/2}$ is the critical value of a normal distribution determining the maximum error of estimate at a specific α level; $\alpha/2$ represents each of the two tails of the standard normal distribution curve; p is the expected proportion of eating raw pig blood at least once in the past one year; and e is the margin of error.

The proportion of eating raw pig blood at least once in the past one year among these young people was presumed to be about 5% and 2.5% in the rural and urban setting, respectively. The minimum sample size of 70 in Ba Vi and 38 in Dong Da are required to detect the above assumed proportions among people aged <20 years with a 5% margin of error and 95% confidence interval. Based on previous surveys, I expected only about 1% of the respondents fall into this age group. As a result, the total sample size required for the quantitative survey is 7,000 respondents in Ba Vi and 3,800 respondents in Dong Da district.

Respondents were sampled following the existing cohorts in the two surveillance sites, each containing approximately 11,000 households. Within the routine interview schedules, field interviewers visited all households in the areas they were responsible for

and conducted the regular interview. The pig blood survey was added at the end of the routine survey and was administered to only one person per household (the person who responded to the routine interview). The pig blood survey was stopped when a sufficient number of households had been reached. All complete data forms were checked by field supervisors and data clerk before data entry. A total of 6,993 respondents in Ba Vi and 3,991 respondents in Dong Da were interviewed (no households refused) across all geographical areas of the cohorts. After persons for whom no data were available regarding age and sex were excluded, 6,943 (99.3%) persons in Ba Vi and 3,921 (98.2%) in Dong Da were included in the analysis.

3.2.3.2 Focus group discussions

The follow-up qualitative study employed a grounded theory embedded design to uncover the experiences of participants related to raw pig blood eating and build the concepts from the data to explain the behavior as described elsewhere [104]. This was conducted from April through June 2013 with 5 focus groups (FGs) in Ba Vi, including FG1-4 at participants' house and FG5 at the meeting room of the FilaBavi office located in the district hospital, and 5 (FG5-10) in Dong Da which were organized at the commune health centers in the participants' vicinity. I moderated all FGs in Vietnamese using the question guide (Appendix 2). The question guide was designed to include 5 categories of questions to maintain the flow of the discussion: opening, introductory, transition, key, and ending [105]. Main aspects discussed are: contexts in which the dish was consumed, attributes of the dish (likes and dislikes), factors influencing the choices of eating, perceived health benefits and health risks associated with the dish, feasibility of relevant public interventions, and recommendations for better implementation. Participants were invited to share their experiences of related public interventions. The subsequent discussion focused four main types of interventions: 1) Information, Education and

Communication (IEC) activities, 2) the ban on selling raw animal blood, 3) inspection of animals and animal products, and 4) vaccination of pigs. The question guide was adjusted after each FG when there was new information identified. Each FG lasted 1.5 to 2 hours and was audio-taped with consent from the participants. One research assistant was present in each FG for note taking and logistics support. At the end of each FG, the moderator summarized the content discussed and checked with the participants if the moderator has correctly understood the participants' responses.

I employed the focus group interviewing method to gather in-depth information associated with consumption of raw pig blood in the community. Focus group (FG) has been used extensively in community health research, and this technique provides a more natural environment which resembles real-life situations where individual behaviors and attitudes tend to influence and be influenced by others' [105]. The question guide with open-ended questions helped to guide the discussion on the specific issues in a comfortable, permissive and non-judgmental environment. To identify the factors associated with consumption and perceptions on interventions from different perspectives – male versus female, rural versus urban, and government workers versus the general population, I designed five FGs in each surveillance site as follows:

- 1 FG of men who never ate *tiet canh*
- 1 FG of men who frequently ate *tiet canh*
- 1 FG of women who never ate *tiet canh*
- 1 FG of women who frequently ate *tiet canh*
- 1FG of government workers (containing both people who ate and never ate *tiet canh*)

Eighty-one people were purposefully selected from the two surveillance sites to participate in 10 focus groups (FGs). The community participants were selected by the field interviewers based on their experience of communication during their routine interviews. The participants were met and invited individually by the field interviewers. The field interviewers also identified the government workers through their network and invited them individually. These included health workers, women's union staff, and culture and information staff. Each FG had from 6-9 participants.

3.2.4. Data analysis

Quantitative data was analyzed in SPSS software version 19.0 (IBM Corp., New York, U.S.). I calculated the proportions of respondents eating *tiet canh* with 95% confidence intervals (CI). Associations between tiet canh consumption and socio-demographic variables were examined using univariate logistic regression with gender and age adjustment. Independent variables included gender, age, education (years of schooling), occupation, household economic status (HES: quintiles), and marital status. Odds ratios (OR) with 95%CIs are calculated for each variable. Model selection was performed by forcing all variables into a model and removing step-by-step the least significant variable. I used the Nagelkerke R Square statistic which can range from 0 to 1 to select the most parsimonious model with the highest explanatory power and smallest number of variables [106].

All qualitative FG data were transcribed and uploaded to the NVivo software version 10 (QSR International Pty Ltd., Victoria, Australia) for cleaning and analysis. I read all the transcripts to identify initial categories. These transcripts were then reread in detail, and each quote examined to see if it fitted within existing categories or a new category was required. Towards the end of the process, all transcripts were reviewed again to group responses into broader categories. Results were verified again by a

Vietnamese expert in qualitative study design, who was part of the research team. Validity of the categories were reviewed again by all team members. Representative quotes from each category are provided in Appendix 3.

3.2.5. Ethical considerations

The study was approved by the Oxford Tropical Research Ethics Committee of University of Oxford (OXTREC Reference: 509-13) and the HMU Review Board (Appendix 3). Household participants were part of the demographic surveillance cohorts for which informed consent was obtained.

3. 3. Results of community survey

3.3.1. Characteristics of survey respondents

Demographic and socioeconomic profiles of the respondents participating in the community survey are described in Table III-0-1. Respondents in rural Ba Vi and urban Dong Da significantly differ in gender (24.6% versus 34.5% male, respectively), educational status (21.9% versus 74.3% with at least 10 years of education) and occupation (2.4% versus 29.6% office workers).

Table III-0-1. Socio-demographic characteristics and *tiet canh* consumption practice of respondents in the quantitative survey in two districts of Ha Noi

Characteristic		Ba Vi (rural) (N = 6943)	Dong Da (urban) (N = 3921)
Male gender		1710 (24.6%)	1353 (34.5%)
Age (year, mean (95%CI))*		47.0 (46.6-47.3)	48.3 (47.8-48.8)
	<20	123 (1.8%)	49 (1.2%)
	20-29	725 (10.4%)	448 (11.4%)
	30-39	1398 (20.1%)	781 (19.9%)
	40-49	1796 (25.9%)	753 (19.2%)
	50-59	1639 (23.6%)	927 (23.6%)
	≥60	1262 (18.2%)	963 (24.6%)
Education#	≤5	1110 (16.0%)	148 (3.8%)
	6-9	4310 (62.1%)	852 (21.9%)
	10-12	1351 (19.5%)	1574 (40.4%)
	>12	165 (2.4%)	1321 (33.9%)
Occupation	Office workers	168 (2.4%)	1155 (29.6%)
	Manual laborers†	666 (9.6%)	339 (8.7%)
	Farmers	4474 (64.7%)	2 (0.1%)
	Services and sales	561 (8.1%)	923 (23.7%)
	Others	260 (3.8%)	56 (1.4%)
	Not in the workforce‡	786 (11.4%)	1424 (36.5%)

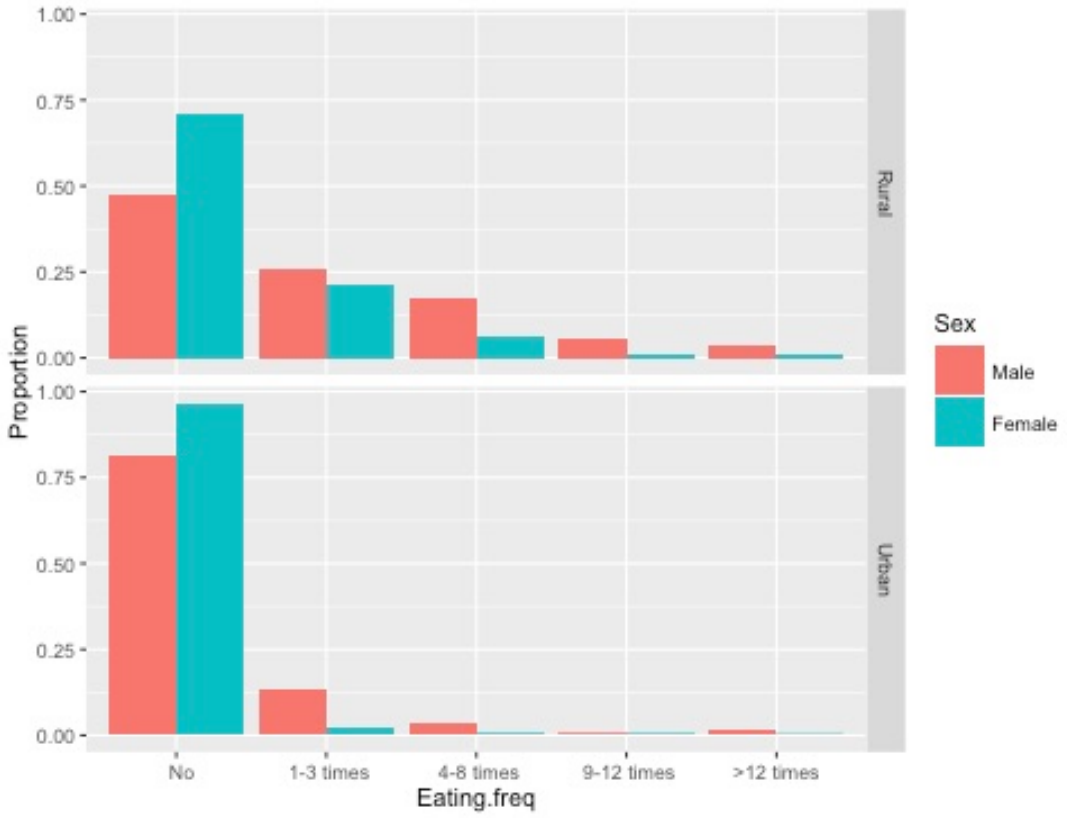
Characteristic		Ba Vi (rural) (N = 6943)	Dong Da (urban) (N = 3921)
HES (quintiles)	Lowest	1140 (16.5%)	654 (16.5%)
	Second	1474 (21.3%)	797 (20.4%)
	Third	1628 (23.6%)	837 (21.4%)
	Fourth	1521 (22.0%)	827 (21.1%)
	Highest	1148 (16.6%)	806 (20.6%)
Marital status	Married	5745 (82.7%)	2923 (74.5%)
	Non-married	1198 (17.3%)	998 (25.5%)

HES: household economic status. *Age range was similar between two sites: 8-97 in Ba Vi and 9-102 in Dong Da. #number of schooling years. †Manual labor includes construction, factory work, casual manual work on call, handicraft work, and mining. ‡Not in the workforce category includes children, housewives, elderly, retired.

3.3.2. Consumption of raw pig blood

2 427 respondents (35.0%, 95%CI 33.8-36.1) in the rural area reported eating *tiet canh* made from raw pig blood in the past year, which was four-fold higher than the proportion of respondents reporting consumption in urban areas (n=338, 8.6%, 95%CI 7.7-9.5%) (Figure III-0-2). Among people who reported consumption, most of them consumed the dish less than 4 times in the past year (63.4% out of 2 427 and 71.0% out of 338 respondents in rural and urban districts respectively).

Figure III-0-2. Proportion of respondents reporting raw pig blood consumption by eating frequency, sex and site



Tiet canh made from duck blood was consumed in 8.0% (n=557) and 8.3% (n=324) of respondents in the rural and urban district respectively. Other, but less common sources of animal blood included goat, cow, dog, horse and rabbit (comprising 0.14% and 0.48% of total reported consumption in rural and urban areas, respectively). The subsequent analyses were restricted to the practice of eating *tiet canh* made from pig blood (and *tiet canh* was used to refer to raw pig blood dish in the remainder of this chapter).

3.3.3. Factors associated with consumption

Gender, age and occupation were significantly associated with consumption patterns in both rural and urban settings by univariate analysis (Table III-0-2).

Table III-0-2. Factors associated with the consumption of *tiet canh* in the past year among the respondents in two districts of Ha Noi

Factor	Ba Vi district (rural)			Dong Da district (urban)		
	Eat		OR* (95%CI)	Eat		OR* (95%CI)
	1-3 times n (%)	≥4 times n (%)		1-3 times n (%)	≥4 times n (%)	
Gender						
M	439 (25.7)	461 (27.0)	**3.0 (2.7–3.4)**	180 (13.3)	70 (5.2)	**6.4 (4.9–8.2)**
F	1 099 (21.0)	428 (8.2)	1	60 (2.3)	28 (1.1)	1
Age (y)						
<20	15 (12.2)	2 (1.6)	**0.4 (0.2–0.7)**	1 (2.0)	1 (2.0)	0.8 (0.2–3.7)
20-29	131 (18.1)	78 (10.8)	**1.5 (1.2–1.8)**	27 (6.0)	10 (2.2)	**1.7 (1.1–2.7)**
30-39	328 (23.5)	207 (14.8)	**2.3 (2.0–2.8)**	43 (5.5)	25 (3.2)	**2.2 (1.5–3.4)**
40-49	474 (26.4)	285 (15.9)	**2.6 (2.2–3.0)**	60 (8.0)	25 (3.3)	**2.8 (1.9–4.1)**
50-59	383 (23.4)	210 (12.8)	**1.9 (1.6–2.2)**	80 (8.6)	24 (2.6)	**2.6 (1.8–3.8)**
≥60	207 (16.4)	107 (8.5)	1	29 (3.0)	13 (1.3)	1
Education (y)						
≤5	199 (17.9)	104 (9.4)	1.5 (1.0–2.1)	7 (4.7)	3 (2.0)	2.2 (1.0–4.5)
6-9	1 055 (24.5)	582 (13.5)	**1.7 (1.2–2.5)**	64 (7.5)	33 (3.9)	**2.0 (1.5–2.8)**
10-12	257 (19.0)	184 (13.6)	1.3 (0.9–1.9)	82 (5.2)	42 (2.7)	1.1 (0.9–1.5)
>12	26 (15.8)	18 (10.9)	1	84 (6.4)	19 (1.4)	1
Occupation						
Office workers	25 (14.9)	16 (9.5)	1	70 (6.1)	18 (1.6)	1

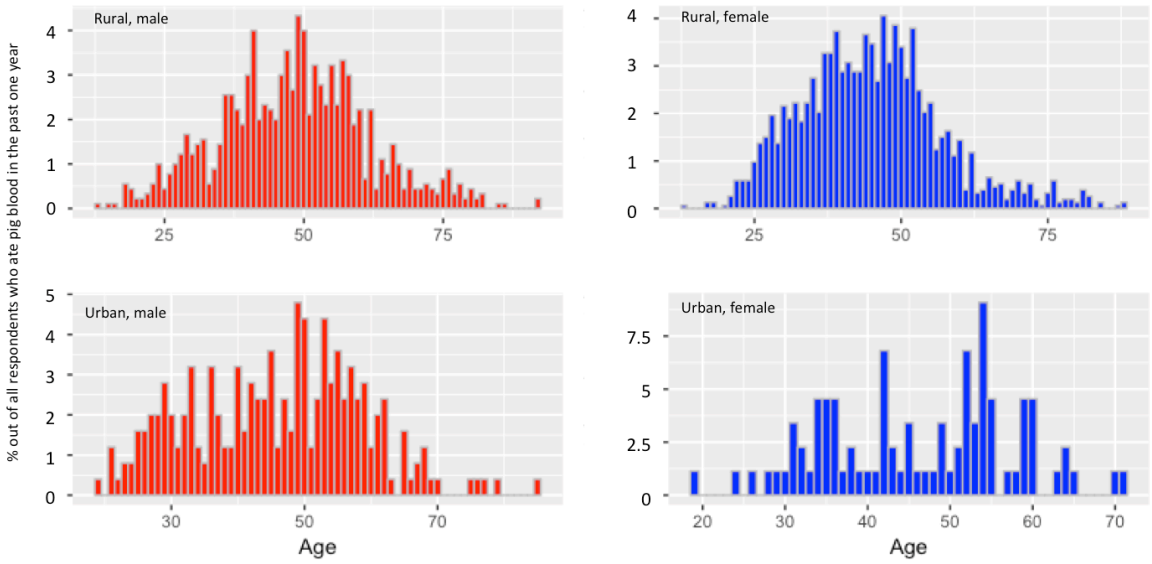
Factor	Ba Vi district (rural)			Dong Da district (urban)		
	Eat		OR* (95%CI)	Eat		OR* (95%CI)
	1-3 times n (%)	≥4 times n (%)		1-3 times n (%)	≥4 times n (%)	
Manual laborers†	155 (23.3)	122 (18.3)	**1.5 (1.0-2.3)**	32 (9.4)	23 (6.8)	**1.9 (1.3-2.8)**
Services and sales	113 (20.1)	82 (14.6)	**1.6 (1.0-2.3)**	76 (8.2)	37 (4.0)	**1.9 (1.4-2.5)**
Farmers	1 085(24.3)	564 (12.6)	**2.0 (1.4-2.9)**	-	-	-
Others	52 (20.0)	46 (17.7)	1.4 (0.9-2.3)	10 (17.9)	2 (3.6)	**2.1 (1.1-4.3)**
Not in the workforce‡	101 (12.8)	55 (7.0)	1.1 (0.7–1.6)	51 (3.6)	18 (1.3)	1.2 (0.8-1.9)
HES (quintiles)						
Lowest	239 (21.0)	107 (9.4)	0.9 (0.7–1.0)	45 (7.0)	26 (4.0)	**2.1 (1.4–3.0)**
Second	311 (21.1)	161 (10.9)	0.9 (0.7–1.0)	80 (10.0)	20 (2.5)	**2.2 (1.5–3.1)**
Third	381 (23.4)	236 (14.5)	1.1 (0.9–1.3)	41 (4.9)	17 (2.0)	1.1 (0.8–1.7)
Fourth	341 (22.4)	220 (14.5)	1.0 (0.9–1.2)	41 (5.0)	13 (1.6)	1.1 (0.7–1.6)
Highest	256 (22.3)	160 (13.9)	1	33 (4.1)	22 (2.7)	1
Marital status						
Married	1 363 (23.7)	823 (14.3)	**1.6 (1.4–1.9)**	188 (6.4)	72 (2.5)	0.8 (0.6–1.1)
Others	175 (14.6)	66 (5.5)	1	52 (5.2)	26 (2.6)	1

Bold face: significant finding ($p<0.05$). HES: household economic status. *Odds ratio for Yes (include ate 1-3 times and >3 times) versus No (Did not eat), adjusted for gender and age. †Manual labor includes construction, factory work, casual manual work on call, handicraft work, and mining. ‡Not in the workforce category includes children, housewives, elderly, retired.

Significantly more men than women reported consuming raw pig blood, and this difference was more profound in the urban than rural setting: age-adjusted ORs 6.4 (95% CI 4.9-8.2) and 3.0 (95%CI 2.7-3.4) respectively. Rural men were the major consumer group with over half of them eating the dish four times or more per year. Consumption was most common in the 40-49 age group (42.1% in rural and 11.3% in urban district). Rural residents tended to start this practice at a younger age than urban residents: 13 and 19 years of age respectively (Figure III-0-3).

Consumption appeared to be more common among people with lower education levels compared with those with higher education. Occupations reporting more consumption included: farmers, manual laborers, service providers and sales people, whilst the practice was less common among office staff and those not in the workforce such as children, older people and housewives (Table III-0-2).

Figure III-0-3. Distribution of respondents reporting raw pig blood consumption by age, sex and site



Married people were more likely to report raw pig blood consumption than others in the rural area, OR1.6 (95%CI 1.4 – 1.9, gender and age adjusted). Conversely,

Household Economic Status (HES) was significantly associated with the practice among urban residents (OR>2.0 for the two lowest quintiles). Among the rural respondents, I examined the consumption between farmers and non-farmers and revealed an interaction effect between occupation and HES on eating behavior (Figure III-0-4). Higher HES among farmers was associated with an increased tendency to eat raw pig blood, ORs of 1.4 (95%CI 1.2-1.8), 1.5 (95%CI 1.2-1.9), and 1.5 (95% CI 1.2-2.0) for the 3rd, 4th and 5th quintile of HES respectively, adjusted for gender and age.

The effects of all variables remained significant in the multivariable logistic regression, except for education (Table III-0-3). The effect of education found in the univariate analysis might have been confounded by the effect of household economic status, which was significant in multivariable models for the rural farmers and urban group. The final model including all variables could explain 24.2% of the variability in the observed data of eating raw pig blood in the community (Nagelkerke $R^2 = 0.242$)

Figure III-0-4. Proportion of respondents reporting raw pig blood consumption in the rural district by household economic status and type of occupations

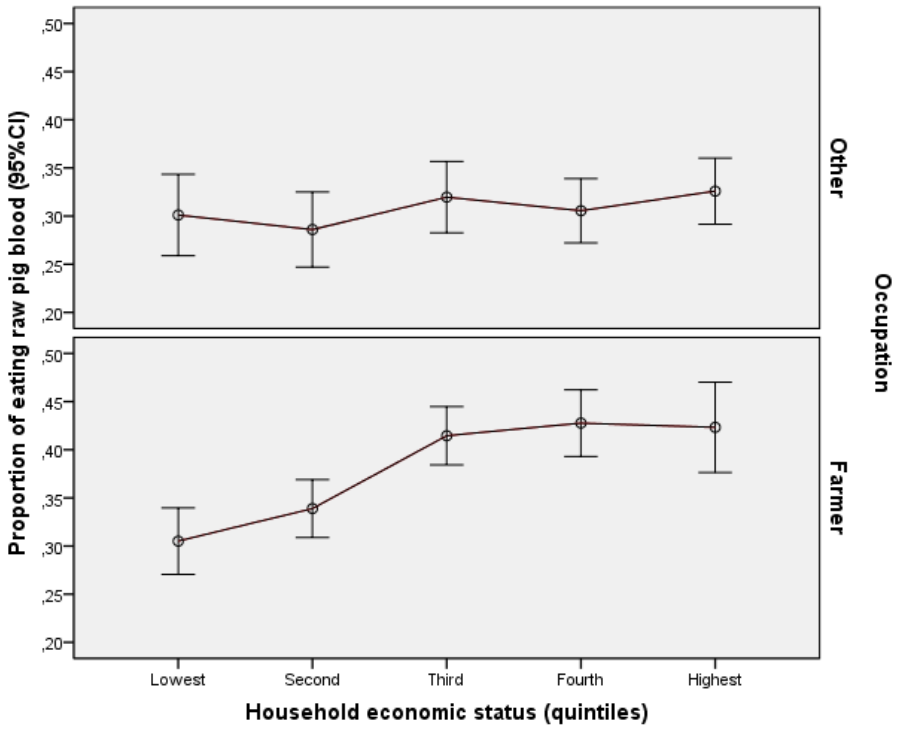


Table III-0-3. Variables in the models predicting the behavior of eating *tiet canh*

Group	Variables in the final model*	Nagelkerke R^2†
Rural people	Gender, age, occupation, marital status, HES	0.123
Rural farmers	Gender, age, marital status, HES	0.086
Rural non-farmers	Gender, age, occupation, marital status	0.168
Urban people	Gender, age, occupation, HES	0.185
Both rural and urban people	Gender, age, occupation, marital status, location (rural versus urban)	0.242

Abbreviations: HES, household economic status. *Model selection: First, all variables were forced into logistic regression. Then each variable with non-significant p-value (≥0.10) was taken out step by step until all the remaining variables were significant (p<0.10) in the model. †Nagelkerke R Squared statistic - Higher value indicates a stronger model.

3. 4. Results of focus group discussions

3.4.1. Characteristics of focus group participants

Characteristics of FG participants are summarized in Table III-0-4. Participants in the urban district tended to be older and less likely to report consumption of raw pig blood than the rural participants. However, consumption of raw duck blood was higher among the former group.

Table III-0-4. Characteristics of 81 participants in 10 focus group discussions

	Ba Vi district (rural) N=40	Dong Da district (urban) N=41
Male gender	18 (45)	16 (39)
Mean age (95%CI)	39.9 (37.1-42.8)	49.6 (45.1-54.2)
Type of participants		
General community	34 (85)	33 (81)
Health workers	4 (10)	3 (7)
Other community workers	2 (5)	5 (12)
Consumed raw pig blood	23 (57.5)	6 (14.6)
Consumed raw duck blood	8 (20)	21 (51.2)

Data are presented as number (percentage), or otherwise specified.

3.4.2. Summary of focus group discussion findings

The content of 10 focus group discussions could be classified into 3 main themes which might have influenced the individual practice in consuming raw pig blood dish:

food characteristics, the social contexts where the eating behavior was first experienced and later maintained, perceived food safety and risks (Table III-0-5).

Table III-0-5. Factors influencing participants' choice of eating *tiet canh* explored from focus groups discussions

Category	Descriptions
Food characteristics	Taste, appearance Perceived benefits: cooling the body's heat, prevent headache, anemia, treat dysentery, mouth ulcers, coughing, clear the lungs, clean the body internal system, bring good luck if eating on the 1st day of the lunar month
Social contexts	Weddings (in rural area), parties, slaughtering events, drinking encounters (best matched food for alcohol drinking) Available in social parties; Available in shops at affordable price; High profit made from selling the dish
Safety and risk	Safe food source: Home grown/ self-made, free-range, rural, wild boar, trustworthy/ known source; Drinking alcohol: to reduce the bloody smell of the dish, and to kill bacteria, disinfect and neutralize the dish (hot and cold food) Reduce/ stop eating *tiet canh* during pig disease outbreaks, eating again after outbreak; Perceived that risk is somewhere else, Belief that death is fate/ chance or disease cannot happen to them;

Food characteristics

All participants who expressed their preference for raw pig blood dish described positively the common aspects of the food including its freshness, softness, appealing appearance and taste. In addition, 44% (n=36) participants indicated the aspects that they perceived to be the benefits of the dish. One frequently mentioned characteristic was that raw pig blood was a 'cooling' food which could ease internal body heat or be helpful in treating mouth ulcers (n=24, mainly in urban FGs). Other perceived benefits included preventing anemia (because it was made of raw blood), treating headache, cough and dysentery, detoxification, or clearing 'minute dust' in the lungs (e.g. 'hair clippings'). Raw pig blood was also considered a 'red' food associated with good luck (n=13, mainly in urban FGs).

Social context

As with all food consumption patterns, raw pig blood consumption can begin at a very young age and is influenced by parents (e.g. the dish may be offered as a special treat for children, or consumption may be encouraged due to belief that it provides improved immunity). The food practice was most commonly learned in social occasions such as rural weddings, New Year celebrations and other special gatherings. On these occasions pigs were slaughtered at the houses of families hosting the celebration. Several male respondents expressed pride and fond memories of their experience participating in such slaughtering events. Raw pig blood was sometimes served at these functions expressly to demonstrate evidence that the slaughtered pig was healthy. Since pigs were often slaughtered in the early morning, raw pig blood was made and consumed as soon as possible within the morning when it was still fresh. Weddings were the most frequently reported occasion for raw pig blood consumption in the rural area. Indeed, among young

rural women, their first reported instance of the food consumption was often at their own wedding.

Raw pig blood could be found in most markets in both urban and rural settings, and was relatively cheap (~10-20,000Viet Nam Dong, equivalent to <$US 0.5-1). Many small shops sold fresh raw pig blood in the morning, however it was also sold for lunch and dinner. Since raw pig blood was affordable and easily available, the food practice was especially common among urban low-income workers. This was also supported by results from the community survey as described in the previous section.

Food safety and risks

Raw pig blood consumption by men was frequently associated with rice wine in both the rural and urban contexts. Drinking alcohol while eating raw pig blood was perceived to help mitigate possible health risks the food might contain. Several men expressed the belief that rice wine could 'disinfect' raw pig blood and thereby made it safe for consumption. Perceptions of safety were also influenced by whether the source of the pig or the person preparing the dish was known to the consumer (mentioned by 61% and 50% of rural and urban participants, respectively). Many expressed more confidence in the dish when prepared by someone they knew and trusted, versus an unknown commercial source. The word 'clean' was used by a substantial number of consumers (31%, n=16) to describe deliberate choices on whether to eat raw pig blood in a given context, and it conveyed two different meanings: the source was known or was perceived as prepared under trusted 'hygienic' conditions. Other sources considered relatively safe were home-grown pigs, wild boar, or local pig breeds (known as '*lợn Mán', 'lợn Mường',* which are typically free-range scavenging and raised by ethnic minorities).

Concerns regarding the risk of diseases associated with raw pig blood were raised in each of the FGs. The presence of ongoing disease outbreaks in pigs negatively influenced consumption patterns, and discouraged some people from eating. This effect was noted more frequently among rural participants, and in communes with a history of pig outbreaks. Nevertheless, very few participants could mention specific named diseases transmissible to humans through raw pig blood consumption.

Risk underestimation through optimism bias was common among the participants. Optimism bias occurs when a person's expectation of the likelihood of an event is different from the actual outcome [107]. In this study, participants expressed the optimistic attitudes such as 'diseases can't happen because I am resistant', 'diseases happen somewhere else but not here'. Confidence in the relative safety of the raw pig blood dish were expressed by many participants based on repeated personal experience: 'I've never had any bad experience after eating *tiet canh*'. Fatalistic attitudes were also shared in the group setting: "death is our fate." After showing participants pictures of severe symptoms of *S. suis* infection, men were less likely than women to indicate a future change in behavior such as reducing or stopping consumption of raw pig blood (8.8%, n=3 versus 42.5%, n= 20). Some participants, despite being aware of health risks and having stopped consumption for some time, resumed eating raw pig blood again in situations where they believed the risk was low (e.g. a woman in FG10 ate when she was given raw pig blood prepared from a '*lợn Mán'*).

Perceptions about existing public interventions

Perceptions about the existing public interventions in controlling the risk practice and disease transmission are summarized in Table III-0-6. Overall, participants expressed

some degree of uncertainty and lack of confidence in existing public interventions in controlling the risk practice or disease transmission.

Mass media was not perceived as an effective method to reach certain populations such as mobile manual workers, men, heavy drinkers and older generations. It was suggested that communication messages should be repeated frequently and sustained over time in order to be most effective. Pig vaccination overall was perceived as an effective measure, but most farmers were not aware of *S. suis* bacteria in pigs. This was probably due to the unavailability of an effective vaccine for this disease in the local market and thus lack of information on this specific pathogen. In addition, some concerns were expressed about current government programs for animal vaccination, including possible disease spread due poor biosecurity practices of field teams responsible for vaccination, insufficient vaccine supply and limited coverage.

Table III-0-6. Perceptions about current public interventions and recommendations

Intervention	Participants' perceptions	Recommendations
Mass media	Important, most effective and feasible compared to other programs; Currently too short and superficial; Can only reach the general population;	Should be tailored to target at specific high-risk groups; Should be continuous and show more details; Show pictures of infected patients; Target children at school so they will not engage in this practice;
Food inspection	Ineffective; Unofficial payment to get inspection stamps; Lack of trust	Should be stronger, stricter, continuous and at a larger scale;

Intervention	Participants' perceptions	Recommendations
	in foods that have been through inspection;	
Ban on selling/ consuming raw animal blood	Currently not much effectiveness; Difficult to implement; Uncontrollable (small scale pig slaughtering, tiet canh sale and consumption); Large benefits from selling tiet canh; Uncontrollable at individual households; Farmers do not want to destroy diseased pigs but sell to illegal collectors; Sick/ dead pigs were moved illegally to other areas;	Law reinforcement need to be stronger and comprehensive; Punishments should be stricter including closing down the restaurants; Impose high tax on selling activities;
Pig vaccination	Important; Several issues related to coverage and administration of the government funded program: lack of vaccines, misunderstanding; Lack of awareness from the farmers on *Streptococcus suis* vaccine;	Periodical vaccination programs from government; Consistent information and implementation; Increase coverage of the government program;

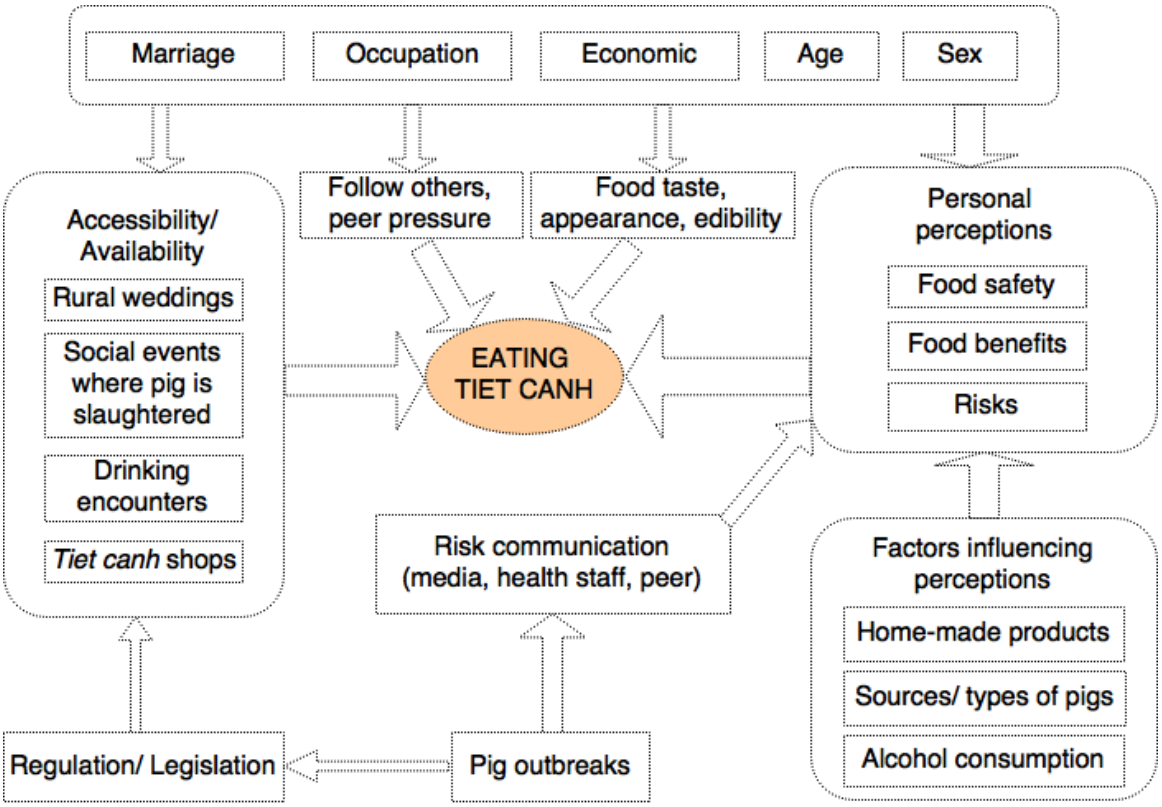
The proposed ban on selling raw animal blood was considered to be infeasible and ineffective amongst participants in all FGs. Both the monetary profit from selling raw pig blood and the consumer demand were considered as key features that could perpetuate this traditional dish. Furthermore, trade in raw pig products was too widespread and

decentralized, and the food chain from pig producers to pork consumers too complex to enable regulation or enforcement of trade bans. Participants discussed lack of confidence in the standards of food inspection programs. In particular, many urban participants complained that sellers of unsafe or contaminated products were able to bypass inspections and they did not trust certified food.

3.4.3. Conceptual framework of factors influencing consumption

The main themes revealed from the qualitative and quantitative survey are summarized in a conceptual framework for understanding the phenomenon of consuming raw pig blood in the northern context of Viet Nam (Figure III-0-5).

Figure III-0-5. Conceptual framework of factors contributing to the practice of eating raw pig blood drawn from the study findings



Personal perceptions about the food safety and benefits and risk of disease transmission appear to play an important role in the individual's decision whether to eat the dish in a given context. Information through risk communication also can affect individual perceptions about the food but this seems to only happen in reaction to pig outbreaks and when cases of *S. suis* are admitted to hospital. The cultural practice of preparing *tiet canh* in social events and celebrations and the easy accessibility of the dish at shops are also factors that reinforce the food behavior in the community. To a lesser extent, people are forced to eat because of the feeling of social pressure. The taste and appearance of *tiet canh* can either positively or negatively influence individual eating behavior. Certain characteristics including age, gender and socioeconomic factors can also predispose to *tiet canh* consumption.

3. 5. Discussion

Eating uncooked or undercooked pork dishes are important risk factors for infectious disease transmission, including *S. suis* [17, 21, 22, 47, 108-112]. This study investigated the cultural practices associated with consumption of *tiet canh*, a traditional Vietnamese dish prepared from coagulated raw pig blood. Our survey revealed that raw pig blood consumption was widespread among rural residents (35%), but less common among urban (8.6%). The quantitative analysis showed significant associations between *tiet canh* consumption and certain sociodemographic factors. However, the low explanatory power (low Nagelkerke R^2) implicates the influence of other unmeasured factors on the practice. This was also reflected in the focus group discussions which showed important contributions of sociocultural factors and personal perceptions.

Participants in FGs articulated strong confidence in the safety of raw pig products when source of the pig was known to the consumer and the pig appeared healthy. The key

perception that apparently healthy pigs do not pose health risks to consumers should be noted, as this is in conflict with prevalence studies showing high carriage rates of *S. suis,* even in apparently healthy pigs and pig products [41, 113]. Importantly, the perception that free-ranging/ scavenging pigs are relatively 'safer' than pigs reared in confinement is in conflict with the literature on agroecology and transmission of diseases (e.g. cysticercosis [114] and trichinellosis [112, 115, 116]).

Our study revealed that raw pig blood consumption practices are closely linked with traditional family celebrations, in particular wedding ceremonies, and in some cases these traditions are a source of pride and social bonding among community members. Understanding the cultural contributions of this raw pig blood dish will be necessary to developing effective communication messages to reduce health risks associated with the practice. Beliefs about potential benefits were widespread among community members, whereas understanding of health risks from infectious agents or contaminants were often vague, and risks were dismissed or overlooked.

The qualitative finding that raw pig blood and alcohol are strongly associated supports clinical observations that *S. suis* patients in Viet Nam are predominantly adult men with a history of drinking alcohol [16]. However, one case-control study showed that alcohol drinking was not independently associated with the risk of infection [21], and there is currently no evidence to support a causal association between alcohol drinking and *S. suis* infection. In Cambodia where raw blood is not as widely consumed, the infection profile of *S. suis* indicates a relatively higher proportion of women [117], suggesting possible transmission through processing of contaminated meat.

Unlike outbreaks of PRRSV in pigs and avian influenza [118-120], there was a low level of community awareness about *S. suis* infections. This is expected since these

diseases occur frequently with large impacts on the livestock and public health sectors in Viet Nam. However, the public is unaware of the presence of *S. suis* bacteria commensally living in pigs. These bacteria can induce severe infections in the animals and may be more common in herds infected with PRRSV [30, 121]. Therefore, knowledge about *S. suis* infection and prevention methods need to be transferred to farmers, workers who come into direct contact with pig and pig products, and consumers of dishes such as raw pig blood. By taking simple strategies such as safe practices of culling, slaughtering, cooking and eating, transmission to humans can relatively easily be prevented. In addition, efforts to reduce *S. suis* infection within pig populations through vaccination targeting *S. suis* or other pathogens which can increase the incidence of *S suis*, such as PRRSV, or other farming practices are needed and would benefit production output, livelihoods of farmers, as well as public health.

This study revealed raw pig blood consumption was more common among adult working-age men, certain occupation groups (mainly outdoor workers), low-income urban inhabitants and married people in the rural area. Interventions specifically targeting these population groups are necessary to reduce the burden of infectious diseases acquired from raw pig blood consumption. Disease surveillance and reporting should be improved to better monitor the burden of zoonotic infections among these populations. Changes in education, urbanization, and rising income levels will no doubt impact social and behavioral attitudes towards raw pig blood consumption in the future. Meanwhile, food safety research could benefit consumers by exploring methods of raw pig blood preparation designed to reduce infectivity of any pathogens present in raw blood while preserving desired texture or taste characteristics. Finally, food inspection programs need to be strengthened and auditing systems put in place to ensure certified food can indeed be trusted.

Limitations of this study included the participation of more women than men in the quantitative survey; given that men are more frequent consumers of raw pig blood our data may underestimate the overall proportion of raw pig blood consumption. Secondly, the qualitative phase was conducted after the quantitative phase, and therefore many socio-psychological and environmental factors were not included in our model analysis. Although the sample size in the qualitative study did not allow for any statistical inferences, the focus group discussions reached a point of saturation, and generated highly valuable observations that greatly informed all aspects of the research topic [122]. Saturation was indicated from the fact that there were no new codes, categories or themes emerging during the data analysis towards the last FGD in each location.

3. 6. Conclusion

The findings of this study provided an indepth understanding on the sociodemographic and cultural patterns and community perceptions regarding consumption of raw pig blood, a food practice that has been associated with an increased risk of human *S. suis* infection in Viet Nam. I showed that the practice was more common in certain groups and reinforced by a tapestry of social and cultural factors together with widespread misperceptions on the food safety and risk of disease transmisison in the community. Targeted communication interventions to facilitate behavioural change and public interventions to reduce the prevalence of pathogenic bacteria in the food chain are required to prevent the disease transmission from pigs to human.

Chapter IV

Long-term health outcomes of human *Streptococcus suis* infection among patients admitted at the National Hospital for Tropical Diseases, Viet Nam

4. 1. Introduction

4.1.1. Rationale

A number of studies have reported clinical sequelae of *S. suis* infection, mostly on the occurrence of hearing loss and, to a lesser extent, vestibular dysfunction [12, 22]. Despite the availability of data in the literature, a lot of uncertainties exist. Hearing loss was usually noted during admission, sometimes prior to admission or after discharge. Reported prevalence of hearing loss vary greatly between studies, from 6% up to 100% [84, 123] with the average of around 39%. For vestibular dysfunction, the average reported prevalence was around 23%, ranging from 3 to 60% of the cases [24, 124]. In Viet Nam, two studies at the Hospital for Tropical Diseases in the south showed different prevalence proportions of hearing loss: 66.4% [20] and 27.3% [125]. However, the prevalence of profound hearing loss (>80dB) in the former study was 24.3% for all patients and 33.3% for patients without dexamethasone treatment. One other study conducted at the National Hospital for Tropical Diseases in the north reported a hearing loss prevalence of 32% [16]. Vestibular dysfunction was only reported in the third study with a low prevalence of 4%.

It is difficult to compare the rates between studies as the difference might just be due to variations in methods of ascertaining and evaluating hearing loss or vestibular dysfunction. Unfortunately, for the majority of cases, evaluation methods were not described in the reports. In addition, patients might experience both hearing and vestibular dysfunction, although this co-morbidity is not well understood from the existing literature. Hearing loss is often described in patients with signs of vestibular impairment in both case reports and large case series. However, it is difficult to establish the proportion of patients who develop multiple disabling outcomes following *S. suis* infection since reported data were often incomplete. Added to this uncertainty is a scarcity of follow-up of *S. suis* survivors and assessing the longer-term impact, both health and economic aspects of such disabling sequelae on the patients. Mai et al [20] evaluated hearing loss at 6 month follow up and found only a small proportion of patients having improved hearing.

More data on sequelae and its progress over time are needed to fully understand and quantify the impact of *S. suis* infections on patients and society. This is particularly important for justifying and prioritizing policy and intervention actions aiming to reduce the burden of *S. suis* infection in an endemic country such as Viet Nam.

4.1.2. Objectives

This study aims to assess the long-term health effects of *S. suis* infection on the patients in comparison to a control group. Specific objectives are to:

1. Describe the patterns and progress of sequelae due to *S. suis* infection among patients who survived from the infection;

2. Assess the impact of *S. suis* infection and its sequelae on the health and quality of life of patients with the infection;
3. Compare the levels of health and health related quality of life among survivors of the *S. suis* infection with that of the control persons.

From this study, some key epidemiological parameters were calculated and used as input data for the estimation of the annual burden of disease due to *S. suis* infection in Chapter V of this thesis.

4. 2. Methods

4.2.1. Study design and setting

This study was conducted at the National Hospital for Tropical Diseases (NHTD) in Ha Noi, Viet Nam. The prospective component entailed the identification and follow up of a cohort of patients with laboratory confirmed *S. suis* infection. The period of enrollment of prospective patients was one year (November 2014 - October 2015), with each patient being followed up for nine months after hospital discharge. This follow-up time period was longer than the follow-up time period for *S suis* patients in the previous prospective study [20, 57]. I assumed that a follow up period of nine months would allow us to determine which sequelae, such as hearing and vestibular loss, are non-reversible and the impact of these disabilities on the patient.

However, it is also important to evaluate if there were any differences in sequelae in *S. suis* survivors beyond 9 months post-discharge compared to the patterns observed among the prospectively enrolled patients followed-up for 9 months. Therefore, this study also recruited a retrospective cohort of patients who were admitted to NHTD in 2013-

2014 prior to the start of the prospective cohort to assess their health outcomes at the time of follow-up (time from hospital discharge to follow-up would be >9 months).

This study included a comparison group of non-patient visitors, who visited the hospital during the study period and were invited to participate in the study. I aimed to select controls that are matched with patients in terms of sex, age (10-year age band), and residential status (rural/ urban) at the ratio of 4 controls per *S. suis* case (see statistical considerations). However, I had difficulty in recruiting controls for older aged patients since there were very few non-patient visitors in the similar age band. On the completion of the study, matching was successfully achieved for 85% of all patients (more details in section 4.2.3).

NHTD is a referral treatment centre for infectious diseases; usually receiving patients from the north and middle parts of Viet Nam. Since diagnostic capacity is limited at provincial and district levels, patients with invasive infection such as meningitis are usually referred from these lower levels to NHTD within a short period after illness onset.

4.2.2. Study sample size

The purpose of this study was to assess the excess health burden of *S. suis* infection in comparison with an ambulatory group of controls. Main study outcomes under investigation include hearing loss and vestibular dysfunction. In the follow-up study in the south of Viet Nam, the proportion of patients who had hearing impairment of greater than 30dB in at least one ear at 6 months follow-up after hospital discharge was 47.7% [20]. For the general adult population (age ≥ 15 years), the estimated hearing loss prevalence was approximately 17% for males and 12.5% for females in the Asia Pacific region (using a 35dB cutoff in the better ear) [126]. Assuming a prevalence of 48% of hearing impairment among patients and 14% of hearing impairment among controls, with

a 2-sided significance level of 5% and a power of 90% to detect a significant difference in hearing loss between two groups, a minimum of 37 patients each for prospectively enrolled and retrospectively enrolled patient group and 148 controls are needed for this study (including an allowance of 5% loss to follow-up).

Prevalence of vestibular dysfunction is more uncertain due to the high variability in evaluation approaches. The rate of vestibular dysfunction based on a simple balance test was approximately 25% among adults in the age between 40-60 years in the National survey in the U.S. [127]. Assuming a similar rate of 25% among the controls and a rate of 50% among patients with *S. suis* infection, a minimum of 50 patients for each patient group and 200 controls are required to have 90% probability at a significant difference in vestibular dysfunction based on the balance test (including an allowance for 5% loss to follow-up).

However, since my overall aim was to estimate the total burden of disease caused by *S. suis* infection, I decided to recruit all patients who were admitted within the timeframe specified for the prospective part and retrospective part and met the selection criteria specified in the following sections.

4.2.3. Recruitment of patients and controls

Prospective patients:

From patients with signs of sepsis, routine blood cultures are collected and from patients with signs of meningitis, both blood and CSF specimens are routinely collected for culture at NHTD. The clinical microbiology laboratory identifies *S. suis* using colony morphology and biochemical tests. Real time PCR test was sometimes requested by the attending doctor but not routinely. Each patient with culture and/or PCR confirmed *S.*

suis infection was approached and invited to participate in the study. Case patients were followed up for a period of nine months from the date of hospital discharge.

Inclusion criteria:

- Admitted at NHTD and is currently receiving treatment for *S. suis* infection in the hospital;

AND

- Culture or PCR confirmation of *S. suis* infection;

AND

- The patient or legal representative signs the consent form to participate in the study.

Exclusion criteria:

- Died in hospital or is discharged home to die.

Retrospective patients:

Patients were identified through archived laboratory and medical records. All patients who had contact details available were contacted and invited to participate in the study.

Inclusion criteria:

- Admitted at NHTD in 2013-2014 and not included in the prospective cohort;

AND

- Culture or PCR confirmation of *S. suis* infection;

AND

- The patient or legal representative signs the consent form to participate in the study.

Exclusion criteria:

- Have no contact information available in hospital records or unable to contact the patient using the recorded contact details;

- Died in the hospital or discharged home to die.

Control group:

Eligible non-patient visitors at NHTD during the study recruitment period were approached by study nurses and invited to participate in the study as a control in the comparison group with a targeted ratio of 4 controls per 1 case patient. This was applied to all patients in both prospective and retrospective case groups.

Inclusion criteria:

- Has the same sex, age band, and residential status (urban/ rural) as the case patient;
- Signs the informed consent form to participate in the study.

Exclusion criteria:

- Recall any previous diagnosis of *S. suis* infection or meningitis

Since cases of *S. suis* infection were widely distributed in terms of geographical locations, it was difficult to determine the base population and therefore a population based control recruitment was not feasible. It was also not practical to conduct community based assessment for controls due to technical requirements for outcome measurement (audiometry in sound-proof booth). Therefore I decided to use hospital based controls and assumed that non-patient visitors could represent the base population where the study patients came from, and that both patients and controls were similar in terms of the baseline risk of hearing and vestibular dysfunction, except for the status of having prior *S. suis* infection. Controls recruited from the pool of non-patient visitors might be healthier than the general population. However, the effect of this was expected to be minor because people with non-severe conditions of hearing loss and vestibular dysfunction still can participate in daily social activities including visiting the hospital.

Severe conditions of hearing loss and vestibular dysfunction are relatively uncommon in the community compared to the non-severe levels.

Initally I decided to use matching to increase the study's efficiency by ensuring the distribution of cases and controls was similar in terms of age, sex and rural/ urban location. The matching variables were chosen because they might be a potential confounder (for age and sex) affecting the health outcomes. Rural/ urban location was matched to ensure the cases and controls are relatively similar in terms of geographical location, improving the representativeness of the controls with the base population. However, due to difficulty of enrolling older aged controls, full matching was only achieved for 66 out of 78 case patients (85%). The remaining 12 patients (aged 55-77 years) did not have sufficient number of controls. Therefore, I decided to use all controls for both prospectively and retrospectively enrolled cases and included age and sex adjustment in all the analysis.

4.2.4. Data collection and instruments

Patients and controls were assessed using the same tests and instruments as detailed in Table IV-0-1, except for clinical examination, which was only performed for patients. Detailed Standards Operating Procedures (SOP) were developed for audiometry, vestibular and visual acuity test. Study staff were provided training on protocol and SOPs for the tasks that they were responsible for.

Table IV-0-1. Study assessments and instruments for patients and controls

Type of participant	Clinical Exam.	Audiometry, Balance, Visual acuity test	MMSE	Questionnaire (HHIA, VSS, DHI, EQ-5D)
Prospective patient				
Discharge	X	X	X	
3 months follow-up	X	X	X	X
9 months follow-up	X	X	X	X
Retrospective patient (one follow-up 11-34 months after discharge)	X	X	X	X
Control		X	X	X

MMSE: Mini Mental State Examination; HHIA: Hearing Handicap Inventory for Adults; VSS: Vertigo Symptoms Scale; DHI: Dizziness Handicap Inventory, EQ-5D: a short questionnaire on generic health status.

Clinical examination:

This includes an evaluation of mental status, motor function and balance, sensory examination, reflexes, cranial nerve evaluation (for patients only). Study doctors performed clinical examination on patients at hospital discharge (prospective patients) and at follow-up visits (all patients).

Audiometric assessment:

Audiometry was conducted according to a standard protocol by trained staff. Air conduction testing was done in a sound-proof booth (for all patients and controls visiting the hospital) or in a quiet place (for patients who were visited at home) following a

modified Hughson Westlake procedure under the automated mode of the audiometer (Interacoustics AD226, Interacoustics, Eden Prarie, MN). Hearing thresholds on both ears were tested for frequencies from 0.5 to 8kHz with the first threshold tested at 1kHz. For quality control, a second measure at 1kHz was included to check if the discrepancy between two threshold measurements was within 10 decibels. Hearing level was defined as hearing threshold averaged over four frequencies 0.5, 1, 2 and 4kHz in the better ear. All hearing thresholds are reported in dB HL (American National Standards Institute, 2004). Categories of hearing loss severity were based on recommendations by the Global Burden of Disease Expert Group [126] (normal <20dB, mild 20-34dB, moderate 35-49dB, moderately severe 50-64dB, severe 65-79dB, profound 80-94dB, complete ≥95dB), and further collapsed to simplify the analysis.

Impact of hearing loss:

I used the 25-item Hearing Handicap Inventory for Adults (HHIA) [128] questionnaire to assess the emotional (13 items) and social/ situational (12 items) problems associated with hearing loss. For each item, a score of 0, 2 or 4 was assigned to the corresponding response as “no”, “sometimes” or “yes”, making the total score ranging from 0 to 100. Total HHIA scores are polytomized into three categories:

- No handicap: score between 0-16
- Mild-Moderate handicap: 18-42
- Significant handicap: 44+

Vestibular assessment:

The Modified Clinical Test of Sensory Interaction and Balance (m-CTSIB) test [129] was used to assess participants' vestibular function. This test was designed to test the participant's ability to stand unassisted with feet together (closed stance) and arms folded across the waist with both hands holding the elbows, under 4 successive test conditions: eyes open on firm surface, eyes closed on firm surface, eyes open on foam surface and eyes closed on foam surface (Table IV-0-2). A Sunmate medium density foam pad (40cm x 45cm x 8cm) was used in test condition 3 and 4.

The test was scored on a pass/ fail basis. It was scored as fail if the participant moved feet or hands off the starting position, began to fall or required assistance to maintain balance or opened their eyes in the conditions that required eyes closed. Each participant was allowed a repeat trial at any condition if he/ she did not pass that condition during the first attempt. The test was discontinued whenever a participant failed to pass a condition in both the first and repeat attempt.

Table IV-0-2. Four test conditions of the Modified Clinical Test of Sensory Interaction and Balance (m-CTSIB) test used for *S. suis* patients and controls

Test condition	Description	Sensory input
Condition 1	Eyes open, stand on firm surface, 15 seconds	Visual, proprioceptive, vestibular system
Condition 2	Eyes closed, stand on firm surface, 15 seconds	Proprioceptive, vestibular system
Condition 3	Eyes open, stand on foam surface, 30 seconds	Visual, vestibular system

Test condition	Description	Sensory input
Condition 4	Eyes closed, stand on foam surface, 30 seconds	Vestibular system

These conditions examine the function of three sensory inputs contributing to balance including the vestibular system, vision, and proprioception. Balance disorder was determined as a failure to complete the test at any condition, while vestibular dysfunction was determined as a failure to pass the test condition 4 only (excluding those who did not pass the previous 3 conditions and thus did not participate in condition 4).

This testing protocol has been used in an on-going cross-sectional national health and nutrition examination survey in the U.S. [130]. Previous studies have shown that inability to remain standing on the foam with eyes closed indicate a vestibular dysfunction with 90% sensitivity and 95% specificity [131].

Vestibular symptoms:

History of vestibular symptoms was assessed using Vertigo Symptoms Scale (VSS) [132]. It consists of 34 items asking each patient/ control to recall their experience in the past 12 months, a score between 0 (never experienced) to 4 (quite often) was given to each symptom. The maximum score was 136. Subscale scores and total VSS score were calculated by summing the corresponding items. These were compared between patient groups and control group.

Three specific symptoms of balance dysfunction were also examined: vertigo (a feeling that things are spinning or moving around), dizziness (a feeling of being light-headed, ‘swimmy’ or giddy), and unsteadiness (feeling unsteady, about to lose balance). Severity of balance dysfunction was also assessed based on two items: balance related falls and incapacity to stand or walk without support (severe unsteadiness).

Impact of dizziness:

Dizziness Handicap Inventory (DHI) [133] was used to assess the perceived handicap caused by vestibular dysfunction. Three domains were examined: functional (9 items about the impact of dizziness on their daily activities), emotional (9 items about the impact of dizziness on the person's emotional wellbeing), and physical (7 items about the impact of physical activities on the person's sense of instability). Scoring was the same as HHIA. Following categories are analyzed:

- No handicap: score between 0-14
- Mild handicap: 16-34
- Significant handicap: 36+

Visual acuity test:

Visual acuity was tested using a locally available Snellen chart (letter C) at 5-meter distance. Vision impairment was defined in two categories as used by the Vision Loss Expert Group in the Global Burden of Disease study [134]:

- Moderate and severe vision impairment: defined as visual acuity in the better eye $< 6/18$ but $\geq 3/60$ at presentation
- Blindness: defined as visual acuity in the better eye $< 3/60$ at presentation

If the subject was wearing correction at presentation, visual acuity while wearing correction was used.

Mental status:

Mental status was assessed using the Mini Mental State Examination (MMSE) [135] on various areas of cognitive function including orientation, registration, attention,

calculation, recall, and language[1]. It has been widely used for assessing cognitive mental status in both clinical and research settings because of its brevity and ease in administration [136]. Maximum score was 30. A score of 24-30 was considered as no cognitive impairment, 18-23 mild cognitive impairment, and below 18 severe cognitive impairment [137].

Generic health status (EQ-5D):

I used EQ-5D, a brief self-reported questionnaire measuring the current health status that has been used many health economic and population health studies [138]. Participants were asked to classify their current health in 5 dimensions (Mobility, Self-Care, Usual Activities, Pain/Discomfort, and Anxiety/Depression) with 5 levels of functioning for each (no problems, slight problems, moderate problems, severe problems and unable to/extreme problems). The overall health status was also assessed using a visual analogue scale (VAS) with points from 0 (worst health state) to 100 (best health state). Percentages of respondents reporting any problems under each dimension and the central estimate of VAS score were used to compare the health status between groups.

4.2.5. Data management

Data from completed Case Record Forms (CRFs) and questionnaires were entered into CliRes, an internally controlled internet-based computerized data entry system. Single data entry was used. I verified the quality of data entry for all participants for the main study outcomes including hearing thresholds, m-CTSIB and visual acuity results, and vestibular symptoms by checking the entry values against the values in the original

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CRFs. For other variables, random check was done for 10% of all study participants to ensure the quality of data entry. Complete datasets were extracted from CliRes and data cleaning was processed in the R software (version 3.2.2) [139].

4.2.6. Statistical analyses

The primary objectives of the study were to compare the prevalence of disabling outcomes between patients with *S. suis* infection and non-patient controls, and to evaluate the change of the main outcomes over time. Prevalence of hearing impairment (by severity categories), balance disorder (fail any m-CTSIB test condition), vestibular dysfunction (fail m-CTSIB test condition 4), reported vestibular symptoms (vertigo, dizziness, unsteadiness), and suboptimal health status (for each EQ-5D domain) are presented as proportion for each study group. The study also assessed visual acuity, cognitive performance, and neurological outcomes.

Baseline characteristics including sex, age (years), location (rural versus urban), education (years of schooling), occupation (farming/ manual work versus others), and pre-existing medical conditions (include one or more of the following: arthritis, diabetes, hepatitis/ cirrhosis, renal disease, hypertension, heart disease, chronic lung disease, seizure, brain injury, paralysis, splenectomy, currently on steroids treatment, depression, HIV, malignancy, or any other immunosuppressed conditions) were compared between patient groups and controls and included in main analyses for outcomes. Corticosteroid treatment during hospital admission was also recorded for patients only based on their hospital charts (having treatment or not, regardless of time and dosage).

To compare rates of outcomes between patients and controls, I used log-binomial models and calculated adjusted prevalence ratios (PR) [140], which was the ratio between the probability of an outcome for patients and the probability of the outcome for controls

after considering potential confounders. I examined possible confounding effects of all baseline characteristics by adding each separately to the core model of exposure (disease status) and outcome, however only age was significant for some outcomes. I presented here PRs adjusted for age and sex for all outcomes except for some outcomes when the models failed to reach convergence.

To evaluate the change in hearing thresholds among patients over time, I excluded patients with no response to sound stimuli during audiometry and ran a linear mixed model that controlled for between-patient variations (random effect) over the main effect of interest (assessment time-points) using the lme function within the nlme package [15]. Hearing threshold was included in the model in a log scale to improve data normality. Assumption about normality was assessed by checking the distribution of the residuals of the model. Baseline characteristics were included as potential confounders, although no significant effect was observed. Missing data on hearing threshold for patients who did not have audiometry were omitted in this analysis. I used one-way ANOVA to compare the mean of VSS score (on a log-transformed scale to improve data normality) between groups defined by levels of hearing impairment, with Levene's test to check the assumption of homogeneity of variances.

All statistical analyses were done with R software (version 3.2.2) [139].

4.2.7. Ethical considerations

The study protocol, patient/ participant information sheet, informed consent document, and relevant supporting information were submitted to the Institutional Review Board of NHTD and Oxford Tropical Research Ethic Committee (OxTREC) and were approved before the start of study recruitment and data collection (Approval number

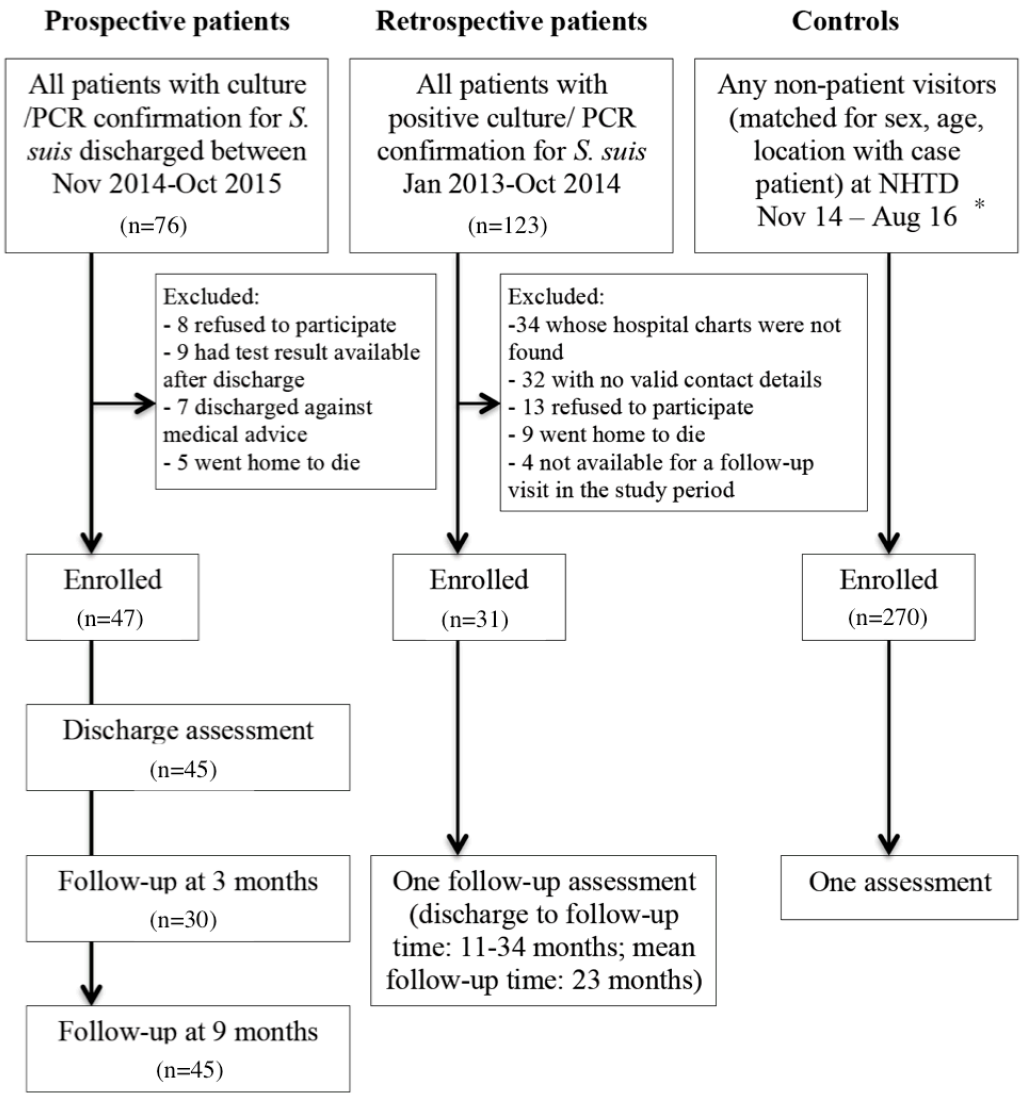
72/ HDDD – NHTD dated 22 August 2014 and OxTREC Reference 48-14 dated 15 September 2014) (Appendix 5).

4. 3. Results

4.3.1. Characteristics of study patients and controls

From 76 patients with culture or PCR confirmation of *S. suis* infection at NHTD between November 2014 and October 2015, 47 were eligible and enrolled into the study (Figure IV-0-1). An additional 31 patients were included retrospectively from those with laboratory confirmation between January 2013 and October 2014. A total of 270 controls were recruited. Patients and controls were similar in terms of sex and location but not age; retrospectively enrolled patients were significantly older than the controls (Table IV-0-3). Prospectively enrolled patients also had less schooling than the controls. History of alcohol drinking and smoking was recorded less for both patient groups compared to controls, although this information for the patients are likely to be missed during evaluation of patient history by the treating doctors. Patients who died or were discharged home to die were excluded from this analysis of long-term outcomes.

Figure IV-0-1. Patient and control recruitment and assessment process



Note: *Controls were recruited using convenience sampling. Any visitor who met matching criteria was invited to participate by nurses on duty in the wards. Number of refusals were not recorded.

Table IV-0-3. Charateristics of study patients and controls

Charateristics	Prospective patients (n=47)	Retrospective patients (n=31)	Controls (n=270)
Male gender	38 (80.8)	28 (90.3)	232 (85.9)
Mean age (SD)	51.3 (12.1)	55 (12.6)	48.7 (10.5)
Living in rural area	37 (78.7)	23 (74.2)	208 (77.0)
Occupation			
Pig-related jobs	7 (14.9)	3 (9.7)	22 (8.1)
Farmers	8 (17.0)	7 (22.6)	79 (29.3)
Manual workers	17 (36.2)	10 (32.3)	46 (17.0)
Others	9 (19.1)	4 (12.9)	96 (35.6)
Retired	6 (12.8)	7 (22.6)	27 (10.0)
Education*	7.8 (3.1)	8.9 (3.6)	9.0 (3.8)
Household income per capita# (median, IQR), thousand VND	1 500 (1 066.7)	1 500 (1 522.7)	2 000 (2 333)
Pre-existing medical conditions	10 (21.3)	3 (9.7)	44 (16.3)

*Number of years spent on schooling (mean (SD)). #For patients, they were asked about their income before hospitalization due to *S. suis* infection.

4.3.2. Hearing impairment

Hearing impairment was found in 31.8% (profound to complete) and 52.2% (moderate to severe) of the patients based on audiometry evaluated at the time of hospital discharge within the prospective cohort (Table IV-0-4). This prevalence fell to 28.8% and 28.9% respectively at 9 months after discharge.

Table IV-0-4. Proportion of subjects with hearing impairment

Hearing impairment level	Prospective patients			Retrospective patients[§] (n=31)	Controls (n=270)
	At discharge[*] (n=44)	3 months follow-up[#] (n=30)	9 months follow-up (n=45)[$]		
No impairment	2 (4.5)	3 (10)	1 (2.2)	2 (6.5)	66 (24.4)
Mild	5 (11.4)	7 (23.3)	18 (40.0)	9 (29.0)	163 (60.4)
Moderate	13 (29.5)	5 (16.7)	8 (17.8)	6 (19.4)	35 (13.0)
Moderately severe	7 (15.9)	2 (6.7)	2 (4.4)	4 (12.9)	2 (0.7)
Severe	3 (6.8)	2 (6.7)	3 (6.7)	2 (6.5)	3 (1.1)
Profound	3 (6.8)	0 (0)	2 (4.4)	2 (6.5)	0 (0)
Complete	11 (25.0)	11 (36.7)	11 (24.4)	6 (19.4)	1 (0.4)

*Audiometry could not be done for 3 patients at hospital discharge. One female patient could not follow the instructions for audiometry; this patient was diagnosed with psychiatric disorder and continued to have on-going treatment at the Psychiatric Department. Audiometry performed 9 months after hospital discharge for this patient showed moderate hearing impairment. The other two patients were still too weak to sit up and move upstairs for audiometry in the hearing booth. Audiometry at 9 months after hospital discharge revealed a complete level of hearing loss for one patient and mild hearing loss for the other patient. #Only 30 patients (63.8%) turned up at the hospital for the follow-up visit at 3 months after hospital discharge. $Follow-up assessment was performed for 45 patients (95.7%), of which 27 patients visited the hospital and 18 patients were followed up at their home. §The mean time from discharge to follow up assessment for retrospective patients was 23 months (ranging from 11-34 months).

Table IV-0-5 and Table IV-0-6 present the unadjusted and age adjusted PRs for hearing loss using three different cut-off thresholds. Using the cut-off of 35dB (having at least moderate impairment), patients had a five times higher prevalence of hearing loss at discharge (adjusted PR 5.01 for moderate or worse impairment, 95%CI 3.59-7.07) compared to controls. This ratio decreased at follow-up assessments, indicating some

recovery of hearing impairment among the patients. However, more improvement occurred by the first 3 months after discharge (PR reduced to 3.68 at 3 months) and less between 3 and 9 months (PR 3.24 at 9 months). The prevalence of hearing loss among retrospective patients was similar with that at 9 months for prospective patients (PR 3.06, 95%CI 2.12-4.45).

Table IV-0-5. Unadjusted prevalence ratios for hearing impairment among *S. suis* patients in comparison with controls (n=270)

Hearing impairment	Prospective patients			Retrospective
	Discharge (n=44)	3 month (n=30)	9 month (n=45)	patients (n=31)
HL ≥35dB (moderate impairment or worse)	5.53 (4.09-7.64)	4.39 (2.94-6.37)	3.80 (2.58-5.53)	4.25 (2.83-6.19)
HL ≥50dB (moderately severe impairment or worse)	24.54 (11.50-63.31)	22.50 (10.02-59.31)	18.00 (8.05-47.51)	20.32 (8.89-54.06)
HL ≥65dB (severe impairment or worse)	26.08 (10.24-87.53)	29.25 (11.20-98.97)	24.00 (9.32-80.96)	21.77 (7.81-75.78)

HL: Hearing level. Data presented are prevalence ratios (PR) and corresponding 95% confidence intervals. PRs were estimated using log-binomial model in *glm* package.

Table IV-0-6. Adjusted prevalence ratios for hearing impairment among *S. suis* patients in comparison with controls (n=270)

Hearing impairment	Prospective patients			Retrospective
	Discharge (n=44)	3 month (n=30)	9 month (n=45)	patients (n=31)

Hearing impairment	Prospective patients			Retrospective
	Discharge (n=44)	3 month (n=30)	9 month (n=45)	patients (n=31)
HL ≥35dB (moderate impairment or worse)	5.01 (3.59-7.07)	3.68 (2.53-5.36)	3.24 (2.21-4.75)	3.06 (2.12-4.45)
HL ≥50dB (moderately severe impairment or worse)	22.55 (10.49-58.49)	21.11 (9.26-56.20)	16.26 (7.21-43.24)	13.53 (5.71-37.64)
HL ≥65dB (severe impairment or worse)	23.92 (9.32-80.76)	26.38 (10.0-90.0)	21.57 (8.30-73.25)	14.96 (5.06-55.20)

HL: Hearing threshold level. Data presented are prevalence ratios (PR) and corresponding 95% confidence intervals adjusted for age. Age, but not sex was significantly associated with hearing impairment. Other variables including location, education, occupation, medical history, were not significant in the univariate check. PRs were estimated using log-binomial model in *glm* package.

Among 13 patients with complete deafness (mostly having no response to sound stimuli), no improvement was observed for all except one patient. This patient had no hearing at all frequencies except at one threshold at 120dB in the right ear. By 9 months, he showed response to sound at all frequencies for the right ear with an average threshold of 111dB while remained completely deaf in the left ear. Figure IV-0-2 shows mean hearing level by frequency and ear for patients at different time-points compared to controls, restricted to those who were still responsive to sound stimuli. Patients had much higher levels of hearing loss than controls across all frequencies. Hearing loss occurred at all measured frequencies among patients, however more severe loss was found in the high-frequency range.

Figure IV-0-2. Mean hearing level by frequency and ear for patients and controls

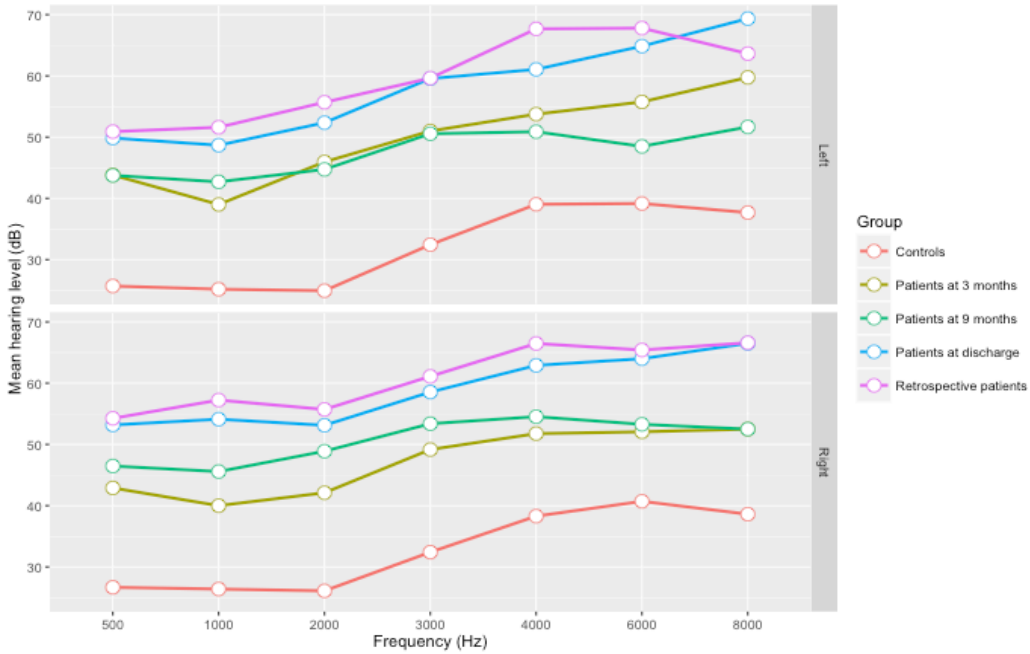
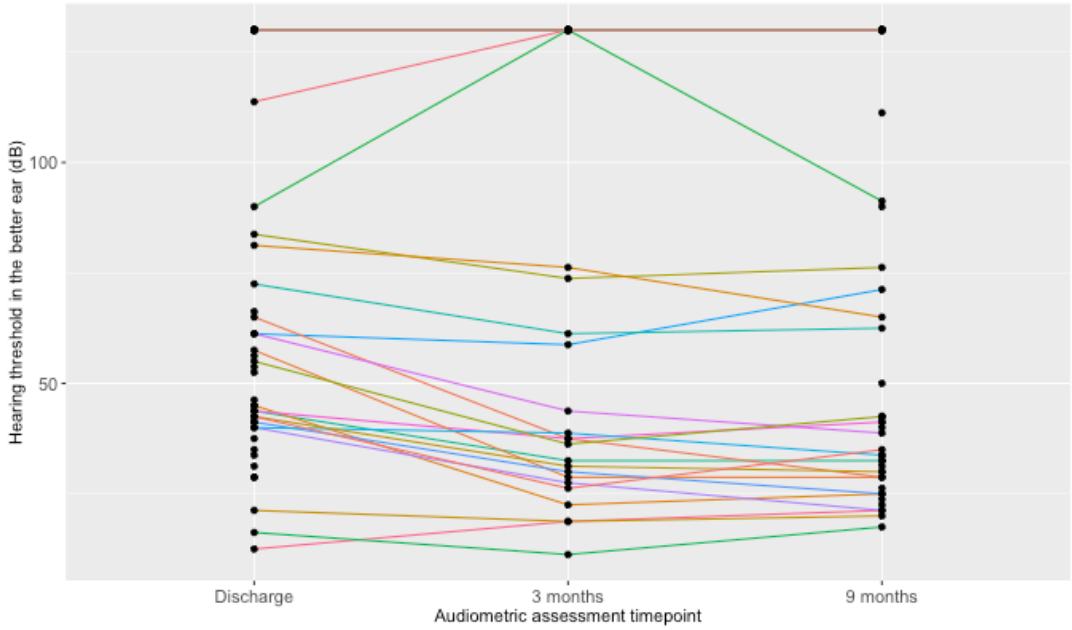


Figure IV-0-3. Change in hearing level over time among prospectively enrolled patients



Each line represents one patient at 3 assessment time-points: at hospital discharge, at 3 month and at 9 month after hospital discharge. Higher hearing threshold level indicates a more severe level of hearing impairment.

Hearing improved over time for 18 out of 29 patients after 3 months (62.1%) and 29 out of 42 after 9 months (69%) compared to discharge, although it deteriorated for some other patients (Figure IV-0-3). Among patients who were still responsive to sound stimuli at discharge, there was a small improvement of 11.1% in hearing thresholds measured at 3 month follow-up compared to those measured at hospital discharge (95%CI: 7.0-15.1%) among patients who were still responsive to sound stimuli (Table IV-0-7). A similar mean change was found at 9 months compared to discharge (10.6%, 95%CI 7.1-14.0%), indicating no significant improvement between 3 month and 9 month time-point. Improvement appears to occur across all frequencies, with higher change at frequency 2000Hz (Table IV-0-8). I found no significant effect of age, sex, corticosteroid treatment and other factors on hearing improvement over time among this prospective cohort.

Table IV-0-7. Analysis of change in hearing thresholds among prospectively enrolled patients at 3 months and 9 months compared to discharge using linear mixed model controlling for between-patient variation

Model	Fixed effects	Mean change* (95%CI)	AIC
Main effect with no adjustment	Assessment timepoints**		-87
	3 month follow-up	11.1% (7.0-15.1)	
	9 month follow-up	10.6% (7.1-14.0)	
Main effect with adjustment for age and sex§	Assessment timepoints**		-73
	3 month follow-up	11.2% (7.0-15.1)	
	9 month follow-up	10.5% (7.1-13.9)	

AIC=Akaike information criterion, a measure of the relative quality of statistical models. * The amount of reduction in the hearing thresholds at 3 months and 9 months as compared to the threshold measured at hospital discharge. **Discharge time-point was the reference for comparison. § Effect of age and sex was non-significant. I also examined the effect of other variables including corticosteroid treatment, preexisting medical conditions, occupation and education on hearing improvement, however no statistical significance was observed.

Table IV-0-8. Relative mean change at 3 months and 9 months compared to discharge in hearing level at each frequency

Frequency	Left ear		Right ear	
(Hz)	3 months	9 months	3 months	9 months
500	11.1 (5.3-6.6)	10.0 (5.1-14.6)	10.0 (3.9-15.8)	9.6 (4.6-14.4)
1000	10.9 (4.7-16.6)	13.8 (8.8-18.4)	10.6 (5.2-15.6)	10.9 (6.6-15.1)
2000	15.0 (9.3-20.3)	16.0 (11.3-20.4)	12.8 (5.1-19.8)	13.0 (6.7-18.8)
3000	11.1 (6.3-15.6)	11.9 (8.0-15.6)	11.1 (2.9-18.6)	12.1 (5.5-18.2)
4000	9.6 (1.8-16.7)	8.8 (2.3-14.9)	8.5 (2.7-13.8)	9.1 (4.5-13.5)
6000	10.1 (2.6-17.0)	14.2 (8.2-19.8)	10.0 (2.2-17.2)	12.5 (6.2-18.3)
8000	9.8 (0.8-17.9)	14.6 (7.5-21.1)	11.8 (3.4-19.6)	12.6 (5.7-18.9)

Data are presented as percentage of change compared to the corresponding value at discharge, adjusted for age and sex.

4.3.3. Handicap due to hearing impairment

The mean HHIA score was 33.4 for patients assessed at 3 months after discharge (median: 16), 25.0 for patients at 9 months (median: 0), 25.0 for retrospectively enrolled patients (median: 8), and 3.0 for controls (median: 0). Among those with hearing loss (at least mild impairment), mean HHIA scores were 64.7 at 3 months after discharge (median: 73), 68.2 at 9 months (median: 73), 58.4 for retrospectively enrolled patients (median: 54) and 38.6 for controls (median: 38), respectively. Perceived handicap increased with the increased levels of hearing impairment severity (Figure IV-0-4). 45.8% of patients at 3 months, 34.1% of patients at 9 months and 26.9% of retrospectively enrolled patients experienced significant level of handicap, compared to 3.4% among controls (excluding those with no hearing impairment) (Table IV-0-9).

Figure IV-0-4. Hearing handicap level (HHIA score) by subject group (patients at 9 months follow-up versus controls) by impairment category (HearingCat)

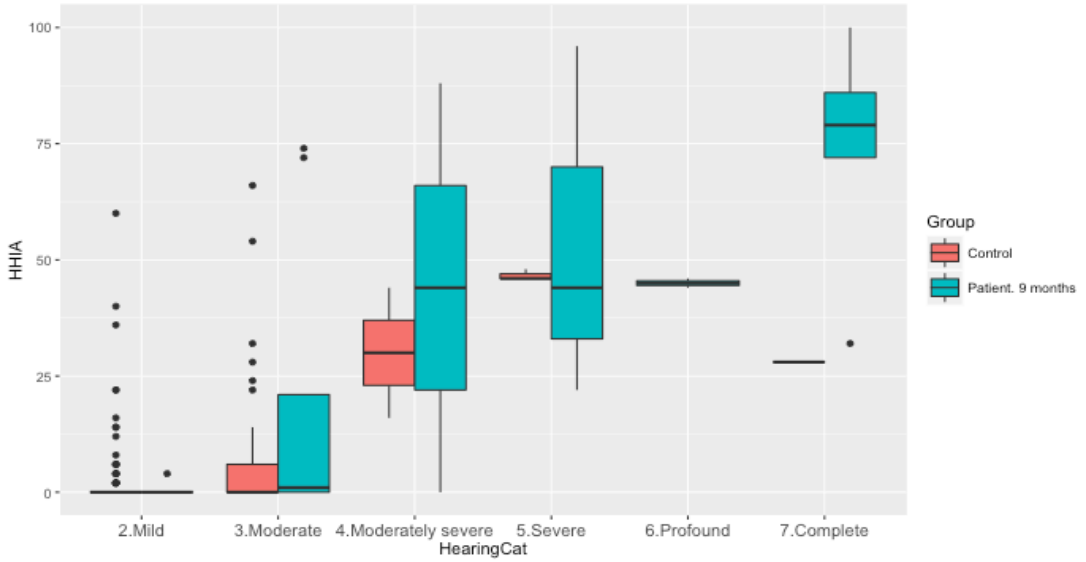


Table IV-0-9. Frequency of handicap among subjects who have mild or worse hearing impairment

Level of handicap due to hearing loss	Prospective patients		Retrospective patients (n=26)	Controls (n=204)
	3 months after discharge (n=24)	9 months after discharge (n=41)		
Mild to moderate handicap	3 (12.5%)	2 (4.9%)	4 (15.4%)	9 (4.4%)
Significant handicap	11 (45.8%)	14 (34.1%)	7 (26.9%)	7 (3.4%)

4.3.4. Vestibular dysfunction

Approximately two thirds of patients had balance disorder, as determined by failure at any condition in the mCTSIB, and this prevalence was consistently observed among both prostectively and retropectively enrolled patient groups (Table IV-0-10). Compared to controls, prospectively enrolled patients were about 3 times more likely to have imbalance at discharge (adjusted PR 2.79, 95%CI 2.22-3.50) and twice as likely at 3 and 9 month follow-up visits (PR 2.26, 95%CI 1.59-3.01 and PR 2.19, 95%CI 1.62-2.86, respectively. A similar increase in the prevalence was also found for retrospectively enrolled patients (PR 2.08, 95%CI 1.45-2.83) (Table IV-0-11).

The prevalence of vestibular dysfunction, as determined by a failure at test condition 4, was more common among patients (51.6% at 9 months follow-up) than the controls (28.2%), and was approximately 1.76 times more common after sex and age adjustment (95%CI 1.13-2.51). A similar ratio was observed among retrospectively enrolled patients (PR 1.83, 95%CI 1.13-2.65).

Table IV-0-10. Proportions of patients and controls failed by each test condition in the m-CTSIB test

Test condition*	Prospective patients			Retrospective patients (n=31)	Controls (n=270)
	Discharge (n=45)	3 months (n=30)	9 months (n=45)		
Condition 1	10 (22.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Condition 1 or 2	13 (28.9%)	1 (3.3%)	1 (2.2%)	1 (3.2%)	4 (1.5%)
Condition 1, 2, or 3	21 (46.7%)	5 (16.7%)	14 (30.4%)	7 (22.6%)	4 (1.5%)
Condition 1, 2, 3, or 4**	37(82.2%)	20(66.7%)	29(64.4%)	20(64.5%)	79 (29.3)
Condition 4 only#	16/24 (66.7%)	15/25 (60%)	16/32 (50.0%)	13/24 (54.2%)	75/266 (28.2%)

*Quantity in each row included the quantity in the previous condition, except for the last row. **This represent the total proportion of patients with balance disorder. #This only counted those who took part in this condition, removing those alerady failed at the previous conditions.

Table IV-0-11. Prevalence ratios of balance and vestibular dysfunction

Fail m-CTSIB test	Prospective patients			Retrospective patients (n=31)
	Discharge (n=44)	3 month (n=30)	9 month (n=45)	
Balance dysfunction*				
Unadjusted PR	2.81 (2.21-3.53)	2.28 (1.60-3.05)	2.22 (1.65-2.92)	2.20 (1.54-2.96)
Adjusted PR	2.79 (2.22-3.50)	2.26 (1.59-3.01)	2.19 (1.62-2.86)	2.08 (1.45-2.83)
Vestibular dysfunction#				
Unadjusted PR	2.36 (1.60-3.22)	2.13 (1.39-2.98)	1.77 (1.14-2.54)	1.92 (1.19-2.77)
Adjusted PR	2.44 (1.66-3.27)	2.19 (1.42-3.11)	1.76 (1.13-2.51)	1.83 (1.13-2.65)

* determined by failure at any condition in the m-CTSIB; # determined by a failure at test condition 4 in the m-CTSIB

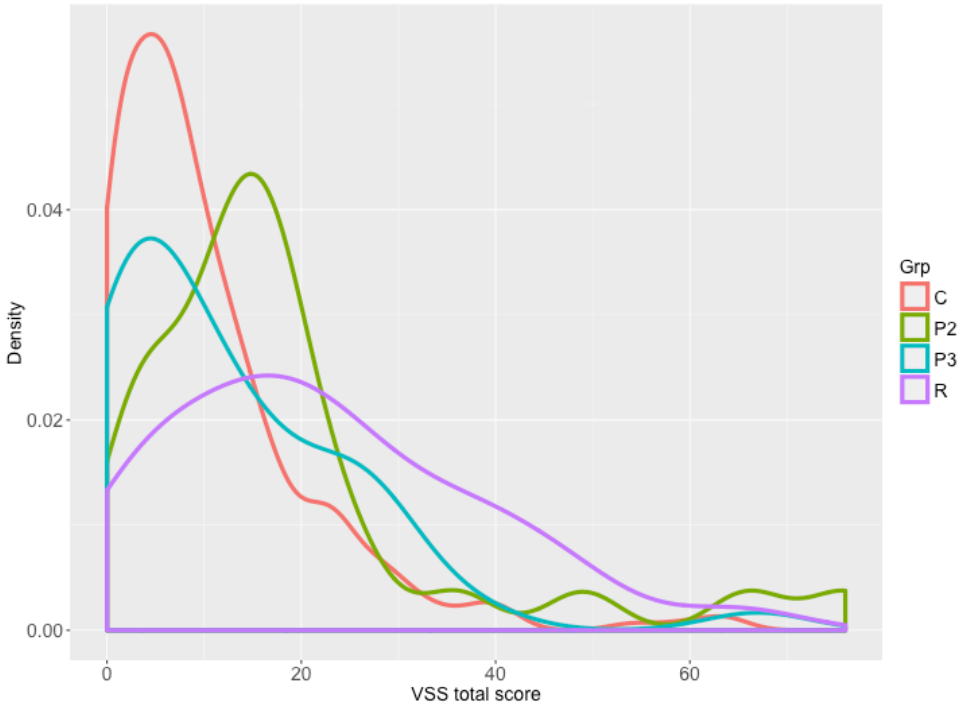
Table IV-0-12 shows the prevalence of imbalance (determined as a failure at any m-CTSIB condition) among patients with hearing impairment at 9 months. All patients with imbalance had some degree of hearing loss. Imbalance occured in 65.9% in patients with mild hearing loss and 84.6% in patients with moderate hearing loss. Vestibular dysfunction also occured in all patients with severe to complete deafness. Only 1 patient had vestibular dysfunction/ imbalance but no hearing loss.

Table IV-0-12. Prevalence of imbalance among hearing impaired patients at 9 months

Hearing impairment category	Patients with hearing impairment, n(%)	Patients with hearing impairment + imbalance, n(%)
HTL ≥ 20dB (mild or worse)	44/45 (97.8)	29/44 (65.9)
HTL ≥ 35dB (moderate or worse)	26/45 (57.8)	12/26 (84.6)
HTL ≥ 50dB (moderately severe or worse)	18/45 (40)	18/18 (100)
HTL ≥ 65dB (severe or worse)	16/45 (35.6)	16/16 (100)

Patients also reported more symptoms associated with vestibular function than controls as shown in Figure IV-0-5 with the distribution of VSS score shifted towards to the higher end of the scale. The mean VSS score for patients was 18.72 (95%CI 12.47-24.98) at 3 months, 12.66 (95%CI 8.81-16.5) at 9 months, and 22.1 (95%CI 16.14- 27.72) for retrospectively enrolled patients, while that for controls was 10.4 (95% 9.13-11.67) (Table IV-0-13).

Figure IV-0-5. Distribution of VSS scores for patients and controls



C: Control, P2: Patients at 3 months, P3: Patients at 9 months, R: Retrospective patients

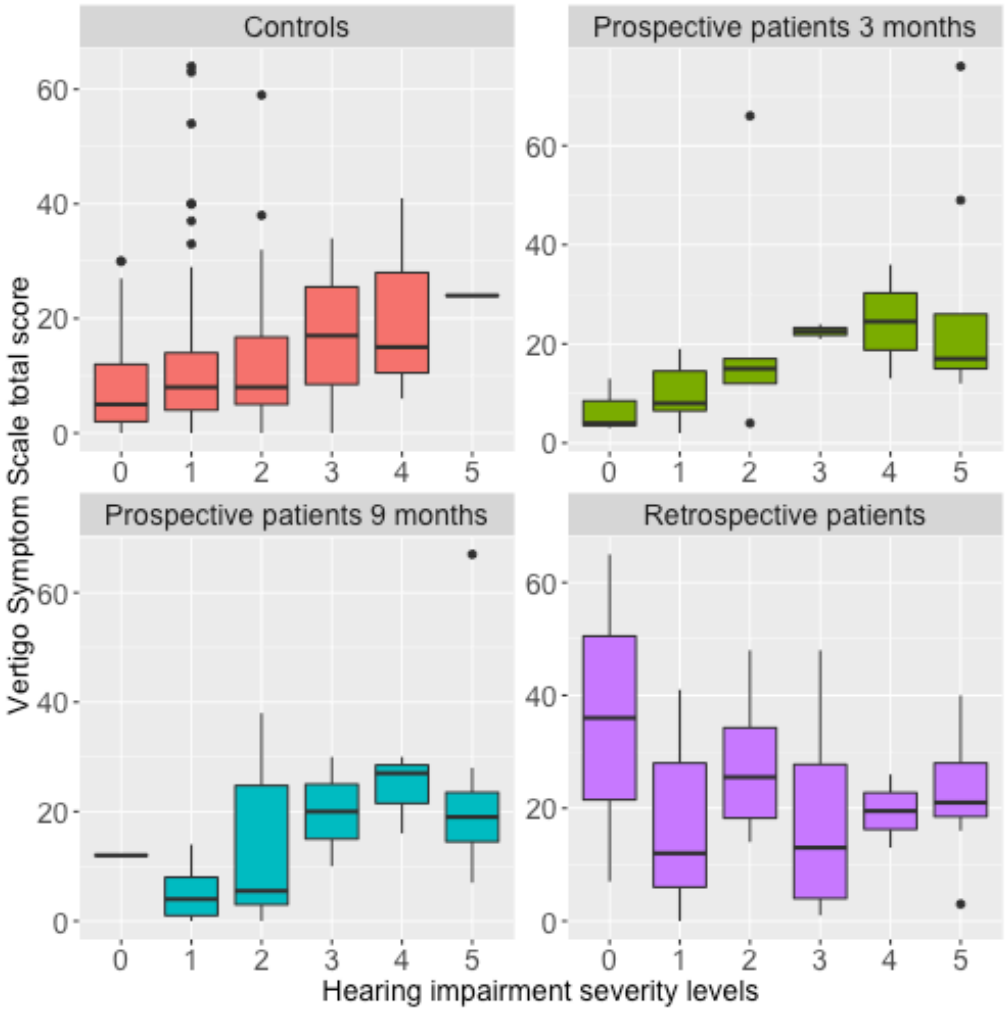
Table IV-0-13. Mean total score (95%CI) for VSS subscales and total score

Subscale	Prospective patients		Retrospective	Controls
	3 months (n=30)	9 months (n=45)	patients (n=31)	(n=270)
Vertigo	6.13(3.97-8.29)	3.93(2.46-5.40)	6(4.02-7.98)	1.74(1.29-2.18)
Anxiety	12.66(8.14-17.17)	8.82(6.11-11.53)	16.3(12.18-20.41)	8.67(7.73-9.62)
Total	18.72(12.47-24.98)	12.66(8.81-16.5)	22.1(16.14-27.72)	10.4(9.13-11.67)

In the multivariable linear regression including the effect of sex, age, and pre-existing medical conditions, VSS scores were 1.80 times (95%CI 1.26-2.57) higher for patients at 3 months after discharge and 2.09 times (95%CI 1.45-3.0) in retrospectively

enrolled patients compared to controls. High VSS scores were associated with increased severity of hearing impairment among prospectively enrolled patients at both 3 months (ANOVA test, p=0.06) and 9 months (p=0.001) (Figure IV-0-6). No significant association between VSS scores and severity of hearing impairment was found for retrospectively enrolled patients and for controls.

Figure IV-0-6. VSS scores by hearing impairment severity for patients and controls



For specific symptoms, patients were significantly more likely than controls to report episodes of vertigo at 3 months (age adjusted PR 2.07, 95%CI 1.19-3.22) but not at 9 months (Table IV-0-14 and Table IV-0-15). Similarly, no difference was found between

retrospectively enrolled patients and controls in vertigo experience after controlling for age. Unlike vertigo, dizziness and unsteadiness were significantly more frequent among prospectively enrolled patients at both follow-ups and among retrospectively enrolled patients compared to controls.

Table IV-0-14. Prevalence of specific self-reported vestibular related symptoms among patients and controls

Symptom	Prospective patients		Retrospective patients (n=31)	Controls (n=270)
	3 months after discharge (n=30)	9 months after discharge (n=45)		
Vertigo	12 (40.0)	10 (22.2)	12 (38.7)	55 (20.4)
Dizziness	20 (66.7)	22 (48.9)	18 (58.1)	63 (23.3)
Unsteadiness	16 (53.3)	18 (40.0)	15 (48.4)	25 (9.3)
Balance related falls	4 (13.3)	7 (15.6)	4 (12.9)	11 (4.1)
Incapacity to stand or walk without support	5 (16.7)	2 (4.4)	4 (12.9)	9 (3.3)

Table IV-0-15. Prevalence ratios of specific self-reported vestibular related symptoms among patients compared to controls (unadjusted and adjusted for age and sex)

Symptom	Prospective patients		Retrospective
	3 months after discharge (n=30)	9 months after discharge (n=45)	patients (n=31)
Vertigo	2.03 (1.16-3.18)	1.14 (0.59-1.95)	1.90 (1.08-3.00)
	2.07 (1.19-3.22)	1.08 (0.56-1.86)	1.84 (1.04-2.92)
Dizziness	2.96 (2.06-4.03)*	2.19 (1.48-3.09)	2.49 (1.65-3.51)
		2.07 (1.40-2.95)#	2.16 (1.41-3.15)#
Unsteadiness	5.96 (3.55-9.77)	4.52 (2.66-7.54)	5.23 (3.02-8.74)
	5.13 (3.08-8.49)#	3.94 (2.27-6.74)	3.72 (2.16-6.38)#
Balance related falls	3.39 (0.99-9.22)	4.00 (1.55-9.60)	3.17 (0.92-8.66)
	3.36 (0.98-9.20)#	4.15 (1.59-10.06)#	2.77 (0.79-782)
Incapacity to stand	5.17 (1.69-13.97)	1.40 (0.22-5.20)	3.87 (1.10-11.16)
or walk without	4.72 (1.53-12.94)	1.27 (0.20-4.75)	2.53 (0.71-7.62)
support			

In each row, data in the first line are unadjusted, data in the second line are adjusted for age and sex. * Unadjusted only. Cannot find adjusted value due to convergence problem. # Adjusted for age only. Cannot find value adjusted for sex due to convergence problem.

Combining the three symptoms of vertigo, dizziness and unsteadiness, 73.3% and 51.1% of patients at 3 months and 9 months after discharge respectively reported at least one of these compared to 36.7% among the controls. Patients were also more likely to experience all three symptoms, with 30% and 20% of patients at 3 months and 9 months

respectively and 29% of retrospectively enrolled patients, while only 4.4% of controls reported all 3 symptoms.

Having hearing loss and at least one of the three above symptoms was observed in 58.6% of prospectively enrolled patients at 3 months (PR 6.49 95%CI 3.89-11.0, age adjusted) and 44.2% at 9 months (PR 5.16 95%CI 2.98-8.95, age and sex adjusted), and 45.2% of retrospectively enrolled patients (PR 4.09 95%CI 2.26-7.4, age adjusted) compared to 7.8% of controls.

4.3.5. Impact of dizziness

Among those who reported experience of dizziness, patients tend to report more significant level of handicap than controls (Table IV-0-16). Combining mild and significant handicap levels, patients have increased rates of reporting handicap than the controls (3 months: PR2.39 95%CI 1.23-5.22, age and sex adjusted; 9 months: PR 1.87 95%CI 0.97-3.43, age adjusted; retrospective: PR 2.24 95%CI 1.3-3.97, age adjusted).

Table IV-0-16. Frequency of handicap among subjects who reported dizziness

Level of handicap due to dizziness	Prospective patients		Retrospective patients (n=17)	Controls (n=61)
	3 months (n=19)	9 months (n=20)		
Mild handicap	5 (26.3)	4 (20.0)	9 (52.9)	13 (21.3)
Significant handicap	5 (26.3)	6 (30.0)	3 (17.6)	3 (4.9)

Data are presented as count (percentage).

4.3.6. Visual disturbance

I found no significant difference in the prevalence of visual disturbance between patients and controls (Table IV-0-17 and Table IV-0-18). Patients at 9 months follow-up

showed some increase in the prevalence, however this was diminished after adjusting for age.

Table IV-0-17. Prevalence of visual disturbance among patients and controls

Visual impairment	Prospective patients			Retrospective patients (n=31)	Controls (n=270)
	Discharge (n=45)	3 months (n=30)	9 months (n=45)		
Moderate impairment	3 (6.7)	4 (13.3)	6 (13.3)	2 (6.5)	12 (4.4)
Severe impairment	2 (4.4)	0 (0)	1 (2.2)	0 (0)	1 (0.4)
Blindness	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.7)

Data are presented as count (percentage).

Table IV-0-18. Prevalence ratios of visual impairment among patients compared to the controls

Visual impairment	Prospective patients			Retrospective patients (n=31)
	Discharge (n=45)	After 3 month (n=30)	After 9 month (n=45)	
Unadjusted PR	2.00 (0.68-4.88)	2.40 (0.72-6.13)	2.80 (1.12-6.25)	1.16 (0.19-3.87)
Age adjusted PR	1.70 (0.60-3.97)	2.11 (0.67-4.99)	2.35 (1.0-4.94)	0.68 (0.11-2.23)

Data are presented as PR (95%CI).

4.3.7. Cognitive impairment (MMSE)

Mean MMSE scores were similar between patient groups and controls (Table IV-0-19). However, more patients at discharge were categorized as having either mild or severe cognitive impairment based on the MMSE scores than controls. Age adjusted PR for any impairment among patients at discharge was 1.83 (95%CI 1.12-2.75) (Table IV-0-20). No further difference was found for patients at all follow-up time-points after discharge.

Table IV-0-19. Mean MMSE score and proportions of patients and controls with mild and severe cognitive impairment

MMSE	Prospective patients			Retrospective patients (n=31)	Controls (n=270)
	Discharge (n=40)	3 months (n=26)	9 months (n=42)		
MMSE score (mean, SD)	23.57 (3.42)	25.12 (2.12)	25.62 (2.76)	24.84 (3.56)	25.37 (3.06)
Mild cognitive impairment (n, %)	13 (32.5%)	5 (19.2%)	9 (21.4%)	6 (19.4%)	54 (20.0%)
Severe cognitive impairment (n, %)	3 (7.5%)	0 (0%)	1 (2.4%)	2 (6.5%)	5 (1.9%)

Table IV-0-20. Prevalence ratios of cognitive impairment among patients compared to controls

Cognitive impairment	Prospective patients			Retrospective
	Discharge (n=40)	3 month (n=26)	9 month (n=42)	patients (n=31)
Unadjusted PR	1.83 (1.13-2.76)	0.88 (0.33-1.77)	1.09 (0.56-1.85)	1.18 (0.57-2.07)
Age and sex adjusted PR	1.83* (1.12-2.75)	0.93 (0.35-1.89)	1.09 (0.56-1.85)	1.20 (0.57-2.15)

*Adjusted for age only.

4.3.8. General health status (EQ5D)

There was no significant difference in self-rated health status on the Visual Analog Scale of the EQ5D between patients and controls (Table IV-0-21). On specific health domains, patients reported significantly more problems than controls with mobility, self-care and usual activities both at 3 months and 9 months after discharge (Table IV-0-21 and Table IV-0-22). No significant result was observed for retrospectively enrolled patients in terms of self-care problem. All patients who reported having some problem with mobility, self-care, and usual activities were those who had both hearing and vestibular impairment (except for one retrospectively enrolled patient who only had hearing loss). Pain and discomfort was significantly more common only among patients at follow-up after 3 months compared to controls. Patients reported less anxiety or depression than the controls on the EQ-5D scale.

Table IV-0-21. Self-rated health status overall and by specific health domains among patients and controls using EQ5D scale

	Prospective patients		Retrospective	Controls
	3 months (n=30)	9 months (n=45)	patients (n=31)	(n=270)
Self-rated health* mean (SD)	81.5 (17.3)	80.3 (18.6)	71.8 (19.1)	84.0 (14.4)
Problem with specific health domain, n(%)				
Mobility	8 (26.7)	8 (17.8)	6 (19.4)	5 (1.9)
Self-care	4 (13.3)	6 (13.3)	1 (3.2)	1 (0.4)
Usual activity	6 (20.0)	7 (15.6)	2 (6.5)	1 (0.4)
Pain/ discomfort	11 (36.7)	12 (26.7)	10 (32.3)	50 (18.5)
Anxiety/ depression	10 (33.3)	10 (22.2)	2 (6.7)	132 (48.9)

* subjects rated their heatlth on a 0-100 scale using the Visual Analog Scale to best describe their health on the present day.

Table IV-0-22. Prevalence ratios for having any problem in each health domain of the EQ5D scale among patients compared to controls

Having any problem with	Prospective patients		Retrospective patients (n=31)
	3 months after discharge (n=30)	9 months after discharge (n=45)	
Mobility	14.4 (5.15 – 45.17)	9.6 (3.36-30.59)	10.45 (3.34-34.5)
	14.5 (5.14-45.75)*	9.27 (3.22-29.69)*	6.77 (2.15-22.9)*
Selfcare	36.0 (5.55-692.14)	36.0 (6.35-671.72)	8.71 (0.35-216.66)
	64.16 (8.10-1672.2)	35.52 (6.25-663.56)*	8.46 (0.31-229.97)*
Usual activity	54.0 (9.67-1002.5)	42.0 (7.73-775.85)	17.42 (1.72-368.62)
	53.5 (9.51-996.0)*	42.48 (7.78-785.96)*	18.13 (1.80-380.81)#
Pain/ discomfort	1.98 (1.09-3.21)	1.44 (0.79-2.38)	1.74 (0.92-2.91)
	1.88 (1.04-3.04)*	1.25 (0.70-2.05)	1.48 (0.78-2.51)*
Anxiety/	0.68 (0.37-1.07)	0.45 (0.24-0.74)	0.14 (0.02-0.40)
depression	0.70 (0.38-1.09)	0.44 (0.23-0.72)	0.13 (0.02-0.39)

Data presented: First line are unadjusted prevalence ratios and confidence intervals. Second line are prevalence ratios adjusted for age and/or sex. *Adjusted for age only. #Adjusted for sex only.

4. 4. Discussion

I found significantly increased proportions of hearing and vestibular impairment among patients at all follow-up time-points compared to the control group. Profound and complete hearing loss (hearing threshold ≥80dB in the better ear) was common among patients and in most cases hearing did not improve over 9 months post-discharge. There was a strong possibility that such severe hearing loss persists beyond 9 months given the similar proportions between prospectively enrolled patients and retrospectively enrolled patients. Improvement was observed mostly among patients with non-severe hearing loss

(below 80dB). This finding is consistent with the current literature on hearing recovery after bacterial meningitis [141] and in other types of sensorineural hearing loss [142], that improvements were more often reported among cases with less severe impairment. In our study improvements occurred quickly after hospital discharge (3 months); no significant change continued beyond this timeframe through to 9 months. This evidence implies that hearing loss caused by *S. suis* infection tends not to be reversible in the long term, regardless of impairment severity.

Proportions of profound and complete hearing loss in our study were comparable to the proportions reported among *S. suis* patients without dexamethasone treatment (33.3% at discharge and 21.7% after 6 months) in a previous study conducted by Mai et al in the south of Viet Nam [20]. However, the authors of this study used a broader definition of severe hearing loss (having a hearing threshold >80dB in at least one ear). If I used that same definition in my study, instead of hearing threshold >80dB in the better ear, I would expect higher proportions of severe hearing loss than those reported here. Dexamethasone was reported to be beneficial in hearing loss outcome in the previous study by significantly reducing the prevalence of hearing loss among those receiving this adjuvant treatment compared to those on placebo [20]. However, Mai et al did not analyse if the recovery progress between the two groups was different. The results of our study did not substantiate this, I found no evidence of an effect of corticosteroid treatment either on the prevalence of deafness outcome between those receiving treatment and those not receiving treatment or the within-patient recovery process at follow-up time-points. However, my study was not powered to examine the effect of corticosteroid treatment on these outcomes. Corticosteroid treatment was indicated in 64% of the medical records of the prospectively enrolled patients following the local routine clinical practice for meningitis patients in this study. More research is still needed to evaluate the

effectiveness of corticosteroid treatment on the long-term hearing outcome among *S. suis* patients.

I used both objective and self-reported methods to examine vestibular outcomes in patients and controls. Approximately one in two patients had evidence of vestibular dysfunction compared to just over one in four in the normal controls, as determined by a simple postural metric m-CTSIB. This metric assesses the ability of an individual to use sensory inputs to maintain balance, and was reported to have 90% sensitivity and 95% specificity in patients with vestibular disorders [143]. Its usefulness in clinical settings for diagnosis of vestibular dysfunction has been shown as non-inferior compared to computerized dynamic posturography instruments [144]. Comparing to the previous studies in *S. suis* infection, our estimates were in the high range of the reported rates [8], although this comparison is problematic due to differences and lack of clarity in evaluation methods. However, in our study the proportions of self-reported vertigo, dizziness and unsteadiness were comparable to the proportions of vestibular dysfunction as measured by m-CTSIB in our study for both patients and controls, indicating the validity of our estimates. Using a similar test to m-CTSIB, the reported prevalence among adults aged 40 years and older was 35.4% in the national health and nutrition survey in the U.S. [127]. Proportions of self-reported vertigo in community samples in the U.K. and Finland were between 21-29% [145-147]. The proportions of vestibular dysfunction and symptoms in our control group were comparable to these community-based rates, further supporting the validity of the evaluation methods used in the current study.

Suppurative labyrinthitis and cochlea inflammation have been recognized as the site of lesion causing deafness and vestibular dysfunction in *S. suis* infection [90, 148]. An experimental study in pigs showed that deafness occurred in cases of *S. suis* meningitis with evidence of cochlear involvement, where bacteria passing through the

cochlear aqueduct to enter and damage the cochlea [53]. A human temporal bone study showed that the cochlea was affected in all cases with suppurative labyrinthitis, which has usually been thought to cause permanent hearing loss [149]. On the other hand, in patients with evidence of serous labyrinthitis, hearing loss was more likely to be reversible [149]. Direct cochlear invasion by bacteria during septicemia has also been suggested independent of meningitis, explaining deafness in patients without signs of meningism [148]. Most patients in our study had meningitis, except for three patients with septicemia only. Profound hearing loss and severe balance disorder was found in one of these septicemia patients. This indicates that the damage pathway in *S. suis* infection could be at different sites and consequently lead to varied levels of impairment severity. Few reports in the literature discuss vestibular complications, although existing evidence suggests a link between labyrinthitis and vestibular complications [150]. I found that vestibular dysfunction tends to occur more often in patients with severe to complete loss to the hearing system. This observation in *S. suis* patients is also in agreement with previous reports for different etiologies, that peripheral vestibular impairment was found mainly among those with residual deafness [151] or co-incidence of balance system dysfunction was more frequent in profound hearing loss [152].

Consistent with previous literature on general health and quality of life among patients after bacterial meningitis [153-156], I found significantly lower health status among patients in comparison to the controls. This lower quality of life could be attributed to the long-term hearing and vestibular impairments, which mostly caused difficulties in mobility and usual activities for patients. There was a tendency of adaptation among patients in terms of self-care and pain/ discomfort over time, reflected in the diminishing impact for patients at 9 months and for retrospective patients at 11-34 months after discharge in these domains.

One limitation of our study was the selection of controls, who were non-patient visitors. Emotional distress is one of the problems that caregivers are faced with while providing care to patients. This expectedly resulted in higher proportions of anxiety/ depression among controls compared to patients. Therefore, I could not assess the emotional impact of *S. suis* infection in this study. Nonetheless, patients still scored worse for emotional and social domains than the controls on the hearing and dizziness specific scales. Higher levels of perceived hearing handicap were seen among patients in more severe hearing loss in our study. However, quality of life could not be predicted based on hearing impairment severity alone but also dependent on individual coping strategies in the adaptation process [157], which emphasizes the importance of training programs to support patients to cope with hearing loss after hospital discharge.

In addition, the use of non-patient visitors as controls is likely to cause an overestimation in the results on health outcomes because these visitors are generally healthier than the general population. However, the prevalence of hearing loss and vestibular dysfunction among the control group was comparable to the general population, indicating the representativeness of the controls with regards to the main study outcomes. This was also likely because people with non-severe hearing loss and vestibular dysfunction would still be expected to participate in the normal activities including visiting the hospital and caring for sick family members. On the other hand, the rates of severe hearing and vestibular dysfunction are quite low in the general population. One other issue is that matching was not fully achieved in our study, resulting in a group of controls with a slightly younger mean age than the cases. However, both age and sex were included as a potential confounder in all our analyses.

Another limitation is that I could not follow-up the prospectively enrolled patients for longer period of time to evaluate if any further change happen in the recovery process

of hearing and vestibular impairment after 9 months. To gain more understanding of sequelae in the longer term, the study included retrospectively enrolled patients who had been discharged for a longer period of time.The findings from this patient group supported the findings from the prospectively enrolled group. However, the findings from the retrospective group should be intepreted with caution because of possible selection bias arising from the process of recruitment. Patients with severe sequelae might be more likely to participate in the study than those who had non-severe sequelae or had been fully recovered. This bias might have lead to an overestimation of severe conditions among retrospectively enrolled patients, but also underestimation of non-severe conditions. Therefore, no definitive comparisons could be made between the prospective and retrospective group.

Finally, I did not formally measure tinnitus. Some of our patients with profound to complete hearing loss complained about disturbing ringing or buzzing in their ears or head all day. Tinnitus and hearing loss are often correlated, although the rate of tinnitus among cases with hearing loss varied greatly in the literature [158]. Among noise-exposed workers, the rates of tinnitus increased with the severity levels of hearing loss, ranging from 5-10% in mild loss to 12-30% in profound to complete loss [159]. Among the retrospective patients I contacted, tinnitus was reported in 14.3% of those who also reported hearing impairment after hospital discharge.

4. 5. Conclusion

In conclusion, this study provides strong evidence that a large proportion of patients who survive *S. suis* infection suffer serious hearing and vestibular impairment, which tend to persist over years after hospital discharge with little improvement. Patients also experienced multiple symptoms, which aggravates the impact of disease sequelae on

their health and quality of life. Further research is required to understand the infection pathways of the bacteria in causing hearing and vestibular impairment and investigate the effectiveness of different treatment regimens to reduce the incidence and severity of the damage. In the meantime, patients could benefit from appropriate training and rehabilitation programs to reduce the impact of symptoms and help them adapt to everyday situations with hearing loss and balance problems.

Chapter V

Annual incidence and burden of disease of *Streptococcus suis* infection in humans in Viet Nam

5. 1. Introduction

Effective disease control and prevention of *S. suis* infection requires data on the disease burden that reflects the multifaceted consequences of the disease on affected individuals and their community, as well as on the health system. For the purposes of health priority setting and decision-making, such data should also be comparable with data on other diseases or from different geographic regions. Traditional ways of summarizing disease burden involving simple indicators such as incidence, prevalence and mortality are widely used, but these quickly become unmanageable when more and more health conditions are to be monitored and compared [160].

This chapter estimated the health burden of *S. suis* infection in Disability Adjusted Life-Years (DALY) and the economic impact of the disease in Viet Nam. DALY is a common population health summary measure that has been used in the Global Burden of Disease (GBD) study since the late 1980s and in many other burden of disease studies in specific countries. In this estimate, I measured the number of years lost due to premature deaths and disability caused by *S. suis* infection and its sequelae following an incidence based approach. In addition to the damage to health and quality of life, *S. suis* infection potentially imposes high financial costs on the patients, their families and society due to: the tertiary care often required for this severe disease; the long-term health impacts

including deafness and loss of balance; and the fact that the affected group is predominantly men of working age. Illness could affect patients and their family through increasing household expenditures on health, reduced level of productivity, reduced non-health expenses (such as clothes, social activities or durable goods), or reduced investments in people (such as education, health) [161]. To date there has been no evaluation of the economic impact of human *S. suis* infection. In Viet Nam, economic studies of illness and diseases are rare, particularly for infectious diseases that cause long-term effects on patients' capacity to join social and economic activities.

5. 2. Objectives

This study aims to estimate the disease burden and economic impact of human *S. suis* infection in Viet Nam. Specifically, it aims to:

- Estimate the annual incidence rate of human *S. suis* infection in Viet Nam from existing surveillance data.
- Estimate the total annual number of DALYs lost due to human *S. suis* infection in Viet Nam.
- Assess the medical costs and non-medical costs associated with *S. suis* infection and its long-term health sequelae from a patient and societal perspective.
- Identify the indirect costs due to premature mortality and morbidity caused by *S. suis* infection and its long-term health sequelae.

5. 3. Methods

5.3.1. Summary

The estimation of the burden of disease caused by *S. suis* infection was performed through a number of sequential steps. Firstly, I estimated the annual incidence of disease

based on existing surveillance data using a model-based imputation approach to account for data missingness. The DALY burden of disease was then calculated using a pathogen and incidence based approach utilizing the outcome tree for *S. suis* infection and incorporating both the impact of premature mortality and morbidity. The economic impact was evaluated in terms of both direct costs and productivity loss (indirect costs) associated with premature mortality and disability caused by the disease using an incidence-based cost-of-illness approach.

5.3.2. Estimation of annual incidence

Incidence data on human *S. suis* infection was obtained from the national surveillance system, which had required mandatory reporting of incident *S. suis* cases since 2011. However, data from surveillance and notification systems often suffer from issues of under-ascertainment and under-reporting of cases [162]. Reasons for under-reporting are many, including the lack of diagnostic capacity in hospitals at the provincial level, lack of a rigorous system to follow up patients after referral to central hospitals, and overlaps or dis-coordination in the reporting responsibilities between health care levels. Of particular concern is the large number of provinces that are not reporting *S. suis* cases to the national surveillance each year. For example, 36 out of 63 provinces (57.1%) did not report any *S. suis* cases in 2011. Similar rates of non-reporting were observed in subsequent years (Figure V-0-1). Non-reporting could mean the real incidence in those provinces was zero case or there were cases but these were under-ascertained or under-reported. Among these non-reporting provinces, some were in the areas with pig production (Figure V-0-2), indicating possible disease occurrence. In addition the fact pork is consumed by >98% of households in Viet Nam [4] suggests that the risk of *S. suis* infection through pork exposure is not restricted in some areas. Incidence data from these

provinces should therefore be treated as missing data and imputed using systematic and valid approaches to incorporate uncertainty in the estimate.

Figure V-0-1. Distribution of *S. suis* cases reported to the national surveillance system 2011-2014 (Data source: NIHE)

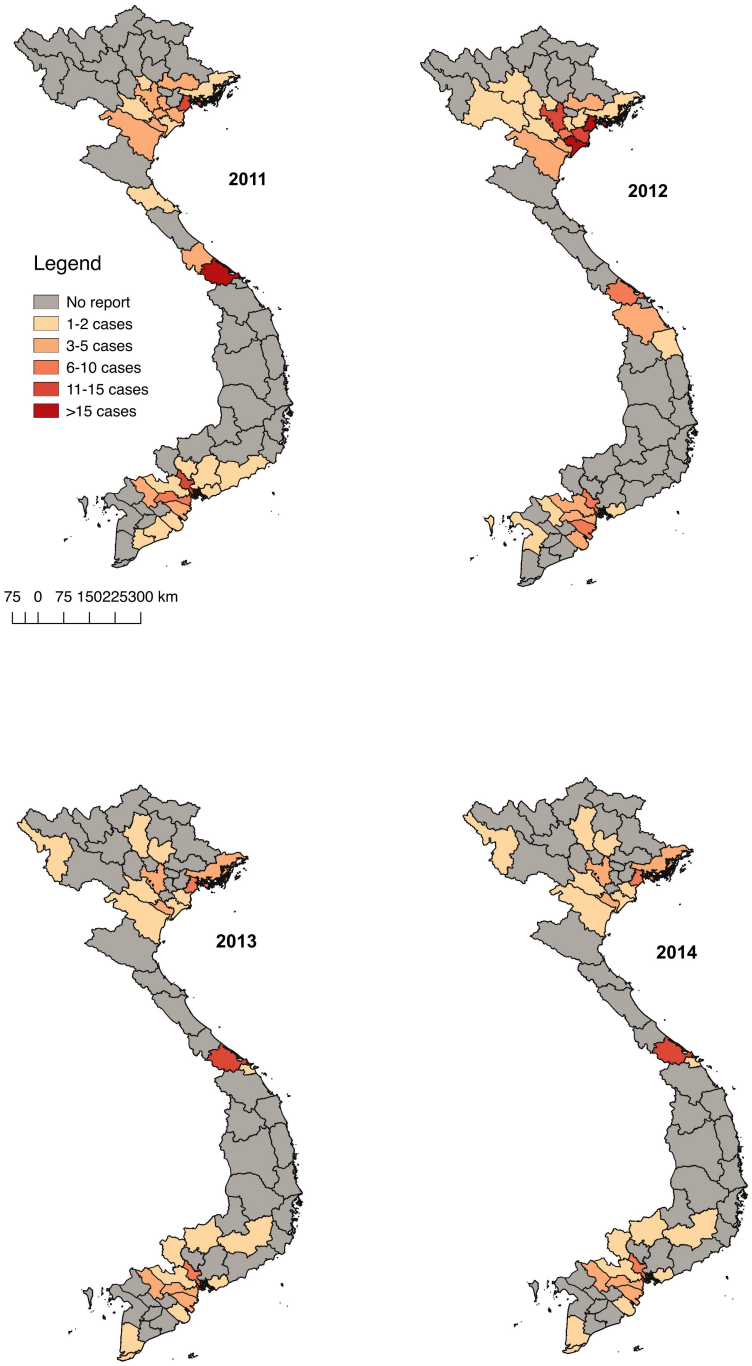
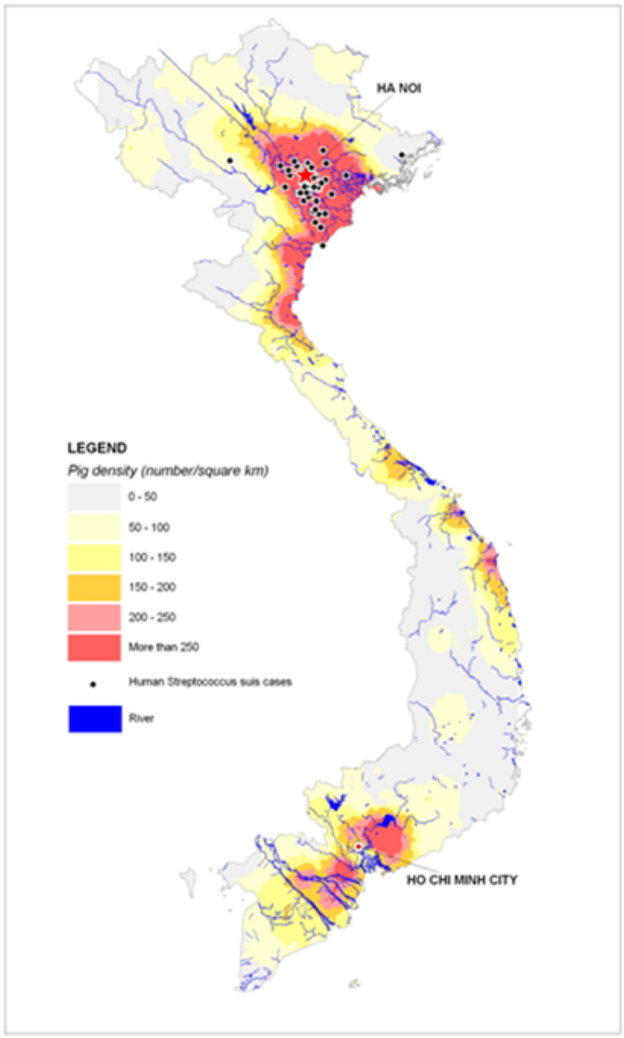


Figure V-0-2. Map of pig density by province in Viet Nam



Source: Wertheim et al [1]

Highest level of pig density in areas with red color, lowest level of pig density in areas with light yellow or grey color. Black dots were human cases admitted at NHTD in 2007.

Choice of data imputation method

Two types of methods have been outlined in the proliferating literature on handling missing data in the last two decades, including “ad hoc” (simple technique such as using complete cases only or imputing single value) and “statistically principled” methods [163]. It is often not recommended to use the former methods due to a high potential for bias and ignorance of statistical uncertainty in the missing data. Much development has been devoted to the later group of methods, which essentially use a

formal modeling approach to account for the missing-data induced uncertainty in the analysis based on observed data and explicit assumptions about missing-ness mechanisms. Maximum likelihood and Bayesian procedures are the two major approaches commonly used in the modern model-based methods [164].

Choice of methods for dealing with missing data depends on the missing mechanisms, which are generally grouped into three categories following the typology developed by Rubin [165]: missing completely at random (MCAR), missing at random (MAR) and missing not at random (MNAR). The MAR assumption holds when probability of missingness depends on observed data but not on the unobserved data (i.e. the value of missing data itself). MCAR occurs when this distribution does not depend on either observed or unobserved data. When neither MCAR nor MAR holds, missing data are said to be MNAR, in which predicting the missing values becomes difficult. Departures from the MAR assumption in reality does not usually cause serious problems in the result of modelling missing data [164]. For the feasibility of implementation, I assume that missing data of *S. suis* cases in the existing surveillance data follow a MAR mechanism in my analysis. For *S. suis* disease incidence, missing data may be more likely to occur in provinces with poor health care resources. Less populous provinces, provinces with less pigs or provinces with low disease incidence might also be more likely to have missing data. These provincial charateristics therefore could be used to predict the incidence of disease.

Both likelihood and Bayesian procedures in dealing with missing data work with the assumption that data missingness is MAR [164]. However, likelihood methods generally require large sample sizes to achieve approximately unbiased and normally distributed estimates. There are 63 provinces in the existing *S. suis* surveillance system, and over half of these have missing data each year. Previous works suggested that small

sample sizes can be handled in methods using Bayesian principles [164]. Therefore I selected methods based on applying Bayesian principles to the missing data problem in estimating the total *S. suis* incidence and its variance over the years 2011-2014.

I chose a multiple imputation approach (using Bayesian principles, details are described in the next section) for imputing missing data on disease incidence because it could incorporate more explanatory variables in the imputation model than other approaches given the limited number of observations. It is recommended that more explanatory variables are added to the imputation model to minimize bias and increase certainty of the data imputed [166, 167]. In addition, including more variables predictive of data missingness could reinforce the plausibility of MAR assumption [168]. I also explored the possibility of applying Bayesian regression with a spatial random effect. However, only a limited number of explanatory variables could be included in these spatial models (models did not run properly when more variables were added due to small number of observations), the produced estimates were smaller with larger uncertainty intervals, and the sets of explanatory variables were different across the years under prediction (see Appendix 4 for details on the implementation of this method).

Choice of model for disease incidence

Disease incidence has a discrete count distribution and a Poisson process is commonly used to model this type of data with an assumption of equal mean and variance for the dependent variable. However, the variance is often larger than the mean in practice, indicating over-dispersion or excess variability around the fitted values under the conventional Poisson model assuming that the systematic part of the model has been correctly specified [169]. The existence of over-dispersion can lead to underestimation of standard errors of the parameters if an inappropriate model is applied. A negative

binomial model is usually used to accommodate over-dispersion problem in such count data [169, 170]. Table V-0-1 shows the mean of provincial incidence data for 4 years with very high variances, indicating an evidence of apparent over-dispersion.

Table V-0-1. Mean and variance of provincial *S. suis* incidence cases per year

Year	Mean	Variance
2011	4.15	27.13
2012	4.81	24.62
2013	4.0	12.93
2014	3.08	9.99

I used three methods for modeling the distribution of incidence: negative binomial, Poisson, and predictive mean matching (*pmm*). Missing values were imputed based on the observed values through a regression model that encompassed both the structural part and the assumed error distribution [167, 168]. The *pmm* method is a "general purpose semi-parametric method" with the imputed values restricted to the observed values. This method is easily operationalized and can still work even when the structural part of the model is incorrectly specified. However, it might result in small variation in the pooled estimate if there is a limited number of predicting variables [167].

Multiple imputation

Multiple imputation is an increasingly popular method employing Bayesian principles in estimating the missing data by using information available within the dataset [171]. It is fundamentally a repeated imputation process taking *m* draws from a posterior predictive distribution for the missing data under a specific imputation model [172]. Each

of these resulting *m* complete datasets are analyzed using the same analysis model and the results are then combined to produce pooled estimates and variances that incorporate the uncertainty in the missing data. Various tools have been proposed for special types of data. For *S. suis* incidence, which exhibits a count data type, I chose the MICE (multiple imputation by chained equations) package with add-on functions from the countimp package [167, 173]. MICE provides a flexible framework for extending procedures to handle special data types such as over-dispersed count data [174]. It also provides helpful tools to assess convergence and quality of data imputation [173]. Under this framework, missing data are assumed to be missing at random (MAR). However, it is also possible to evaluate the influence of alternative scenarios under a MNAR mechanism in this framework [167]. The imputation model under MAR assumption is appropriate if after controlling for all available data in the model, 'any remaining missingness is completely at random' [175].

Imputation model:

Disease incidence was modeled conditional on a set of predictor variables for which data were available at the provincial level from the national general statistics office of Viet Nam. These variables were selected based on their possible association with disease incidence or with the probability of data missingness. The initial list included geographical region, human population density, pig density, proportion of rural population, number of hospitals, income, immigration rate, outmigration rate, and proportion of skilled workers.

All 9 predictors were included in the imputation model which generated m=100 imputed datasets with 10 iterations for each imputation cycle. Total disease incidence was calculated for each imputed dataset separately and the results were pooled across 100

imputed datasets to produce the mean incidence with a variance that took into account both within-dataset and between-dataset variation (using *pool.scalar* function). If the model ran with warnings produced, one variable was removed from the predictor list and the imputation process was started again for the updated list of predictors. To assess convergence of the imputation process, the mean and variance for each imputed dataset (sequence) were plotted against the iterations. Convergence ass reached when the sequences did not show any definite trends and the variance between sequences was not larger than the variance within the sequences [176]. I used density plot to check the distributional discrepancy between observed and imputed values and check the residuals of the missing data model conditional on propensity score for missing probability. These distributions should be similar if the data imputation model was appropriate [167].

5.3.3. Burden of disease due to *S. suis* infection

Disability Adjusted Life Year (DALY) is a health gap measure widely used to estimate the total burden of diseases at national, regional and global levels. This is a time metric expressing years of life lost to premature mortality and years lived with suboptimal health states due to specific causes [177]. It reflects the disparity between the observed amount of healthy years lived and the expected goal for the target population. Therefore, measuring disease burden in DALYs can provide a comparable output measure for priority setting in healthcare and health research as well as intervention planning and evaluation [178]. However since its first introduction by the Global Burden of Disease and injury study team, this measure has been criticized for not capturing the long-term aspects of non-fatal infectious diseases, among several other limitations [179]. In 2009, the European Centre for Disease Prevention and Control launched the Burden of Communicable Diseases project with an aim to improve the DALY methodology for

better capturing the true burden of infectious diseases based on a pathogen based and incidence approach [180].

Pathogen based and incidence approach

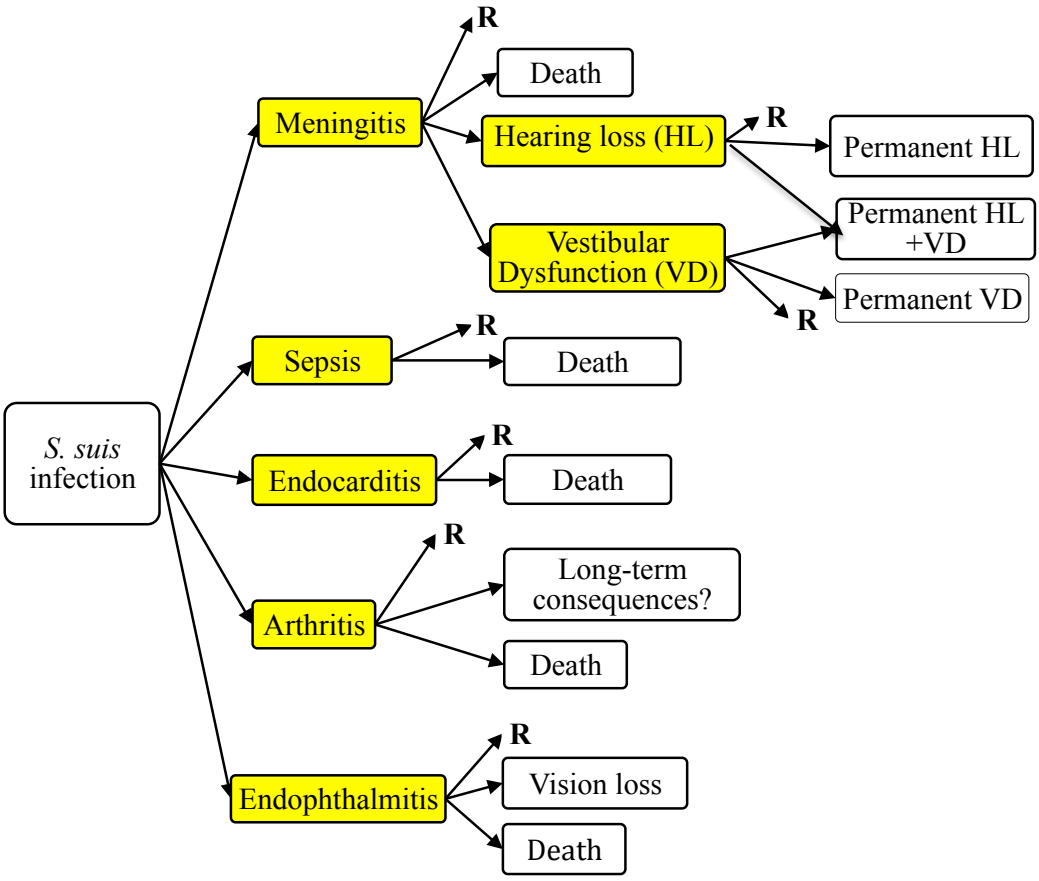
I employed a pathogen based and incidence approach for estimating the burden of disease caused by *S. suis* infection. Using the pathogen-based approach allows the inclusion of short-term and long-term related sequelae in the estimation of burden of disease due to an infectious agent, unlike the outcome-based approach [181]. This requires correct identification of health consequences attributable to an infection through an outcome tree, which describes the causal relationships between these consequences and the initial infection. Taking an incidence approach also means attributing the impact of all these health consequences, including their severity and duration, to one point in time when the infection occurs [182]. Therefore, all DALYs associated with current and future health outcomes are assigned to the year of the initial infection event using this pathogen- and incidence based approach.

Outcome tree for human S. suis infection

Figure V-0-3 represents qualitatively the disease progression over time for *S. suis* infection in humans. As summarized in the systematic review in Chapter II, the most common syndromes included meningitis, sepsis, arthritis, endocarditis, and endophthalmitis. These syndromes were included in the outcome tree. I excluded spondylodiscitis because it was a rare syndrome among patients with *S. suis* infection with limited clinical evidence available. Similarly, TSLS had only been reported in two outbreaks in China and one study in Thailand, and in 5 anecdotal reports. However, this syndrome was included in the sepsis category when pooling data to calculate relevant average estimates for patients with *S. suis* sepsis.

Figure V-0-3. Outcome tree for *S. suis* infection in humans

(R: Recovered)



As shown in the systematic review in Chapter II, the majority of patients fall into the meningitis category (68%). Two main sequelae identified among meningitis patients included hearing loss and vestibular dysfunction, which could be long-term and irreversible. Patients who developed hearing loss often also reported problems with vestibular functioning. This was observed in my patient cohort and in the case reports literature. The proportion of patients who had no hearing loss but reported balance problems was very low, and thus the exclusion of this group would have little impact on the overall burden estimation. Also from our observation, reversible hearing loss and vestibular disorders tended to resolve in a short period of approximately 1-3 months during their usual recovery process after the acute infection period. Therefore, the impact

of temporary hearing loss was not assessed separately but considered to be counted within the burden that a usual recovery process from an infectious episode might cause to the individual patients.

Although arthritis has been reported to develop in patients with *S. suis* infection in a number of large case series (at a median of 16%) [8], literature on long-term consequences such as joint damage is not available. Pathogenesis of acute arthritis depends on the host immune response to the bacteria, and this interaction can lead to joint destruction [183]. One patient had *S. suis* positive joint fluid culture and continued to have joint pain and swelling persisting post-infection with a plan for knee replacement [184] However, it is premature to draw conclusions about the impact of any outcomes of this clinical pathway on patients, and therefore I excluded the long-term outcomes of acute arthritis from the estimation of the disease burden caused by *S. suis* infection. For endophthalmitis, some associated vision loss has been reported in the literature and also observed in 2 patients within our patient cohort. However, this condition was rare among patients and was included in the non-meningitis group in general without affecting too much the total burden of disease.

The simplified outcome tree is presented in Figure V-0-4. I considered two main long-term outcomes among patients affected by *S. suis* infection: hearing loss with vestibular dysfunction and hearing loss without vestibular dysfunction. All other temporary states were grouped under the post-infection phase category. Death from all syndromes was counted in one group with the overall mortality rate. Probabilities of transitioning from one state to the next were summarized in Table V-0-2. For each health state other than death, two more parameters were required to quantify the burden: these were the duration for each health state and a disability weight, which described the severity of the condition.

Figure V-0-4. Simplified outcome tree for *S. suis* infection in humans

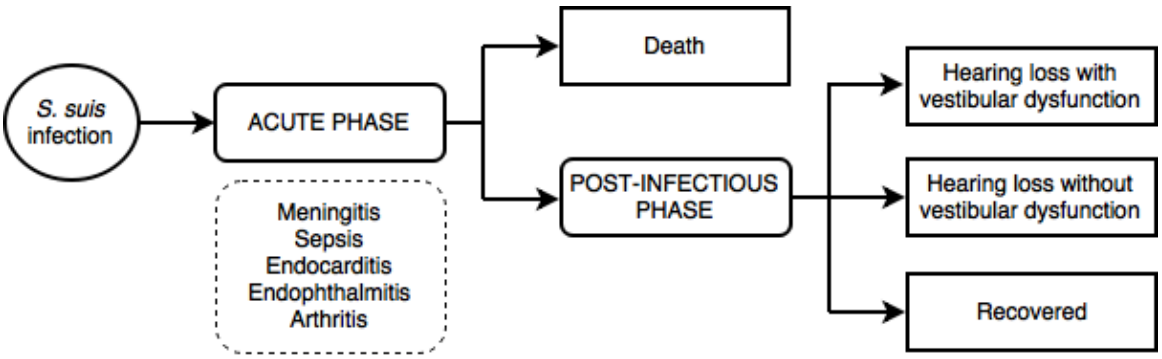


Table V-0-2. Epidemiological parameters for different health states and outcomes

Health state	**Probability**	**Duration**	**Disability weight**
Acute phase of infection	-	d_c	w_c
Hearing loss with vestibular dysfunction	p_{hv}	d_{hv}	w_{hv}
Hearing loss without vestibular dysfunction	p_h	d_h	w_h
Post-acute phase	p_p	d_p	w_p
Death	p_d	-	-

All health states other than acute phase are transitioned from the acute phase.

DALYs calculation

The basic formulae for calculating DALYs is the summation of years lived with disability (YLDs) and years of life lost due to premature mortality (YLLs) [185].

For each age and sex group in a one year period with an annual incidence *I*:

YLD = Number of cases × duration of health state × disability weight

$= I \times (d_c \times w_c + p_{hv} \times d_{hv} \times w_{hv} + p_h \times d_h \times w_h + p_p \times d_p \times w_p)$

YLL = Number of deaths × life expectancy at the age of death (*L*)

$= I \times p_d \times L$

Calculations was performed using the DALY Calculator - a Graphical User Interface for stochastic calculation of DALY developed in the R environment [186]. Estimates were specifically for the following age groups by sex: 15-44, 45-59 and ≥60 (*S. suis* infection has not been reported in children in Viet Nam). Uncertainty was performed through Monte Carlo simulations by iteratively simulating random values for each parameter according to its predefined distribution.

Annual incidence was estimated from modeling based on the available national surveillance system as described in the previous section. Gamma distribution was assumed for incidence of disease and subsequent health states with shape and scale parameters derived from the estimated mean and uncertainty interval. Transitioning probabilities (fixed values) (Table V-0-3) and time/ duration of different health states (average and range) (Table V-0-4) were obtained from national data if a reasonable estimate was available. If no national figures existed, estimates were then obtained from the literature through the systematic review.

Table V-0-3. Transitioning probabilities of health states from acute *S. suis* infection

Health state	Probability	Source
Hearing loss (All)		Cohort[§]
Mild	40.0%	
Moderate	17.8%	
Severe[*]	11.1%	
Profound/ Complete	28.8%	
Hearing loss with vestibular dysfunction[*]		Cohort[§]
Mild	26.6%	
Moderate	15.1 %	
Severe[**]	11.1%	
Profound/ Complete	28.8%	
Post-acute phase	87.2 %	Systematic review[¶]
Death	12.5%; 6.1%; 10.3%; 12.2%	Surveillance data from NIHE for 2011; 2012; 2013; 2014

*The product of rate of hearing loss and rate of imbalance among hearing impaired patients at 9 months; **Include moderately severe category; ¶Systematic review and meta-analysis of global literature published by 2012. Probability of post-infectious phase was calculated as the complement of the probability of death, considering all survived patients would go through the post-infectious process. An averaged probability of death derived from systematic review (12.8%) was used to estimate this parameter [8]. §Cohort study of *S. suis* patients admitted at the NHTD, Viet Nam 2011-2015; vestibular dysfunction was defined as failure at the test condition 4 of the m-CTSIB.

Table V-0-4. Time/ duration of health states associated with *S. suis* infection

Health state	Average	Range	Source
Age at acute infection	48.2	46.5-49.9	Systematic review*
Acute phase duration (days)	17.2	13.0-19.2	Systematic review*
Post-acute phase duration (days)	90	15-270	Cohort§

*Systematic review and meta-analysis of global literature published by 2012 [8]. Mean age of acute infection was pooled from meta-analysis of Viet Nam based studies, random effect model; §Cohort study of *S. suis* patients admitted at the NHTD, Viet Nam 2011-2015. Time duration from the point of hospital discharge to the point of patient returning to work was considered as post-acute phase.

For disability weights, I examined and compared the values from the updated values from the World Health Organization (WHO), the Global Burden of Disease (GBD) 2010 project, and the European study [187-189]. The updated set of disability weights generated by the GBD 2010 was the largest set generated for a diverse range of health states and populations. However, there have been criticisms regarding the low weights reported from this study for certain conditions including hearing impairment and vision loss [190-192]. In the recent WHO global health estimates (GHEs), modified weights for these conditions predicted based on individual EQ-5D profiles were used instead of the GBD 2010 values [187]. As part of the study on burden of communicable diseases in Europe, the study team replicated the GBD 2010 disability weight measurement study and provided an updated set of values with uncertainty intervals [188]. For hearing impairment, I used the updated values from the European study, which are close to the modified values used by WHO GHEs. For the co-existent health state of hearing loss with vestibular dysfunction, a multiplicative method [193, 194] was used to derive a combined disability weight as follow:

$$w_{hv} = 1 - (1 - w_{hearing\ loss}) \times (1 - w_{vestibular\ dysfunction})$$

Table V-0-5. Disability weights for calculating DALYs loss due to *S. suis* infection

Health state	Mean	95% Uncertainty interval (UI)	Source of data	Shape parameters** *(alpha, beta)*
Acute phase	0.210	0.139 – 0.298*	GBD2010	49.4, 185.8
Post-acute phase	0.254	0.170– 0.355	GBD2010	50.4, 147.9
Hearing loss without vestibular dysfunction				
Mild	0.011	0.007-0.014	Europe¶	87.9, 7903.8
Moderate	0.037	0.028-0.045		164.2, 4273.3
Severe	0.152	0.125-0.187		183.3, 1022.8
Profound/ Complete	0.235	0.197-0.274		256.3, 834.3
Vestibular dysfunction♯	0.097	0.079-0.119	Europe	191.1, 1778.7
Hearing loss with vestibular dysfunction (calculated using multiplicative method)				
Mild	0.107	0.085-0.131		173.8, 1450.8
Moderate	0.130	0.105-0.159		181.4, 1213.9
Severe	0.234	0.194-0.284		186.2, 609.5
Profound / Complete	0.309	0.260-0.360		237.2, 530.5

*weight for a severe acute episode of infectious disease was used because *S. suis* infection was considered to be a severe systemic infection. ** Shape parameters selected for a beta distribution assumed for disability weights based on the available mean and uncertainty interval. ¶European study. ♯This was to combine with disability weight for hearing loss to derive the combined weight for hearing loss with vestibular dysfunction. The value of vertigo and balance disorder from the European study was used to describe vestibular dysfunction: 0.097 (95%UI: 0.079-0.119). Combined estimates were estimated based on the formulae described in the text.

Values of disability weights used for calculating burden of disease due to *S. suis* infection are listed in Table V-0-5. The description of each health state is provided in Appendix 5. The age and sex structure used for this calculation was obtained from the case series data from the NHTD over 5 years (Table V-0-6).

Table V-0-6. Patient age and sex structures

Sex	Age group (years)			
	15-44	45-59	60+	All
Male	85 (28.7%)	133 (44.9%)	30 (10.1%)	248 (83.8%)
Female	9 (3.0%)	16 (5.4%)	23 (7.8%)	48 (16.2%)
All	94 (31.8%)	149 (50.3%)	53 (17.9%)	296 (100%)

Data from the series of *S. suis* cases admitted at NHTD between 2011-2015

A summary of values used for input parameters are listed in Appendix 6. In this calculation of DALYs, neither age weighting nor discount rate was applied. Sensitivity analysis was performed using linear regression on the overall DALY estimates against the simulated values for the input parameters to identify those parameters that caused significant uncertainty in the overall DALY estimates.

5.3.4. Economic impact of human *S. suis* infection

Analysis perspective and setting

This is an incidence-based cost-of-illness analysis from a patient and household perspective. Cost of illness approach is frequently used to establish the costs for illness and diseases by identifying all associated cost-generating components and attributing a monetary value to them [195]. Costs for these components at a detailed level throughout the life-time of a new case of *S. suis* infection was assessed and summed up to obtain the total economic impact incurred by the disease. Costs included direct medical costs (costs

for health care services at any health facilities for disease detection, treatment, continuing care, rehabilitation and terminal care related to the infection episode or associated sequelae), direct non-medical costs (costs for travelling, food, accommodation and any other costs related to the infection episode and associated sequelae), and indirect costs (productivity losses of the patients and caregivers related to the infection episode and associated sequelae).

Data collection of this study was conducted as part of cohort study of prospective patients confirmed with *S. suis* infection admitted at NHTD (see Chapter IV). NHTD is a public hospital receiving government funding for overhead costs including labour costs and assets. Operational costs are covered by health insurance and patient out-of-pocket payments. Insurance coverage in Viet Nam is not high especially among near-poor people (people having income up to 1.5 times above the poverty threshold) and the groups that do not receive any subsidy for health insurance (11% and 21% respectively, compared to 58% on average) [66].

Costs data collection

Cost data were collected through patient record reviews and interviews of patients and their family members. Formal hospital fees covered by patients and insurance were collected from hospital discharge records at NHTD. From interviews with patients and/ or family members/ caregivers, the following data were sought: informal fees (any fees paid outside of standard hospital financial regulations), medical costs paid to health facilities prior or after hospitalization at NHTD, costs of other drugs or medical goods purchased outside the hospital and health facilities, non-medical costs arising from the hospitalization episode such as food, transport, and accommodation, amount of income loss due to sickness and care-giving activities for both patients and caregivers, patient's

time spent on paid and unpaid work before and after illness, patient's income earned before and after illness, and other impacts caused by the illness and its sequelae.

Annual incidence of *S. suis* cases is predicted based on surveillance data as described in Chapter V.

Calculation of direct costs

The average direct cost per typical hospitalization (Q) was calculated by summing the average direct medical cost including formal fees and informal fees paid at NHTD, medical costs paid at other health facilities and costs of drugs and other medical goods purchased outside the hospital and the average direct non-medical cost per new infection.

The annual direct cost of human *S. suis* infection for the whole country is the product of the annual number of *S. suis* cases (n) and the average direct cost for treating one typical episode of *S. suis* infection (Q).

$$Direct\ Cost = n \times Q$$

Calculation of indirect costs

The indirect component encompasses patient's productivity losses related to short-term morbidity (acute infection, post-discharge recovery) ($IC_{morbidity\ ST}$), premature mortality ($IC_{mortality}$), and long-term morbidity due to sequelae ($IC_{morbidityLT}$).

$$Indirect\ Cost = IC_{mortality} + IC_{morbidityST} + IC_{morbidityLT}$$

The calculation of indirect costs was based on human capital approach which values the production losses due to morbidity and premature mortality by multiplying the total amount of time off work with the average wage rate [196]. To value human life in economic terms, I used the same methodology that has been recently used to calculate the

indirect costs of smoking in Viet Nam [197] from a human capital approach as described by Max et al [198]. Present Value of Lifetime Earnings (PVLE) was estimated taking into account age and sex specific life expectancy and the proportions of individuals potentially active in the workforce following the formula:

$$PVLE_{y,s} = \sum_{y}^{85+} P_{y,s}(m) * Y * E_s(m) * (1+r)^{m-y}/(1+v)^{m-y}$$

where $PVLE_{y,s}$ is the present discounted value of lifetime earnings for a person of the present age *y* and sex *s*; $P_{y,g}(m)$ is the probability that a person of age *y* and sex *s* will survive to age *m* (obtained from Life Table for Viet Nam 2008); *Y* is per capita family income (obtained from Viet Nam Household Living Standard Survey in 2010); $E_s(m)$ is the proportion of the population of sex *s* and age *m* that are employed in the labor market (average of values from Viet Nam Labour and Work Surveys 2011-2014, 82% for males and 73% for females); *r* is the growth rate of labour productivity (assumed at 6%, which approximates the average real growth rate of gross domestic product in Viet Nam between 2011-2014); *v* is the discount rate (selected at 3% to convert future earnings into current worth).

Indirect cost due to mortality

$$IC_{mortality} = CFR \times \sum(n_{sa} \times PVLE_{sa}),$$

where n_{sa} is the number of *S. suis* cases per year by sex (*s*) and 5-year age group (*a*) and *CFR* is average case fatality rate of *S. suis* infection. $PVLE_{sa}$ is the present value of lifetime earnings for each sex and age group. A fixed case fatality rate was applied for all sex and age groups each year.

Indirect cost due to short-term morbidity

$$IC_{morbidity\ ST} = n \times P_{ST}$$

where P_{ST} is the average income loss per episode of hospitalization due to lost productive time spent for treatment of acute infection in hospital. This was estimated from the patient cohort at NHTD as described in the previous chapter. One common approach of calculating productivity loss is to multiply the average amount of time lost with the national average per capita income. However, this methodology has been criticized for overestimating the economic impact of sick days and a friction cost has been proposed to count only the production loss while finding a replacement worker [161]. However, this later approach is not suitable for estimating the cost of illness to the affected households and for non-formal work such as household farming which are the main occupation among *S. suis* patients.

Indirect cost due to long-term morbidity

Indirect cost due to long-term conditions (e.g. loss of hearing and loss of balance) was estimated in a similar approach used for premature mortality, with an additional assumption that the earning level of person with a specific health condition would be less than that of a normal person. The level of earning reduction for patients survived with hearing loss was based on the existing literature. Mohr et al estimated that people with severe to profound hearing loss in the U.S. earn 50-70% of normal people [199]. The following formula was used for calculation of indirect costs due to long-term hearing loss:

$$IC_{morbidity\ LT} = V \times HLR \times \sum(n_{sa} \times PVLE_{sa}),$$

where *V* was fixed at 0.4 as an average value of earning reduction from Mohr et al report for people with severe to complete hearing loss compared to an average person in the population, and *HLR* is the rate of patients with severe to complete hearing loss among those who had *S. suis* infection. In this case, I assume that people with non-severe hearing loss work normally as an average person in the community, and that all patients would be able to return to paid work if I follow them for a longer period of time.

For comparison, I also used the rate of patients who were unable to resume paid work after hospital discharge to calculate the productivity loss. With this approach, indirect costs due to long-term morbidity were calculated as follows:

$$IC_{morbidity\ LT} = U \times \sum(n_{sa} \times PVLE_{sa}),$$

where *U* is the rate of patients who were unable to return to paid work after hospital discharge. The observed value for U at 9 months after discharge was 0.439 in our prospective cohort study at NHTD. The values for *U* was varied from 0.439 to 0.3, 0.2 and 0.1 and calculations was made accordingly to examine the change in the indirect costs due to long-term morbidity. For this method, I assume that patients who returned to paid work after hospital discharge have the same earnings as an average person in the community, and that the duration from the time of discharge to the time of returning back to paid work is minimal compared to the remaining working time their life.

Costs are summarized as mean, median, min and max in both Vietnamese Dong (VND) and U.S. Dollar (USD). Costs are estimated at the prices of 2014-2015 (during the time of prospective cohort study). An average exchange rate of 21.563 VND per 1 USD for the costs in years 2014-2015 (http://usd.fx-exchange.com/vnd/exchange-rates-history.html, accessed on 09 November 2016). A discount rate of 3% was applied to adjust for differences in the timing of costs when calculating for other years. Differences

in costs were tested using the Welch two-sample t-test with unequal variances on a log scale. All data processing and analysis was performed in the R software (version 3.2.2) [139].

5. 4. Results

5.4.1. Annual incidence rate of S. suis per 100 000 population in Viet Nam

Annual incidence rate based on the estimated incidence of *S. suis* infection from multiple imputation are presented in Table V-0-7.

Table V-0-7. Annual incidence per 100 000 population* calculated from the incidence estimates generated from multiple imputation

Year	Estimated rate based on imputation	95% Uncertainty Interval**	Rate based on the actual surveillance data
Negative binomial			
2011	0.394	0.368-0.420	0.128
2012	0.315	0.267-0.363	0.147
2013	0.452	0.226-0.677	0.130
2014	0.470	0.122-0.818	0.082
Poisson			
2011	0.438	0.418-0.459	0.128
2012	0.270	0.263-0.276	0.147
2013	0.322	0.284-0.360	0.130
2014	0.231	0.221-0.241	0.082
Predictive mean matching			
2011	0.318	0.306-0.331	0.128

Year	Estimated rate based on imputation	95% Uncertainty Interval**	Rate based on the actual surveillance data
2012	0.324	0.313-0.335	0.147
2013	0.255	0.250-0.261	0.130
2014	0.249	0.238-0.261	0.082

*Source of denominator population data: Statistical Yearbook of Viet Nam for the years 2011-2014, published by the General Statistics Office of Viet Nam; **Higher and lower limits of the uncertainty intervals for the incidence rate were obtained from higher and lower limits of the total number of incident cases estimated through multiple imputation.

As the predictive mean matching method yielded more consistent and stable results, the incidence rates estimated from this method were used for DALY estimation. I estimated an annual rate between 0.25-0.32 cases per 100 000 population during the period from 2011-2014 using the predictive mean matching method, compared to the very low rate of 0.08-0.15 cases per 100 000 as captured by the surveillance system.

5.4.2. Disability Adjusted Life Years lost to *S. suis* infection in Viet Nam

The estimated numbers of DALYs lost due to human *S. suis* infection in Viet Nam are 1 810, 1 373, 1 323, and 1 401 for the years 2011, 2012, 2013 and 2014, respectively (Table V-0-8). The high number of cases in 2011 explained the higher number of DALYs estimated for this year, while the low case fatality rate reduced the DALY burden in 2012. Overall, deaths due to acute infection accounts for the largest proportion of DALYs lost every year, except the year 2012 (Figure V-0-5). If an average case fatality rate of 9.95% was applied for the year 2012 instead of 6.15%, the DALY burden of disease for this year mounted to a total of 2 021 years (95%UI: 1 957-2 086) of which mortality accounted for 1 107 years (95%UI: 1 094-1 120).

Years of life lived with profound/ complete hearing loss with vestibular dysfunction accounted for for the second large share of DALYs attributable to human *S. suis* infection. The DALY burden was disproportionately distributed towards men and people of working age (Table V-0-9). On average, the mean number of DALYs lost per case of human *S. suis* infection was 5.88 (95%CI 5.73-6.04). At a population level, the disease claimed between 1.5 to 2 DALYs per 100 000 lost to mortality and morbidity each year.

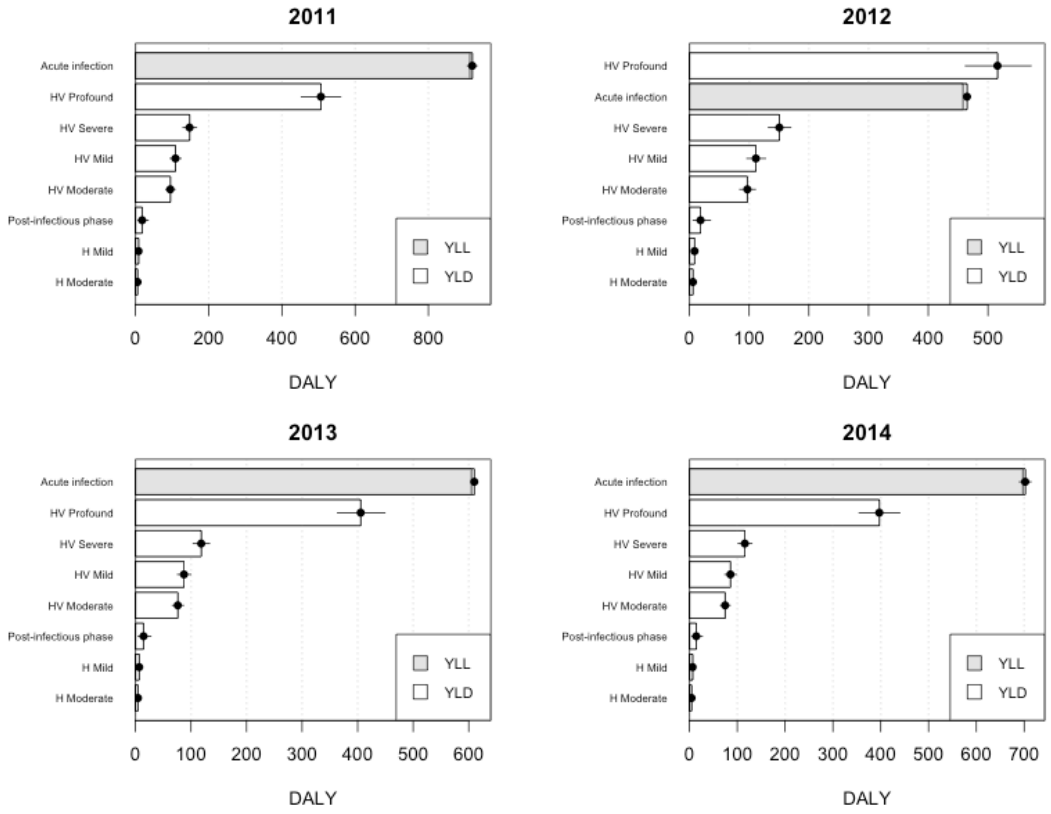
Uncertainty in the disability weights of profound/ complete hearing loss has the greatest influence on the overall uncertainty in the values of DALYs estimated, followed by the disability weights for other conditions (Figure V-0-6). Variation in duration of post-infectious phase also has significant impact on the uncertainty of DALYs estimate.

Table V-0-8. Number of DALYs, YLDs, and YLLs lost due to human *S. suis* infection

Year	Annual total (years)			DALYs per 100 000 population	DALYs per 100 000 adults (≥15 years old)
	YLL	**YLD**	**DALY**		
	913 (900-927)	897 (836-962)	1 810 (1 747-1 876)	2.06 (1.99-2.14)	2.73 (2.64-2.83)
2012	458 (453-464)	914 (852-979)	1 373 (1 310-1 437)	1.55 (1.48-1.62)	2.05 (1.96-1.44)
2013	605 (600-610)	718 (669-769)	1 323 (1 274-1 374)	1.48 (1.42-1.53)	1.96 (1.88-2.03)
2014	697 (685-709)	704 (655-754)	1 401 (1 350-1 453)	1.55 (1.49-1.60)	2.05 (1.97-2.12)

Data are presented as number of years (95% uncertainty interval); YLL: Year of life lost due to premature death; YLD: Year of life lived with disability; DALY: Disability adjusted life year. Neither age weighting nor discount rate was applied in the calculation.

Figure V-0-5. DALYs lost due to *S. suis* infection by condition

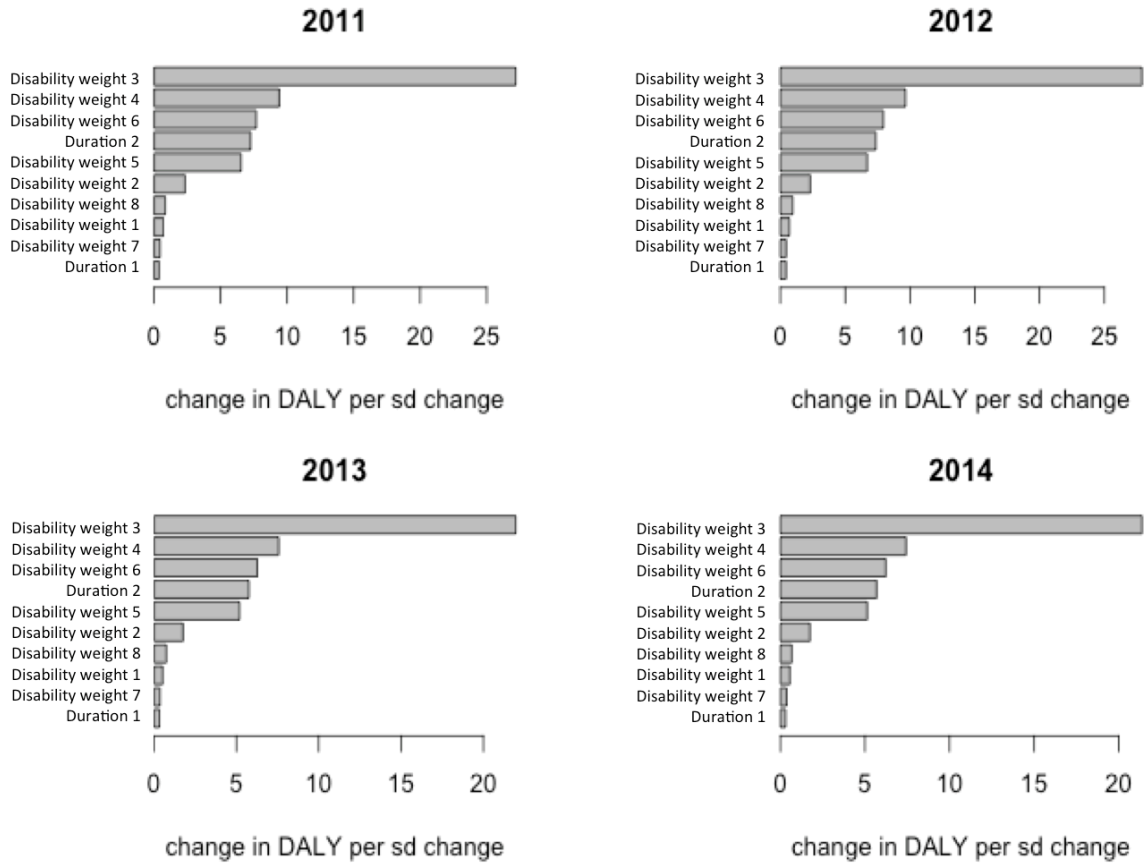


(H: Hearing loss, HV: Hearing loss and Vestibular dysfunction)

Table V-0-9. DALY, YLD, YLL estimates by health states associated with *S. suis* infection

Year	Factor	Subgroup	YLL (95%CI)	YLD (95%CI)	DALY (95%CI)
2011	Sex	Male	783 (140-1 165)	781 (36-1 332)	1564 (178-2 398)
		Female	130 (101-153)	116 (40-164)	246 (141-313)
	Age	15-44	400 (89-724)	474 (96-894)	874 (187-1 606)
		45-59	431 (98-778)	396 (96-733)	828 (196-1 499)
		60+	81 (67-97)	27 (24-31)	109 (94-125)
2012	Sex	Male	393 (71-583)	796 (37-1 356)	1188 (108-1 890)
		Female	65 (51-76)	119 (40-167)	184 (92-241)
	Age	15-44	201 (46-362)	482 (97-911)	684 (144-1 267)
		45-59	216 (49-389)	404 (98-747)	620 (148-1 129)
		60+	41 (34-49)	28 (24-32)	69 (61-78)
2013	Sex	Male	519 (94-765)	625 (29-1 065)	1144 (123-1 770)
		Female	86 (67-101)	93 (32-131)	180 (100-230)
	Age	15-44	265 (60-475)	379 (77-715)	644 (137-1 185)
		45-59	286 (66-511)	317 (77-587)	603 (144-1 092)
		60+	54 (45-63)	22 (19-25)	76 (66-87)
2014	Sex	Male	598 (107-893)	613 (28-1 045)	1211 (137-1 860)
		Female	99 (76-117)	91 (31-128)	190 (108-241)
	Age	15-44	306 (68-555)	371 (75-701)	666 (144-1 245)
		45-59	329 (74-597)	311 (75-576)	640 (151-1 161)
		60+	62 (51-75)	21 (19-24)	84 (72-97)

Figure V-0-6. Sensitivity analysis for estimation of DALYs lost due to *S. suis* infection



1: Acute infection;
2: Post-infectious phase;
3: Hearing loss with vestibular dysfunction/ profound to complete;
4: Hearing loss with vestibular dysfunction/ severe;
5: Hearing loss with vestibular dysfunction/ moderate;
6: Hearing loss with vestibular dysfunction/ mild;
7: Hearing loss without vestibular dysfunction/ moderate;
8: Hearing loss without vestibular dysfunction/ mild;

5.4.3. Direct costs of *S. suis* infection per hospitalization

The mean direct cost of *S. suis* infection was 35 249 thousand VND (USD 1 635) per confirmed *S. suis* hospitalization calculated based on data from 48 prospective patients, of which the medical cost was 22 559 thousand VND (USD 1 046) and non-medical cost was 12 688 thousand VND (USD 588). Table V-0-10 and Table V-0-11 present the

detailed direct costs broken down by insurance coverage and hearing impairment severity.

Table V-0-10. Direct medical costs of *S. suis* infection by healthcare insurance coverage and hearing impairment

	Direct medical (thousand VND)				Direct medical (USD)			
	Mean	Median	Min	Max	Mean	Median	Min	Max
All patients	22 559	20 018	1 602	66 717	1 046	928	74	3 094
Insurance coverage								
Yes	14 634	12 656	1 602	46 880	679	587	74	2,174
No	30 830	27 983	7 483	66 717	1 430	1 298	347	3 094
Hearing impairment at discharge								
Threshold ≤64dB	19 069	17 068	1 602	60 271	884	792	74	2 795
Threshold >64dB	27 558	27 983	7 483	66 717	1 278	1 298	347	3 094
Hearing impairment at 9 months								
Threshold ≤64dB	19 811	17 068	1 602	60 271	919	792	74	2 795
Threshold >64dB	28 507	24 784	7 483	66 717	1 322	1 149	347	3 094

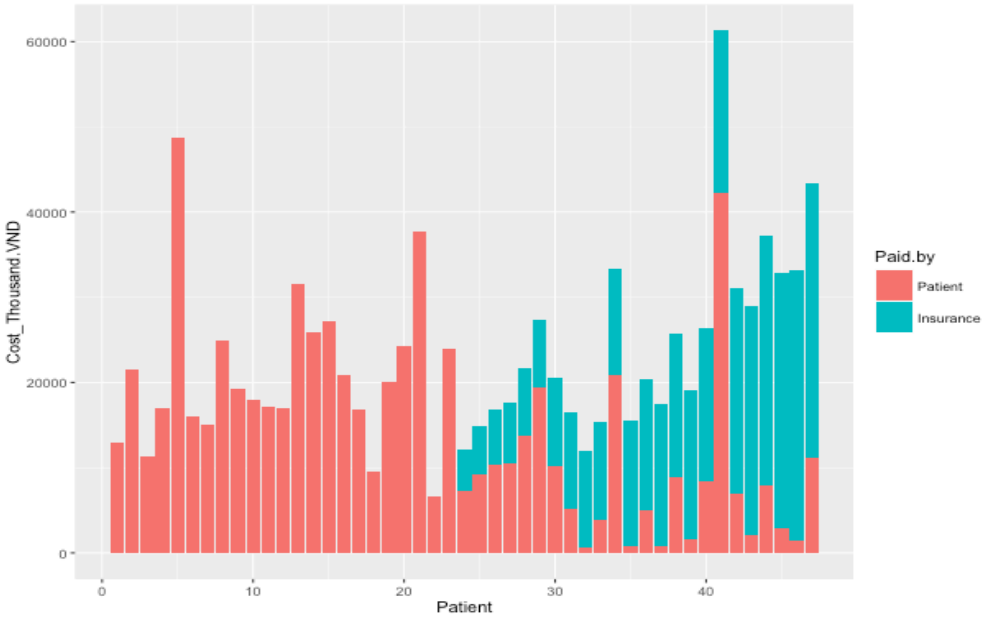
Only half of the patients had insurance coverage (24/47), and amongst those insured, however coverage levels varied between patients from 29% to 96% of the total hospital costs (Figure V-0-7). Patients without health insurance coverage had to pay about twice higher medical costs than those with coverage (30 830 versus 14 634 thousand VND, significant difference by p-value <0.001 from Welch two sample t-test with unequal variances on a log scale). Patients with higher hearing impairment severity also paid significantly higher medical costs (16 483 versus 10 917 thousand VND for patients with

hearing threshold > 64dB and ≤64dB at 9 months follow-up respectively, p-value = 0.03). However, no significant difference was observed for direct non-medical costs between groups by insurance and by hearing impairment severity.

Table V-0-11. Direct non-medical costs of *S. suis* infection by healthcare insurance coverage and hearing impairment

	Cost (thousand VND)				Cost (USD)			
	Mean	Median	Min	Max	Mean	Median	Min	Max
All patients	12 688	9 000	0	40 000	588	417	0	1 855
Insurance coverage								
Yes	12 250	9 500	0	40 000	568	441	0	1 855
No	11 727	7 000	600	37 500	544	325	28	1 739
Hearing impairment at discharge								
Threshold ≤64dB	10 264	8 000	600	30 200	476	371	28	1 401
Threshold >64dB	15 536	12 420	0	40 000	720	576	0	1 855
Hearing impairment at 9 months								
Threshold ≤64dB	10 917	8 800	600	29 000	506	408	28	1 345
Threshold >64dB	16 483	10 710	0	40 000	764	497	0	1 855

Figure V-0-7. Direct medical costs paid by patient and insurance



5.4.4. Total annual direct costs of human *S. suis* infection in Viet Nam

The total direct cost of human *S. suis* infection was approximately 10.7 billion VND (~500 000 USD) in 2011 and 2012 and about 8 billion VND (~370 000 in 2013 and 385 000 USD in 2014) (Table V-0-12).

Table V-0-12. Total annual direct costs of *S. suis* infection

Year	Annual direct cost (million VND)			Annual direct cost (USD)		
	Estimated total cost	Lower bound	Upper bound	Estimated total cost	Lower bound	Upper bound
2011	10 773	3 067	25 972	499 622	142 241	1 204 480
2012	10 755	3 083	25 771	498 771	142 982	1 195 135
2013	8 321	2 414	19 708	385 913	111 963	913 971
2014	7 977	2 256	19 338	369 932	104 631	896 826

Costs were calculated at the 2014 monetary value. Discount rate of 3% was applied.

5.4.5. Productivity change after hospitalization

Twenty-three out of 41 patients (56.1%) who were active in the paid work market before hospitalization returned to paid work by 9 months after hospital discharge (Table V-0-13). Among these, eight had to change their job due to their health conditions which did not allow them to perform heavy duties such as construction work. The mean duration from the time of hospital discharge to the time of resuming paid work was about 3 months. The reported number of hours spent on paid work per week was also significantly reduced (mean reduction of 13.65 hours, 95%CI 2.61-24.69, p-value = 0.018 paired t-test). Average income earned by patients and per-capita family income per month appeared to be lower by 9 months after discharge compared to that before hospitalization, however this change was not statistically significant.

Patients who had hearing impairment (threshold >34dB) had a 2.57 times higher risk of unemployment at 9 months after discharge compared to other patients (63.6% versus 23.5%, age adjusted PR 2.57, 95%CI 1.17-7.86). 62.5% of patients with balance problem (failure at any condition of the m-CTSIB) were also more likely to be unemployed at 9 months compared to other patients (20%) (age adjusted PR 2.93, 95%CI 1.19-11.30).

Table V-0-13. Work and income of patients before hospitalization and 9 months after hospital discharge

Variable	Summary values*
Patients engaged in labour force before hospitalization	41/47 (87.2%)#
Patients returned to paid work at 9 months after discharge	23/41 (56.1)#
Severe-complete hearing loss	5/14 (35.9)§
Non-severe hearing loss	16/25 (64.1)
Duration from time at discharge to time returning to work**	3.22 (2.34)
(months)	3 (0.5-9)
Number of hours spent on paid work per week before	51 (20.7)
hospitalization (All patients)	56 (14-84)
Number of hours spent on paid work per week before	50.5 (22.8)
hospitalization (Among those returning to work by 9 months)	56 (14-84)
Number of hours spent on paid work per week by 9 months	40.5 (19.2)
after discharge	45 (14-70)
Income earned by patient per month before hospitalization	6 026 (7 692)
(thousand VND) (All patients)	3 000 (200-32 000)
Income earned by patient per month before hospitalization	7 271 (8 495)
(thousand VND) (Among those returning to work by 9	4 000 (840-32 000)
months after discharge)	
Income earned by patient per month by 9 months after	5 106 (4 229)
discharge (thousand VND)	4 530 (560-16 000)
Per-capita family income per month before hospitalization	1 663 (1 022)
(thousand VND) (All patients)	1 500 (100-4 500)

Variable	Summary values*
Per-capita family income per month before hospitalization (thousand VND) (Among those returning to work by 9 months)	2 048 (923) 2 000 (667-4 000)
Per-capita family income per month by 9 months after discharge (thousand VND)	1 758 (1 115) 1 667 (167-5 200)

Data presented in this table were from the cohort of prospectively enrolled patients at NHTD described in Chapter IV; *Values in each row are mean (sd) in the first line and median (min-max) for the second line or otherwise specified; # count/denominator (%);§Two patients did not have any audiology test. Chi-square test for difference in probability to return to paid work by 9 months had a p-value of 0.09. **Among those who already returned to work by 9 months after hospital discharge

5.4.6. Indirect costs due to mortality caused by *S. suis* infection in Viet Nam

The total indirect cost due to premature deaths caused by *S. suis* infection was highest in 2011 (25 796 million VND) and lowest in 2012 (13 050 million VND) (Table V-0-14, Table V-0-15, Table V-0-16, and Table V-0-17). Indirect costs were sensitive to the number of incident cases and the case fatality rate each year. The case fatality rates applied were the reported rates from the surveillance system, and it was only 6.15% in 2012 compared to 10.34% in 2013, 12.16% in 2014 and 12.50% in 2011. If an average rate of 9.95% was applied for 2012, the indirect cost due to premature deaths would be 21 114 million VND, comparable to the costs calculated for the other years. On average, the indirect cost due to premature mortality per case of hospitalization was 74.9 million VND. As expected, men and working age groups constituted dominant shares in indirect costs due to premature deaths in all years.

Table V-0-14. Annual indirect costs of premature mortality caused by *S. suis* infection in 2011

Factor	Subgroup	Cost (million VND)			Cost (thousand USD)		
		Estimated total cost	Lower bound	Upper bound	Estimated total cost	Lower bound	Upper bound
All		**25 796**	**24 773**	**26 820**	**1 196**	**1 149**	**1 244**
Sex	Male	22 041	21 166	22 915	1 022	982	1 063
	Female	3 756	3 607	3 905	174	167	181
Age	15-44	13 127	12 606	13 648	609	585	633
	45-59	11 067	10 628	11 507	513	493	534
	60+	1 602	1 539	1 666	74	71	77

Table V-0-15. Annual indirect costs of premature mortality caused by *S. suis* infection in 2012

Factor	Subgroup	Cost (million VND)			Cost (thousand USD)		
		Estimated total cost	Lower bound	Upper bound	Estimated total cost	Lower bound	Upper bound
All		**13 050**	**12 619**	**13 486**	**605**	**585**	**625**
Sex	Male	11 150	10 782	11 522	517	500	534
	Female	1 900	1 837	1 963	88	85	91
Age	15-44	6 641	6 421	6 862	308	298	318
	45-59	5 599	5 414	5 786	256	251	268
	60+	811	784	838	38	36	39

Table V-0-16. Annual indirect costs of premature mortality caused by *S. suis* infection in 2013

Factor	Subgroup	Cost (million VND)			Cost (thousand USD)		
		Estimated total cost	Lower bound	Upper bound	Estimated total cost	Lower bound	Upper bound
All		**17 486**	**17 112**	**17 860**	**811**	**794**	**828**
Sex	Male	14 940	14 621	15 260	693	678	708
	Female	2 546	2 491	2 600	118	116	121
Age	15-44	8 898	8 708	9 088	413	404	421
	45-59	7 502	7 342	7 662	348	340	355
	60+	1 086	1 063	1 109	50	49	51

Table V-0-17. Annual indirect costs of premature mortality caused by *S. suis* infection in 2014

Factor	Subgroup	Cost (million VND)			Cost (thousand USD)		
		Estimated total cost	Lower bound	Upper bound	Estimated total cost	Lower bound	Upper bound
All		**20 304**	**19 370**	**21 228**	**942**	**898**	**984**
Sex	Male	17 348	16 550	18 137	805	768	841
	Female	2 956	2 820	3 090	137	131	143
Age	15-44	10 332	9 857	10 802	479	457	501
	45-59	8 711	8 311	9 107	404	385	422
	60+	1 261	1 203	1 318	58	56	61

5.4.7. Indirect costs due to morbidity caused by *S. suis* infection in Viet Nam

Combining both short-term and long-term conditions, the total indirect cost due to morbidity was 33 980 million VND in 2012, 34 939 million VND in 2012, 27 845 million VND in 2013 and 27 492 million VND in 2014 using the rate of productivity reduction of 40% among people with severe to profound hearing loss (Table V-0-18, Table V-0-19, Table V-0-20, Table V-0-21). On average, the indirect cost due to morbidity per case of hospitalization was 117.8 million VND. The total morbidity induced indirect cost was only sensitive to the number of incidence cases across 4 years.

In the second method using the rate of patients returning to paid work after hospital discharge, the estimated indirect costs were about three times higher than those calculated with the method 1, assuming 43.9% of patients unable to return to work after illness (Figure V-0-8). The costs calculated by this method were still higher than that estimated by method 1 even when the rate of patients unable to return to paid work was reduced to 20%.

Table V-0-18. Annual indirect costs of morbidity caused by *S. suis* infection in 2011 using the rate of 40% reduction in earnings

Factor	Subgroup	Cost (million VND)			Cost (thousand USD)		
		Estimated total cost	Lower bound	Upper bound	Estimated total cost	Lower bound	Upper bound
All		**33 980**	**32 631**	**35 328**	**1 576**	**1 513**	**1 638**
Sex	Male	29 015	27 863	30 167	1 346	1 292	1 399
	Female	4 964	4 767	5 162	230	221	239
Age	15-44	17 091	16 413	17 769	793	761	824
	45-59	14 656	14 075	15 238	680	653	707
	60+	2 232	2 143	2 321	104	99	108

Table V-0-19. Annual indirect costs of morbidity caused by *S. suis* infection in 2012 using the rate of 40% reduction in earnings

Factor	Subgroup	Cost (million VND)			Cost (thousand USD)		
		Estimated total cost	Lower bound	Upper bound	Estimated total cost	Lower bound	Upper bound
All		**34 939**	**33 785**	**36 106**	**1 620**	**1 567**	**1 674**
Sex	Male	29 835	28 849	30 831	5 105	4 936	5 275
	Female	1 384	1 338	1 430	237	229	245
Age	15-44	17 574	16 993	18 161	815	788	842
	45-59	15 070	14 573	15 573	699	676	722
	60+	2 295	2 219	2 372	106	103	110

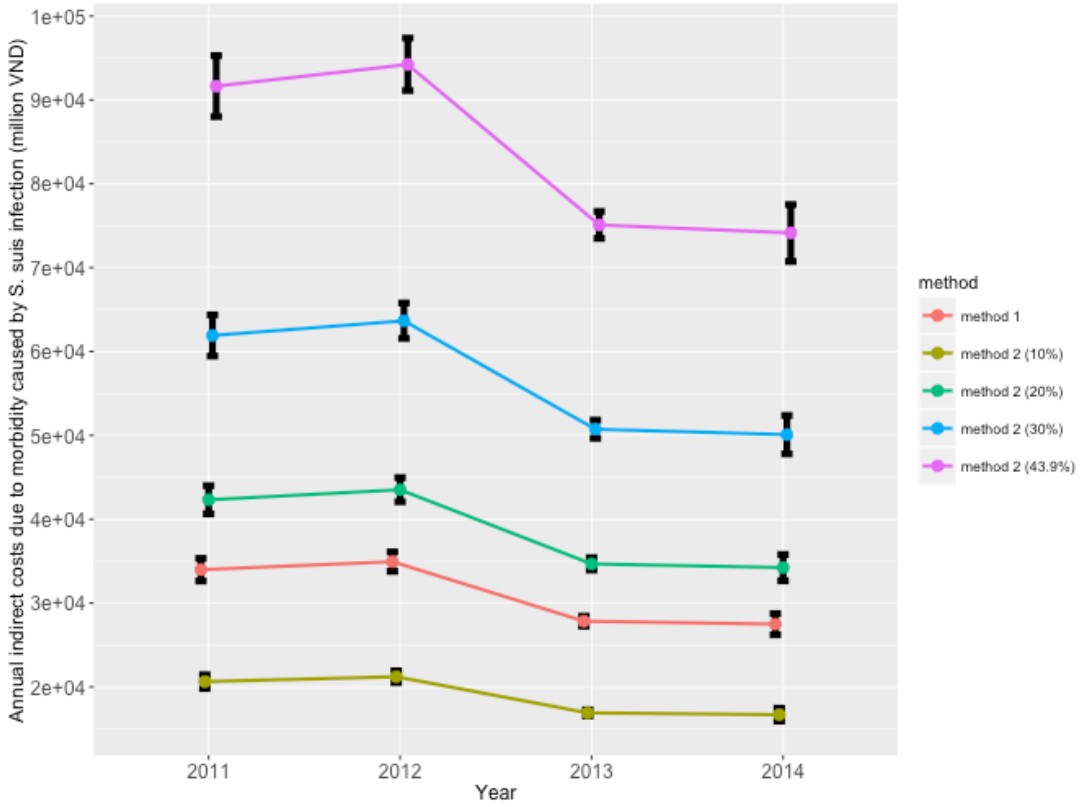
Table V-0-20. Annual indirect costs of morbidity caused by *S. suis* infection in 2013 using the rate of 40% reduction in earnings

Factor	Subgroup	Cost (million VND)			Cost (thousand USD)		
		Estimated total cost	Lower bound	Upper bound	Estimated total cost	Lower bound	Upper bound
All		**27 845**	**27 249**	**28 440**	**1 291**	**1 264**	**1 319**
Sex	Male	23 776	23 268	24 285	1 103	1 079	1 126
	Female	4 068	3 981	4 155	189	185	193
Age	15-44	14 005	13 706	14 305	650	636	663
	45-59	12 010	11 753	12 267	557	545	569
	60+	1 829	1 790	1 868	85	83	87

Table V-0-21. Annual indirect costs of morbidity caused by *S. suis* infection in 2014 using the rate of 40% reduction in earnings

Factor	Subgroup	Cost (million VND)			Cost (thousand USD)		
		Estimated total cost	Lower bound	Upper bound	Estimated total cost	Lower bound	Upper bound
All		**27 492**	**26 229**	**28 744**	**1 275**	**1 216**	**1 333**
Sex	Male	23 476	22 397	24 544	1 089	1 039	1 138
	Female	4 017	3 832	4 199	186	178	195
Age	15-44	13 828	13 193	14 458	641	612	670
	45-59	11 858	11 313	12 398	550	525	575
	60+	1 806	1 723	1 888	84	80	88

Figure V-0-8. Indirect costs due to morbidity estimated by two methods of calculating productivity loss caused by *S. suis* infection

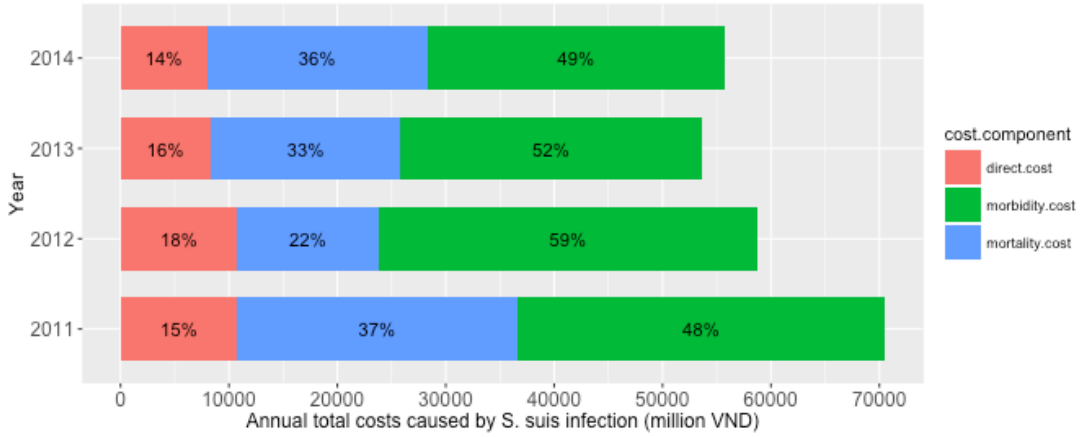


Method 1: based on the rate of 40% reduction in earnings due to hearing loss reported by Mohr et al; Method 2: based on rate of not returning to paid work after hospital discharge (data from prospective cohort: 43.9% observed at 9 months after hospital discharge, varied to 30%, 20% and 10% accordingly)

5.4.8. Total annual costs of *S. suis* infection in humans in Viet Nam

Total costs of *S. suis* infection per year by cost component is presented in Figure V-0-9. The estimated total cost was about 54-70 billion VND (2.5-3.3 million USD) every year in Viet Nam, which was highest for 2011 and lowest for 2013. Indirect costs due to premature deaths and morbidity account for over 80%, and the costs due to morbidity constitute approximately half of the total costs.

Figure V-0-9. Annual total costs of *S. suis* infection by cost component



5.4.9. Other impacts of *S. suis* infection on the patients and their family

When interviewed at hospital discharge, 95.6% of respondents (patients or their family caregivers in case the patients could not attend to the interview) stated that *S. suis* infection left a great impact on their family (Table V-0-22). This proportion declined by 3 months and 9 months after hospital discharge. However, the great negative impact of the disease was still expressed by many patients by 9 months (43.5%). Reduced productivity in paid work was the leading factor causing negative impact on the patients and their family at all interview time-points, followed by the increase in health related expenses (Table V-0-23). Taking out loans was another factor that many families had to resort to in the event of hospitalization due to the disease, and for some families they still had to continue to pay loan interest even by 9 months after discharge.

Other impacts and issues described qualitatively include poverty (some families requested certificate of disabling health conditions to get government's subsidies for the poor); affected economic activities (a patient who was a pork seller at a local wet market complained that after illness her earnings were reduced because the local residents knew

about her illness and lost trust in her products, affected agricultural outputs due to lack of farm labor); health insurance issues (many patients had no health insurance cover; some patients discharged after a short time period of admission without insurance and then readmitted again after getting insurance coverage); reduced food expenses (milk for children, normal food for other family members); psychological impact (patient was deaf and dizzy and became irritable with other family members, family was also affected psychologically, worried about health and future); impact on social communication and relationships (due to deafness and dizziness).

Table V-0-22. Negative impact of *S. suis* infection on affected families rated by patients and their family members at hospital discharge and at 3 months and 9 months after hospital discharge

Interview time-point	Great impact	Some impact	No impact
At discharge (n=45)	43 (95.6%)	2 (4.4%)	0 (0%)
3 months (n=31)	18 (58.1%)	6 (19.4%)	7 (22.6%)
9 months (n=46)	20 (43.5%)	11 (23.9%)	15 (32.6%)

Table V-0-23. Proportion of patients and their families reported specific negative impacts due to *S. suis* infection at different time-points

	Discharge (n=45)	3 months (n=24)	9 months (n=31)
Reduced productivity in paid work	36 (80%)	23 (95.8%)	27 (87.1%)
Reduced productivity in unpaid work	18 (40%)	8 (33.3%)	8 (25.8%)
Increased expenses in health matters	34 (75.6%)	11 (45.8)	12 (38.7%)
Cutting back on clothing	10 (22.2%)	9 (37.5%)	5 (16.1%)
Cutting back on leisure/ social activities	6 (13.3%)	8 (33.3%)	7 (22.6%)
Cutting back educational expenses	2 (4.4%)	1 (4.2%)	2 (6.5%)
Use the savings	16 (35.6%)	0 (0%)	1 (3.2%)
Take out loans	33 (73.3%)	9 (37.5%)	9 (29%)
Sell capital assets	6 (13.3%)	2 (8.3%)	2 (6.5%)

5.5. Discussion

5.5.1. Incidence of human S. suis infection in Viet Nam

Despite the fact that reporting of human *S. suis* cases to the national surveillance system has been mandatory for several years, the quality of surveillance data is less than optimal. Using the reported incidence data from this system, the incidence rate would be 0.128, 0.147, 0.130 and 0.082 cases per 100 000 population in 2011, 2012, 2013 and 2014 respectively. This rate is rather low, compared to the rates estimated in a prospective observational study in twelve selected provinces in the southern part of Viet Nam during 2070-2010 [19]. They estimated an annual incidence rate of 0.57 cases per 100 000 person-years (95%CI 0.47-0.70) in the adult population, equivalent to 0.422

cases per 100 000 person-years for the whole population. It is likely that the rates based on surveillance data are underestimated. Underestimation occurs when a surveillance system fails to capture the true incidence of infections in the targeted population, and it can affect the efficiency and reliability of the system in monitoring disease trends and outbreaks [162].

I used a statistically principled method to estimate the true values for incidence rates based on reported data from the existing surveillance system with the assumption that provinces with a zero value potentially represent missing data. With the simplicity and flexibility in applying the imputation model across different years in the context of limited amount of data, the multiple imputation approach was preferred to other approaches (Bayesian regression approach, see Appendix 7). Incidence estimates based on both count models including Poisson and negative binomial model sometimes generated invalid values of disease incidence and very large variances. In addition, imputation models sometimes failed to find appropriate values for parameters in the alternating process. The predictive mean matching method appeared to work stably for data in all four years, and therefore could be an effective method for predicting the incidence of *S. suis* infection in the context of poor data quality and data scarcity. With this method, from 9 auxiliary variables, the annual incidence rate of *S. suis* infection in the general population in Viet Nam was estimated at approximately between 0.25 to 0.32 per 100 000 population in 2011-2014. This estimate is closer to the observed rate reported in the prospective study in southern part of Viet Nam in previous years [19].

5.5.2. Health burden of disease due to human S. suis infection in Viet Nam

The DALY estimate in this study provides a summary snapshot of the total annual health impact of human *S. suis* infection on population health in Viet Nam. Both the impact of premature deaths and long-term disability are integrated into this outcome

measure, each accounting for approximately half of the total burden. The lower share of mortality in 2012 was explained by a significantly lower case fatality rate reported from the surveillance system. If the average case fatality rate as was applied, I observed a similar picture of mortality and morbidity impact in the DALY estimate as in the other years. Interestingly, uncertainty in the annual incidence rate and mortality rate did not significantly affect the DALY estimate as shown in the sensitivity analysis. The sensitivity analysis was based on regression of the DALY estimate against epidemiological parameters and disability weights, which were randomly generated through Monte Carlo simulations according to their defined distributions. Uncertainty in this sensitivity analysis only takes into account estimation error[2] [200], but not the natural variability from year to year in the epidemiological parameters including incidence and mortality. This uncertainty obviously affects the total annual impact of the disease as shown in the large differences in DALYs between the years 2011-2014 in this analysis.

This highlights the importance of including a time effect in the analysis of disease burden, which I could not do within the scope of this thesis due to the inadequacy of historical incidence data as well as a lack of data in a number of important factors that can affect the annual incidence and mortality of *S. suis* infection in humans in Viet Nam. These include, but are not limited to, the occurrence of pig disease outbreaks, regulation and control of sick/dead pigs and related contaminated products, consumption patterns of high-risk pig/ pork products, changes in bacterial virulence, activity and pathogenicity, vigilance (or lack of) in monitoring and surveillance practices at all levels for this zoonosis, or the implementation of public health interventions for prevention of disease

[2] Estimation uncertainty is resulted from not knowing the true population parameters perfectly due to not having an infinite number of observations, as opposed to fundamental uncertainty, which represents the stochastic component in the model chosen to predict the outcome of interest.

transmission or medical improvements for treatment and prevention of long-term sequelae.

The use of an incidence-based approach is appropriate for studying the health burden of infectious diseases with long-term sequelae such as *S. suis* infection [180]. Using a prevalence-based approach it would have been more difficult to include the impact of hearing loss with and without vestibular dysfunction because calculating prevalence of such sensory problems attributable to a specific cause is impossible in the context of Viet Nam. The incidence approach has been used previously for enteric pathogens [180, 201-203]. To estimate the incidence of subsequent health outcomes caused by the infection, two approaches are commonly applied. The first is to use conditional transitioning probabilities and multiply with the number of original cases of infection. The second approach is to apply etiology fractions to the incidence of the health outcomes identified through the general syndrome surveillance.

The use of the latter approach is time-consuming and thus not efficient for timely monitoring of the burden of disease [180]. I used conditional probabilities following an outcome tree for *S. suis* infection to derive the number of incidence cases with hearing loss and vestibular dysfunction of different severity. These probabilities were obtained from local data sources including the surveillance system and a prospective longitudinal cohort study, and from the systematic review. To update the burden of disease every year, only the incidence rate of annual *S. suis* infection needs to be updated. This process can easily be done by using the incidence report from the existing surveillance system and using multiple imputation to predict the true incidence as described above.

A national burden of disease and injury for Viet Nam for the year 2008 has been conducted using the GBD methodology [204]. The total burden of disease in 2008 was

estimated to be 12.3 million DALYs with about 13% of these attributed to infectious diseases, neonatal and maternal conditions. Long-term consequences of infectious diseases are often neglected in the assessments of disease burden [179, 205]. In the above report, the impact of mortality constituted more than 3 quarters of the DALYs caused by such communicable conditions in Viet Nam in 2008. This implies a lack of data for long-term morbidity of infectious diseases in this country. As shown in our study, long-term morbidity contributes half of the total burden induced by *S. suis* infection. Despite causing a small number of cases compared to other common infectious diseases, the total burden caused by *S. suis* infection is comparable to one sixth of the burden estimated for diarrhea in adult men in the Viet Nam burden of disease in 2008 (12 727 DALYs) [206]. The total burden of pneumonia and upper respiratory among adult men was 90 697 and 6 301 DALYs respectively in 2008. *S. suis* caused a similar amount of DALYs in Viet Nam compared to the burden caused by *Campylobacter* spp. and non-typhoidal *Salmonella* spp. in the Netherlands [180].

The disease burden of infectious diseases such as *S. suis* infection was not included in the national burden of disease analysis, largely due to the perception that such diseases affect a small number of cases compared to other diseases, as well as the unavailability of data. It is important to note that with the rapidly changing agricultural practices and urbanization, the risk for emerging zoonotic infections can increase in magnitude and severity [205]. These diseases might not currently present a great burden compared to other diseases but monitoring the trends and impact of these zoonotic infections is necessary to improve our preparedness for outbreaks and to reduce the impact on the population. Improving health information systems including disease monitoring and surveillance is crucial for effective planning and for prioritizing scarce resources.

5.5.3. Economic consequences of human S. suis infection in Viet Nam

The economic impact of human *S. suis* infection has not been examined previously. I followed up a prospective cohort of 47 patients with confirmed *S. suis* infection at a national referral hospital to determine the direct and indirect costs associated with the disease from a patient perspective. I found a significant direct cost associated with *S. suis* infection, on average 35.2 million VND (USD 1,635) per hospitalization of which two thirds were out-of-pocket payment for direct medical costs. This amount is higher than average annual income of 31,680 thousand VND per capita in Viet Nam in 2014 as reported in the country's Statistical Yearbook. It is also much higher than the per-capita family income as reported by our cohort members, which was only approximately 24,000 thousand VND per annum. Over two thirds of our cohort was employed in non-skilled work including farming and manual labour. In addition, most of the patients were the main income source of their family. The cost of hospitalization caused by *S. suis* infection created a sudden increase in the health related expenditures for patients and their family. Added to this cost burden was the significant loss in productivity for many patients due to disability caused by long-term conditions including hearing loss and dizziness. The impact of reduced productivity in the longer term is on average 6 times higher than the direct cost associated with acute infection.

One study quantified the treatment costs of invasive bacterial diseases among children in a provincial hospital in Viet Nam, from a health system perspective and reported an average treatment cost ranging from USD44 per suspected meningitis case to USD164 per definite meningitis case in 2006 [207]. They also estimated that the cost for meningitis was about twice that for pneumonia. In another study in children at a tertiary central hospital, the mean direct medical cost per case of meningitis was USD 300 from a health sector perspective in 2012 [208]. The mean direct medical cost for an adult case

with meningitis as calculated from our study cohort is more than three times the costs determined for children in these studies. Comparison of the costs across different studies might be limited due to different frames of cost analysis, year of estimation, and level of healthcare settings. However, the higher hospital costs reported in my study are likely to reflect the higher levels of disease severity and sequelae of *S suis* infection. I only calculated the direct medical cost from a patient perspective, excluding the costs shared by insurance and the labour and capital costs supported by the government.

The direct costs for *S. suis* infection is also higher than the costs for several other common acute infections. The average direct medical and non-medical costs were USD32.7 and 28.7 respectively in 2005 for dengue hemorrhagic fever observed at a children hospital in Ho Chi Minh City [209]. In one recent study at NHTD (the same hospital as in our study) in 2013, the direct medical cost per episode of foodborne illness was USD67.7 while the direct non-medical cost was USD62.4 on average [210]. The direct cost per case of hospitalization cause by *S. suis* infection is comparable to the annual direct cost of chronic hepatitis B infection and cirrhosis in Viet Nam. The average annual direct medical cost per patient in 2008 was USD 450, USD 690, USD 1 114, and USD 1 883 for chronic hepatitis B infection, compensated cirrhosis, decompensated cirrhosis and hepatocellular carcinoma, respectively [211].

The direct medical costs did not include long-term audiologic assessment and fitting of assistive devices, while special education and rehabilitation including vocational training for hearing impaired people is not counted under the indirect costs. However such services are not common in most low-income settings in Viet Nam. In addition, such costs tend to be minimal compared to the costs of productivity loss. The relatively high rates of case fatality and long-term impairment in *S. suis* patients contribute to the high indirect costs of the disease which account for well over 80% of the estimated total costs.

Patients with more severe hearing loss were less likely to return to paid work after their illness as suggested in our cohort data. Our data also showed that hearing loss reduces patient's mobility and active participation in the society (as shown in Chapter IV). Approximately 50% of the annual total costs are caused by productivity loss due to severe to profound hearing loss using the rate of reduced productivity reported by Mohr et al e [199]. In their analysis from a societal perspective, productivity loss accounts for over 85% compared to 11% incurred by associated medical costs and costs of assistive devices for hearing loss acquired at the working age [199]. In our analysis, I did not include potential productivity loss associated with non-severe hearing loss. The large burden created by lost productivity has also been shown in many other diseases such as malaria, TB, and AIDS [212].

It is also important to note that families affected by illness often need to make decisions about treatment and manage their financial resources to pay for treatment and replace the lost income as a result of absence from work [212]. It is often argued that estimating productivity loss without taking into account such coping strategies will overestimate the indirect costs of illness [161]. In some settings where work is collectively responsible for by the whole household, workdays lost by the patient could be filled by other household members. Nonetheless, coping strategies are often not costless. In our study, taking out loans was the main immediate response by the families in order to have money to pay off the hospitalization costs. This is particularly the case for families who do not have their own savings and and for whom farming is their primary work for subsistence. Studies in developing countries indicated that families' coping capacity depend on their established social networks, and the poorest families often have the weakest network support for finding financial resources they need in these

circumstances [212-214]. Poor families are often forced to borrow money from high-interest sources as reported in some families in our study.

5.5.4. Study limitations and implications

Findings from our estimation of health and economic burden should be interpreted in light of its potential limitations. First, some epidemiological parameters for long-term health outcomes were drawn from our prospective cohort, which followed up patients for 9 months. Health outcomes might still change after 9 months, and thus affect the transitioning probabilities and thereby the incidence of hearing loss and vestibular dysfunction and the estimated number of DALYs and the amount of economic costs. However, when I compared health outcomes among patients after 9 months and patients who had been discharged for a longer time period, I found similar rates (Chapter IV). Therefore any change in the burden estimates caused by change in the transitioning probabilities would likely be minimal.

One might also argue that such probabilities might not be representative for the whole country since the cohort was based in the national hospital for tropical diseases located in the north. However, the rate of severe to profound hearing loss from our cohort was comparable to the rate reported in one other study in a hospital for tropical diseases located in the south of Viet Nam [20] (see also Chapter IV for further discussion). Data from our follow-up cohort also allowed us to have more solid estimates for some epidemiological parameters than can be obtained from the existing literature such as duration of post-acute phase, rate of co-existing hearing loss and vestibular dysfunction, and some other productivity related parameters. The cohort data therefore lend more strength to our analysis of disease burden and economic burden of *S. suis* infection, especially in the context of limited health information systems with poor data quality.

Another potential limitation was that I used the average per-capita monthly income reported in the national statistical yearbook to calculate the potential value of lifetime earnings for both males and females. Consequently, I might have underestimated the productivity cost caused by premature mortality and morbidity because *S. suis* patients are predominantly men, while men are often reported to have higher levels of earnings than women [215]. In addition, I did not have data to estimate the value of lifetime earnings attributed to housework activities by a person as suggested in the original approach [198] following the human capital approach. I followed the same methodology to calculate the lifetime earnings for estimating indirect costs of smoking in Viet Nam [197].

I used an incidence-based approach to quantify the burden of disease and economic impact and assigned the impact of all consequences to the year of infection. Incidence-based approach can provide useful information on the costs that interventions can save if they could prevent the disease and its sequelae from happening [196]. It is a reasonable approach for costing an illness with long-term sequelae such as *S. suis* infection, especially in the context where data are lacking to monitor the causes of certain conditions such as hearing loss. One related difficulty was estimating the incidence of *S. suis* infection based on the incomplete surveillance data. I have tried several statistically principled methods, however, due to the large quantity of missing data and small number of observations, more sophisticated approaches did not work well. As a result, I had to opt for the simplest approach to data imputation using predictive mean matching technique. With this approach I still can incorporate some uncertainty in the incidence estimate, although the variation was relatively small and might not capture the true incidence in the population. Nevertheless, our estimates were comparable to the estimated incidence in an observational study for the previous years [19]. The strength in our

approach is that it can be easily applied to any year based on the current surveillance data, and therefore the burden of disease and economic impact can be easily updated accordingly.

5.6. Conlusion

In conclusion, this analysis provided a comprehensive picture of the health and economic burden of *S. suis* infection in humans in Viet Nam. Data were presented in DALYs, a common summary population health metric that can be used to compare across different health conditions and therefore can support decision-making and prioritizing scarce health resources. The economic cost analysis also showed a large impact of this disease from a patient perspective, both in terms of direct costs associated with hospitalization and indirect costs caused by premature mortality and long-term hearing loss.

Chapter VI

Discussion and conclusion

The bacterium *Streptococcus suis* can cause serious and life-threatening infections in humans and result in long-term disabling conditions. *S. suis* infection is a zoonotic disease that is generally transmitted from pigs to humans through close contact or consuming contaminated pig-derived products. This zoonosis is endemic in Viet Nam and in several other regions in Asia where pig rearing and consumption are common. The improved understanding about the disease through rigorous research has not yet brought about a reduction in the disease burden. The complex epidemiological patterns of the infection, the role of human behaviour, lack of awareness, unknown source of transmission among a proportion of patients, and unresolved issues regarding bacterial virulence and disease pathogenesis [216] complicate the development of effective prevention and control strategies. In addition to these, the lack of a thorough assessment of the burden of disease hampers efforts to plan, justify and implement policies. Decision-making on policy and programs to control disease transmission and disease burden necessitates data on the impact of disease on the patients and society. Prevention and control also requires an understanding of local practices that put the community at an increased risk of acquiring the infection.

In this context, this thesis set out to answer the following specific questions that could provide practical understanding on the burden of disease due to mortality and

morbidity caused by *S. suis* infection in humans and provide a stimulus to policy development:

- Question 1: Is the consumption of raw pig blood common in the community and can it be changed or modified to reduce the risk of disease transmission?
- Question 2: Do hearing loss and vestibular dysfunction resulting from *S. suis* infection improve after hospital discharge?
- Question 3: What are the health and economic consequences of *S. suis* infection and its sequelae on the patients and their family?
- Question 4: What is the annual burden of disease caused by *S. suis* infection and its sequelae at a population level measured in years of life lost and in monetary value?

6. 1. Eating raw pig blood as an important risk factor

As shown in our systematic review (Chapter II), the risk patterns of *S. suis* infection differ between regions. In many parts of Asia, the traditional practice of consuming raw or undercooked pig products appears to play an important role in the high burden of disease locally. In the case-control study in the south of Viet Nam, high-risk pig dishes including raw pig blood and internal organs were consumed by up to one in two patients with *S. suis* infection, while occupational exposure was reported in only 27% [21]. After controlling for other potential confounders such as age, sex and medical history, high-risk consumption was significantly associated with infection with an odds ratio of 4.4 (95%CI 2.15–9.15). A higher odds ratio was estimated for exposure to pigs/ pork with skin injuries in the past 2 weeks (OR 16.0, 95%CI 3.0–85.7). However, since high-risk consumption practices are observed among a larger group of patients and is a

relatively common behavior in Viet Nam, the magnitude of the disease burden due to this risk factor is likely to be higher than less frequent risk factors in the Viet Nam context.

To gain more insights into this consumption practice for the purpose of informing the prevention of disease transmission, I implemented a mixed-methods study consisting of a quantitative survey and a qualitative assessment focusing on *tiet canh* (raw pig blood) consumption in the community. The findings from this study show that eating *tiet canh* is common, particularly in rural areas. About 30% in rural and 10% in the urban area of Ha Noi eat *tiet canh* at least once in the previous one year. This is an important finding, since it confirms that raw pig blood consumption is common in the community despite Government regulation aimed at stopping this practice. The contribution of a given risk factor to the burden of disease depends on both the risk of having the disease among people exposed relative to those without the exposure, and the frequency of the risk factor in the target population where cases come from [217]. In order to estimate the relative contribution of consuming raw pig blood or other high-risk pig dishes to the total burden of *S. suis* infection, a relative risk is required. However, a cohort study to establish the risk of *S. suis* infection among people with exposure and those without exposure has not been conducted. For a risk factor that is prevalent in the population such as raw pig blood consumption, the attributable fraction for *S. suis* infection at a population level will likely be large. Using the odds ratio reported in the above mentioned case-control study [21] as an approximation of the relative risk (with the rare disease assumption [218]), I estimated that approximately 35% of human *S. suis* cases in Viet Nam could be attributed to the consumption of high-risk pig dishes including raw pig blood. On the other hand, the fraction attributable to occupational exposure and pig/ pork contact in the presence of skin lesions is about 16% and 19%, respectively (unpublished data). I also observed a higher frequency of consuming high-risk pig dishes other than raw pig blood, such as

tongue, tonsils, intestines, stomach or uterus, in the urban setting of Dong Da (data not reported in this thesis). However, the risk of disease transmission from these dishes might be lower than from raw pig blood dishes because preparation of these dishes often includes an element of heating (e.g. boiling, although the tissue is usually not completely cooked to avoid chewiness), therefore their contribution to the disease burden is likely lower.

Tiet canh consumption and the consequence of *S. suis* infection is attracting increasing attention in the media. A simple search on two phrases "tiet canh" and "lien cau lon" (Vietnamese word for *S. suis*) in Google Viet Nam generated over 700,000 results containing both phrases. Television news items often feature *S. suis* cases after eating *tiet canh*, particularly during the peak season in summer time. The control and prevention of *S. suis* infection via consumption of risk foods requires knowledge of the behaviors, attitudes and awareness of the targeted communities, as well as the individual, environmental and contextual factors that predispose or reinforce those behaviors. Food choice and determinants have been widely explored for different food aspects in the literature. For example, Meiselman identified three broad areas that may interact when an individual consumes a food [219], including the food context (e.g. combinations with other foods, culinary tradition and culture), the eating situation (social and physical environment), and the individual (food likes and dislikes, personal tendencies e.g. to try new things, food expectations).

As part of the qualitative methods, I conducted focus group discussions and found that all three areas intertwined and shaped the practice of raw pig blood consumption in the community. While banning the consumption practice might not be easy due to the complex deeply rooted cultural interactions, communication strategies can aim at reducing the associated health risks through behavioral modifications for a safer practice.

Firstly, widespread misperceptions about the safety of the raw pig blood dish (and raw blood dish made from any other animals) and the risk of disease transmission (e.g. apparently healthy pigs or free-ranging pigs do not pose risk to consumers, or alcohol can kill all bacteria in the dish) need to be addressed in these communication messages. In addition, balanced information is needed about the risks of disease transmission to humans from sick/ dead pigs as well as from apparently healthy pigs during outbreak and non-outbreak times. Simple preventive strategies including safe hygiene practices while slaughtering, cooking, and eating pig products should be clearly demonstrated in these health education programs.

Another important issue is the timing and sustainability of such communication campaigns, which currently appeared to be ad hoc and insufficient as reflected in our discussion groups. Mass media are still an effective channel to reach the majority of the target population [220]. However, targeted campaigns are required for specific groups with higher risk of *S. suis* transmission, particularly men who are excess alcohol drinkers and manual workers whose work forces them to change their locations regularly. As the data suggested, higher consumption of raw pig blood was reported among these groups since the food price is reasonable and it is widely available in the food shops or social events. On the other hand, they are also a population that is hard to reach by mass media campaigns. Multiple communication channels can be used to increase the chance and frequency of exposure to the messages and lead to behavior change in the target audience [221]. Besides behavior change communication, other public interventions are also needed to achieve a reduction in the incidence of *S. suis* infection in humans, including interventions aiming at reducing the prevalence of bacteria in the pig populations and ensuring the quality of pork products in the production and supply chain as discussed in Chapter III.

6. 2. Progress of long-term health sequelae

As shown in the systematic review and in the cohort study, hearing and vestibular impairment are the two main sequelae. In the meta-analysis, the pooled prevalence of reported hearing loss in patients with *S. suis* infection, regardless of severity, is 39.1% with a narrow 95% CI between 31% to 47.8%. The pooled rate of recovery from hearing loss calculated from a limited number of studies was 15.4%, with a high degree of uncertainty reflected in a large CI between 5.3-37.3%. I could not calculate the specific rates for different hearing impairment levels in this review since few data reported severity levels of the impairment. The difference in the impact of non-severe hearing loss and that of profound to complete deafness is large; their corresponding disability weights are 0.011-0.037 versus 0.235 on a 0 to 1 scale [188]. Therefore, to quantify the burden of disease due to *S. suis* infection, a specific rate for each hearing impairment level is crucial. Another issue is the lack of follow-up data on long-term health outcomes in patients surviving *S. suis* infection including recovery of hearing loss. For vestibular dysfunction, I calculated a pooled prevalence of 22.7% with a 95%CI between 5.6-32.0% for this outcome. However, data from the review show a wide variation in the outcome measures and their definitions, mostly limited to symptoms such as: ataxia, vertigo, or loss of balance. More importantly, no data exist for the rate of co-incidence of hearing and vestibular impairment in patients with bacterial meningitis and *S. suis* infection.

The cohort study conducted at NHTD provided answers to the above questions. I found that severe to complete hearing loss occured in 54.5%, 50.1%, 39.9% and 45.3% in prospectively enrolled patients at discharge, 3 months and 9 months and retrospectively enrolled patients at 11 to 34 months after discharge. After adjusted for age using log binomial regression against the controls, I found a gradual decrease in the prevalence ratios of hearing loss along these follow-up time-points. This finding confirms that

hearing loss caused by *S. suis* infection is generally irreversible but improves a little after acute illness. The long-term prevalence of severe to complete hearing loss tends to stabilize at around 40%, a rate similar to the pooled rate found in my meta-analysis. A similar trend was found for loss of balance and vestibular dysfuntion, which occured in 65.2% and 51.6% of the patients at 9 months, respectively (similar rates were found for retrospectively enrolled patients after adjusted for age and sex).

Another important finding is that vestibular dysfunction tends to occur more often in patients with severe to complete loss to the hearing system. All patients with severe to complete hearing impairment also had vestibular dysfunction. Co-incidence of vestibular impairment in severe hearing loss has been reported previously [151, 152]. Nakashima and Yanagita [152] also observed that less hearing recovery occured in high-frequency impairment among those with vertigo compared to those without vertigo. I could not evaluate this effect due to the small sample size, however I also found that little hearing improvement was observed for profound to complete impairment compared to non-severe cases. This illustrates that severe hearing loss remains relatively stable after short-term improvements, particularly in those with vestibular dysfunction. This observation needs to be included in the analysis of burden of disease and economic consequences of *S. suis* infection in humans.

6. 3. Impact of *S. suis* infection in Viet Nam

Quantifying the health burden and economic impact of disease is important for setting health priorities and making evidence based decisions on resource allocation. In a resource-constrained enrivonment such as Viet Nam, the costs and consequences of inappropriate policy and planning are likely to be significant [65]. *S. suis* has been identified as a neglected zoonotic disease despite it causing major problems in the swine

industry worldwide [3]. Lack of prioritization of healthcare resources for zoonotic diseases has been largely attributed to the lack of awareness of the disease and its long-term chronic sequelae among clinicians and policy makers, lack of diagnositic capacity at local levels, and fragmentary data collections on zoonotic infections by unconnected sectors, and a lack of reliable data on the burden of such diseases in endemic countries [222]. *S. suis* infection, as with many other neglected infectious diseases, has not received adequate attention in the research agenda for policy and public health actions and was excluded from the national burden of disease study [206]. However, as demonstrated in this analysis of disease burden and economic impact, the consequences of diseases such as *S. suis* infection are not negligible.

In 2011, the total estimated burden of disease caused by *S. suis* infection in humans combining both mortality and morbidity was 1810 DALYs, equivalent to 2.73 DALYs per 100,000 adult population. This was translated into a cost of over 70 billion VND (USD 3,3 million) per year combining both direct costs and productivity loss associated with the infection. This was estimated for an annual incidence rate of 0.318 cases per 100 000 population and a case fatality rate of 12.5%. Estimations for other years were lower as a result of lower incidence and lower case fatality rate. This work clearly shows the importance of having a functional disease surveillance system in order to provide more accurate and complete national data on the incidence and case fatality. Current surveillance practices appear to suffer from serious under reporting and ascertainment of human cases with *S. suis* infection, which will require political commitment and financial investments to improve. Nevertheless, statistical and modelling techniques can be used in the mean time to quantify the burden of disease as shown in my analysis.

Another salient issue is the disease impact from a societal point of view. The burden of human *S. suis* infection might not be as high as other infectious diseases such as influenza, but the impact on the affected households can be devastating. As implicated in the findings from interviews of patients and their relatives, the sudden high costs associated with hospitalization and long-term disability including hearing and vestibular impairment caused by *S. suis* infection create a great burden on mostly poor families, forcing them to resort to high-cost resolutions such as taking high-interest loans, pushing them further into poverty. The disease-driven poverty trap with infectious diseases [223] is clearly demonstrated in this case, as *S. suis* infection disproportionately affects low-income households in the community. The national health insurance scheme includes programs for the poor and near-poor groups, however covering the rural and informal populations remains a challenge [224]. Indeed, a large proportion of the patients in our study are not covered with any insurance or prepayment program. Improving access to health insurance for these disadvantaged groups in the population remains a key financial risk protection for them to cope with health shocks caused by diseases such as *S. suis* infection.

The health and economic burden of disease caused by *S. suis* infection in this thesis is confined to the disease and its sequelae on humans only. This does not mean the impact of this disease in animals is minimal or should be tackled in isolation. To create a stronger advocacy basis for policy and planning of resources towards prevention and control of *S. suis* infection, the impact of disease in both humans and animals needs to be combined. The fact that both public health and veterinary experts joined together in a workshop organized by the Vietnamese Ministry of Health in December 2012 to discuss the development of guidelines on the surveillance, prevention and control of human *S. suis* infections showed the recognition of the importance of multi-disciplinary efforts to

tackle this zoonosis. Indeed, it requires more than these two sectors to achieve effectiveness in the surveillance and control of zoonotic diseases, others include agriculture, education, wildlife and the environment sector [222]. However, a functional surveillance system for *S. suis* in pigs is lacking and no incidence data are available in Viet Nam. This poses a great challenge to efforts to evaluate the full burden of the disease in both health and economic terms and to influence public policy and planning.

6. 4. Conclusions and future directions

An important overriding finding from this thesis is that the significant impact of *S. suis* infection on the patients and their family is not only due to acute infection and associated hospitalization, but also the long-term consequences caused by premature mortality and disabling sequelae. Severe to complete hearing loss and vestibular dysfunction both persisted in many patients surviving the infection, doubling the disease burden. The economic consequences are also considerable, particularly in the context of the low-income status of the majority of patients affected by *S. suis* infection. Currently, neither human vaccine nor pig vaccine is available, but the disease in human is preventable through simple preventive measures including safe slaughtering and handling pigs or pork products, and avoiding consumption of raw or undercooked pork derived dishes. Interventions targeting behaviour change still plays an important role in the efforts to reduce the incidence of *S. suis* infection. These necessitate a thorough understanding of the local practices including the habit of consuming high-risk pig dishes, such as raw pig blood, as the leading risk factor for disease transmission in the country.

Based on the findings and discussion from this thesis, I propose the following directions for the future work to support the prevention and control of *S. suis* infection in humans:

1. Strong surveillance is the foundation for effective control of many diseases including *S. suis* infection. A close examination of the current surveillance systems for both human and animal *S. suis* disease is needed to identify the weaknesses and bottlenecks that affect data quality and areas for improvement. More research is needed in this area to provide recommendations for policy makers and advocacy for investments.

2. There needs to be a comprehensive evaluation of economic burden of *S. suis* infection taking into account both costs incurred by disease in humans and disease in the swine population. This will provide stronger evidence to influence policy and planning of resources for investing in reducing the burden of this disease. For more effective decision-making, further economic evaluation of the cost-effectiveness of public interventions can also be performed based on this burden of disease analysis.

3. Studies on pathogenesis of *S. suis* infection in humans and its mechanisms of causing severe to complete hearing impairment with vestibular dysfunction are required. Better understanding on these mechanisms can support the development of appropriate treatment regimes to minimize the damage of the infection on the hearing and vestibular system. Further research is also needed to evaluate the effectiveness of corticosteroid treatment to prevent hearing and vestibular impairment and to support the recovery of such impairment.

4. Given the considerable impact of hearing and vestibular impairment following hospital discharge, rehabilitation and education programs should be developed to support patients and their family in the recovery process both physically and psychologically. In addition, follow-up of patients post-discharge is particularly important since patients might still suffer from symptoms of dizziness and imbalance that might require clinical interventions.

5. Besides the consumption of undercooked pig dishes, occupational exposure and daily contacts with pig/ pork products also present a considerable risk of infection and need to be explored further in settings like Viet Nam. Poor hygiene practices together with the lack of awareness about potential disease transmission along the food chain from meat production to meal preparation might put people at risk. The fact that many patients could not recall risk exposure might be explained by their ignorance on the events that can increase the risk of disease transmission in their everyday life activities. Further investigations on the risk factors are recommended for the development of culturally appropriate interventions to bring about a sizable reduction in the burden of *S. suis* infection.

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Appendices

Appendix 1. Details of 177 included publications in review of the epidemiology, clinical manifestations, and outcome of *Streptococcus suis* infection in humans

Publication ID	Author/year/country/city	Study design	Study population	Recruitment period	No. *S. suis* cases	Included
7	Agass/1977/England/Oxford	Case report		ND	1	In single dataset
8	Alonso-Socas/2006/Spain/Tenerife	Case report			1	In single dataset
9	Arends/1995/Netherlands/Leiden	Case report		1994	1	In single dataset
11	Arends/1988/Netherlands/Amsterdam	Case series	We only included the Netherlands' cases	1968–1984	30	As a large study
12	Asensi/2001/Spain/Oviedo	Case report		1998	2	In single dataset
13	Aspiroz/2009/Spain/zaragoza	Case report		ND	1	In single dataset
14	Atterholm/1985/Sweden/Lund	Case report		ND	1	In single dataset
15	Baddeley/1995/UK/Gloucestershire	Case report		ND	1	In single dataset
16	Bahloul/2008/France/Salon de Provence	Case report		ND	1	In single dataset
24	Berlit/1989/Gemany/Mannheim	Case report		ND	1	In single dataset
25	Bezian/1996/France/Bordeaux	Case report		ND	1	In single dataset
28	Bouchaud/1997/France/Elbeuf	Case report		Feb 1995	1	In single dataset
29	Busova/2010/Czech Republic/	Case report		Jul 2005	1	In single dataset
31	Braun/2007/Germany/Augsburg	Case report		ND	1	In single dataset
34	Bungener/1989/Federal Republic of Germany	Case report		1984	1	In single dataset
35	Cammaert/1990/Belgium/	Case report		ND	1	In single dataset
36	Camporese/2007/Italy/Pordenone	Case report		ND	1	In single dataset
37	Caumont/1996/France/Reims	Case report		ND	1	In single dataset
38	Chan/2002/Singapore	Cross section	Bacterial meningitis	1993–Jun 2000	1	In single dataset
39	Chang/2006/Japan	Case series		1994–2006	7	As a large study
41	Chattopadhyay/1980/England/London	Cross section	Meningitis	1971–1978	1	In single dataset
42	Chau/1983/China/Hong Kong	Case series		1978–1981	8	As a large study
43	Cheng/1987/China/Hong Kong	Case report		ND	1	In single dataset
44	Chotmongkol/1999/Thailand	Case report		1997	1	In single dataset
47	Clarke/1991/UK/London	Case report		ND	1	In single dataset
48	Clements/1982/UK/Cambridge	Case report		ND	2	In single dataset
52	Colaert/1985/Belgium	Case report		1984	1	In single dataset
55	Coolen/1989/Belgium	Case report		ND	1	In single dataset
56	D'Agostino/2009/Italia/Rome	Cross section	Vertebral osteomyelitis	ND	1	In single dataset
58	De La Hoz/2005/Spain/Cadiz	Case report		ND	1	In single dataset
60	Desjars/1987/France/Nantes	Case report		Nov 1986	1	In single dataset
61	Dickie/1987/New Zealand	Case report		Nov 1986	1	In single dataset
62	Donsakul/2003/Thailand/Bangkok	Cross section	Bacterial meningitis	1993–1999	8	As a large study
63	Doube/1988/England/Bath	Case report		ND	1	In single dataset
64	Dragojlovic/2005/Serbia/Belgrade	Case series		2003	5	As a large study
65	Duong/2004/VietNam/HoChiMinh city	Case report			1	In single dataset
66	Dupas/1992/France/Nantes	Case report		Apr 1991	1	In single dataset
67	Durand/2001/France/Grenoble	Case report		ND	1	In single dataset
69	Faucqueur/1983/France/Chateaudun	Case report		Oct 1981	1	In single dataset
70	Fauveau/2007/France/Blois	Case report		ND	1	In single dataset
73	Feng/2009/China/Shenzhen City and Sichuan	Case report		Jul–Aug 2007	3	In single dataset
75	Fernandez/2011/Spain/A Coruna	Case report		ND	1	In single dataset
76	Fittipaldi/2009/US/Hawaii	Case report			1	In single dataset
78	Fongcom/2009/Thailand/Lamphun	Case series		1999–2000	10	As a large study
79	Fongcom/2009/Thailand/Lamphun	Case series		Apr 2001–Apr 2002: Jul 2005–Jul 2007	53	As a large study
80	Francois/1998/France/Limoges	Case report		1996	1	In single dataset

Publication ID	Author/year/country/city	Study design	Study population	Recruitment period	No. *S. suis* cases	Included
81	Galbarro/2009/Spain/Huelva	Case report		ND	1	In single dataset
84	Garau/1995/Itali/Cagliari	Case report		ND	1	In single dataset
85	Geffner/2001/Spain/Castellon	Case report		ND	2	In single dataset
95	Grebe/1997/Germany/Heidelberg	Case report		ND	1	In single dataset
97	Halaby/2000/Netherlands/Maastricht	Case report		ND	1	In single dataset
98	Haleis/2009/Canada/Manitoba	Case report		Oct 2007	1	In single dataset
99	Hantson/1991/Belgium/Brussels	Case report		ND	1	In single dataset
100	Hay/1989/UK/Edinnburgh	Case report		Apr–1987	2	In single dataset
102	Heidt/2005/Germany/Giessen	Case report			1	In single dataset
103	Helbok/2006/Thailand/Bangkok	Cross section	Chronic meningitis	1993–1999	2	In single dataset
105	Hidalgo/2007/Spain/Malaga	Case report			1	In single dataset
106	Ho/1990/China/Hong Kong	Case report		Aug 1989	1	In single dataset
108	Hoa/1998/Vietnam/Ho Chi Minh	Cross section	Community acquired septicemia	1 Jun 1993–30 May 1994	4	In single dataset
110	Hu/2000/China/Jiangsu	Outbreak investigation		Jul 1998	25	As a large study
112	Huang/2005/China/Taiwan/Taipei	Case report		Mar 2000, Sep 2002	2	In single dataset
113	Huh/2011/Korea/Seoul	Case report			1	In single dataset
114	Hui/2005/China/Hongkong	Cross section	Bacterial meningitis	1992–2001	6	As a large study
115	Ibaraki/2003/Japan/Nagaoka	Case report		Aug 2002	1	In single dataset
116	Ip/2007/Hongkong	Case series		1995–2005	10	As a large study
121	Juncal/1997/Spain/La Coruna	Case report		ND	1	In single dataset
122	Kaufhold/1988/Germany/Koln	Case report		Sep 1987	1	In single dataset
125	Kay/1995/China/Hong Kong	Case series		1984–1993	25	As a large study
126	Kennedy/2008/Australia/New South Wales	Case report		Oct 2006, Jan 2008	2	In single dataset
127	Kerdsin/2009/Thailand	Cross section	Unidentified streptococcal isolates	Jan 2006–Sep 2008	165	As a large study
128	Kerdsin/2011/Thailand/Uttaradit	Case report		Jun and May 2007	2	In single dataset
129	Kerdsin/2009/Thailand	Cross section	Confirmed *S. suis*	Jan 2006–Sep 2008	12	As a large study
130	Khin Thi/1985/China/HongKong	Case series		1982–1984	30	As a large study
131	Kim/2011/Korea/Chungcheong	Case report		ND	1	In single dataset
134	Kohler/1989/Germany/	Case report		Jun 1987	1	In single dataset
135	Kopic/2002/Croatia/Slavonski Broad	Case report		Nov 2000	2	In single dataset
137	Kowalik/2007/Poland/Gdansk	Cross section	Community acquired bacterial meningitis	Jan 1999–Dec 2001	3	In single dataset
138	Laohapensang/2010/Thailand/Chiang Mai	Case report		ND	1	In single dataset
139	Lecuyer/2004/France/Saint Quentin	Case report		ND	1	In single dataset
140	Lee/2008/US/San Francisco	Case report		2003	1	In single dataset
141	Leelarasamee/1997/Thailand/bangkok	Case report		ND	3	In single dataset
145	Lopreto/2005/Agentina/La Plata	Case report		ND	1	In single dataset
146	Luengo/2006/Spain/Caceres	Case report		2004	1	In single dataset
149	Lutticken/1986/Germany/Munich	Case report		1985	1	In single dataset
150	Ma/2008/China/HongKong	Case series		1 Jan 2003–31 Jul 2005	21	As a large study
152	Maher/1991/UK/Leeds	Case report		ND	1	In single dataset
153	Mai/2008/VietNam/HCMC	Cross section	Bacterial meningitis	Nov 1996–Jun 2005	151	As a large study
155	Manzin/2008/Italy/Sardinia	Case report		Nov 2007	1	In single dataset
156	Martimez Aviles/1994/Spain/Madrid	Case report		ND	1	In single dataset
157	Matano/1984/Italy/Caserta	Case report		Jan 1983	1	In single dataset
158	Matsuo/2003/Japan/Nagasaki	Case report		Feb 2002	1	In single dataset
159	Mazokopakis/2005/Greece/Crete	Case report		ND	1	In single dataset
160	McLedon/1978/Leicester/UK	Case report		1976	1	In single dataset
161	McNeil/1986/London/UK	Case report		ND	1	In single dataset
162	Meecham/1992/England/Chester	Case report		ND	1	In single dataset
163	Michaud/1996/Canada/Quebec	Case report		1994	1	In single dataset
165	Nagel/2008/Agentina/Santa Fe	Case report		ND	1	In single dataset
166	Navacharoen/2009/Thailand/Northern	Case series		Jan 2003–Jan 2007	40	As a large study
167	Nghia/2008/VietNam/Long An	Case report		2001	1	In single dataset

Publication ID	Author/year/country/city	Study design	Study population	Recruitment period	No. *S. suis* cases	Included
168	Nghia/2011/VietNam/Ho Chi Minh city	Case control	Hospital cases and controls, community controls	May 2006–Jun 2009	101	As a large study
172	Pedroli/2003/France/Montbrison	Case report		Nov 2000	1	In single dataset
173	Peetermans/1989/Netherlands/Leiden	Case report		1988	1	In single dataset
174	Perseghin/1995/Italy/Sondalo	Case report		ND	1	In single dataset
175	Piech/2009/France/Clermont-Ferrand	Case report		ND	1	In single dataset
177	Poggenborg/2008/Denmark/Copenhagen	Case report		2006	1	In single dataset
179	Pychova./2011Czech/Brno	Case report		2008; 2009	2	In single dataset
180	Plotek/2007/Poland/Kalisz	Case report			1	In single dataset
183	Rao/2008/UK/Kent	Case report		ND	1	In single dataset
186	Riquelme/2008/Spain/Santander	Case report			1	In single dataset
190	Rosenkranz/2003/Germany	Case report		ND	1	In single dataset
191	Rosenstingl/2008/France/Coulommiers	Case report		ND	1	In single dataset
192	Rusmeechan/2008/Thailand/Phitsanulok	Case series		2001–2006	41	As a large study
197	Seidel/1995/Germany/Munster	Case report		Jan 1994	1	In single dataset
198	Shneerson/1980/UK/London	Case report		ND	1	In single dataset
199	Sia/2006/China/Hong Kong	Case report		2004	1	In single dataset
202	Soemirien/2010/Netherlands/Amsterdam	Case series	Adult community-acquired bacterial meningitis	Mar 2006–Jan 2009	2	In single dataset
204	Spiss/1999/Austria/Innsbruck	Case report		ND	1	In single dataset
206	Strangmann/2002/Germany/Oldenberg	Case report		ND	1	In single dataset
208	Suankratay/2004/Thailand/Bangkok	Case series		Jan 1997–May 2002	12	As a large study
209	Taipa/2008/Portugal?Porto	Case report		ND	1	In single dataset
212	Tambyah/1997/Singapore	Case report		ND	1	In single dataset
213	Tan/2010/Singapore/	Case report		ND	1	In single dataset
214	Tang/2006/China/Sichuan	Outbreak investigation		Jun 2005	204	As a large study
216	Taradas/2001/Spain/Castellon	Case report		ND	2	In single dataset
217	Tayoro/1996/France/Tours	Case report		1994	1	In single dataset
219	Thwaites/2002/Viet Nam/Ho Chi Minh city	Cross section	Bacterial meningitis of tuberculous meningitis	1997–2000	31	As a large study
222	Tramontana/2008/Australia/Melbourne	Case report		2007	1	In single dataset
224	Tran/2011/Viet Nam/HCMC	Cross section	Baterial meningitis	May 2006–Jun 2009	110	As a large study
225	Trottier/1991/Canada/Quebec	Case report		ND	1	In single dataset
226	Tsai/2005/China/Taiwan	Case report		ND	1	In single dataset
227	Twort/1981/England/Chichester	Case report		ND	1	In single dataset
230	van de Beek/2008/Netherlands/nationwide	Cross section	Community-acquired bacterial meningitis	Oct 1998–Apr 2002	4	In single dataset
231	van Jaarsveld/1990/Netherlands/Nijimegen	Case report		April 1989	1	In single dataset
232	Van Ooteghem/1988/Belgium/Gent	Case report		Mar1986	1	In single dataset
235	Vilaichone/2002/Thailand/Bangkok	Case series		1994–2001	17	As a large study
236	Voutsadakis/2006/Greece/Larissa	Case report		ND	1	In single dataset
237	Walsh/1992/UK/	Case series		1975–1990	35	As a large study
240	Wangkaew/2006/Thailand/ChiangMai	Case series		May 2000–Dec 2002	41	As a large study
241	Wangsomboonsiri/2008/Thailand/Nokornsawan	Case series		Jan 2005–Oct 2007	66	As a large study
242	Watkins/2001/UK/Leeds	Case report		Aug 1999	1	In single dataset
244	Wertheim/2009/Viet Nam/Ha Noi	Case series		Jan–Dec 2007	50	As a large study
245	Wilenburg/2006/US/	Case report		ND	1	In single dataset
248	Woo/1987/China/Hongkong	Case report		ND	2	In single dataset
252	Yamaguchi/1994/Japan	Case report		ND	1	In single dataset
253	Yang/2005/China/Taiwan	Case report		ND	1	In single dataset
254	Yang/2009/China	Case report		Jul 2005	4	In single dataset

Publication ID	Author/year/country/city	Study design	Study population	Recruitment period	No. *S. suis* cases	Included
257	Ye/2009/China/	Outbreak investigation		2005	38	As a large study
259	Yen/1994/China/Taiwan/Kaohsiung	Case report		Apr1991	1	In single dataset
261	Yu/2006/China/Sichuan	Outbreak investigation		Jun–Aug 2005	215	As a large study
269	Chau/1983/China/Hong Kong	Case report		ND	1	In single dataset
270	Koldkjaer/1972/Danmark/Odense	Case report		ND	1	In single dataset
271	Perch/1968/Denmrk/Hillerød	Case report		1960–1966	3	In single dataset
272	Hickling/1976/England/London	Case report		ND	1	In single dataset
273	Kloppenburg/1975/Netherlands/Groningen	Case report		ND	1	In single dataset
281	Khadthasrima/2008/Thailand/Phayao	Outbreak investigation		12 April–11 May/2007	50	As a large study
282	Joynson/1980/Wales/West Glamorgan	Case report		ND	1	In single dataset
283	Peel/1979/UK/York	Case report		ND	1	In single dataset
290	Damodaram/2011/India/Andhra Pradesh	Cross section	Myositis	2006–2011	1	In single dataset
295	Isaradisaikul/2010/Thailand	Cross section	Vertigo	April 2005–December 2007	1	In single dataset
296	Bronstein/1995/UK/London	Case report		ND	1	In single dataset
297	Pachirat/2012/Thailand	Cross section	Infective endocarditis	January 2010–December 2011	3	In single dataset
298	Takeuchi/2012/Thailand/Phayao	Cross section		2010	31	As a large study
299	Moon/2011/Korea	Case report			1	In single dataset
300	Nghia/2012/Vietnam	Cross section	CNS infections	August 2007–April 2010	147	As a large study
302	Boonyagars/2010/Thailand/	Case report		ND	1	In single dataset
303	Choi/2012/Korea/Gwangju	Case report		ND	1	In single dataset
304	Tsai/2012/China/Taiwan/	Case series		2000, 2002, 2005, 2007, 2009, 2010, 2010, 2011	8	As a large study
305	Thayawiwat/2012/Thailand/Phayao	Cross section	Sepsis, meningitis, IE, pneumonia, arthritis	January 2009–December 2011	31	As a large study
307	Taylor/2012/Vietnam/Hanoi	Cross section	CNS infections	May 2007–December 2008	48	As a large study
308	Hanterdsith/2012/Thailand/	Case report			1	In single dataset
309	Paul/1977/France/	Case report			1	In single dataset
310	Avril/1977/France/Paris	Case report			2	In single dataset
311	Perch/1971/Denmark/Copenhagen	Case report			1	In single dataset
312	Yoon/2012/Korea/Jeju	Case report		ND	1	In single dataset
313	de Ceuster/2012/Netherlands	Case report		ND	1	In single dataset
314	Zalas-Wiêcek/2012/Poland/Bydgoszcz	Case report		ND	1	In single dataset
315	Vela/2012/Spain/	Case report		ND	1	In single dataset

Appendix 2. Question guide for moderating focus groups discussions

Type of question	Content
OPENING (5 minutes)	• *For people who consume tiết canh*: Tell us who you are, what is you occupation, and when was the last time you eat tiết canh. • *For people who do not consume tiết canh:* Tell us who you are, what you do, and when was the last time you eat tiết canh, if ever.
INTRODUCTION (10 minutes)	• What is the first thing that comes to mind when you hear the word "tiết canh"? What are the main ingredients for a plate/ bowl of tiết canh? Where do the main ingredients come from? Describe the process of making pig tiet canh. • *For people who consume tiết canh*: Think back to your last experience related to pig tiết canh, could you describe that experience (where, where, with whom, how were your feelings)? • *For people who do not consume tiết canh:* Think back to your last experience related to pig tiết canh (even when you did not eat it), could you describe that experience (where, with whom, your feelings)? • *For women who do not eat tiết canh:* Does your husband eat pig tiết canh? How do you feel about that?
TRANSITION (15 minutes)	• Who often eat tiết canh, what type of tiết canh, with what food/ drink and when/ where do they often eat it (***context***)? *Context might include: breakfasts, special traditional occasions (wedding, funeral, annual commemoration of death…), friend/ family gathering…* *Explore further if these traditional occasions/ friend or family gatherings are mentioned:* What attributes of tiết canh have made it a common food to serve at these events?
KEY Total: 75 minutes	***1. Attributes of tiết canh*** *(10 minutes)* • What do you like about pig tiết canh? • What do you dislike about pig tiết canh?
	2. Influences to consume tiết canh *(15 minutes)* • *For people who consume tiết canh*: Please tell us the three most important factors that often make you decide to eat pig tiết canh. • *For people who do not consume tiết canh*: Please tell us the three most important factors that often make you avoid eating pig tiết canh. *Moderator/ assistant write all factors mentioned in the flipchart*

Type of question	Content
	• Now, look at the list of all these factors, which factor is the most important for you (not necessary the one that you have told us before)?
	3. Health risks associated with tiết canh *(30 minutes)*
	• What are the good things to your health from eating pig tiết canh? • What are the bad things to your health from eating pig tiết canh? • Should children eat tiết canh? What are the reasons for children eat/ not to eat pig tiết canh? • Should women eat pig tiết canh? What are the reasons for women eat/ not to eat pig tiết canh? *Show a picture of a patients with severe Streptococcus suis infection with skin necrosis.* • Look at this picture, what do you think has happened to this patient? And how? *Explain what happened to the patient: Streptococcus suis infection after eating pig tiết canh. People can get this disease not only through eating tiết canh, but also through eating other undercooked pig products.* • *For people who consume tiết canh*: Knowing this possible consequence of eating pig tiết canh, will you still eat it, and what are the reasons for your choice? • *For all participants*: Pig organs such as uterus, intestines, tonsils or tongue are also the foods that can contain *S.suis* bacteria, and these dishes are often served undercooked. What are the reasons for these foods to be undercooked? From your experience, is this easy to prepare these foods well enough to kill harmful bacteria?
	4. Recommendations for interventions *(20 minutes)*
	• Should we do something to prevent *S.suis* infection through eating pig tiết canh and other undercooked pig products? If yes, what should be done and how? (by individual, by government) *Moderator list all suggestions from participants on the flipchart.* • Following is a list of possible programs that might be carried out to prevent *S.suis* infection through eating pig tiết canh and other undercooked pig products, please choose the most effective intervention that you think can work for your community. Provide reasons for your choice. *Moderator shows the following list of interventions on a flipchart*

Type of question	Content
	to the participants, and add any new interventions mentioned by the participants in the previous question: - Public education campaign (via TV, radio, newspaper, loudspeakers…) to advice the public not to prepare or eat raw/ undercooked pig derived dishes - Public education programs to provide information for communities of how to prepare safer pig dishes from the high-risk pig products. - Public ban on selling tiết canh products and implementation of this ban - Vaccination of pigs against *S.suis* - Education at school on the health risks of eating raw/ undercooked pig products - Prohibition of selling, processing and consumption of sick/ dead pigs - ???
ENDING (10 minutes)	*Moderator summarizes the main content of the focus group.* • Is there anything else you would like to add or mention before we wrap- up?

Appendix 3. Ethics approval letters from OxTREC and HMU for the qualitative study on consumption of raw pig blood in Ha Noi

Oxford Tropical Research Ethics Committee

University of Oxford
Joint Research Office
Churchill Hospital, Oxford OX3 7LE
Tel. +44 (0) 1865 (5)72346 fax +44 (0) 1865 (5)72228
E-mail: jacqueline.gerencser@admin.ox.ac.uk



Vu Thi Lan Huong MPH
Centre for Tropical Medicine
Oxford University Clinical Research Unit
78 Giai Phong,
Hanoi,
Vietnam

26 February 2013

Dear Huong Vu

Full Title of Study: Prevention of human Streptococus suis infection in Vietnam: A qualitative study on the consumption of tiet canh and other undercooked pig products

OXTREC Reference: 509-13

In addition to your application, the documents reviewed were:

Documentation	Version	Date
Protocol	V1.0	20/02/13
PI Signature Page		20/02/13
Participation Information Sheet	V1.0	20/02/13
Informed Consent Form	V1.0	20/02/13

The OXTREC executive team reviewed the above application on the 22 February 2013 and gave approval for this study. Approval is given for the first five years and subject to receiving a copy of local ethical approval.

This approval will be fully implemented upon final consent of the full committee at the meeting on Thursday 28 March 2013.

Yours sincerely

Dr Mary Warrell
OXTREC Chairman

MINISTRY OF HEALTH
HANOI MEDICAL UNIVERSITY
No. 126/HMU IRB

SOCIALIST REPUBLIC OF VIETNAM
Independence-Freedom-Happiness

Issue: Approval of HMU IRB

Hanoi, April 09, 2013

CERTIFICATE OF APPROVAL

- Basing on the Decision No.766/QĐ-ĐHYHN dated March 18, 2011 by the President of Hanoi Medical University about the foundation of the HMU Review Board and secretariat for reviewing the ethical issues in Bio-medical researches ;
- Basing on the approval no. IRB 00003121 to Hanoi Medical University by Office for Human Research Protection USA on June 16, 2009;
- Basing on summary minute dated April 08, 2013 of the Hanoi Medical University Review Board (HMU IRB) and petition for the approval of project's principal investigator.

HANOI MEDICAL UNIVERSITY REVIEW BOARD (HMURB) IN BIO-MEDICAL RESEARCH

Approve the ethical issues of the following research proposal

Research title: **Prevention of human *Streptoccocus suis* infection in Vietnam: a qualitative study on the consumtion of "tiết canh" and other undercooked pig products**

- **Funding source:** OUCRU
- **Principal investigator:** Trần Khánh Toàn, MD.PhD
- **Research Institution:** Hanoi Medical University, Vietnam
- **Site for research:** Filabavi, DodaLab (Hanoi, Vietnam)
- **Research period :** 03/2013 – 07/2013
- **Date of approval:** 09 April, 2013

IRB Standing Member
Hanoi Medical University

Assoc. Prof. Ngo Van Toan MD., PhD

VICE PRESIDENT OF
HANOI MEDICAL UNIVERSITY
IRB CHAIRMAN

TRƯỜNG ĐẠI HỌC Y HÀ NỘI

Prof. Ta Thanh Van MD., PhD

Appendix 4. Illustrative quotes taken from the transcripts of 10 focus group discussions in Ba Vi and Dong Da, Hanoi, Vietnam

Factor	Representative quotes
Food characteristics	*'I feel it is very delicious and cool. It is more special than other dishes...'* (F, age 43, rural, FG3)
	'Tiet canh is a favorite food. Why? Putting a piece of tiet canh into the mouth there is a buttery taste, not much bloody taste, crunchy, secondly it feels cool. There is that feeling in the mouth, and with some herbs, some lemon, some chilly, it feels sour and hot...' (M, age 63, urban, FG6).
	'The main ingredient is pig raw pig blood. Also include pig cartilages which is chopped, roasted onion, mint leaves,...' (M, age 41, rural, FG2).
	'Good food is often ordered for parties including pig tiet canh which contains throat meat, liver, and lung... These are boiled, roasted, chopped and fried, then added to tiet canh bowl so it will be very tasty, crunchy and has good flavor.' (F, age 49, rural, FG3).
	'I often have internal body heat, regularly eating fruits but no effect. It is too unbearable when you have internal body heat. Eating tiet canh you feel it is eased right away...' (F, age 56, urban, FG10).
	'*These days, in the north, on the 1st day of the lunar month, staff go to that restaurant to eat either steamed or raw tiet canh for good luck in their jobs... it has become a trend. My niece who opens a restaurant there can sell 500 bowls in a morning...*' (M, age 65, urban, FG6).
Social contexts	'*When I got married I ate tiet canh. I followed my husband to come here, the elderly slaughtered pigs and made tiet canh... then I got familiarized with the dish.*' (F, age 29, rural, FG3).
	'*My children eat it every Saturday and Sunday... if they have enough time in the morning they will eat it for breakfast.*' (F, age 33, rural, FG3).
	'*Generally going out to eat chao long tiet canh* (soup with pig intestines and raw pig blood dish) *has to be in a group of two to three people so you feel fun and the dish is delicious, otherwise I don't wanna go*.' (F, age 48, urban, FG9).
	'*...we often eat it together, gather together, they eat so I also eat...*' (M, age 46, rural, FG5).
	'*I think I do not really like it, just sit together and eat together for fun, eat a little not much and not frequently*' (F, age 29, rural, FG3).
	'*At the time near Tet* (Lunar new year celebration) ... *we made tiet canh. Three to four households for example shared one pig (for slaughtering) and then we had to prepare food for the men sitting together, on bowl tiet canh each and rice wine*' (F, 40, urban, FG9).
	'*Most people eat it, little or a lot, never say I never eat it. Thirty days in a month, twenty-seven days with weddings already, how can I avoid tiet canh?*'

	(M, age 45, rural, FG4).
	'Wake up in the morning, they already sit there at tables on the roads, in the market in B20.' (F, age 69, urban, FG7).
	'Not only it is men's favorite, but selling it make a lot of money, high profit so in any circumstances they will sell it.' (F, age 47, rural, FG1).
	'If eating out, tiet canh is the most suitable food for our pocket money' (M, age 45, rural, FG4).
	'*...laborers often eat tiet canh more. In my opinion, it's simply because it is low-price*.' (M, age 48, urban, FG8).
Food safety and risk	'*I will eat provided that I know its source, whether it is home grown or from a clean source and secondly it was made carefully, and cleanly by the family then I will eat two bowls, not one*.' (M, age 63, urban, FG6).
	'*I went to the highlands, pig from the forest, slaughtered and the tiet canh was yummy, I still crave for it...*' (F, age 56, urban, FG10).
	'*The thing I don't like about tiet canh is during outbreaks so I don't eat. And hearing from the mass media, radio, newspaper about the outbreaks, all types of outbreaks such as flu, then I never eat... until things are normal. When the news announce that outbreaks are over, we start to eat again*' (M, age 41, rural, FG2).
	'*Death is our fate, now restaurants here do not make tiet canh for us to eat, only in town they still make it, not here, for a long time already*' (M, age 45, rural, FG4).
	'If there are outbreaks, we only eat tiet canh when knowing where the pig comes from, if no outbreaks we go to restaurants to eat a bowl of tiet canh everyday' (M, age 45, rural, FG4).
	'*Even when they heard about it* (disease outbreak in pigs)*, nothing changes. Obviously hand-foot-mouth and blue ear pig outbreaks in the village, they still continue shared slaughtering. Last year, Mrs. N, the blue ear outbreak was in the village, (pigs) died in nearly all households, but in the last household where pigs were not yet diseased they still shared to slaughter a pig of 70-80kg, 7-8 households*' (F, age 47, rural, FG1).
	'I like it because it is tasty, but thinking of many outbreaks and diseases make me scared... I stop eating because of too many disease outbreaks.' (F, age 50, urban, FG10).
	'*Eating tiet canh will be good for your blood, secondly it has the cooling effect, but now there are too many diseases so we need to be alert, reduce it, should not eat*.' (M, age 63, urban, FG6).
	'the mass media report that tiet canh has many diseases so I avoid tiet canh' (M, age 22, rural, FG2).
	'*Firstly it is unhygienic. Secondly the problem that they* (the sellers) *want to get more profit so they make it from rubbish ingredients, no conscience, so we are afraid*.' (F, age 41, urban, FG7).
	'*migrant workers, low awareness, don't care anything, neither their health...*

	they still eat' (F, age 48, urban, FG10)
	'*I see that nothing bad happens* (after eating tiet canh)*... They can eat it then I can also eat it. There is nothing happening after eating, not die then it is ok*' (F, age 47, rural, FG1)
	'*Nobody eats tiet canh by itself, at least you've got to have a cup of rice wine*' (M, age 41, rural, FG2).
	'Frequent eaters are those who can drink rice wine, those who can only have sugary drinks (with tiet canh) *will probably get diarrhea*'(M, age 22, rural, FG2).
	'*I think bacteria will die when there is rice wine*'(M, village health worker, age 41, rural, FG5).
Perceptions about public interventions	
Information, Education, Communication	'*Communication campaigns can only reach more perceptive people, especially the mass media... The remaining groups including mobile manual laborers, low awareness, don't care anything... they still eat*' (F, age 48, urban, FG10).
	'*A while ago TV mentioned about eating tiet canh and streptococcus suis infection, at that time I stopped eating. Then when it no longer talked about it, I thought the outbreak was over... so TV, radio and newspapers should continue talking about this, eating tiet canh will decrease*' (F, age 33, rural, FG3).
	'*I think communication campaigns should be longer. Not one period with one or two days, need to repeat, one week, ten days or more, if someone could not hear it today they can hear it at another time*' (M, age 48, urban, FG8).
Food inspection	'*To prevent* (infections), *firstly, the commune units for animal products inspection need to continue to inspect the hygiene and safety of food products regularly'* (M, age 63, urban, FG6).
	'*Firstly inspection, have you done it right? ...We don't know if there is an outbreak, we sell, the pig blood looks still clean, so we still eat. Who will check if this pig has enough qualities for sale or it is diseased and therefore not allowed for selling?*' (M, age 45, rural, FG4)
	'in the early mornings the inspector goes to check (the meat products in the market)*,* (the seller) *gives him money then he will give a stamp* (on the product)*, he doesn't care what is what* (the quality)*...'* (F, age 29, urban, FG10).
	'*About inspection, in reality they* (the sellers/ transporters) *still transport many, one truck with hundreds of pigs, then to the market, clearly there are stamps on the pork but do they really know what the quality is, stamp then collect money... we still need to go to the market, don't know which pig is good, which one is sick.*' (F, age 48, urban, FG9).

Ban on selling raw animal blood	'*The trade is everywhere at small scales in this rural area, can't control. Even when they come, they did not have strong actions on the people they know*' (F, age 38, rural, FG1).
	'*Ban it* (tiet canh*) but they* (the sellers) *still sell it illegally... They keep it in the fridge inside, anyone who wants to eat can ask and they will bring it out*' (F, age 50, urban, FG10).
	'*There is still a demand, then no one able to control, can you control it both day and night? So you can prohibit them now, they continue it another time, you punish them with big money they still keep the practice*' (M, age 63, urban, FG6).
Pig vaccination	'*All farmers who breed pigs vaccinate. Even in the small households, if one or two pigs they won't, but if three four or more pigs they vaccinate. Not much for streptococcus suis vaccine, but other vaccines...*' (M, age 54, rural, FG2).
	'*Vaccination is not a guarantee, there was one staff going from door to door with just only one syringe, not safe...*' (F, age 48, rural, FG1).
	'*My household has 30 pigs, they gave us just one dose for one pig, they told me to inject the pigs myself... Drugs were provided abundantly somewhere else but not here...*' (M, age 45, rural, FG4).
	'I think to maintain the shops selling tiet canh and pork intestines porridge, the most important thing is to ensure the good quality products from the breeders and pig farming. Secondly they need to vaccinate the pigs carefully. I think in this case, it is ok to eat tiet canh.' (M, middle age, urban, FG8).

F: Female, M: Male; FG: Focus Group

Appendix 5. Ethics approval letters from OxTREC and NHTD for the study of long –term outcomes in S. suis infection in human

Oxford Tropical Research Ethics Committee

University of Oxford
Joint Research Office, Block 60
Churchill Hospital, Oxford OX3 7LE
Tel. +44 (0) 1865 (5)72346, fax +44 (0) 1865 (5)72224
E-mail: jacqueline.gerencser@admin.ox.ac.uk

Dr Heiman Wertheim
Oxford University Clinical Research Unit,
National Hospital for Tropical Diseases
78 Giai Phong, Dong Da, Ha Noi,
Viet Nam

08 August 2014

Dear Dr Wertheim

Full Title of Study: Health and economic impact of Streptococcus suis infections in humans in Vietnam

OXTREC Reference: 48-14

Thank you for your email of the 05 August 2014, in which you have responded to the committees request for further clarification:

Documents:	Version:	Date:
Protocol	V1.0	09/05/14
Informed Participant Information Sheet (Community)	V1.1	05/08/14
Informed Participant Information Sheet (Patient)	V1.1	23/07/14
Questionnaires		13/06/14
Peer Review Form by Juliet Bryant		05/06/14
PI Signatory Page		10/06/14

I am therefore pleased as Chairman for OxTREC to give approval for this study.

This is given for the first five years and is subject to receiving the local ethical approval.

Any subsequent changes to the application must be submitted to the Committee as an Amendment. This should include a letter to give the reasons for the proposed modifications and all modified documents with changes tracked.

Please submit a completed Annual Report form on every anniversary of this approval and a final End of Study Report.

Yours sincerely,

Dr Mary Warrell
OXTREC Chairman

Direct Line Tel: +44 (0)1865 (5)72224
Fax: +44 (0)1865 (5)72228 Email: jacqueline.gerencser@admin.ox.ac.uk
Web: www.admin.ox.ac.uk/rso/

BỘ Y TẾ
BỆNH VIỆN BỆNH NHIỆT ĐỚI TRUNG ƯƠNG

CỘNG HOÀ XÃ HỘI CHỦ NGHĨA VIỆT NAM
Độc lập – Tự do - Hạnh phúc

Số: ...12.../HĐĐĐ-NĐTƯ
V/v: Chấp thuận cho thực hiện nghiên cứu 12ZN tại bệnh viện

Hà nội, ngày 22 tháng 8 năm 2014

CHẤP THUẬN CỦA HỘI ĐỒNG ĐẠO ĐỨC TRONG NGHIÊN CỨU Y SINH HỌC BỆNH VIỆN BỆNH NHIỆT ĐỚI TRUNG ƯƠNG

- Căn cứ Quyết định số 69/QĐ-NĐTW ngày 06 tháng 04 năm 2010 của Bệnh viện Bệnh Nhiệt đới Trung ương về việc thành lập Hội đồng Đạo đức trong nghiên cứu Y sinh học;
- Căn cứ vào số đăng ký IORG 0006480 được cấp bởi ORHP chấp thuận thành lập Hội đồng đạo đức Bệnh viện Bệnh Nhiệt đới Trung ương thời hạn đến ngày 28/5/2016 ;
- Căn cứ vào biên bản họp Hội đồng đạo đức ngày 01 tháng 08 năm 2014 và phiếu giải trình chỉnh sửa đề cương nghiên cứu của Chủ nhiệm đề tài ngày 15/08/2014,

Chủ tịch Hội đồng Đạo đức trong nghiên cứu Y sinh học Bệnh viện Bệnh Nhiệt đới Trung ương chấp thuận về khía cạnh đạo đức trong nghiên cứu đối với đề tài nghiên cứu sau:

1. Tên đề tài: ***"Đánh giá gánh nặng về sức khỏe và kinh tế trên bệnh nhân mắc liên cầu lợn tại Việt Nam"(Mã số đề tài 12ZN).***
2. Chủ nhiệm đề tài: PGS.TS Nguyễn Văn Kính
3. Đơn vị chủ trì đề tài: Bệnh viện Bệnh Nhiệt đới Trung ương
4. Đơn vị triển khai nghiên cứu: Bệnh viện Bệnh Nhiệt đới Trung ương
5. Đơn vị phối hợp thực hiện: OUCRU
6. Thời gian thực hiện nghiên cứu: Từ 1/9/2014 đến 1/9/2016
7. Các tài liệu phê duyệt được đính kèm theo phiếu chấp thuận này.
   Ngày chấp thuận: ngày 22 tháng 8 năm 2014.

Nghiên cứu viên chính và nhóm nghiên cứu có trách nhiệm thực hiện đúng các nguyên tắc về đạo đức đã được ghi trong đề cương nghiên cứu, các yêu cầu báo cáo về an toàn của Hội đồng và các qui định hiện hành về nghiên cứu lâm sàng đã ban hành của Bộ Y tế, tuân thủ tuyên ngôn Helsinki và hướng dẫn thực hành lâm sàng tốt của Hội nghị Hợp nhất Quốc tế (ICH GCP).

KT CHỦ TỊCH HỘI ĐỒNG
PHÓ CHỦ TỊCH

Nguyễn Hồng Hà

Appendix 6. Data imputation using Bayesian regression model including spatial random effect

Geographically correlated data usually have spatial autocorrelation implicating that neighboring areas tend to share similar disease risks, which can also be a cause for over-dispersion in the disease count data [225]. In this method, I included a spatial random effect via a conditional auto-regression approach (CAR) in a Bayesian regression approach [226].

This model included a CAR model in the model for disease incidence which was assumed to follow a negative binomial distribution. The covariates were selected using a generalized linear model to identify the combination of covariates that produced the model with the smallest value of Akaike Information Criteria (AIC) in predicting disease incidence.

For both models below, *S(i)* represents a spatial random structure which is specified using a conditional spatial autoregressive (CAR) model. Spatial variation is usually described in terms of neighborhood, the relationship between one area and its neighbors. The most common and simplest way of defining neighborhood is by existence of any shared border between areas. And the weight of the relationship equals 1 if two areas are adjacent, or 0 otherwise. The prior mean for each *S(i)* is defined as a weighted average of the other *S(j)* ($i \neq j$) and its prior variance is proportional to the number of neighbors [225].

$y_i \sim Negative\ Binomial\ (\pi, r)$ with $\lambda = \frac{r(1-\pi)}{\pi}$

$$log\lambda_i = \beta_0 + \sum \beta_j x_{ij} + S(i)$$

where y_i being the count for province *i,* and x_{ij} being a covariate *j* with regression coefficient β_j. Disease count following this distribution has a mean λ and variance $\frac{r(1-\pi)}{\pi^2}$ with (1-π) being the probability of observing y_i disease cases in province *i* after ($r + y_i$) attempts, *r* is an integer valued parameter.

An advantage of using a Bayesian approach is that additional information about the data can be incorporated in the model by specifying priors for the random parameters and hyper-parameters [163]. Total disease incidence and its variance at the national level were predicted from the posterior distributions through Markov Chain Monte Carlo (MCMC) method using the R2OpenBUGS package [227] in R software version 3.2.5 (R Foundation for Statistical Computing, Vienna, Austria). Two chains were initiated of 10,000 posterior samples each, with the first 10% discarded. Standard graphical checks for convergence were performed.

Table. Disease incidence of *S. suis* infection predicted based on Bayesian regression with a spatial random effect

Year	Method*¶	Model	Predictors	Incidence	95%UI
2011	Bayesian regression	Poisson	Skilled workers + Rural population	303.3	190.0-606.0
		NB	Skilled workers	260.4	190.0-404.1
2012	Bayesian	Poisson	Pig density + Rural	266.5	191.0-418.2

Year	Method*¶	Model	Predictors	Incidence	95%UI
	regression		population + Immigration#		
		NB	Pig density + Rural population	232.1	185.0-302.0
2013	Bayesian regression	Poisson	Region + Population density	219.3	189.0-253.0
		NB	Region + Population density	217.6	188.0-258.0
2014	Bayesian regression	Poisson	Rural population + Immigration#	198.0	143.0-299.0
		NB	Rural population	182.8	141.0-235.0

*All multiple imputations (MI) were run in MICE package with the add-on countimp package for 100 repeated imputations and 10 iterations for each imputation.

¶ Posterior distributions were derived through MCMC method in R software. Vague priors were used for parameters and hyper-parameters).

§Imputation model was run with the warning "In glm.nb(form, data = dat): alternation limit reached".

Immigration was included on a log-scale to improve the model fit and prediction.

Appendix 7. Annual number of incident cases of S. suis infection in Viet Nam

Annual numbers of incident cases of *S. suis* infection for the period from 2011 to 2014 estimated from multiple imputation using three methods are presented in the table below**Error! Reference source not found.**. Overall, imputation based on a negative binomial model generated estimates with higher mean and larger variance. The estimations often run to the limit in the alternating estimation process (warnings produced despite continued imputation process). This model appears to work well for 2011 but not for other years (some very large values imputed) (Figure a**Error! Reference source not found.**). Smaller uncertainty intervals were observed for the estimates produced based on the Poisson model, however the imputation was not good for 2013 and 2014 as very large values were imputed. Comparing the distributions of the residuals in the observed and imputed data conditional on the propensity score for missing probability, both largely overlapped, which indicates that the overall imputation process in all 3 methods were appropriate (Figure b). Nevertheless, imputation based on the predictive mean matching method generated the most stable and consistent estimates across the 4 years with good convergence of different streams of the imputed datasets.

Table. Disease incidence of *S. suis* infection predicted based on multiple imputation

Year	Explanatory variable	Estimated incidence	95%UI	Reported incidence*
Negative binomial				
2011	All 9 variables§	345.4	322.2-368.6	112
2012	All 9 variables	278.9	236.4-321.3	130
2013	All 9 variables§	404.3	202.1-606.5	116
2014	All 9 variables	425.7	110.2-741.1	74
Poisson				
2011	All 9 variables	384.4	366.8-402.0	112
2012	All 9 variables	238.8	232.7-244.9	130
2013	All 9 variables	288.0	253.9-322.1	116
2014	All 9 variables	209.1	200.4-217.9	74
Predictive mean matching (pmm)				
2011	All 9 variables	279.7	268.6-290.8	112
2012	All 9 variables	287.6	278.1-297.2	130
2013	All 9 variables	229.2	224.3-234.1	116
2014	All 9 variables	226.3	215.9-236.6	74

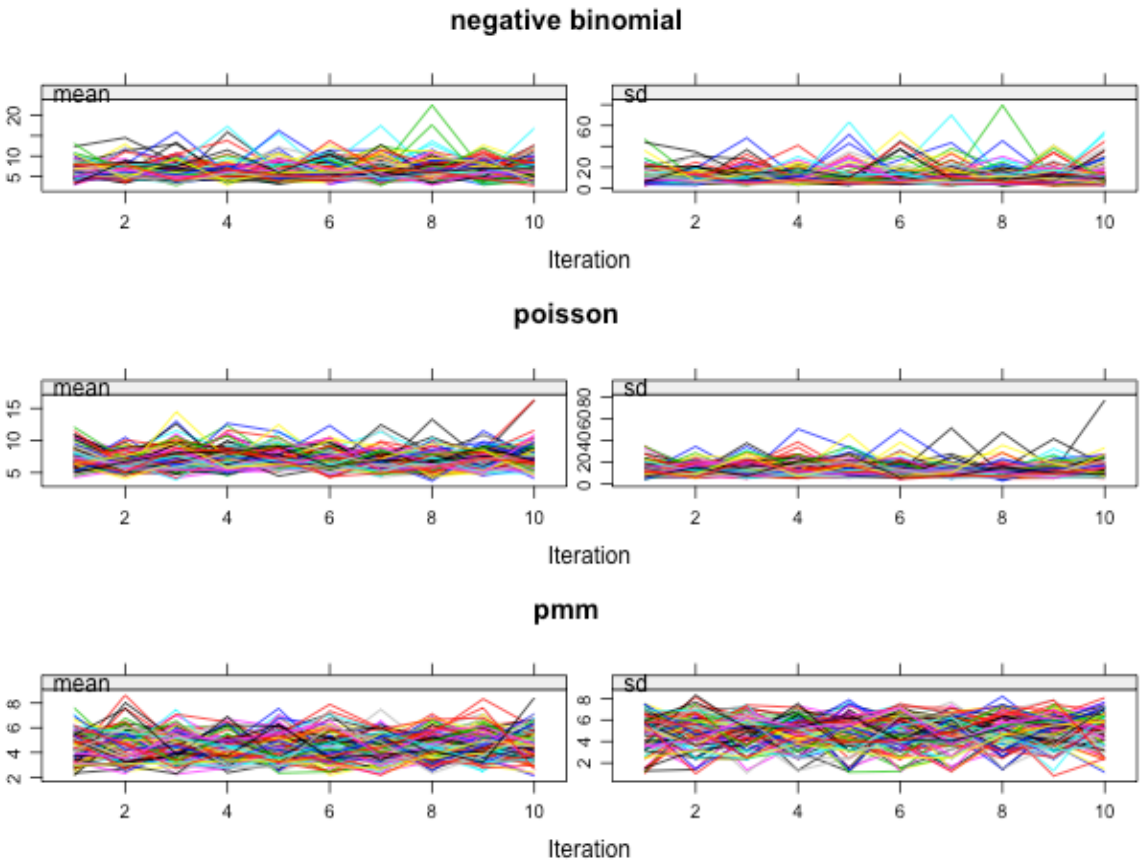
All multiple imputations were run in MICE package with the add-on countimp package for 100 repeated imputations and 10 iterations for each imputation.

*Reported from the existing surveillance system; §Imputation model was run with the warning "In glm.nb(form, data = dat): alternation limit reached".

Figure a. Imputed values for incidence of *S. suis* infection.

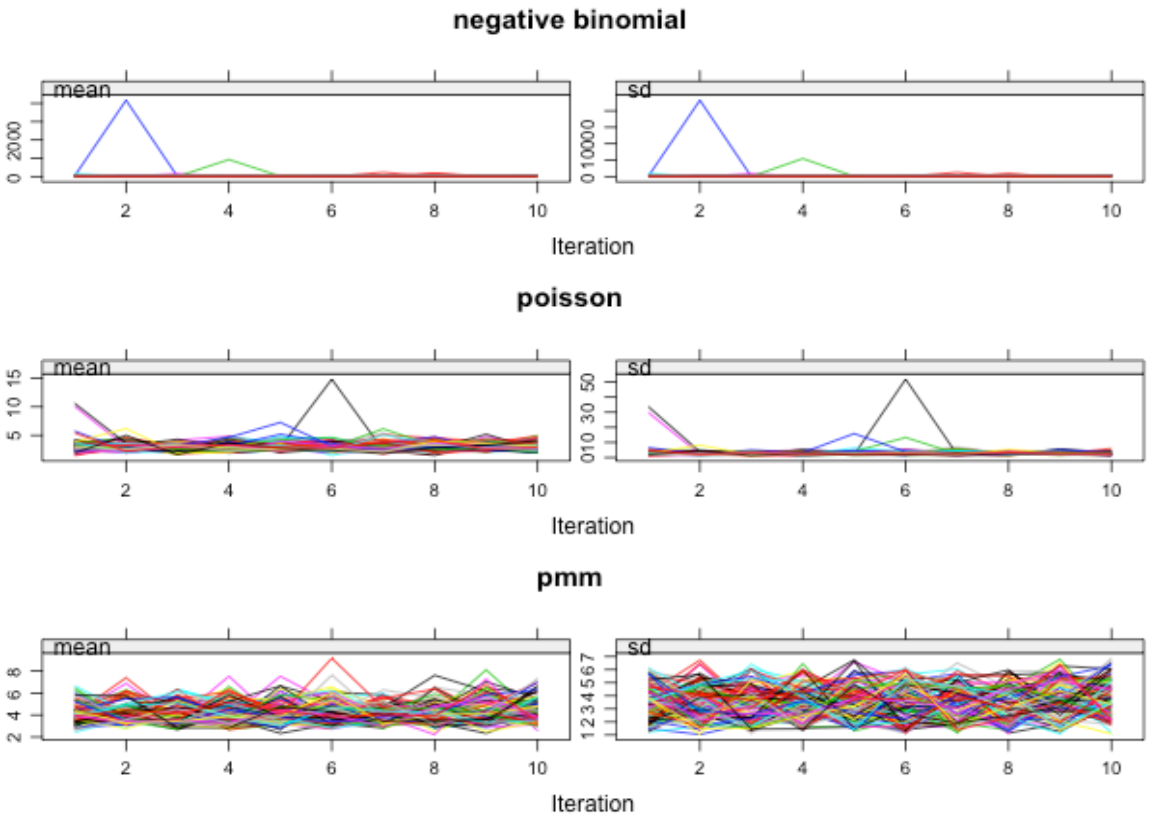
Note: Imputed values for incidence of *S. suis* infection (mean and sd) were plotted against iteration number for the imputed data by 3 imputation methods. A good imputation method should show little trend and the streams should mingle well together.

a1. 2011



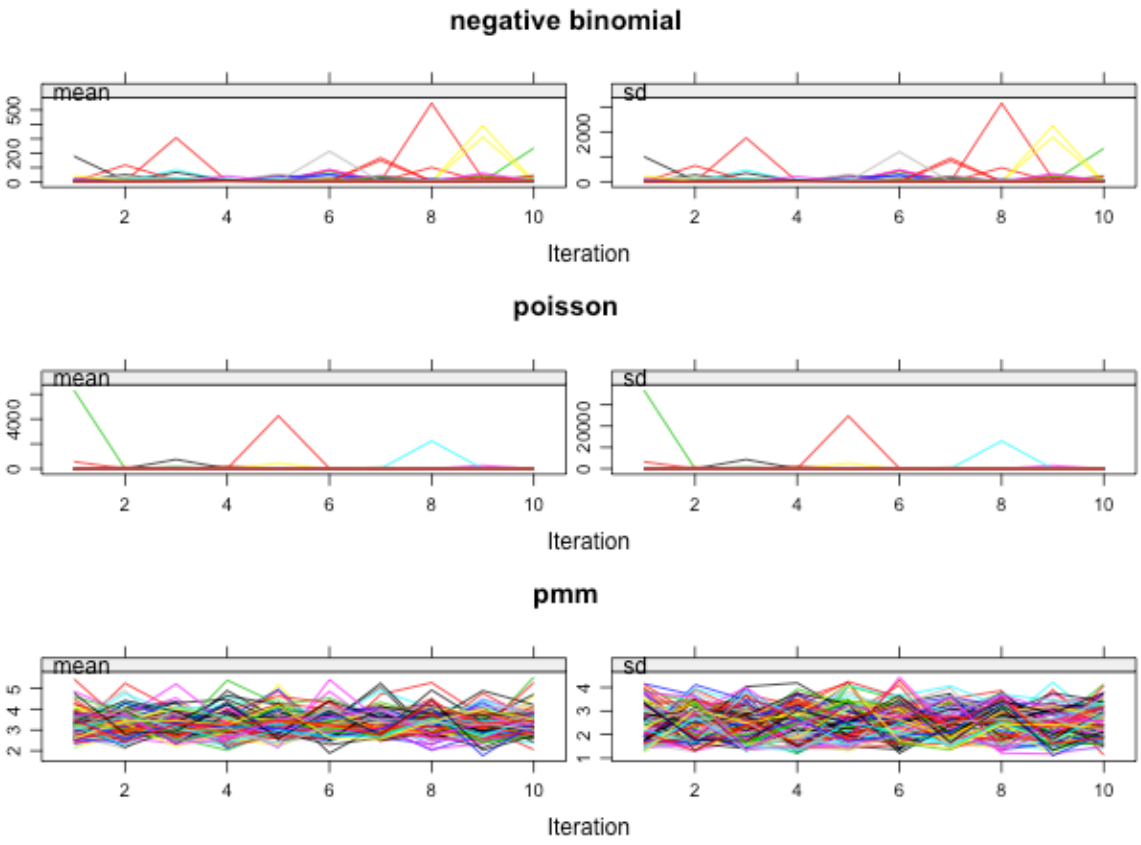
For 2011, all three models yielded relatively good data imputation. The model using pmm method generated more conservative data with lowest mean and standard deviation (sd).

a2. 2012



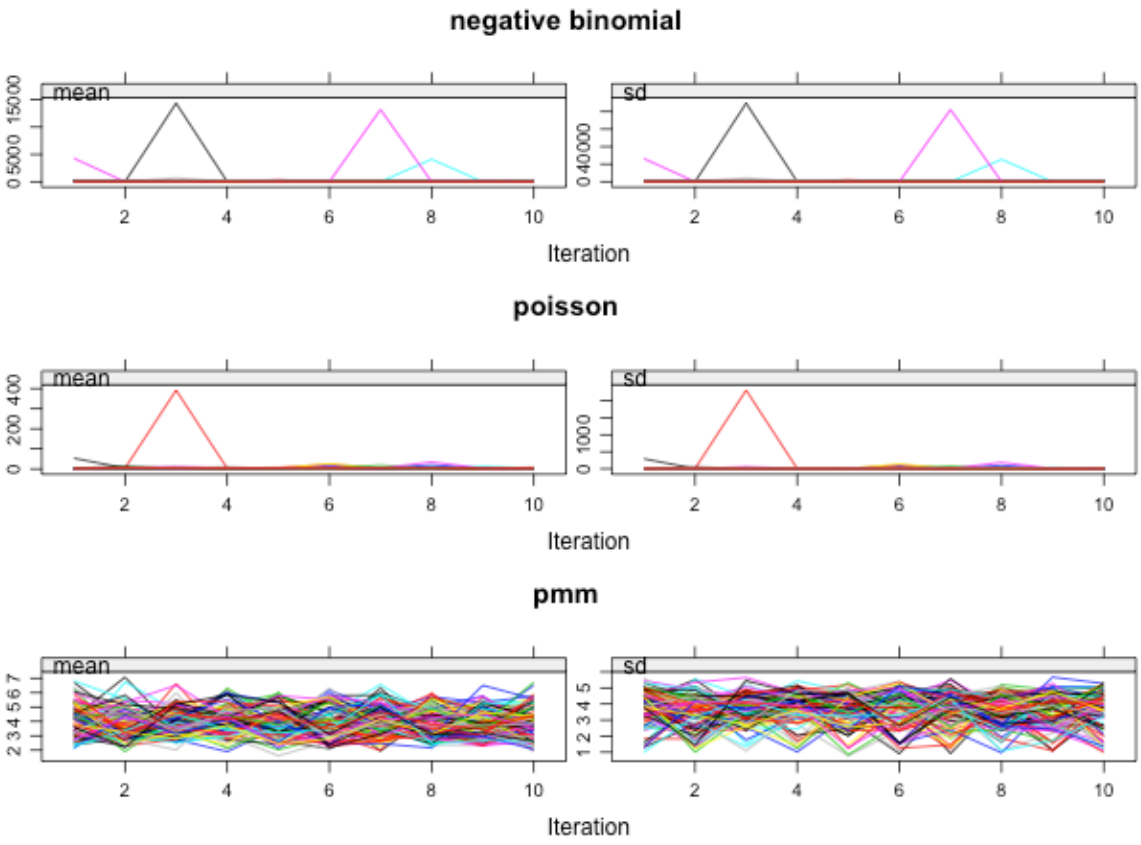
For 2012, only the model using pmm yielded good data imputation. The other two models generated values that appear to be out of range.

a3. 2013



For 2013, only the model using pmm yielded good data imputation. The other two models generated values that appear to be out of range.

a4. 2014

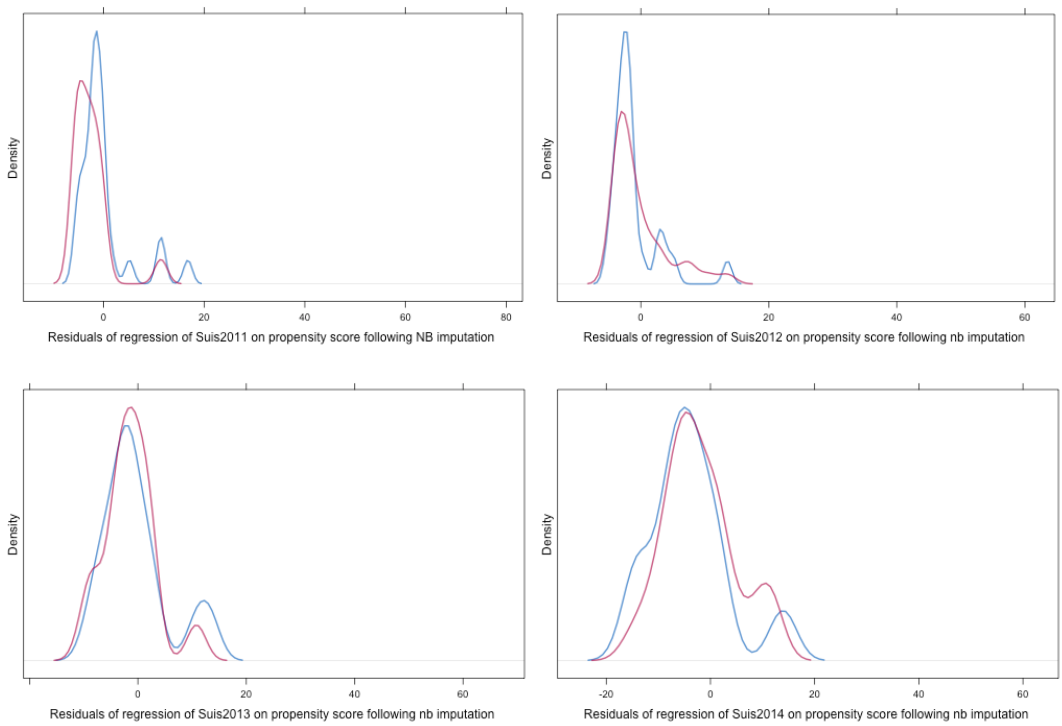


For 2014, only the model using pmm yielded good data imputation. The other two models generated values that seemed to be out of range.

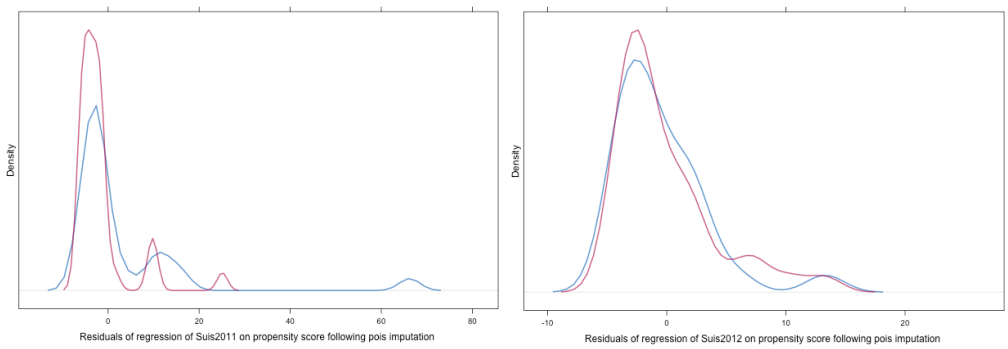
Figure b. Distributions of the residuals of the missing data model

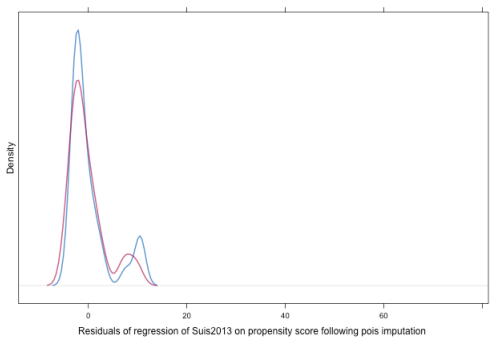
Note: Distributions of the residuals of the missing data model conditional on pooled propensity score for missing probability were plotted for observed data (blue) and imputed data (red). Data imputation was for *S. suis* disease incidence in 2011-2014 using all 9 predictors.

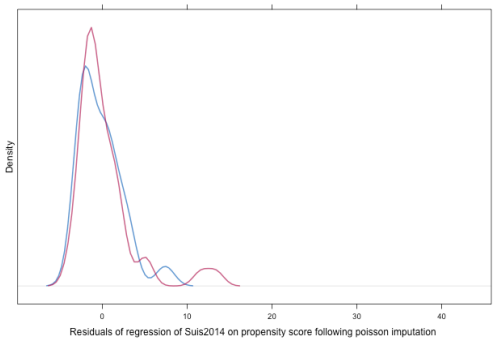
Negative binomial



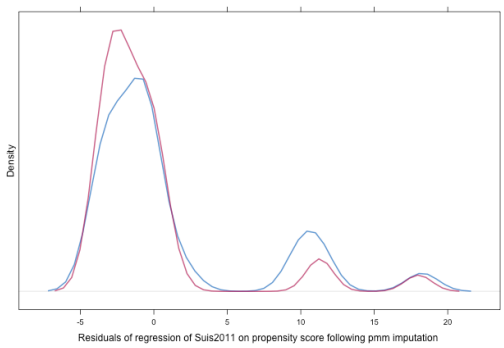
Poisson

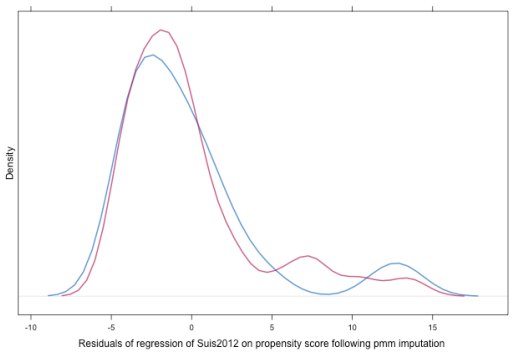


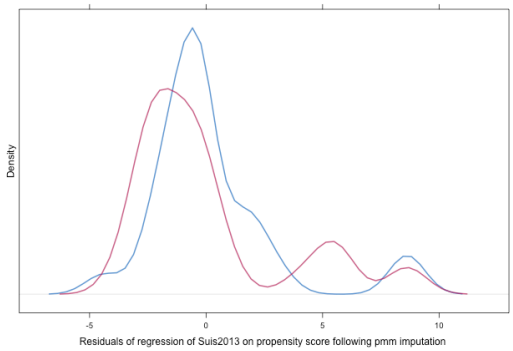


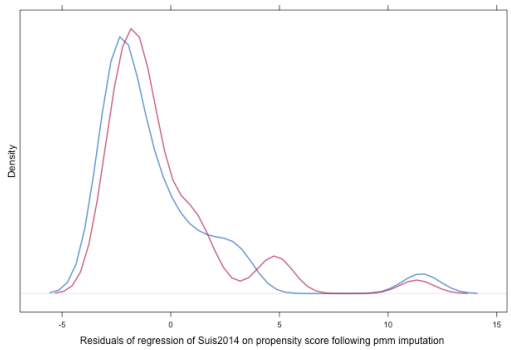


Predictive mean matching









Appendix 8. Description of health states used in the estimation of disease burden caused by *S. suis* infection

Health state	Lay description	Source
Acute infectious phase	has a high fever and pain, and feels very weak, which causes great difficulty with daily activities.	GBD 2010*
Post-infectious phase	is always tired and easily upset. The person feels pain all over the body and is depressed.	GBD 2010*
Mild hearing loss	has great difficulty hearing and understanding another person talking in a noisy place (for example, on an urban street).	Europe
Moderate hearing loss	is unable to hear and understand another person talking in a noisy place (for example, on an urban street), and has difficulty hearing another person talking even in a quiet place or on the phone.	Europe
Severe hearing loss	is unable to hear and understand another person talking, even in a quiet place, and unable to take part in a phone conversation. Difficulties with communicating and relating to others cause emotional impact at times (for example worry or depression).	Europe
Profound hearing loss	is unable to hear and understand another person talking, even in a quiet place, is unable to take part in a phone conversation, and has great difficulty hearing anything in any other situation. Difficulties with	Europe

Health state	Lay description	Source
	communicating and relating to others often cause worry, depression or loneliness.	
Complete hearing loss	cannot hear at all in any situation, including even the loudest sounds, and cannot communicate verbally or use a phone. Difficulties with communicating and relating to others often cause worry, depression or loneliness.	Europe
Vestibular dysfunction[§]	has short spells of dizziness and loss of balance; between spells the person is worried the spells will occur again.	Europe

[*]Used in Global Health Estimates 2000-2011 [187] by World Health Organization. [§]Definition and disability weight for vertigo and balance disorder provided by the European study is used for this category.

Appendix 9. Input parameters for the estimation of DALYs lost due to human *S. suis* infection in 2011-2014

Year	Sex	Age	Case incidence rate (per 1000 population)			Case fatality rate	Hearing loss with vestibular dysfunction				Hearing loss without vestibular dysfunction		Post-acute phase
			Average	Lower bound	Upper bound		Complete/ Profound	Severe	Moderate	Mild	Moderate	Mild	
2011	ALL	ALL	0.00318	0.00306	0.00331	12.50%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2011	M	15-44	0.00348	0.00335	0.00362	12.50%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2011	M	45-59	0.02016	0.01940	0.02099	12.50%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2011	M	60+	0.00943	0.00907	0.00981	12.50%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2011	F	15-44	0.00038	0.00036	0.00039	12.50%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2011	F	45-59	0.00218	0.00209	0.00226	12.50%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2011	F	60+	0.00474	0.00456	0.00493	12.50%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2012	ALL	ALL	0.00324	0.00313	0.00335	6.15%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2012	M	15-44	0.00354	0.00342	0.00366	6.15%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2012	M	45-59	0.02054	0.01984	0.02124	6.15%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2012	M	60+	0.00960	0.00928	0.00993	6.15%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2012	F	15-44	0.00038	0.00037	0.00040	6.15%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2012	F	45-59	0.00222	0.00214	0.00229	6.15%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2012	F	60+	0.00483	0.00466	0.00499	6.15%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2013	ALL	ALL	0.00255	0.00250	0.00261	10.34%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2013	M	15-44	0.00278	0.00273	0.00285	10.34%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2013	M	45-59	0.01614	0.01582	0.01652	10.34%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2013	M	60+	0.00754	0.00740	0.00772	10.34%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2013	F	15-44	0.00030	0.00030	0.00031	10.34%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2013	F	45-59	0.00175	0.00171	0.00179	10.34%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%

Year	Sex	Age	Case incidence rate (per 1000 population)			Case fatality rate	Hearing loss with vestibular dysfunction				Hearing loss without vestibular dysfunction		Post-acute phase
			Average	Lower bound	Upper bound		Complete/ Profound	Severe	Moderate	Mild	Moderate	Mild	
2013	F	60+	0.00381	0.00373	0.00390	10.34%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2014	ALL	ALL	0.00249	0.00238	0.00261	12.16%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2014	M	15-44	0.00273	0.00261	0.00286	12.16%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2014	M	45-59	0.01583	0.01513	0.01659	12.16%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2014	M	60+	0.00740	0.00707	0.00776	12.16%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2014	F	15-44	0.00029	0.00028	0.00031	12.16%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2014	F	45-59	0.00170	0.00162	0.00178	12.16%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%
2014	F	60+	0.00370	0.00354	0.00388	12.16%	28.8%	11.10%	15.10%	26.60%	2.70%	13.40%	87.20%

Appendix 10. List of publications arising directly from or linked with this thesis

HuongVTL, Long HB, Kinh NV, Ngan TTD, Dung VTV, Nadjm B, van Doorn R, Hoa NT, Horby P, Wertheim HFL. Long-term outcomes of patients with *Streptococcus suis* infection in Viet Nam: a case-control study. Journal of Infection. 2017. https://doi.org/10.1016/j.jinf.2017.09.019 (in press).

Huong VTL, Thanh LV, Phu VD, Trinh DT, Inui K, Tung N, Oanh NTK, Trung NV, Hoa NT, Bryant JE, Horby PW, Kinh NV, Wertheim HFL. Temporal and spatial association of *Streptococcus suis* infection in humans and porcine reproductive and respiratory syndrome outbreaks in pigs in northern Vietnam. Epidemiology & Infection. 2016, vol 144 (1): 35–44.

Huong VTL, Hoa NT, Horby P, Bryant JE, Kinh NV, Toan TK, Wertheim HFL. Raw pig blood consumption and potential risk for *Streptococcus suis* infection, Vietnam. Emerging Infectious Diseases. 2014, vol 20 (11), 1895-8.

Huong VTL, Ha N, Huy NT, Horby P, Nghia HDT, Thiem VD, Zhu X, Hoa NT, Hien TT, Zamora J, Schultsz C, Wertheim HFL, Hirayama K. Epidemiology, clinical manifestations, and outcomes of *Streptococcus suis* infection in humans. Emerging Infectious Diseases. 2014, vol 20 (7), 1105-14.

Hoa NT, Chieu TT, Do Dung S, Long NT, Hieu TQ, Luc NT, Nhuong PT, **Huong VT**, Trinh DT, Wertheim HF, Van Kinh N, Campbell JI, Farrar J, Chau NV, Baker S, Bryant JE. *Streptococcus suis* and Porcine Reproductive and Respiratory Syndrome, Vietnam. Emerging Infectious Diseases. 2013, vol 19 (2), 331-3.