Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science

Host risk factors in relation to the occurrence of Japanese encephalitis virus in pigs and dogs in northern Vietnam

Linnea Gustafsson

Uppsala

2020

Degree Project 30 credits within the Veterinary Medicine Programme

Host risk factors in relation to the occurrence of Japanese encephalitis virus in pigs and dogs in northern Vietnam

Linnea Gustafsson

Supervisor: *Johanna Lindahl, Department of Clinical Sciences*

Assistant Supervisor: *Dr. Nguyen Viet Hung, International Livestock Research Institute*

Examiner: *Ulf Magnusson, Department of Clinical Sciences*

Degree Project in Veterinary Medicine

Credits*: 30 HEC*
Level*: Second cycle, A2E*
Course code*: EX0869*
Course coordinating department: *Department of Clinical Sciences*

Place of publication*: Uppsala*
Year of publication*: 2020*
Online publication*: https://stud.epsilon.slu.se*

Key words*: Japanese encephalitis, JEV, pig, dog, Vietnam, antibodies, risk factors.*

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science
Department of Clinical Sciences

SUMMARY

Japanese encephalitis is the leading cause of encephalitis in Asia. The disease is caused by Japanese encephalitis virus (JEV), a vector-borne virus that has an enzootic cycle involving mosquitoes, pigs and birds. Surveillance programs for detection of virus activity can function as an early warning system of increased risk of transmission and disease outbreak. Surveillance of JEV infection is often performed using sentinel animals like pigs. Dogs have recently been shown to be good sentinels as well. The objective of this study was to determine the seroprevalence and to assess the host factors in pigs and dogs favoring JEV infection in Vietnam. For this purpose, 114 blood samples were collected from dogs in rural areas in the provinces of Hai Duong and Thai Binh in northern Vietnam. Ten blood samples were retrieved from pigs in the same areas. In addition, 250 blood samples were collected from pigs originating from all over Vietnam, in a slaughterhouse in Hanoi. Blood samples were tested for antibodies against JEV using a competitive ELISA method. A total of 30% pigs (n =78) and 72% of dogs (n=82) were JEV seropositive. The seroprevalence in pigs found in this study is much lower than the previously found; in the range of 60-100%.This could be indicative of a reduced infection pressure due to the ongoing depopulation of pigs as a result of the recent outbreak of African swine fever in the country. Further, the seroprevalence was lower in the south and south-central areas (19-26%) compared to the north (87%). The seroprevalence among dogs was higher than in earlier studies suggesting that people in these areas are exposed to JEV in high extent. The results from the dog samples show a possible correlation between age and JEV seropositivity. Also breed seems to play a role for the risk of being seropositive.

TABLE OF CONTENTS

INTRODUCTION

Japanese encephalitis is the major worldwide cause of infectious encephalitis in humans with approximately 68.000 cases annually in the 24 JEV- endemic countries in Asia and the Western Pacific (Campbell *et al.,* 2011). Only 1% of people infected develop clinical disease, nonetheless it leads to death in a third of clinical cases and among survivors neurologic sequelae are seen in 30%-50% of patients (van den Hurk *et al.,* 2009; Griffiths *et al.,* 2014; Yin *et al.,* 2015). The disease is caused by Japanese encephalitis virus (JEV) which is a vector-borne RNA-virus and has an enzootic cycle involving mosquitoes, pigs and birds. Pigs are the main component in the transmission cycle as they develop high viremia and serve as amplifying hosts while wading ardeid birds are important for the maintenance of the virus. Humans are dead-end hosts and are not part in the transmission cycle of the virus. Infected humans and horses develop encephalitis while infection in pigs causes reproductive disorders. Many other animals get infected and develop antibodies with no clinical signs of disease (van den Hurk *et al.*, 2009). The main vector *Culex tritaeniorhynchus* breeds in rice fields, making JEV a disease of mostly rural areas (Rosen, 1986). However, competent vectors have also been found in urban homes (Lindahl *et al.,* 2012).

Vaccination campaigns and other control strategies have decreased the incidence of the disease in many countries like Japan and South Korea. However, the incidence is expected to increase in other countries lacking vaccination and surveillance programs and due to population growth, intensified rice farming and increased pork production (Erlanger *et al.*, 2009). In recent years, the virus has expanded to new areas and has the potential to spread to Europe and Africa (Gao *et al.,* 2019).

As the disease is not possible to eradicate, like polio, due to the enzootic cycle there is a need for monitoring and control of the virus (Solomon, 2000). Animal sentinels are important for predicting spill-over of the virus into human population. Pigs have been used for JEV surveillance for many years (Rosen, 1986). Dogs are even better sentinels as they live close to humans and they do not become viremic after infection and thus do not spread the virus to humans as pigs do (Shimoda *et al.,* 2010).

The purpose of this study was to assess the seroprevalence of JEV in pigs and dogs in two provinces in northern Vietnam and to evaluate some risk factors affecting the incidence of JEV infection. Factors studied for dogs were age, sex, breed and keeping conditions and a factor studied for pigs was their origin in Vietnam.

LITERATURE REVIEW

Japanese encephalitis virus

Japanese encephalitis virus (JEV) is a mosquito-borne zoonotic virus of the genus flavivirus belonging to the family *Flaviviridae*. It belongs to the Japanese encephalitis serogroup together with St. Louis encephalitis in North America, Murray Valley encephalitis in Australia and West Nile viruses found in Africa, the Middle East and parts of Europe (De Madrid & Porterfield, 1974; Solomon *et al.*, 2000). Other flaviviruses known to be important human pathogen are dengue, Yellow fever, West Nile and Zika viruses (Rosen, 1986).

JEV is a single-stranded, positive-sense RNA virus with an envelope protein crucial for viral attachment and endocytosis. Humans, swine and equines are the only vertebrate hosts in which JE induces disease although there are many other vertebrate hosts that can be infected with the virus (Rosen, 1986).

JEV has been divided into 5 different genotypes (G1-G5), all initially originated from a virus in the Indonesia–Malaysia region (Solomon *et al.*, 2003). Each genotype has a different geographical distribution, but all are capable of causing disease outbreaks. For example, endemic cases in the tropics are predominantly due to JEV G1, while G3 is more often found in temperate areas (Uchil & Satchidanandam, 2001). The distribution of the different genotypes is changing with both G1 and G3 becoming more widespread and G5 has re-emerged after 50 years with no reported cases (Li *et al.*, 2011).

Epidemiology of the enzootic cycle

JEV is a zoonotic virus and the transmission cycle involves pigs as the amplifying host, ardeid birds as the reservoir host, and *Culex tritaeniorhynchus* as the main vector (figure 1). This makes the interplay between rice cultivation affecting the vector densities, as *Cx. tritaeniorhynchus* breeds in rice fields, and pig rearing close to human habitation very important in the epidemiology. Humans and other mammals like dogs, cats and horses are accidental dead-end hosts not developing a viremia high enough to pass the virus on to a new vector (Mackenzie *et al*., 2006).

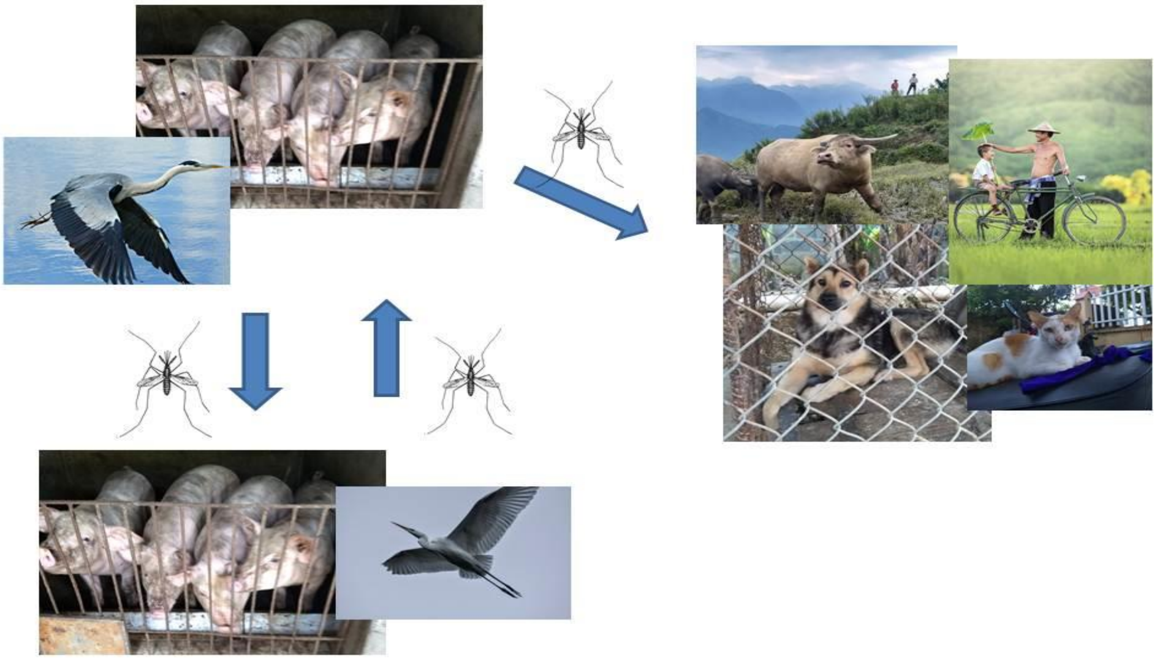

Figure 1. *Transmission cycle of Japanese encephalitis virus.*

Vectors

JEV has been isolated from more than 30 different mosquitoes from different genera including *Culex, Aedes, Anopheles, Armigeres* and *Mansonia* (Pearce *et al.*, 2018). There is evidence that *Cx. tritaeniorhynchus* is the major vector throughout most of Asia, but other species play a role in local and regional transmission, such as *Cx. gelidus, Cx. vishnui, Cx. fuscocephala, Cx. bitaeniorhynchus, Anopheles subpictus, Aedes togoi,* (Rosen, 1986; Mackenzie *et al.*, 2006). Within the *Culex vishnui* subgroup the *Cx. tritaeniorhynchus, Cx. psuedovishui* and *Cx. vishnui* are most important.

The main vector *Cx. tritaeniorhynchus* commonly breeds in rice paddies making JE mainly a disease of rural areas. Seasonal rains with flooding of rice fields leads to an increased mosquito population and transmission of JEV (Solomon, 2004). Also areas with high rice production and flooding-related agricultural practices may have a large influence on *Cx tritaeniorhynchus* numbers (Keiser *et al.*, 2005).

Another factor influencing the transmission of the virus is the feeding behavior of the *Culex tritaeniorhyncus*. In the Indian study by Arunachalam *et al.* (2005) where 3,067 blood-engorged mosquitoes were tested, it was found that a third of mosquitoes had fed on different animal species thus increasing the risk of JEV transmission. In general, cattle seem to be the preferred host, followed by pigs (Arunachalam *et al.,* 2005). The feeding behavior is also influenced by host availability so in the absence of cattle more mosquitoes feed on pigs (Mwandawiro *et al.*, 2000).

Hosts

Although viremia can be detected in several vertebrate hosts, including horses, dogs and humans, only porcine and avian hosts have high enough levels of blood viremia to facilitate transmission to mosquitoes (Pearce *et al.,* 2018). Pigs in this way serve as amplifying hosts. They do also have a high natural infection rate (98%-100%), long lasting viremia (up to 4 days) and mosquitoes prefer to feed on them (van den Hurk *et al.,* 2009). Pig populations do also have a rapid turnover since they are slaughtered at around 6-8 months and replaced by a young and naive population which helps the amplifying of the virus (Ruget *et al.*, 2018; van den Hurk *et al.,* 2009). All these factors make domestic pig rearing close to human habitation an important risk factor for JEV transmission (Solomon, 2006). Amplification in pigs occurs via two cycles. During the initial cycle approximately 20% of pigs are infected with JEV. Mosquitoes become infected after feeding on viremic pigs and transmit the virus to other susceptible pigs after a 7-14 days extrinsic incubation period. This second cycle results in up to 100% seroconversion in pigs. Human cases start to appear after another 7-14 days of incubation in mosquitoes (Peiris *et al.,* 1992; van den Hurk *et al.,* 2009). A recent experimental study has shown a direct transmission route between pigs via oronasal secretions further enhancing their role as amplifiers (Ricklin *et al.*, 2016).

Also birds play an important role in the epidemiology of JEV. More than 90 bird species are recognized as amplifying hosts and reservoir hosts of JEV. Some ardeid birds like egrets (*Egretta garzetta*) and herons (*Nycticorax nycticorax*) seem to be particularly susceptible to infection with JEV and develop high viral titers (van den Hurk *et al.,* 2009; Solomon, 2006). They seem to be important maintenance hosts for JEV and may function as amplifiers in regions with a low number of pigs. They also seem to be the source of virus in outbreaks where pigs are absent (Mackenzie *et al.,* 2006).

Bats, especially fruit bats (megachiroptera) may also function as reservoirs for the virus and are able to carry the virus long distances (Mackenzie *et al.,* 2008).

How the virus survives the winter months is still not fully elucidated. Vector-free transmission between pigs may contribute to the persistence of the virus when mosquito populations decline during the dry season (Diallo *et al.*, 2018). Migratory birds can function as a source of JEV and serve as viral reservoirs and reintroduce the virus each year (van den Hurk *et al.*, 2009). Other theories are that the virus might survive in hibernating mosquitoes or in mosquito eggs after vertical transmission (Mackenzie *et al.,* 2006). In Japan there is evidence that the virus both is continually reintroduced each year and that it also survives locally (Nabeshima *et al.*, 2009).

Epidemiology of human disease

The first clinical case of JE was documented in Japan in 1871. A large outbreak occurred 50 years later, also in Japan, involving more than 6.000 individuals. Following outbreaks were reported in 1927, 1934 and 1935. Today, nearly half of the global population lives in countries where JEV is present (Erlanger *et al.,* 2009).

JE remains the largest cause of viral encephalitis in Asia in humans despite the wide use of vaccination. The intensity and quality of JE surveillance and serologic diagnosis varies in different countries making the global incidence of JE difficult to estimate. Also it is often confused with other types of encephalitides (Campbell *et al.,* 2011). Most infections are asymptomatic or cause a mild febrile illness and less than 1% of human infections progress to clinical disease which leads to underdiagnosis (van den Hurk *et al.*, 2009). Campbell *et al.* (2011) estimated the annual incidence in 2011 in the 24 JE-endemic countries to be 67.900 which is 1.8 cases per 100.000 individuals. According to the estimates by Tsai (2000), disease burden might be closer to 175.000 cases annually (Tsai, 2000). Approximately 50% of the cases occur in China and 75% of JE cases occurs in children aged 0-14 years (Campbell *et al.*, 2011). The case fatality can be as high as 67% although normally it lies between 20% - 40% with children and elderly at the greatest risk of fatal infection. Neurological sequelae occur in 30% - 50% of infected humans that survive the disease (van den Hurk *et al.*, 2009; Griffiths *et al.*, 2014). Previous exposure to one flavivirus leads to development of cross-reacting antibodies that complicates serological diagnosis but also can protect from infection with a second flavivirus. Thus, a prior infection with dengue virus has been shown to be protective for patients with Japanese encephalitis giving a milder form of the disease (Libraty *et al.,* 2002).

JE incidence has increased in recent years in countries with no specific diagnostic centers, vaccination programs or JEV surveillance such as Bangladesh, Cambodia, India and Laos. Conversely, countries like China, Japan, South Korea, Nepal and Thailand, where vaccination programs are used and routine surveillance is pursued, are experiencing a stable or declining incidence of JE (Erlanger *et al.*, 2009).

Clinical features

Human JEV infection and disease is always the result of an infected mosquito bite. The virus is first taken up into dendritic cells (Langerhan´s cells) in the skin. The virus is then carried to the peripheral lymph nodes where replication occurs mainly within macrophages. A short-lived viremia usually <1 week follows, which precedes the entry of the virus into the central nervous system. In the majority of cases the infection resolves here. Clinical disease occurs in 1:200-1:1000 cases after the virus penetrates the blood-brain barrier and infects neuronal cells. Inflammation and oedema of the affected areas causes the cerebral disease (Mackenzie *et al*., 2006; Campbell *et al*., 2011). The first symptoms are very unspecific including lethargy, fever, headache and vomiting and appear after a 5-7 day incubation period. Later symptoms are nuchal rigidity, photophobia, altered consciousness, muscle rigidity, tremors, cranial nerve palsies, paresis and incoordination (Watt & Jongsakul, 2003; Solomon *et al*., 2002). Focal or general seizures are more common in children than in adults and are associated with poorer outcome (Solomon *et al*., 2002). Survivors of the disease often show clinical improvement after about 1 week. Nevertheless, neurologic sequelae like paralysis, hemiplegia and limb movement disorders are common and have been seen in 43.6% of JE survivors 1-2 years after being discharged from hospital (Yin et al., 2015). There is no cure for the disease, and management of the disease is purely symptomatic. For many years Japanese encephalitis was treated with corticosteroids but the randomized placebo-controlled study by Hoke *et al.* (1992) showed that there was no benefit of treatment with dexamethasone in 40 patients (Hoke *et al*., 1992). Also, interferon-alfa has been used against the disease however there are studies showing no effect on the outcome, like the one by Solomon *et al.* (2003) on Vietnamese children with the disease (Solomon *et al*., 2003).

There are effective and safe vaccines for human use that have been used effectively in economically advanced Asian countries (Japan, Taiwan, South Korea) and more recently in moderate and low-income countries (Thailand, Sri Lanka, Nepal). These vaccination programs have significantly reduced the incidence of JE (Halstead & Thomas, 2010; Solomon *et al*., 2004).

JE infection in animals

Many animal species can get infected but only horses and pigs develop disease. Horses are dead-end hosts like humans and can develop encephalitis after infection, but do not seem to play an important role in the transmission cycle of JEV (Rosen, 1986). In pigs, clinical symptoms are seen if infection occurs after sexual maturity around 8 months of age. Infection in pregnant sows may result in abortion, stillbirth and embryonic death (Desingu *et al.,* 2016; Liu *et al.,* 2013). Infected boars may show signs of infertility or orchitis (Teng *et al.,* 2013). In endemic areas, piglets acquire immunity early in life by absorption of antibodies in colostrum which can be measured up to 3 months, after their decline they rapidly get infected (Capelle *et al*., 2016). This means that the virus has little or no effect on pig production since the majority of pigs get infected before sexual maturity and gives immunity before the sows first pregnancy. In endemic areas only young sows under 1.5 years of age seem to yield a lower number of piglets after infection with JEV (Lindahl *et al*., 2012). On the other hand, in epidemic areas like China and Northern Vietnam pigs are affected with reproductive disorders and control measures such as vaccination of pigs should be used (Khan *et al*., 2014).

Cattle do not develop clinical disease but can play a role in the transmission of the virus. As *Cx. tritaeniorhynchus* has cattle as preferred host to feed on bovines may function as passive zooprophylaxis as they do not develop viremia high enough to pass the virus to mosquitoes, thus impeding JEV transmission (Mwandawiro *et al.,* 2000).

Geographical distribution and spreading

Japanese encephalitis virus is present in eastern, south-eastern and southern Asia and has spread to the western pacific including the Indonesian archipelago, Papua New Guinea and Northern Australia as well as Pakistan (Mackenzie *et al*., 2006; van den Hurk *et al*., 2009). Geographical distribution of JE is seen in figure 2.

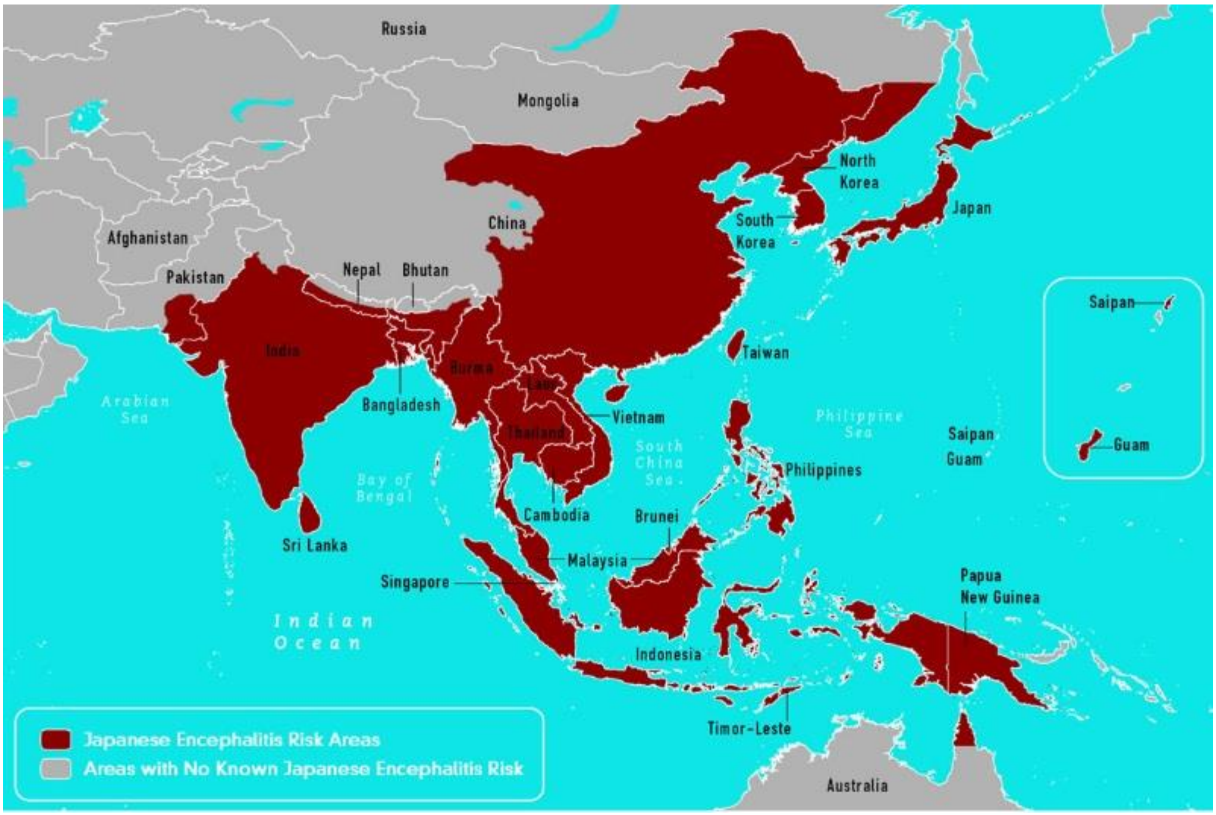

Figure 2. *Geographical distribution of Japanese encephalitis (CDC, 2019) (accessed 2019-10-30 via https://www.cdc.gov/japaneseencephalitis/Maps/index.html) (not adapted).*

Two distinct epidemiological patterns of JE are recognized. In temperate areas such as northern Vietnam, Korea, Japan, China, Nepal and northern India, large epidemics occur during the summer months; in tropical areas like southern Vietnam, southern Thailand, Indonesia, Malaysia, the Philippines and Sri Lanka, JE tends to be endemic with peaks during the rainy season (Solomon *et al*., 2000).

The epidemiology of vector-borne viruses is complex and strongly influenced by environmental factors and other aspects of vector and host ecology. Higher temperatures, for example, are beneficial for the development of mosquito larvae and shorten the time needed for virus replication within mosquitoes helping the mosquito populations to increase and facilitating the transmission of JEV. Also humidity and precipitation promote the growth and spreading of JEV mosquito vectors (Esser *et al*., 2019). With rapid globalization and climatic changes, JE is expanding to new areas. Significant densities of JE vectors, such as *Cx. tritaeniorhynchus* have been found in more northerly latitudes like Greece (Lytra & Emmanouel, 2014) and in highland areas of Tibet and Nepal (Li *et al*., 2011). Strong winds could be a factor facilitating the dispersion of vectors, and has been suggested as a route for introduction of JEV to Australia from Papua New Guinea (Ritchie & Rochester, 2001).

JEV has been isolated from mosquitoes and birds in Italy (Platonov *et al*., 2012) and a JE case was reported in Angola, Africa in 2016 (Simon-Loriere *et al.,* 2017) making Europe and Africa potential areas for new epidemics of JE (Gao *et al*., 2019).

The recent population growth, increase in pig rearing for meat consumption and increase in rice production are all factors affecting the emergence of JE. It is clear that in areas where rice production and pig rearing overlap a higher infection rate is seen compared to areas where the activities are kept separate from each other (Erlanger *et al*., 2009).

Though JEV is generally viewed as a rural disease, competent vectors have been found in urban homes (Lindahl *et al*., 2012) and peri-urban circulation of the virus has been seen in Bangkok, Thailand, Can Tho, Vietnam and Phnom Penh, Cambodia (Olsen *et al*., 2010; Lindahl *et al*., 2013; Capelle *et al*., 2016).

JEV in Vietnam

The first clinical case of JE in Vietnam was reported in the early 1960s (Erlanger *et al*., 2009) Five strains of JE virus genotype I are currently circulating in the country according to a surveillance study carried out between 2006-2008 (Kuwata *et al*., 2013).

According to the estimates by Campbell *et al.* (2011) the annual incidence in Vietnam of JE is 1.5 per 100.000 inhabitants with a case frequency of 8072 per year. Of these cases, 7063 occur in children under the age of 15 (Campbell *et al*., 2011). The study by Lowry *et al.* (1998) concluded that JE was the most common cause of acute encephalitis syndrome in children in Hanoi, northern Vietnam (Lowry *et al*., 1998). A national vaccination program was introduced in 1997 and targeted initially young children in high risk areas, but was expanded in 2007 to include children in 65% of the district of Vietnam (Yen *et al*., 2010). In Vietnam, the pattern of JE is epidemic in the subtropical north with an epidemic season between May and August and is endemic in the south, with reports of disease all year round. Several thousand cases are reported annually, mainly from the delta regions of the Red River in the north and the Mekong River in the south (Solomon *et al.*, 2000).

Factors affecting the incidence of JEV in Vietnam are rice farming and pork production. The rice-paddy area increased 21% between 1990 and 2005 (Erlanger *et al*., 2009). Vietnam produced 44.8 million tonnes of rice in 2017 and is currently the third largest rice exporter exporting around 7 tonnes of rice per year (FAO, 2018). In recent years the livestock sector in Vietnam has been dominated by pigs. In Vietnam, pork production doubled between 2001 and 2011 (Dzung, 2019). In 2018, 28.2 million pigs were raised in Vietnam. 70-75% of all pig production is carried out at small-scale in households (GSO, 2019). The prevalence of JE in pigs was estimated to be 60.4% in northern Vietnam in a study from 2017 (Ruget *et al*., 2018) and 74.5% in another study carried out in 10 provinces in Vietnam (Lee *et al*., 2019).

In February 2019 an outbreak of African swine fever was confirmed in Vietnam with the first cases found at a back-yard pig farm in Hung Yen Province approximately 50 km from Hanoi and 250 km from the China border. The causative strain was 100% identical with viruses isolated in the outbreaks in China (2018) and Georgia (2007) (Le *et al*., 2019). Since the confirmation of the first outbreak in February all 63 provinces/municipalities in Vietnam have reported outbreaks and more than 5.4 million pigs have been culled (FAO, 2019).

Virus detection

Traditionally, virus isolation has been considered the golden standard for detection of vector-borne viruses (Hall *et al.*, 2012). Molecular methods are faster and do not always require the virus to be viable. A molecular method employed to detect the viral RNA of JEV is reverse transcriptase (RT) polymerase chain reaction (PCR) which in the first step transforms RNA to DNA. Virus in blood samples from viremic individuals and virus in mosquitoes can be detected with both of these methods (Williams *et al*., 2001).

Because detection of virus in living vertebrates only is possible during the short viremia period other methods for indirect detection also are used. Serological methods to detect IgG and IgM antibodies used are haemagglutination inhibition test (HI) and the enzyme-linked immune-sorbent assay (ELISA). Indirect IgG ELISAS for JEV in pigs seem to have a good correlation with HI results (Yang *et al*., 2006). There is a risk of cross-reactions between different flaviviruses when using serological analysis nevertheless, ELISA shows a lower risk for cross-reaction between different flaviviruses than HI (De Madrid & Porterfield, 1974). Also ELISA may work semi-quantitatively as the optical density (OD) values measured are correlated to the antibody titres (Robinson *et al*., 2010).

Monitoring of JEV

Surveillance programs for detection of virus activity can function as an early warning system of increased risk of transmission and disease outbreak. Monitoring of JEV activity is often performed using sentinel animals and/or via detection of the virus in mosquito vectors (Hall *et al*., 2012). Surveillance of JEV with sentinel pigs can be used to detect JEV emergence in new areas since confirmation in humans is more challenging. Humans get a transient viremia making detection of the virus only possible during a short time-span rendering RT-PCR and virus isolation methods with a low sensitivity. At the same time IgM detection can be misleading by antigenic cross-reactions and by secretion of antibodies in previously vaccinated patients (Dubot-Peres *et al*., 2015). Pig populations are easier to routinely monitor for the virus by for example collecting blood samples from slaughterhouses in areas with high risk (Capelle *et al*., 2016). In some countries, pigs are often raised in open pens near human habitation and thus can function as an indicator for virus burden. They have been used as sentinel animals for a long time in many countries like Japan, Taiwan, Thailand and India (Mackenzie *et al*., 2006).

Today, in countries using large-scale pig rearing systems located far away from urban areas dogs might function as more sensitive indicators of human risk than pigs. The study by Shimoda *et al.* (2011) where dogs experimentally got infected with JEV showed that dogs do not develop clinical signs after infection but develop high JEV antibody titers (Shimoda *et al*., 2011). Dogs are great sentinels for serological surveys since they are susceptible to infection, they develop antibodies, do not develop high levels of viremia to infect vector mosquitoes, survive the infection and share living space with humans (CDC, 2003).

Also the detection of virus in mosquito vectors is an important part of vector-borne disease surveillance. Traditional methods include collection of vectors with traps, identification to species level and analysis of vector pools for the viruses of interest. Detection of viral RNA is commonly performed with real-time PCR (Hall *et al*., 2012).

JE surveillance and immunization programs are today more widespread. The percentage of countries with JEV transmission risk conducting JE surveillance increased from 75% in 2012 to 92% in 2006 (Heffelfinger *et al.,* 2017).

Control of JEV

Control of JEV is achieved through different strategies like human vaccination, control of the amplifying hosts by either swine vaccination or changes in animal husbandry and mosquito control (van den Hurk *et al*., 2009). The most important control measure to avoid disease in humans is the use of a live attenuated vaccine. Current vaccines are safe and effective but a drawback is that multiple doses are needed for the vaccine to be protective (Solomon, 2006). The use of inactivated and live attenuated vaccines on pigs is protective though not practical in most settings. The study by Khan *et al.* (2014) showed that vaccinating half of the susceptible pigs each year reduces the incidence in pigs by >70% (Khan *et al*., 2014). Another strategy to decrease transmission to humans is the relocation of domestic pigs to farms sited away from human habitations. This has been partially linked to the reduction in the incidence of JE in Japan, Taiwan and South Korea (van den Hurk *et al*., 2009).

Control of the vector population can be done by modifying the breeding environment such as using alternative wetting and drying of rice fields which can reduce vector breeding while saving water (Keiser *et al*., 2005). Mosquito control can also be achieved by using insecticides such as pyrethroids, organophosphates and carbamates, although high level of resistance has reduced their efficacy (Karunaratne & Hemingway, 2000).

Other control measures that should be taken by residents and travellers to endemic areas are personal protection to reduce the number of mosquito bites by minimizing outdoor exposure at dusk and dawn, using insect repellent, wearing covering clothing and sleeping under permethrin-impregnated mosquito nets or in air-conditioned rooms. Yet, all these measures are not practical for residents of endemic areas (Solomon *et al*., 2000; Halstead & Thomas, 2010).

In 2012, immunization programs were used in 50% of the countries with risk of JEV transmission compared to 46% in 2006 (Heffelfinger *et al*., 2017). Japan and South Korea have successful control programs against JE and the low incidence rates have been stable for more than two decades. Strategies used in these countries that might explain the effective decline of JE are large-scale vaccination programs for humans, pig vaccination and separation of pig farms from human habitation, decrease of irrigated land in agricultural practice and improved living standards (Erlanger *et al*., 2009).

Japanese encephalitis is not a disease possible to eradicate globally with vaccination programs like smallpox and polio due to its enzootic nature with different animal species involved, and will remain an important public health problem in the future (Solomon *et al*., 2000).

MATERIAL AND METHODS

Study design

In total, 160 households were chosen in two provinces in northern Vietnam based on the type of rice production system they used. Half of the households used a water-saving method for rice production named alternative wetting and drying and the other half used normal flooded rice production. The reason for this selection was a concurrent project focusing on the influence of the rice production method on the mosquito presence at the households (Norrman, 2020). The households were common for both projects. The owners of the selected households were mainly rice farmers. Blood samples were collected in the ones where dogs and pigs were present. The two provinces were known to use the water-saving method and were thus chosen as study sites.

Study area

In 2018, Vietnam had a population of 94.7 million persons. The urban population was 35.7% and the rural 64.3% (GSO, 2019).

Hai Duong province

Hai Duong province (Thành phố Hải Dương) is situated in the north-east part of Vietnam in the Red River Delta as shown in Figure 3. The total population is estimated to 1.8 million people and the province covers an area of 1668.2 km^2 resulting in average population density of 1083 persons/km^2 (2018) (GSO, 2019). The village of An Duong in the Nam Sach district was one of the study sites.

Thai Binh province

Thai Binh province (Thành phố Thái Bình) is also situated in the north-east part of Vietnam in the Red River Delta. The total population is estimated to 1.8 million people and the province covers an area of 1586,4 km^2 resulting in average population density of 1130 persons/km^2 (2018) (GSO, 2019). The village of Le Loi in Dong Hung district was the second study site.

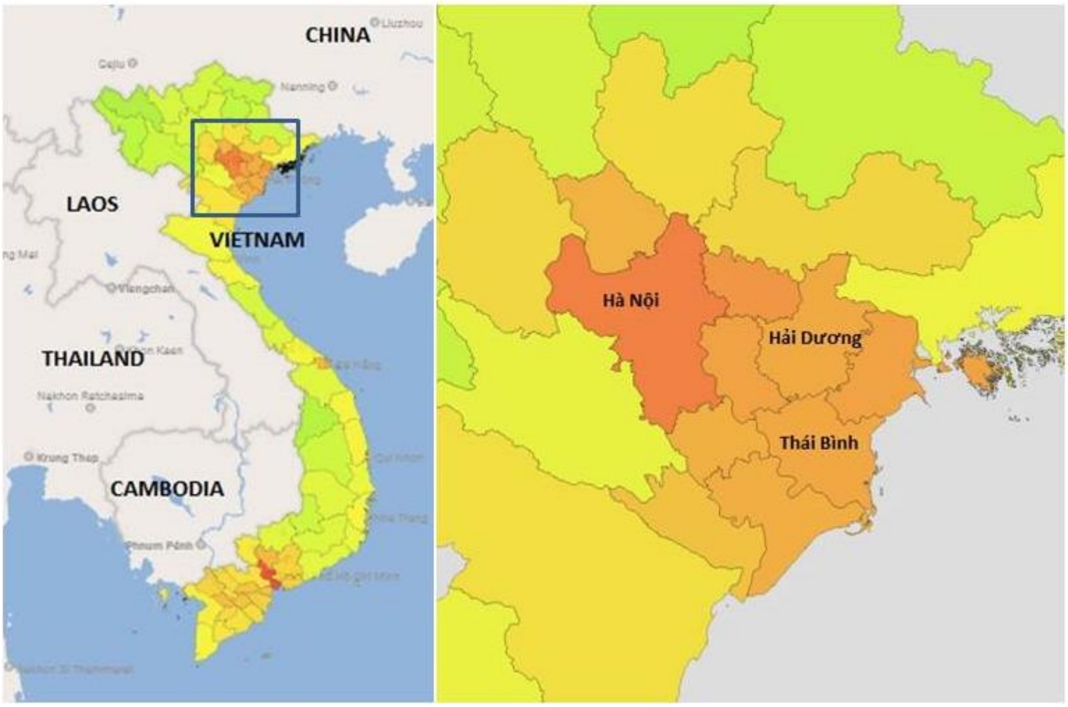

Figure 3. *Map of Vietnam showing the geographic locations of the two provinces of Hai Duong and Thai Binh, the two study sites. (Map modified from Citypopulation.de).*

Collection of pig blood samples

Blood samples from pigs were collected from the two selected villages in Thai Binh and Hai Duong, as well as from Van Phuc slaughterhouse in Hanoi during the months of September and October. Due to the African swine fever outbreak in 2019 in Vietnam, 5.4 million pigs had been culled earlier in the year, largely affecting the pig population in rural areas (FAO, 2019). For this reason only 10 pigs in the selected areas were possible to sample. Another 250 blood samples from the slaughterhouse in Hanoi were collected by staff working at the National Institute of Veterinary Research.

A questionnaire for gathering data about sex, age, symptoms during the last two months, pregnancy status, number of piglets in the last litter and information about earlier abortions or birth of stillborn piglets was prepared for the pigs from the two study sites. Information was collected in connection to the blood sampling by a native Vietnamese using a written questionnaire. Concerning the pigs from the slaughterhouse information about origin was collected. However, for the majority of pigs, it was not always possible to identify the origin and sometimes two provinces were stated as possible place of origin.

Jugular blood samples from pigs were drawn aseptically into serum tubes using vacutainer tubes and needles. Regarding the pigs from the slaughterhouse, blood samples were collected in connection with de-blooding. Blood samples were transferred on ice to the department of virology of National Institute of Veterinary Research (NIVR) in Hanoi for serological analysis. Serum was separated by centrifugation at 5000 rpm for 5 min and kept at -80° C until further processing

Collection of dog blood samples

Blood samples from dogs in the provinces of Hai Duong (56 samples) and Thai Binh (58 samples) were collected during the months of September and October after consent from the owners. Information about the age, sex, breed, symptoms during the last 2 months, indoor or outdoor keeping and purpose of the dog was collected in connection to the blood sampling by a native Vietnamese using a written questionnaire.

Blood was collected aseptically from the cephalic vein in the front limb into serum tubes. The samples were transported on ice to the department of virology of NIVR in Hanoi for serological analysis. Serum was separated by centrifugation at 5.000 rpm for 5 min and kept at -80 °C until further processing. Exclusion criteria were if the dog was newly bought or constituted a risk when handling.

Detection of JEV antibodies using enzyme-linked immunosorbent assay

A commercial enzyme-linked immunosorbent assay (ELISA) kit (ID Screen West Nile Competition Multi-Species kits from IDVet, Montpellier, France) was used to detect antibodies against the envelope protein of West Nile virus (WNV) or of related flaviviruses. Since the WNV envelope shares conserved epitopes with other flaviviruses it can be used for the detection of those belonging to the Japanese encephalitis serocomplex (Beck *et al.*, 2013). The serum samples of both dogs and pigs were tested by using the ELISA according to manufacturer´s protocol. Samples were duplicated and microplates were read at 450 nm after adding the stop solution. The OD was read using ELx808 absorbance microplate reader (BioTek, USA) with Gen 5 version 2.09.1 software. The ELISA procedure was carried out with assistance from the virology department at the National Institute for Veterinary Research.

To avoid differences in incubation times between specimens a 96-well plate was prepared containing the test and control specimens before transferring into an ELISA microplate using a multichannel pipette. All reagents were homogenized and at room temperature before use. All samples and controls were diluted with dilution buffer and then transferred to the ELISA plate. The plate was incubated for 90 min at 26 °C. Wells were then emptied and washed 3 times with 300 µl of wash solution which was prepared earlier by diluting the wash concentrate (20x) to 1/20 in distilled water. After washing, 100 µl of the conjugate (diluted to 1/10 in dilution buffer) was added to each well. The plate was incubated for 30 min at 26 °C, the wells emptied and then washed again 3 more times with 300 µl of wash solution. To each well, 100 µl of substrate solution was added and left to incubate for 15 min at 26 °C in the dark. 100 µl of the stop solution was added to each well in order to stop the reaction. The plate was immediately taken for reading of the OD at 450 nm.

The test was validated following the manufacturer´s instructions. The mean value of the negative control O.D. (OD_{NC}) had to be greater than 0.700 and the mean value of the positive control (OD_{PC}) less than 30% of the OD_{NC}. For each sample the S/N percentage (S/N%) was calculated.

$$S/N\% = OD_{sample} / OD_{NC} \times 100$$

Samples presenting a S/N% less than or equal to 40% were considered positive. A S/N% less than or equal to 50% and greater than 40% were considered doubtful. And samples with an S/N% greater than 50% were considered negative.

Statistics

STATA statistical software 14.2 was used. Descriptive statistics like frequencies, ranges and means were used to evaluate most of the results. In addition, we performed $\chi 2$ and students T-test. Odds ratios were calculated using logistic regression (logit command in STATA).

RESULTS

ELISA results

Pigs

In total, 260 pig samples were analyzed for JEV antibodies. The results show that 30% (n=78) of the pigs had antibodies against JEV. Of the tested pigs from the slaughterhouse, 28% (n=70) were positive while 80% (n=8) of pigs from the study sites were positive.

Regarding the 10 pigs from the study sites, the age varied between 2 and 5 months and 50% (n=5) were boars and 50% gilts (n=5). No pigs had had disease symptoms during the last two months. No sows were sampled and the remaining questions prepared about reproductive health could therefore not be addressed.

The pigs from the slaughterhouse were transported from different parts of Vietnam and had their origin in one of 8 provinces: Phú Thọ, Quảng Ngãi, Đồng Nai, Bình Dương, Vĩnh Phúc, Hà Nội, Khánh Hòa and Hà Tĩnh. To investigate if there is a difference in seropositivity depending on the origin pigs were further divided in the categories North, South and South-central following the given provinces. Pigs that had an unclear origin where, for example one province stated was from the north and the other one from the south of Vietnam were grouped in the category Unknown. The 10 pigs from the two study sites were included in the category North. Results are shown in Table 1 and Figure 4. There was a significantly ($p<0.001$) higher percentage of positive pigs with their origin in the north of Vietnam compared to the south and south-central. Calculation of odds ratio gives a 19.2 higher risk for the pig to be JEV positive if belonging to the North category. The difference between the south and south central groups was not found to be statistically significant.

Table 1. *Number and percentage of pigs that are positive, negative and doubtful for JEV antibodies grouped by geographic origin*

	Positive		Negative		Doubtful		Total n
Geographic location	**n**	**%**	**n**	**%**	**n**	**%**	
North	20	87%	2	9%	1	4%	23
South	25	26%	71	73%	1	1%	97
South-central	12	19%	52	81%	0	0%	64
Unknown	21	28%	55	72%	0	0%	76
Total	**78**	**30%**	**180**	**69%**	**2**	**1%**	**260**

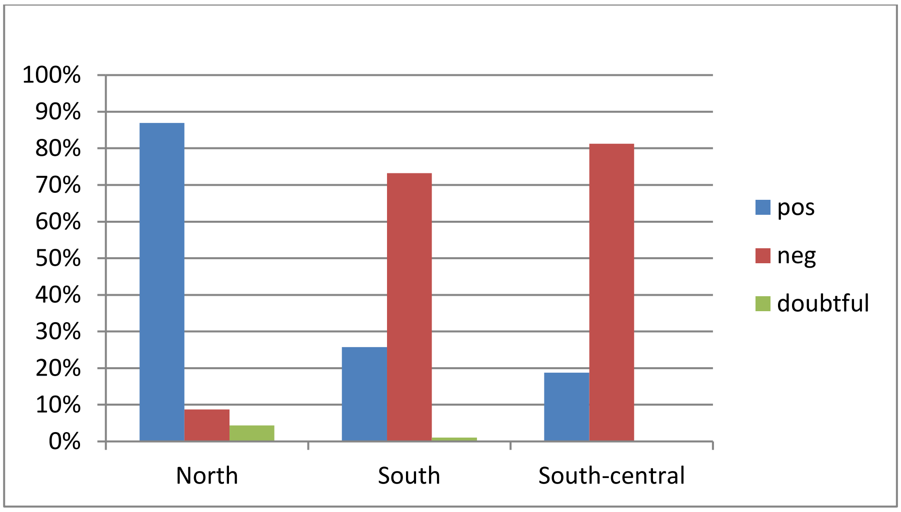

Figure 4. *Percentage of pigs that are positive, negative of doubtful for JEV antibodies divided in geographic areas of Vietnam.*

Dogs

In total, 114 dog samples were analyzed for JEV antibodies. The results show that 72% (n=82) of the samples were positive. The age of the dogs varied between 1 month and 192 months. The average age was 40 months and the median age was 24 months. The mean age for females was 46 months compared to 28 months for males. The average age for a positive dog was 48 months and for a negative dog only 20 months (p=0.01).

According to sex, 65% (n= 74) of the dogs were female and 35% (n=39) were male. The majority, 78 % (n=58) of female dogs were positive compared to 59% (n=23) of male dogs and this difference is significant ($p<0.05$). This gives females a 2.5 higher risk of being JEV positive than males. Results are shown in Table 2.

Table 2. *Number and percentage of dogs that are positive, negative and doubtful for JEV antibodies grouped by sex*

Sex	Positive	Negative	Doubtful	Total no of dogs	Positive (%)
Male	23	12	4	39	59,0
Female	58	15	1	74	78,4
Total	**81**	**27**	**5**	**113**	**71,7**

Regarding breed, 58% (n=64) were listed as Vietnamese crossbreeds, 24% (n=27) were reported to be German Shepherd crossbreeds and 18% (n=20) were classified as other crossbreeds. There was a significantly ($p<0.05$) higher percentage of German Shepherd crossbreeds that were positive compared to the group other crossbreeds. No statistically significant difference was found between the German Shepard crossbreeds and Vietnamese crossbreeds. The results are displayed in Table 3.

Table 3. *Number and percentage of dogs that are positive, negative and doubtful for JEV antibodies grouped by breed*

Breed	Positive		Negative		Doubtful		Total
	n	%	n	%	n	%	n
Vietnamese cross breed	45	70%	16	25%	3	5%	64
German Shepherd cross	23	85%	4	15%	0	0%	27
Other cross breeds	11	55%	7	35%	2	10%	20
Total	**79**	**71%**	**27**	**24%**	**5**	**5%**	**111**

Only one dog had had disease symptoms during the last 2 months. Concerning keeping conditions, 57% (n=65) of the dogs were kept fenced outdoors, 27% (n=31) were tied partly outdoors and 12% (n=14) were kept indoors. Only three of the dogs were kept free roaming. The way of keeping, indoors versus outdoors did not affect the seropositivity of the dogs. When comparing the collection sites, 82% (n=46) of dogs in Hai Duong were positive and 62% (n=36) of dogs in Thai Binh were positive for JEV antibodies. The results divided in provinces are presented in Figure 5. Dogs from Hai Duong have a 2.8 higher risk ($p<0.05$) of being JEV positive compared to the dogs from Thai Binh.

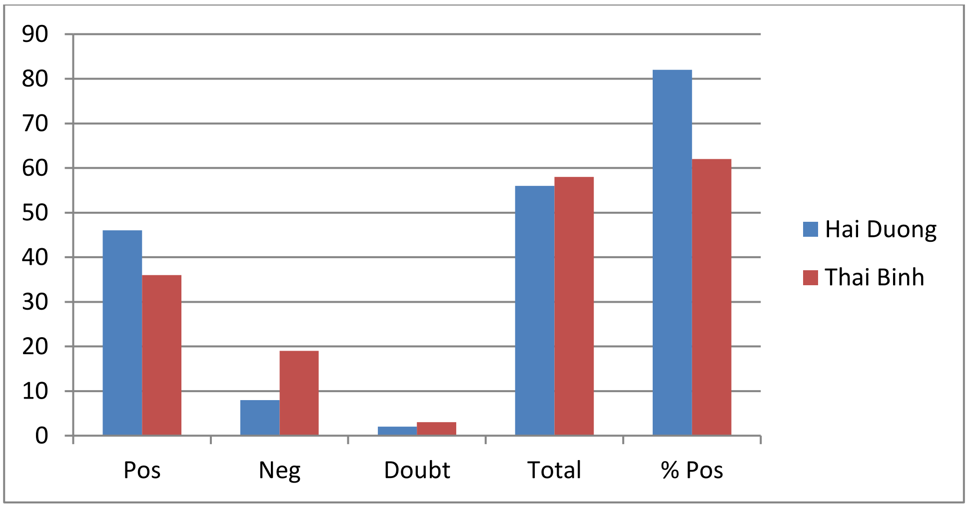

Figure 5. *Number of dog samples that are positive, negative and doubtful for each of the two study sites. The total number of samples and the percentage of positive samples for each study site is also showed.*

DISCUSSION

The purpose of this study was to assess the seroprevalence of JEV in dogs and pigs in some regions of Vietnam, and to establish risk factors in these animals that affect the seroprevalence of infection.

Due to the recent outbreak of African swine fever in Vietnam many animals had been culled and the pig population dropped markedly during 2019. This fact became an obstacle for performing the original project plan in which pigs from the two study sites were supposed to be sampled and information collected about age, sex, breed but also their reproductive health. The lack of pigs in the study sites was replaced with blood samples from the slaughterhouse in Hanoi. Unluckily, the only information possible to gather about these pigs was their place of origin.

Thirty percent of the pig samples were positive for JEV antibodies. This prevalence is much lower than in previous studies performed in Vietnam. In the Mekong delta, the reported seroprevalence is 60 to 99%, although those pigs were older (Lindahl 2012). Lee *et al.* (2019) similarly found a prevalence of 73.45 % among 2000 pigs in 10 provinces and in the study by Ruget *et al*. (2018) 60.4% among 641 pigs were positive. In addition, the study by Di Francesco *et al.* (2018) where sentinel pigs were monitored for JEV shows that all pigs seroconverted before the age of 6 months. Regarding the pigs from the slaughterhouse, since no information is recorded about the age of the pigs, way of keeping, size of the farm they come from, etc. only speculative reasons can be given to explain the lower prevalence found in this study. Causes for the lower seropositivity might be a true lower prevalence if these pigs have been kept in farms with high levels of biosecurity, protected from JEV carrying mosquitoes. Another reason could be if many older and JEV positive pigs had been culled due to the African swine fever, which could result in a decline of the total prevalence. A lower prevalence is seen in pigs between four and five months and born during the months of September and October as described by Ruget *et al.* (2018). As the age of the pigs sent to slaughter is unknown it could be possible that the majority of the samples retrieved belonged to younger pigs that have not been infected yet with JEV or developed antibodies against the virus. However, as the samples were collected during the month of September it is more likely that the pigs were born during spring or early summer and would thus have a predicted seroprevalence of >50% according to the model made by Ruget *et al.* (2018). The prevalence might also be false low due to improper handling of samples or analysis errors.

The results do also show that pigs in the north of Vietnam have a 19.2 higher risk to be JEV positive compared to pigs in the south or south-central areas ($p<0.001$). When only taking into account the pigs from the north, 87% are JEV positive and this prevalence is more consistent with the ones from earlier studies. Lee *et al.* (2017) found a prevalence of 75% among the pigs originating from 5 Northern provinces in Vietnam. An explanation could be a regional variation of the virus burden or that the pigs from the north were older than the pigs from more southern areas and thus more likely carriers of antibodies against JEV. Once again, these are mere speculations as we do not have information about age. Since only 23 pig samples from the north were analyzed a larger set of samples would be needed to draw definitive conclusions.

Concerning the 10 pigs from the study villages, data about sex, age, breed and symptoms during the last two months was collected in connection to the blood sampling but since the sampling size was too small no conclusions about possible risk factors can be drawn.

As dogs live closer to humans than pigs, they might be a better indicator for the infection risk in humans. Regarding dogs, 72% of the samples collected in Hai Duong and Thai Binh were positive for JEV antibodies. A study made in Can Tho, Vietnam found a prevalence of 21% (Nilsson 2013). In the study by Shimoda *et al.* (2010) where serological analysis of 652 blood samples collected from dogs in different regions of Japan was performed, the seroprevalence of JEV was found to be 25%. Another study carried out in Bangkok, Thailand shows a prevalence of 51% (Shimoda *et al.*, 2013). The higher prevalence found in our study suggests a high risk for human infection with JEV in the studied areas.

When comparing the collection sites, 82% (n=46) of dogs in Hai Duong were positive and 62% (n=36) of dogs in Thai Binh were positive for JEV antibodies ($p<0.05$). Why the seroprevalence in Hai Duong is higher than in Thai Binh is an interesting result as the average age of the dogs in Hai Duong is actually lower than in Thai Binh. Perhaps, Hai Duong had a higher pig density before the African swine fever outbreak with a higher JEV burden and more infected dogs. Shimoda *et al.* (2010) found in their study that in districts in southern Japan the seroprevalence was 61% and thus much higher than in northern districts with prevalences of 0% and 9%. This indicates a great regional variation in seroprevalence that is also shown in our study. In addition, Shimoda *et al.* (2010) found a significantly higher seroprevalence among dogs living in rural areas (43%) compared to those living in urban/residential areas (21%) suggesting that pig farming and rice fields in rural areas are associated with JEV. All dogs included in our study lived in rural areas and consequently are at higher risk for JEV infection. The pig population today has decreased markedly leading to a lower circulation of the virus in the environment since the vial for replication is not present. However, the majority of the infected dogs might have become infected years ago thus being indicators for the JEV exposure some time ago. One can hypothesize that if this study would be repeated in 5 years, a lower seroprevalence would be found as long as pig farming is not resumed in rural areas.

In this study, 78% of female dogs were seropositive compared to 59% of males and this difference was found to be significant ($p<0.05$). Furthermore, female dogs were also found to be older with a mean age of 46 months and males were just 28 months old. The mean age of the seropositive dogs was 48 months. It can be hypothesized that the higher seropositivity among females is more likely due to their older age rather than sex. Indeed, in the study by Shimoda *et al*. (2010) no significant difference was found between male and female dogs. In addition, no significant correlation was found between ages of dogs and seropositivity for JEV (Shimoda *et al.*, 2010). There is not much research done in dogs about JEV and it is not possible to completely rule out a correlation between age and seropositivity as an older age means a longer time of exposure and the longer an animal lives the higher the probability to become infected.

The current results do also suggest that German shepherd cross-breeds have a higher seropositivity than “other” cross-breeds. A genetic predisposition might be the cause. Moreover, German shepherd crosses tend to have a shorter hair coat compared to some other breeds, which might be preferred by mosquitoes. Whether this was the case for the dogs sampled in this study is not possible to guarantee since we did not record the physical appearance of the dogs. The keeping conditions outdoors vs indoors have been shown to affect the seroprevalence in earlier studies (Shimoda *et al.*, 2010). However, no significant difference was found in our study. How well the seropositivity of the dogs in this study correlate to the risk for humans to become infected is a complicated question. It is important to consider the measures taken by humans to avoid mosquito bites which may cause dogs to be more exposed. An important factor affecting the seropositivity in humans is the general awareness about mosquito-borne diseases. As reported by Norrman (2020), a questionnaire was used to determine the awareness regarding JEV and other mosquito-borne diseases among rice farmers. These households interviewed were the same where the dog blood samples were collected in this study. 86% of the households interviewed knew what JEV was or had heard of it, showing a considerable general awareness regarding JEV. The percentage of the population having heard or knowing about JE is higher than in the study by Dhakal *et al.* (2014) made in Nepal where pig farmers were asked the same question. In that study only 30% of farmers had heard about JE and 17% knew it is transmitted by mosquitoes (Dhakal *et al.*, 2014). Further, Norrman (2020) presents that 99% of farmers knew at least one adequate method of preventing mosquito bites in their family. Even though people knew about prevention measures the use of them could be improved. Also, as stated by Norrman (2020) the general knowledge about these diseases needs to be increased, for example only 31% knew that rice fields are a breeding site of mosquitoes. This in turn, would lead to a better practice of preventive measures. Mosquito-avoiding practices can be low-cost preventive measures for vector-borne diseases as JE. For example, a case–control study in China found that use of insecticide-treated mosquito nets was associated with significant decline in JE cases (Luo *et al.*, 1994).

The ELISA-kit used is produced to detect antibodies against the envelope protein of WNV or of related flaviviruses. Since the WNV envelope shares conserved epitopes with other flaviviruses it can be used for the detection of those belonging to the Japanese encephalitis sero-group (Beck *et al.*, 2013). However, it has been shown that dengue virus, despite belonging to another sero-group, has a tendency to cross-react with JEV (Madrid & Porterfield, 1974). WNV is present in west Asia but not in Vietnam and thus is not a likely cause of cross-reactions (Lan *et al.*, 2011). However, dengue is endemic in Vietnam and dogs have recently been shown to be able to become infected with dengue virus as demonstrated by Thongyuan & Kittayapong (2017). Also antibodies against dengue have presumably been found in a dog in Thailand by Shimoda *et al.* (2013). Due to this, especially the dog samples should be interpreted with some caution as some could be positive for dengue instead for JEV. To reduce this risk, a virus neutralization test could additionally have been performed which is a highly specific serological assay to distinguish antibodies against related flaviviruses. However, some cross-neutralization among viruses in the same serocomplex can still be observed (Beck *et al.*, 2013). No research confirming that pigs can be infected with dengue was found.

CONCLUSION

In conclusion, this study found a raised level of antibodies towards JEV in dogs than in earlier studies suggesting a high risk for human infection with JEV in the present areas. Possible risk factors found were female sex and older age but further studies are needed to confirm this. Also, German shepherd cross-breeds seem to have a higher risk. In young pigs sent for slaughter, seroprevalence was much lower than expected, which may be indicative of a reduced infection pressure due to the ongoing depopulation of pigs. Also a regional variation was found.

An interesting deepening of this study would have been to assess the seroprevalence among the farmers and to try to make a correlation with the dogs' seroprevalence. Also a follow-up study regarding the seroprevalence in dogs in the same villages if pigs are not reintroduced would be interesting.

ACKNOWLEDGEMENTS

Thank you:

Johanna Lindahl for being a great supervisor, for all your help planning the project and for always being available to answer questions. Your enthusiasm and devotion is inspiring. The National Institute of Veterinary Research, and especially to Dr. Vuong and all the lab staff at the Virology Department for helping us with our samples and for making us feel so welcome. It was all very much appreciated. The International Livestock Research Institute and especially to Mr. Thang Nguyen for all your support. I would also like to thank Dr. Long, Dr. Quynh , the IAE team and everybody joining us during the field trips for the assistance in collecting blood samples from dogs and for the help with the interviews. Also thank you Dr. Son for welcoming us to your lab at the National Institute of Hygiene and Epidemiology and for your patience helping us with the mosquito identification process.

Furthermore, I would like to thank SIDA for the MFS scholarship and for making this research possible.

A very special thank you to Sandra Norrman for being an amazing friend and roommate. For pushing me outside of my comfort zone and for making me laugh every moment along the way.

POPULAR SCIENCE SUMMARY

Japanese encephalitis is the major cause of infectious inflammation of the brain in humans with approximately 68,000 cases annually in Asia and the Western Pacific. Only 1% of infected people develop the disease but it can lead to death in one third of cases. The disease is caused by a virus called Japanese encephalitis virus (JEV) that is spread by mosquitoes. Birds and pigs are involved in the maintenance and the amplification of the virus in the environment. Pigs that get infected may also acquire reproductive disorders. Vaccination against the disease is possible and is used in many countries, however the disease is expected to increase in frequency in some countries with a large population growth, increased pork production and intensified rice production as the mosquitoes breed in flooded rice fields. Monitoring and control of the disease are important. Pigs and dogs can work as sentinels as they can get infected and develop antibodies against the disease that later can be analyzed with a blood sample. A large population of infected animals means a large burden of the virus in the environment and thus a high risk for humans to become infected.

The purpose of this study was to evaluate the quantity of infected pigs and dogs in Vietnam by taking blood samples and analyzing them for antibodies against JEV and to study the factors affecting why some animals get infected and others do not.

Blood samples from 250 pigs from a slaughterhouse in Hanoi were retrieved. The only information collected regarding these pigs was the province of origin. In addition, 10 more blood samples from pigs were collected in two rural provinces in northern Vietnam and 114 blood samples from dogs were collected in the same provinces. Information like age, sex, breed and keeping conditions was collected for these animals.

Results show that 30% of pigs had antibodies against JEV and thus had been infected by the virus. Other studies have shown higher percentages of infected pigs so the low prevalence here may potentially be explained by the reduction in pig population due to the ongoing outbreak of African swine fever. Other interesting result was that the pigs coming from the north of Vietnam had a 19.2 higher risk to be JEV positive. Concerning the dogs, 72% were positive for JEV which is higher than the percentage found in earlier similar studies. It was also found that females were positive in a higher extent than males but they were also older. It can be hypothesized that the higher seropositivity among females is more likely due to their older age rather than sex. Also, in this study German shepherd cross-breeds were positive in higher percentage than other cross-breeds. However, more studies are needed to confirm this.

REFERENCES

Arunachalam, N., Samuel, P.P., Hiriyan, J., Rajendran, R. & Dash, A.P. (2005). Short report: observations on the multiple feeding behavior of *Culex tritaeniorhynchus* (*Diptera: culicidae*), the vector of Japanese encephalitis in Kerala in southern India. *American Journal of Tropical Medicine and Hygiene,* 72: 198–200.

Beck, C., Jimenez-Clavero, M.A., Leblond, A., Durand, B., Nowotny, N., Leparc-Goffart, I., Zientara, S., Jourdain, E. & Lecollinet, S. (2013). Flaviviruses in Europe: Complex circulation patterns and their consequences for the diagnosis and control of West Nile disease. *International Journal of Environmental Research and Public Health,* 10: 6049–6083. https://doi.org/10.3390/ijerph10116049

Campbell, G.L., Hills, S.L., Fischer, M., Jacobson, J.A., Hoke, C.H., Hombach, J.M., Marfin, A.A., Solomon, T., Tsai, T.F., Tsu, V.D. & Ginsburg, A.S. (2011). Estimated global incidence of Japanese encephalitis: a systematic review. *Bulletin of the World Health Organization,* 89: 766–774, 774A-774E.

Cappelle, J., Duong, V., Pring, L., Kong, L., Yakovleff, M., Prasetyo, D.B., Peng, B., Choeung, R., Duboz, R., Ong, S., Sorn, S., Dussart, P., Tarantola, A., Buchy, P. & Chevalier, V. (2016). Intensive circulation of Japanese encephalitis virus in peri-urban sentinel pigs near Phnom Penh, Cambodia. *PLoS Neglected Tropical Diseases,* 10. https://doi.org/10.1371/journal.pntd.0005149

Centers for Disease Control and Prevention (CDC). (2003). Epidemic/Epizootic West Nile virus in the United States: Guidelines for surveillance, prevention, and control. Division of vector-borne infectious diseases, US Department of health and human services, Fort Collins, CO (available at: http:// www.cdc.gov/ncidod/dvbid/arbor).

De Madrid, A.T. & Porterfield, J.S. (1974). The flaviviruses (Group B arboviruses): a cross-neutralization study. *Journal of General Virology*, 23: 91–96. https://doi.org/10.1099/0022-1317-23-1-91

Diallo, A.O.I., Chevalier, V., Cappelle, J., Duong, V., Fontenille, D. & Duboz, R. (2018). How much does direct transmission between pigs contribute to Japanese Encephalitis virus circulation? A modelling approach in Cambodia. *PLoS ONE*, 13, e0201209. https://doi.org/10.1371/journal.pone.0201209

Di Francesco, J., Choeung, R., Peng, B., Pring, L., Pang, S., Duboz, R., Ong, S., Sorn, S., Tarantola, A., Fontenille, D., Duong, V., Dussart, P., Chevalier, V & Cappelle, J. (2018). Comparison of the dynamics of Japanese encephalitis virus circulation in sentinel pigs between a rural and a peri-urban setting in Cambodia. *PLoS Neglected Tropical Diseases,* 12. https://doi.org/10.1371/journal.pntd.0006644

Desingu, P.A., Ray, P.K., Patel, B.H.M., Singh, R., Singh, R.K. & Saikumar, G. (2016). Pathogenic and genotypic characterization of a Japanese encephalitis virus isolate associated with reproductive failure in an Indian pig herd. *PLoS ONE,* 11, e0147611. https://doi.org/10.1371/journal.pone.0147611

Dhakal, S., Joshi, D.D., Ale, A., Sharma, M., Dahal, M., Shah, Y., Pant, D.K. & Stephen, C. (2014). Regional variation in pig farmer awareness and actions regarding Japanese encephalitis in Nepal: implications for public health education. *PLoS ONE* 9, e85399. https://doi.org/10.1371/journal.pone.0085399

Dubot-Pérès, A., Sengvilaipaseuth, O., Chanthongthip, A., Newton, P.N. & de Lamballerie, X. (2015). How many patients with anti-JEV IgM in cerebrospinal fluid really have Japanese encephalitis? *The Lancet Infectious Diseases*, 15: 1376–1377. https://doi.org/10.1016/S1473-3099(15)00405-3

Dzung, N.M. (2013). Pig production and marketing in Vietnam. [Online] available: http://www.angrin.tlri.gov.tw/English/2014Swine/p145-152.pdf [accessed 25102019]

Erlanger, T.E., Weiss, S., Keiser, J., Utzinger, J. & Wiedenmayer, K. (2009). Past, present, and future of Japanese encephalitis. *Emerging Infectious Diseases*, 15: 1–7.

Esser, H.J., Mögling, R., Cleton, N.B., van der Jeugd, H., Sprong, H., Stroo, A., Koopmans, M.P.G., de Boer, W.F. & Reusken, C.B.E.M. (2019). Risk factors associated with sustained circulation of six zoonotic arboviruses: a systematic review for selection of surveillance sites in non-endemic areas. *Parasites & Vectors*, 12. https://doi.org/10.1186/s13071-019-3515-7

FAO (2018) Rice market monitor, volume XXI, issue no.1. [Online] available: http://www.fao.org/3/I9243EN/i9243en.pdf [accessed 21102019]

FAO (2019) Animal production and health. [Online] available: http://www.fao.org/ag/againfo/programmes/en/empres/ASF/situation_update.html [accessed 21102019]

Gao, X., Liu, H., Li, X., Fu, S., Cao, L., Shao, N., Zhang, W., Wang, Q., Lu, Z., Lei, W., He, Y., Cao, Y., Wang, H. & Liang, G. (2019). Changing geographic distribution of Japanese encephalitis virus genotypes, 1935-2017. *Vector-Borne and Zoonotic Diseases*, 19:35–44. https://doi.org/10.1089/vbz.2018.2291

Griffiths, M.J., Turtle, L. & Solomon, T. (2014). Japanese encephalitis virus infection. *Handbook of Clinical Neurology*, 123: 561-576.

GSO, 2019. General Statistics Office of Vietnam. [Online] available: http://www.gso.gov.vn/default_en.aspx? [accessed 24102019].

Hall, R.A., Blitvich, B.J., Johansen, C.A. & Blacksell, S.D. (2012). Advances in arbovirus surveillance, detection and diagnosis. *Journal of Biomedicine and Biotechnology*, 2012 https://doi.org/10.1155/2012/512969

Halstead, S.B. & Thomas, S.J. (2010). New vaccines for Japanese encephalitis. *Current Infectious Diseases Reports*, 12: 174–180. https://doi.org/10.1007/s11908-010-0098-z

Heffelfinger, J.D., Li, X., Batmunkh, N., Grabovac, V., Diorditsa, S., Liyanage, J.B., Pattamadilok, S., Bahl, S., Vannice, K.S., Hyde, T.B., Chu, S.Y., Fox, K.K., Hills, S.L. & Marfin, A.A. (2017). Japanese encephalitis surveillance and immunization - Asia and Western Pacific regions, 2016. *Morbidity and Mortality Weekly Report*, 66: 579–583. https://doi.org/10.15585/mmwr.mm6622a3

Hoke, C.H., Vaughn, D.W., Nisalak, A., Intralawan, P., Poolsuppasit, S., Jongsawas, V., Titsyakorn, U. & Johnson, R.T. (1992). Effect of high-dose dexamethasone on the outcome of acute encephalitis due to Japanese encephalitis virus. *Journal of Infectious Diseases*, 165: 631–637. https://doi.org/10.1093/infdis/165.4.631

Karunaratne, S.H. & Hemingway, J. (2000). Insecticide resistance spectra and resistance mechanisms in populations of Japanese encephalitis vector mosquitoes, *Culex tritaeniorhynchus* and *Cx. gelidus*, in Sri Lanka. *Medical and Veterinary Entomology*, 14: 430–436.

Keiser, J., Maltese, M.F., Erlanger, T.E., Bos, R., Tanner, M., Singer, B.H. & Utzinger, J. (2005). Effect of irrigated rice agriculture on Japanese encephalitis, including challenges and opportunities for integrated vector management. *Acta Tropica*, 95: 40–57. https://doi.org/10.1016/j.actatropica.2005.04.012

Khan, S.U., Salje, H., Hannan, A., Islam, Md. Atiqul, Bhuyan, A.A.M., Islam, Md. Ariful, Rahman, M.Z., Nahar, N., Hossain, M.J., Luby, S.P. & Gurley, E.S. (2014). Dynamics of Japanese encephalitis virus transmission among pigs in northwest Bangladesh and the potential impact of pig vaccination. *PLoS Neglected Tropical Diseases*, 8:e3166.

Kumar, K., Arshad, S.S., Selvarajah, G.T., Abu, J., Toung, O.P., Abba, Y., Bande, F., Yasmin, A.R., Sharma, R., Ong, B.L., Rasid, A.A., Hashim, N., Peli, A., Heshini, E.P. & Shah, A.K.M.K. (2018). Prevalence and risk factors of Japanese encephalitis virus (JEV) in livestock and companion animal in high-risk areas in Malaysia. *Tropical Animal Health and Production*, 50: 741–752. https://doi.org/10.1007/s11250-017-1490-6

Kuwata, R., Nga, P.T., Yen, N.T., Hoshino, K., Isawa, H., Higa, Y., Hoang, N.V., Trang, B.M., Loan, D.P., Phong, T.V., Sasaki, T., Tsuda, Y., Kobayashi, M., Sawabe, K. & Takagi, M. (2013). Surveillance of Japanese encephalitis virus infection in mosquitoes in Vietnam from 2006 to 2008. *American Journal of Tropical Medicine and Hygiene*, 88, 681–688. https://doi.org/10.4269/ajtmh.12-0407

Lan, D., Ji, W., Yu, D., Chu, J., Wang, C., Yang, Z. & Hua, X. (2011). Serological evidence of West Nile virus in dogs and cats in China. *Archives of Virology*, 156: 893–895. https://doi.org/10.1007/s00705-010-0913-8

Le, V.P., Jeong, D.G., Yoon, S.-W., Kwon, H.-M., Trinh, T.B.N., Nguyen, T.L., Bui, T.T.N., Oh, J., Kim, J.B., Cheong, K.M., Van Tuyen, N., Bae, E., Vu, T.T.H., Yeom, M., Na, W. & Song, D. (2019). Outbreak of African swine fever, Vietnam, 2019. *Emerging Infectious Diseases*, 25:1433–1435. https://doi.org/10.3201/eid2507.190303

Lee, H.S., Nguyen-Viet, H., Lee, M., Duc, P.P. & Grace, D. (2017). Seasonality of viral encephalitis and associated environmental risk factors in Son La and Thai Binh provinces in Vietnam from 2004 to 2013. *The American Journal of Tropical Medicine and Hygiene*, 96: 110–117. https://doi.org/10.4269/ajtmh.16-0471

Lee, H.S., Thanh, T.L., Ly, N.K., Nguyen-Viet, H., Thakur, K.K. & Grace, D. (2019). Seroprevalence of leptospirosis and Japanese encephalitis in swine in ten provinces of Vietnam. *PLoS ONE*, 14: e0214701. https://doi.org/10.1371/journal.pone.0214701

Li, M.-H., Fu, S.-H., Chen, W.-X., Wang, H.-Y., Guo, Y.-H., Liu, Q.-Y., Li, Y.-X., Luo, H.-M., Da, W., Duo Ji, D.Z., Ye, X.-M. & Liang, G.D. (2011). Genotype V Japanese encephalitis virus is emerging. *PLoS Neglected Tropical Diseases*, 5. https://doi.org/10.1371/journal.pntd.0001231

Libraty, D.H., Nisalak, A., Endy, T.P., Suntayakorn, S., Vaughn, D.W. & Innis, B.L. (2002). Clinical and immunological risk factors for severe disease in Japanese encephalitis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 96: 173–178. https://doi.org/10.1016/s0035-9203(02)90294-4

Lindahl, J. (2012). *Japanese encephalitis virus in pigs and vectors in the Mekong Delta: with special reference to urban farming*. Diss. Uppsala: Sveriges lantbruksuniversitet.

Lindahl, J., Boqvist, S., Ståhl, K., Thu, H.T.V. & Magnusson, U. (2012). Reproductive performance in sows in relation to Japanese encephalitis virus seropositivity in an endemic area. *Tropical Animal Health and Production*, 44: 239–245. https://doi.org/10.1007/s11250-011-0005-0

Lindahl, J.F., Ståhl, K., Chirico, J., Boqvist, S., Thu, H.T.V. & Magnusson, U. (2013). Circulation of Japanese encephalitis virus in pigs and mosquito vectors within Can Tho City, Vietnam. *PLoS Neglected Tropical Diseases*, 7:e2153

Liu, H., Lu, H.-J., Liu, Z.-J., Jing, J., Ren, J.-Q., Liu, Y.-Y., Lu, F. & Jin, N.-Y. (2013). Japanese encephalitis virus in mosquitoes and swine in Yunnan province, China 2009-2010. *Vector-Borne and Zoonotic Diseases*, 13: 41–49. https://doi.org/10.1089/vbz.2012.1016

Lowry, P.W., Cropp, C.B., Martin, D., Hinh, L.D., Karabatsos, N., Ladinsky, J.L., Truong, D.H. & Gubler, D.J. (1998). Japanese encephalitis among hospitalized pediatric and adult patients with acute encephalitis syndrome in Hanoi, Vietnam 1995. *The American Journal of Tropical Medicine and Hygiene*, 58: 324–329. https://doi.org/10.4269/ajtmh.1998.58.324

Luo, D., Yao, R., Song, J., Huo, H. & Wang, Z. (1994). The effect of DDT spraying and bed nets impregnated with pyrethroid insecticide on the incidence of Japanese encephalitis virus infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 88: 629–631.

Lytra, I. & Emmanouel, N. (2014). Study of *Culex tritaeniorhynchus* and species composition of mosquitoes in a rice field in Greece. *Acta Tropica*, 134: 66–71. https://doi.org/10.1016/j.actatropica.2014.02.018

Mackenzie, J.S., Childs, J.E., Field, H.E., Wang, L.-F. & Breed, A.C. (2008). The role of bats as reservoir hosts of emerging neurological viruses. In: Shoshkes Reiss, C. (Ed.), *Neurotropic Viral Infections*. Cambridge University Press, Cambridge, pp. 382–406. https://doi.org/10.1017/CBO9780511541728.026

Mackenzie, J.S., Williams, D.T. & Smith, D.W. (2006). Japanese encephalitis virus: The geographic distribution, incidence, and spread of a virus with a propensity to emerge in new areas. *Perspectives in Medical Virology*, 16: 201–268

Mwandawiro, C., Boots, M., Tuno, N., Suwonkerd, W., Tsuda, Y. & Takagi, M. (2000). Heterogeneity in the host preference of Japanese encephalitis vectors in Chiang Mai, northern Thailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 94, 238–242. https://doi.org/10.1016/s0035-9203(00)90303-1

Nabeshima, T., Loan, H.T.K., Inoue, S., Sumiyoshi, M., Haruta, Y., Nga, P.T., Huoung, V.T.Q., del Carmen Parquet, M., Hasebe, F. & Morita, K. (2009). Evidence of frequent introductions of Japanese encephalitis virus from south-east Asia and continental east Asia to Japan. *Journal of General Virology*, 90: 827–832. https://doi.org/10.1099/vir.0.007617-0

Nguyen-Tien, T., Lundkvist, Å. & Lindahl, J. (2019). Urban transmission of mosquito-borne flaviviruses – a review of the risk for humans in Vietnam. *Infection Ecology & Epidemiology*, 9. https://doi.org/10.1080/20008686.2019.1660129

Nilsson, S. (2013). *Seroprevalence of Japanese encephalitis virus in pigs and dogs in the Mekong Delta*. Sveriges lantbruksuniversitet. Veterinärprogrammet (Examensarbete, 2013:46).

Norrman, S. (2020). *Alternate wetting and drying and its influence on zoonotic disease vectors in northern Vietnam*. Sveriges lantbruksuniversitet. Veterinärprogrammet (Examensarbete).

Oliveira, A.R.S., Cohnstaedt, L.W. & Cernicchiaro, N. (2018). Japanese encephalitis virus: Placing disease vectors in the epidemiologic triad. *Annals of the Entomological Society of America*, 111(6): 295–303.

Olsen, S.J., Supawat, K., Campbell, A.P., Anantapreecha, S., Liamsuwan, S., Tunlayadechanont, S., Visudtibhan, A., Lupthikulthum, S., Dhiravibulya, K., Viriyavejakul, A., Vasiknanonte, P., Rajborirug, K., Watanaveeradej, V., Nabangchang, C., Laven, J., Kosoy, O., Panella, A., Ellis, C., Henchaichon, S., Khetsuriani, N., Powers, A.M., Dowell, S.F. & Fischer, M. (2010). Japanese encephalitis virus remains an important cause of encephalitis in Thailand. *International Journal of Infectious Diseases*, 14, e888-892. https://doi.org/10.1016/j.ijid.2010.03.022

Pearce, J.C., Learoyd, T.P., Langendorf, B.J. & Logan, J.G. (2018). Japanese encephalitis: the vectors, ecology and potential for expansion. *Journal of Travel Medicine*, 25: 16–26. https://doi.org/10.1093/jtm/tay009

Peiris, J.S., Amerasinghe, F.P., Amerasinghe, P.H., Ratnayake, C.B., Karunaratne, S.H. & Tsai, T.F. (1992). Japanese encephalitis in Sri Lanka--the study of an epidemic: vector incrimination, porcine infection and human disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 86: 307–313. https://doi.org/10.1016/0035-9203(92)90325-7

Platonov, A.E., Rossi, G., Karan, L.S., Mironov, K.O., Busani, L. & Rezza, G. (2012). Does the Japanese encephalitis virus (JEV) represent a threat for human health in Europe? Detection of JEV RNA sequences in birds collected in Italy. *Eurosurveillance*, 17, 20241. https://doi.org/10.2807/ese.17.32.20241-en

Ricklin, M.E., García-Nicolás, O., Brechbühl, D., Python, S., Zumkehr, B., Nougairede, A., Charrel, R.N., Posthaus, H., Oevermann, A. & Summerfield, A. (2016). Vector-free transmission and persistence of Japanese encephalitis virus in pigs. *Nature Communications*, 7, 10832. https://doi.org/10.1038/ncomms10832

Ritchie, S.A. & Rochester, W. (2001). Wind-blown mosquitoes and introduction of Japanese encephalitis into Australia. *Emerging Infectious Diseases*, 7: 900–903. https://doi.org/10.3201/eid0705.017524

Robinson, J.S., Featherstone, D., Vasanthapuram, R., Biggerstaff, B.J., Desai, A., Ramamurty, N., Chowdhury, A.H., Sandhu, H.S., Cavallaro, K.F. & Johnson, B.W. (2010). Evaluation of three commercially available Japanese encephalitis virus IgM enzyme-linked immunosorbent assays. *American Journal of Tropical Medicine & Hygiene*, 83: 1146–1155. https://doi.org/10.4269/ajtmh.2010.10-0212

Rosen, L. (1986). The natural history of Japanese encephalitis virus. *Annual Review of Microbiology*, 40: 395–414. https://doi.org/10.1146/annurev.mi.40.100186.002143

Ruget, A.-S., Beck, C., Gabassi, A., Trevennec, K., Lecollinet, S., Chevalier, V. & Cappelle, J. (2018). Japanese encephalitis circulation pattern in swine of northern Vietnam and consequences for swine's vaccination recommendations. *Transbound Emerging Diseases*, 65: 1485–1492.

Shimoda, H., Ohno, Y., Mochizuki, M., Iwata, H., Okuda, M. & Maeda, K. (2010). Dogs as sentinels for human infection with Japanese encephalitis virus. *Emerging Infectious Diseases*, 16: 1137–1139. https://doi.org/10.3201/eid1607.091757

Shimoda, H., Tamaru, S., Morimoto, M., Hayashi, T., Shimojima, M. & Maeda, K. (2011). Experimental infection of Japanese encephalitis virus in dogs. *Journal of Veterinary Medical Science*, 73: 1241–1242. https://doi.org/10.1292/jvms.11-0142

Simon-Loriere, E., Faye, O., Prot, M., Casademont, I., Fall, G., Fernandez-Garcia, M.D., Diagne, M.M., Kipela, J.-M., Fall, I.S., Holmes, E.C., Sakuntabhai, A. & Sall, A.A. (2017). Autochthonous Japanese encephalitis with yellow fever coinfection in Africa. *New England Journal of Medicine*, 376(15):1483-1485. https://doi.org/10.1056/NEJMc1701600

Solomon, T. (2004). Flavivirus encephalitis. *New England Journal of Medicine*, 351: 370–378. https://doi.org/10.1056/NEJMra030476

Solomon, T. (2006). Control of Japanese encephalitis--within our grasp? *New England Journal of Medicine*, 355: 869–871. https://doi.org/10.1056/NEJMp058263

Solomon, T., Dung, N.M., Kneen, R., Gainsborough, M., Vaughn, D.W. & Khanh, V.T. (2000). Japanese encephalitis. *Journal of Neurology, Neurosurgery & Psychiatry*, 68: 405–415. https://doi.org/10.1136/jnnp.68.4.405

Solomon, T., Dung, N.M., Kneen, R., Thao, L.T.T., Gainsborough, M., Nisalak, A., Day, N.P.J., Kirkham, F.J., Vaughn, D.W., Smith, S. & White, N.J. (2002). Seizures and raised intracranial pressure in Vietnamese patients with Japanese encephalitis. *Brain: a Journal of Neurology*, 125: 1084–1093. https://doi.org/10.1093/brain/awf116

Solomon, T., Dung, N.M., Wills, B., Kneen, R., Gainsborough, M., Diet, T.V., Thuy, T.T.N., Loan, H.T., Khanh, V.C., Vaughn, D.W., White, N.J. & Farrar, J.J. (2003). Interferon alfa-2a in Japanese encephalitis: a randomised double-blind placebo-controlled trial. *Lancet*, 361: 821–826. https://doi.org/10.1016/s0140-6736(03)12709-2

Teng, M., Luo, J., Fan, J.-M., Chen, L., Wang, X.-T., Yao, W., Wang, C.-Q. & Zhang, G.-P. (2013). Molecular characterization of Japanese encephalitis viruses circulating in pigs and mosquitoes on pig farms in the Chinese province of Henan. *Virus Genes*, 46: 170–174. https://doi.org/10.1007/s11262-012-0813-y

Thongyuan, S. & Kittayapong, P. (2017). First evidence of dengue infection in domestic dogs living in different ecological settings in Thailand. *PLoS One*, 12. https://doi.org/10.1371/journal.pone.0180013

Tsai, T.F. (2000). New initiatives for the control of Japanese encephalitis by vaccination. *Vaccine*, 18: 1–25. https://doi.org/10.1016/S0264-410X(00)00037-2

Uchil, P.D. & Satchidanandam, V. (2001). Phylogenetic analysis of Japanese encephalitis virus: envelope gene based analysis reveals a fifth genotype, geographic clustering, and multiple introductions of the virus into the Indian subcontinent. *The American Journal of Tropical Medicine and Hygiene*, 65: 242–251. https://doi.org/10.4269/ajtmh.2001.65.242

van den Hurk, A.F., Ritchie, S.A. & Mackenzie, J.S. (2009). Ecology and geographical expansion of Japanese encephalitis virus. *Annual Review of Entomology*, 54: 17–35. https://doi.org/10.1146/annurev.ento.54.110807.090510

Watt, G. & Jongsakul, K. (2003). Acute undifferentiated fever caused by infection with Japanese encephalitis virus. *American Journal of Tropical Medicine & Hygiene*, 68: 704–706.

Williams, D.T., Daniels, P.W., Lunt, R.A., Wang, L.F., Newberry, K.M. & Mackenzie, J.S. (2001). Experimental infections of pigs with Japanese encephalitis virus and closely related Australian flaviviruses. *American Journal of Tropical Medicine & Hygiene*, 65: 379–387. https://doi.org/10.4269/ajtmh.2001.65.379

Yang, D.-K., Kim, B.-H., Lim, S.-I., Kwon, J.-H., Lee, K.-W., Choi, C.-U. & Kweon, C.-H. (2006). Development and evaluation of indirect ELISA for the detection of antibodies against Japanese encephalitis virus in swine. *Journal of Veterinary Science*, 7: 271–275. https://doi.org/10.4142/jvs.2006.7.3.271

Yen, N.T., Duffy, M.R., Hong, N.M., Hien, N.T., Fischer, M. & Hills, S.L. (2010). Surveillance for Japanese encephalitis in Vietnam, 1998-2007. *American Journal of Tropical Medicine & Hygiene*, 83: 816–819.

Yin, Z., Wang, X., Li, L., Li, H., Zhang, X., Li, J., Ning, G., Li, F., Liang, Xuefeng, Gao, L., Liang, Xiaofeng. & Li, Y. (2015). Neurological sequelae of hospitalized Japanese encephalitis cases in Gansu province, China. *American Journal of Tropical Medicine & Hygiene*, 92: 1125–1129. https://doi.org/10.4269/ajtmh.14-0148