Surveillance of vector-borne diseases in Indonesia

インドネシアにおける節足動物媒介性感染症の疫学調査研究

The United Graduate School of Veterinary Science

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1. GENERAL INTRODUCTION

1.1. Vector-borne diseases

Vector-borne diseases (VBDs) are illnesses that can have serious effects on both human and animal health, it has also significant implications in economic burden. Vectors are living organisms that can transmit infectious pathogens such as virus, bacteria, and parasites, between humans, or from animals to humans (WHO, 2020). Most of these vectors are blood sucking arthropods, for instance, mosquitoes, ticks, flies, sandflies, fleas, triatomine bugs, and lice (McHugh, 1994; WHO, 2020). Several VBDs such as malaria, dengue fever, schistosomiasis, human African trypanosomiasis, leishmaniasis, Chagas disease, yellow fever, Japanese encephalitis (JE) and onchocerciasis are frequently diagnosed in tropical and subtropical countries and cause more than 700,000 deaths every year (WHO, 2020).

Most of VBDs are zoonoses and have a primary vertebrate reservoir, however, humans and domestic animals commonly have taken by the diseases infections incidentally or sometimes became as a dead-end host (Ortigao and Gubler, 2020). Moreover, distribution of VBDs is determined by a complex set of demographic, environmental changes and social factors such as global travel, trade of animals or supplies, and unplanned urbanization (Weaver, 2013). These factors can affect diseases outbreaks by altering biological variables such as vector population size and density, vector survival rates, reservoir hosts, and pathogen reproduction rates (Gage et al., 2008; Guha-Sapir and Schimmer, 2005; Mordecai et al., 2019).

1.2. Tick-borne diseases

Ticks are efficient vectors of multiple pathogens due to their potential interactions with several different vertebrate hosts during their life cycle. The ticks can transmit several diseases to blood-feeding host including wild animals, livestock and humans. At least 10% of the 900 currently known tick species are significant medical or veterinary importance (de la Fuente et al., 2017). Several tick species that have been reported as threat to animal and public health are

*Ixode*s spp., *Dermacentor* spp., *Amblyomma* spp., *Haemaphysalis* spp., *Hyalomma* spp., and *Rhipicephalus* spp. (Jongejan and Uilenberg, 2004; Parola and Raoult, 2001). Besides causing direct damage associated with blood-feeding activities, the ticks can also transmit several microorganisms including viruses, bacteria, protozoa, and helminths (Jongejan and Uilenberg, 2004). The ticks fed on certain groups of wild animals and become problems for livestock when these wild hosts came into and contact with ticks or vice versa (de la Fuente et al., 2017). The exploitation of environmental resources and the increase in human activities has promoted contact with tick vectors, resulting in increased transmission of tick-borne pathogens (de la Fuente et al., 2017). Among the tick-borne pathogens, anaplasmosis, theileriosis, and babesiosis affecting on economic importance particularly for livestock, predominantly in tropical areas (Jongejan and Uilenberg, 2004). However, there is also impact on public health such as relapsing fever (RF), Lyme diseases (LD), rickettsial diseases (Q-fever, spotted fever, Anaplasmosis and Ehrlichiosis), Tularemia, Colorado Tick Fever, Crimean-Congo hemorrhagic fever (CCHF), Tularemia, tick-borne encephalitis virus, and severe fever with thrombocytopenia syndrome (SFTS) (Mansfield et al., 2009; Li, 2013).

1.2.1. Anaplasmosis and Ehrlichiosis

1.2.1.1 History

Anaplasmosis and Ehrlichiosis are one of tick-borne bacterial diseases and caused by *Anaplasma* spp. and *Ehrlichia* spp., respectively. Genera *Ehrlichia* and *Anaplasma* are obligately intracellular gram-negative bacteria and members of family *Anaplasmataceae* (order *Rickettsiales*) (Dumler et al., 2001).

The genus *Anaplasma* was established in 1910 by Sir Arnold Theiler who first recognized that ‘marginal points’ in stained erythrocytes of cattle in South Africa (Theiler, 1910). Because of the ‘marginal points’ located inclusions had been seen frequently in

erythrocytes of anemic cattle, often in those suffering from babesiosis (piroplasmosis), therefore, Smith and Kilborne (1893) had incorrectly concluded that the 'marginal points' were part of the *Babesia bigemina* life cycle. In 1926, Darlington (1926) reported that cattle from southeastern Kansas, had a febrile disease that usually occurs in the late summer and fall of the year (Texas cattle fever) and he concluded that it was the same disease reported by Theiler in South Africa in 1910. Since then, Anaplasmosis were reported commonly infected ruminants such as, cattle, sheep, goats, buffaloes, and some wild ruminants (Atif, 2015).

Anaplasma phagocytophilum was previously described as *Rickettsia phagocytopila* (Foggie, 1951). After that, the agent was included in the tribe *Ehrlichia* of the order *Rickettsiales* (Woldehiwet, 2006). Recently, *A. phagocytophilum* is emended a name replacing three species of granulocytic bacteria, *Ehrlichia phagocytophila, Ehrlichia equi* and the agent of human granulocytic ehrlichiosis (HGE) (Dumler et al., 2001). The prototype of *A. phagocytophilum* was first described in 1940 as causative agent of tick-borne relapsing fever in sheep, cattle, and goats, (Jeffries et al., 2014).

Ehrlichia caffeensis causes of Ehrlichiosis or human monocytic ehrlichiosis (HME) (de la Fuente et al., 2016) was first identified in 1986 in USA (Maeda et al., 1987).

1.2.1.2. Epidemiology

Both Anaplasmosis and Ehrlichiosis have become important tick-borne pathogen in several countries in America, Europe, and Asia, with an increasing number of patients and animals (Beyer and Carlyon, 2015; Paddock and Childs, 2003; Ismail et al., 2010). Clinically, *Anaplasma marginale* is the most important pathogen in veterinary field and *A. phagocytophilum* and *E. caffeensis* are important for human health.

A. marginale is host specific, causes disease primarily in ruminants (Kocan et al., 2010). *A. marginale* has been reported in tropical and subtropical areas throughout the world such as

USA, Mexico, Canada, South Africa, India, and China (Hove et al., 2018; Ashuma et al., 2013; Kocan et al., 2010; Yang et al., 2017). The wide distribution of this disease might be caused by transportation of carrier cattle with subsequent mechanical or biological transmission from asymptomatic infected cattle to susceptible ones (Kocan et al., 2010). *A. marginale* is biologically transmitted primarily by *Rhipicephalus* ticks through trans-stadial (larva-nymph-adult) transmission within tick stages (Atif, 2015). Therefore, the movement of ticks and blood-feeding hosts may contribute to spreading of this disease. Moreover, transmission of *A. marginale* can also be affected mechanically by biting flies or blood-contaminated fomites (Kocan et al., 2003).

Distribution of *A. phagocytophilum* is worldwide primarily in America, Europe, and Asia including Japan and Mongolia (Kocan et al., 2010; Woldehiwet, 2006; Ohashi et al., 2005; Javkhlan et al., 2014). For instance, it was reported to be causes of tick-borne relapsing fever in sheep and cattle in UK (Hudson, 1950), Ireland (Zintl et al., 2017), Scandinavia (Tuomi, 1967) and California (Gribble, 1969). *A. phagocytophilum* is transmitted primarily by genus *Ixodes* such as *I. scapularis, I. pacificus, I. ricinus*, and *I. persulcatus* (de la Fuente et al., 2016). *A. phagocytophilum* has several reservoirs including small mammals such as white footed mice (*Peromyscus Leucopus*), dusky-footed wood rats (*Neotoma fuscipes*), *Apodemus, Microtus* or *Clethrionymus* species, with humans as dead-end hosts (Chapman et al., 2006).

Distribution of *E. caffeensis* is globally such as American, Europe, and Asia (Paddock and Childs, 2003). Since it was found in Rocky Mountain in 1986, the disease was reported in Arkansas, Oklahoma, and Maryland (Tan et al., 2001) consequently. In the other countries, the disease was reported in Argentina (Ripoll et al., 1999), Italy (Nuti et al., 1998), South Korea (Paddock and Childs, 2003), and Thailand (Hepner et al.,1997). *E. caffeensis* is transmitted to human primarily by the lone star tick (*Amblyomma americanum*) and other tick species such as *I. ricinus, Haemaphysalis yeni, Amblyomma testudinarium, Amblyomma maculatum,* and

Dermacentor variabilis (Belongia, 2002; MacLeod and Gordon, 1983; Cao et al., 2000). Trans-stadial transmission of *Ehrlichia* occurs during nymph and adult feeding stages, but it is not maintained by transovarial (female adult-larva) transmission (Ismail et al., 2010).

1.2.1.3. Disease and pathogenesis

Clinical anaplasmosis is more commonly encountered in cattle older than 1 year of age (de la Fuente et al., 2005). It is suggested that cows persistently infected in advanced, however, the pregnancy and/or lactation may relapse and show the clinical manifestations of acute infection (de la Fuente et al., 2005). *A. marginale* cause of bovine anaplasmosis and give an impact on economic significance in the cattle industry (Atif, 2015). Infection of *A. marginale* are characterized by progressive anemia due to extravascular destruction of infected erythrocytes, and infected cattle showed fever, pale mucous membranes, and abortion (Atif, 2015). Besides that, the infection cause of decreasing the milk production, inappetence, loss of coordination, and breathlessness (Atif, 2015).

In human, infection of *A. phagocytophilum* and *E. caffeensis* induce mild to severe illness and the symptom including fever, rash, thrombocytopenia, muscle aches, weakness, and headache (Ismail et al., 2010; Yabsley, 2010). Since 1986 to 2018, more than 1000 people in USA were infected by *E. caffeensis* (CDC, 2018a). General clinical features are fever, headache, myalgias, arthralgias, skin eruption, and rash. However, gastrointestinal symptoms such as nausea, vomiting, abdominal pain, and diarrhea are sometimes occurred (Ismail et al., 2010).

Recently, there were several reports described about detection of uncharacterized *Anaplasma* spp. or *Ehrlichia* spp. from ticks or wild animals (Sumrandee et al., 2016; Parola et al., 2003; Kuo et al., 2017). However, the pathogenesis of these bacteria still unclear.

1.2.1.4. Diagnosis

Diagnosis of bovine anaplasmosis may be made tentatively based on geographic location, season, presenting clinical signs and/or necropsy findings observed in infected animals (de la Fuente et al., 2005; Woldehiwet, 2006). A competitive enzyme-linked immunosorbent assay (cELISA) has been used for diagnosis of *A. marginale* infection in various ruminants including cattle, sheep and deer (Ndungu et al., 1995; Woldehiwet, 2006). Detection of *A. marginale* by PCR have been developed, but a serologic test remains the most practical means of testing large numbers of cattle for evidence of infection (Kocan et al., 2010).

Diagnosis for *Ehrlichia* can be conducted by serologic tests to measure specific antibody titers, detection of morulae in peripheral blood or in leukocytes from cerebrospinal fluid (CSF), detection of Ehrlichial DNA by PCR from whole blood or CSF, direct detection of *Ehrlichia* in tissue samples by immunohistochemistry, and isolation of bacteria (Paddock and Childs, 2003).

1.2.1.5. Prevention and Control

Prevention and control of *Anaplasma* spp. and *Ehrlichia* spp. can be carried out by arthropod control using acaricides, prophylactic administration of antibiotics for animals, and vaccination for arthropod (Ndungu et al., 1995; Woldehiwet, 2006). In human, current control strategies of the diseases are based on the reduction of opportunity of tick infestations.

For animals, vector control program by acaricides is one method to reduce the number of vectors. Vaccination for anaplasmosis in cattle over 6 months of age and new arrivals can reduce the clinical signs of the diseases. Antibiotics such as chlortetracycline can be added in the mineral mix to prevent the disease outbreak. Moreover, good management practice is also an important strategy to prevent the infection due to mechanical transmissions of diseases (Kocan et al., 2010; Ismail et al., 2010; Woldehiwet, 2006).

1.2.1.6. Situation in Indonesia

In Indonesia, *Anaplasma* spp. and *Erhlichia* spp. are the main indigenous tick-borne pathogens affecting cattle and dogs. However, previous study reported that 14.6% (7/48) of human serum collected from Gag island, Papua were positive to *E. caffeensis* by indirect immunofluorescence assay (IFA) (Richards et al., 2003). Bovine anaplasmosis, caused by *A. marginale* is the most common disease in cattle in Indonesia. Sero-epidemiology was conducted in Aceh in 1988 resulted 82% (84/102) cattle in that areas were infected with *A. marginale* (Payne et al., 1988). Several *Anaplasma* spp. were detected in Indonesia such as *Anaplasma platy*, *Anaplasma* spp., in dogs, and cattle (Faisal et al., 2019; Putra et al., 2019). *A. platy* were detected by PCR in blood of dogs (6/56) collected in Yogyakarta in 2018 (Faisal et al., 2019). The surveillance of cattle revealed 3/147 were infected with *Anaplasma* spp. by blood smears in Surabaya, in 2019 (Dyahningrum et al., 2019). Until now, there are a few data available regarding the prevalence of *Anaplasmataceae* in humans, animals, and/or ticks in Indonesia.

1.2.2. Rickettsioses

1.2.2.1. History

Rickettsioses was caused by *Rickettsia* spp., order *Rickettsiales* and family *Rickettsiaceae* (Blair et al., 2004). Rickettsiae are small (0.3 x 1–2 μm) obligate intracellular, gram-negative bacteria that survive freely within cytosol of the host cell and is maintained in animal and/or arthropod (Blair et al., 2004). The diseases represent some of the oldest infectious disease, epidemic typhus was suspected of being responsible for the Athens plaque in 5th century BC and the disease was certainly recognized during the 16 centuries (Raoult and Roux, 1997).

1.2.2.2. Epidemiology

The geographic distribution of rickettsiae is worldwide, Europe, America, Australia, and Asia (Raoult and Roux, 1997). Rickettsiae are associated with arthropods, which may act as vectors, reservoir, and/or amplifiers and ticks considered as the main vectors primarily of spotted fever group (SFG) of Rickettsiae (Raoult and Roux, 1997). Several *Rickettsia* species such as, *R. conorii, R. rhipicephali, R. ricketsii, R. japonica, R. africae,* and *R. belii,* that are transmitted by several tick species genera *Rhipicephalus, Dermacentor, Amblyomma, Haemaphysalis, Hyalomma, Ixodes, Argas* and *Ornithodoros* (Raoult and Roux, 1997). *R. rhipicephali* was first reported from Mississippi in 1975, isolated from *Rhipicephalus sanguineus* (Burgdorfer et al., 1975) and then was detected in France (Drancourt et al., 1992) and Portugal (Burgdorfer et al., 1975) from same tick species. In Asia, *Rickettsia* sp. was detected from pool of *Ixodes* and *Rhipicephalus* ticks in Chiang Mai, Thailand and Pakistan in 1970-1973 (Robertson and Wisseman, 1973). *R. japonica* was isolated from *H. longicornis*, *D. taiwanensis*, *I. ovatus* and *H. flava* in Japan (Uchida, 1993; Takada et al., 1994). *R. aeschlimanni* has been obtained from the Moroccan tick *H. marginatum* (Beati et al., 1997). *R. helvetica* was first isolated from *I. ricinus* in Switzerland in 1979 (Burgdorfer et al., 1975), then France and Sweden (Nilsson et al., 1997), and Japan (Ando and Fujita, 2013). *R. parkeri* was isolated in United State in 1937 from *Am. maculatum* collected from cows (Parker et al., 1939). *R. belii* was detected from several tick species, *Dermacentor spp., O. concanensis, Am. cooleyi* and *H. leporispalustris* in United State (Philip et al., 1983). The typhus group (TG) of Rickettsiae, *R. prowazelii* and *R. typhi*, was distributed worldwide and transmitted primarily by human body lice and rat flea, respectively (Weiss et al., 1975; Gross, 1996).

1.2.2.3. Disease and pathogenesis

According to the host specificity, intracellular location, in vitro growth condition, antigenic characteristic, clinical features and epidemiology, members of the genus *Rickettsia* are divided into two groups, the SFG and TG (Drancourt and Raoult, 1994; Eremeeva et al., 1994; Raoult and Roux, 1997).

SFG consists of 17 species of genus *Rickettsia* that belonged to tick-borne rickettsiae with exception *R. akari* (mite-borne) and *R. felis* (flea-borne) (Tamura et al., 1995). These SFG Rickettsiae caused spotted fever for humans. The incubation period of SFG is up to 2 weeks prior to the onset of clinical symptoms.

There was endemic spotted fever in each area where specific vectors were distributed; Rocky Mountain spotted fever (RMSF) caused by *R. rickettsii* in America, Mediterranean spotted fever (MSF) caused by *R. conorii* in Mediterranean, and Japanese spotted fever (JSF) caused by *R. japonica* in Asia (Walker et al., 1978; Uchida, 1993).

The TG consists of two species *R. prowazekii* and *R. typhi*, while the former scrub typhus group has been moved into one genus, *Orientia tsutsugamushi* (Tamura et al., 1995). *R. prowazekii* and *R. typhi* caused Epidemic typhus and Murine typhus for humans, respectively.

In general, the typical clinical symptoms of Rickettsioses are high fever, headache, myalgias, and rash. A rash can become purpuric on several days after the onset of symptoms (Raoult and Roux, 1997).

1.2.2.4. Diagnosis

Clinical examination and epidemiologic investigation of patients with potential rickettsioses, for instance, the presence of a characteristic rash is critical (Raoult and Roux, 1997). Serological assays Weil-Felix test and IFA are also commonly used. The test is reliable but does not allow differentiation of infected species of SFG rickettsiae (Raoult and Roux,

1997). ELISA was first introduced for detection of antibodies against *R. typhi* and *R. prowazekii* (Halle et al., 1977). This technique is highly sensitive and reproducible, allowing differentiation of immunoglobulin G (IgG) and IgM antibodies (Halle et al., 1977). Rickettsial DNA can also be detected by PCR amplification from samples that include blood, skin biopsy samples, eschar, and arthropod tissues (Raoult and Roux, 1997).

1.2.2.5. Prevention and control

Several methods for prevention of rickettsial infections such as, vector and rodent control, insecticides application, enhancement of the natural immunity acquired by animals in response to tick infestation and vaccination with concealed tick antigens as well as the use of hormones, chemosterilants and genetic manipulation can also be considered (Raoult and Roux, 1997). For short-term high-risk exposure, doxycycline may be an effective prophylaxis of illness but may not prevent infection with scrub typhus or SFG rickettsiae. Recently, for specific prevention by vaccination, only Q fever vaccines are available for common use (Kazar and Brezina, 1991). Because of antigenic diversity or heterogeneity of rickettsial strains causing certain rickettsial diseases, especially scrub typhus (Yamamoto et al., 1986) and RMSF (Anacker et al., 1986), their vaccine is not available.

1.2.2.6. Situation in Indonesia

The presence of rickettsial diseases in human in Indonesia was reported in 2003 with surveillance in Gag island, Papua showed sero-reactivity *R. rickettsii* (5/48, 10.4%), *R. typhi* (1/50, 2%) by ELISA and *R. conorii* (10/49, 20.4%), *O. tsutsugamushi* (5/53, 9.4%), *R. typhi* (1/48, 2.1%) by IFA (Richards et al., 2003). The other study was conducted in East Java in 1994 showed *R. typhi* and *R. felis* were detected from fleas by real-time PCR with 12.8% (5/39) and 5.1% (2/39), respectively (Jiang et al., 2006). Sixteen percent of the *Xenopsylla cheopis*

pools were found positive for *Rickettsia* spp.; four (10.8%) *R. typhi*, one (2.7%) *R. felis*, and one (2.7%) was co-infection of *R. felis* and unknown SFG *Rickettsia* sp. (Barbara et al., 2010). Moreover, surveillance in Malang, East Java in 1997 revealed that 34.7% and 1.3% of 464 human sera were positive to *R. typhi* and *O. tsutsugamushi* by ELISA, respectively (Richards et al., 1997).

1.2.3. Borreliosis

1.2.3.1. History

Borreliosis is tick-borne disease caused by *Borrelia* spp., gram negative bacteria and a member of spirochetes (family: *Spirochaetaceae*) (Radolf et al., 2012; Parola and Raoult, 2001). It is a motile spirochaetal bacteria, helically shaped with tapered ends, diderm membrane architecture (outer surface membrane, periplasmic space, peptidoglycan-cytoplasmic membrane) and periplasmic flagella (n= 4–14), with 0.2–0.3 μm diameter and 10–30 μm in length (Margos et al., 2020).

Generally, Borreliosis is divided into two groups, Lyme diseases (LD) caused by infection of LD borreliae (*B. burgdorferi* sensu lato) and relapsing fever (RF) caused by infection of RF borreliae (*B. recurrentis, B. duttonii, B. turicatae,* etc.) (Reboul et al., 2018; Gil et al., 2005).

RF was described after an outbreak in Edinburgh in the 1840s, but the etiology of the diseases was not understood. Most of the important features of the biology and immunology of the disease were described at the end of the 19th century and in the first half of the 20th century. The elucidation of the pathogenesis and genetics of RF borreliae was developed recently.

Previously, Lyme arthritis was recognized in 1976 with geographical clustering of children with arthritis in Lyme, Connecticut in the United States (Elbaum-Garfinkle, 2011). In

1982, Burgdorfer and colleagues isolated the infectious agent that causes LD which was designated as *Borrelia burgdorferi* (Burgdorfer et al., 1982).

Several borrelial species have been reported to be pathogenic to humans and/or animals such as *B. afzelii, B. bavariensis, B. bissettii, B. burgdorferi* (s.s.), *B. garinii, B. kurtenbachii, B. lusitaniae, B. spielmanii*, *B. valaisiana, B. hermsii, B. parkeri, B. turicatae, B. duttonii, B. miyamotoi,* and *B. hispanica* (Rudenko et al., 2011; Obiegala et al., 2017; Barbour, 1984).

1.2.3.2. Epidemiology

The incidence of tick bites and the case of disease has increased in USA, Europe, and Asia where the diseases distributed (Vrijmoeth et al., 2019; Mendoza-Roldan et al., 2019, Seki et al., 2018; Fukunaga et al., 1995).

Distribution of Lyme borreliosis has been reported in northern hemisphere; North America, Europe, and Asia (Hubalek, 2009). While, distribution of RF is worldwide including Western and Northern Africa (Felsenfeld, 1965), USA, Mexico, Panama, Australia (Barbour, 1984; Reboul et al., 2018).

LD is commonly was transmitted by hard-bodied ticks (Ixodid ticks), while RF is transmitted by soft-bodied ticks (Argasid ticks) (Reboul et al., 2018; Gil et al., 2005). Previous study reported, several tick species were vector of LD, for instance, *I. ricinus, I. persulcatus, I. scapularis* and *I. pacificus.* These ticks commonly fed on the animal host (i.e small mammals, birds, reptile) which was the reservoir of LD (Scott et al., 2010; Gern et al., 1998). While, RF is mainly transmitted by soft ticks primarily genus *Ornithodoros* (Reboul et al., 2018).

1.2.3.3. Disease and pathogenesis

Several animals have been found naturally infected by borreliae without any clinical signs and some of them showed spirochetemia in blood (Reboul et al., 2018). In Europe, more

than 232,000 new cases of LD are reported each year with symptoms from unspecific flu-like symptoms to typical LD clinical signs (e.g erythema migrants, meningitis, and neurological abnormalities) (Jahfari et al., 2017; Stanek and Strle, 2018; Sykes and Makiello, 2016; Hubalek and Halouzka, 1997). In USA, approximately, 300,000 LD cases per year are reported (CDC, 2018b). In addition to the clinical signs in Europe, the arthritis is typical symptoms of *B. burgdorferi* infection (Strle et al., 1999). Clinical manifestations of RF are recurrent high fever, malaise, myalgia, headache and neurologic symptoms (Reboul et al., 2018).

1.2.3.4. Diagnosis

Diagnosis of Borreliosis is usually based on recognition of characteristic clinical features. Detection of antibodies can be determined by ELISA and western blotting for LD (Beard, 2014). Diagnostic tests in common use for LD are all serologic tests that rely on a detectable antibody response (Beard, 2014). Serodiagnostic tests are in sensitive during the first several weeks of infection and PCR-based testing in synovial fluid is often positive in patients with Lyme arthritis (Steere at al., 2016).

For RF, the gold-standard diagnosis is direct microscopic visualization of borreliae in a Giemsa-stained thick blood smears for acute infections. Molecular and serological test based on the observation of glycerophosphodiester phosphodiesterase (*glpQ*) gene which present in RF borreliae and absent in LD borreliae is also useful. Moreover, detection of borrelial flagellin gene (*flaB*) by PCR amplification from the blood is highly sensitive and specific (Trape et al., 1991; Schwan et al., 1996; Jiang et al, 2003).

1.2.3.5. Prevention and Control

Several methods to prevent infection are personal protection, environmental intervention, and prophylactic treatment (Piesman and Eisen 2008; Steere at al., 2016). At

present, there is no vaccine for Borreliosis in humans is available, therefore Borreliosis preventative recommendations currently include activities aimed at reducing exposure to infected ticks including pesticide applications, landscape management, animal management, applying repellants or toxicants to skin or clothing, tick checks, showering after being in tick habitat, and host-targeted interventions (Piesman and Eisen 2008).

1.2.3.6. Situation in Indonesia

In Indonesia, there is no information about the presence of *Borrelia* sp. in human, animals and/or tick vectors to date.

1.3. Mosquito-borne diseases

Mosquitoes are most major vectors of human pathogens. They live in high density in close association with the human environment globally, and carries micro-organisms which causes severe diseases with high morbidity and mortality. The mosquitoes belonged to the family of *Culicidae* and encompass 3556 species and divided within subfamilies *Culicinae* and *Anophelinae* (Harbach, 2013). Several mosquito species such as *Aedes aegypti, Aedes albopictus, Culex* spp., and *Anopheles* spp. play an important role in the transmission of diseases. They can transmit many kinds of arthropod-borne virus (Arbovirus) and parasites, e.g. malaria. These arboviruses were belonging to families *Flaviviridae*, *Togaviridae*, *Phenuiviridae*, and *Rhabdoviridae* (Arrigo et al., 2016). Several mosquito-borne viral diseases have been reported, dengue fever, Japanese encephalitis, zika fever, and chikungunya fever were caused by dengue virus (DENV), Japanese encephalitis virus (JEV), Zika virus (ZIKV), and Chikungunya virus (CHIKV), respectively (WHO, 2020; Gould et al., 2017).

1.3.1. Pathogenic Flaviviruses endemic in Indonesia

Genus *Flavivirus* (family: *Flaviviridae*), are one of the most important arbovirus and possesses public health significance (Bhatt et al., 2013; Chambers et al., 1990). Most flaviviruses are transmitted to vertebrate hosts by arthropod vectors primarily mosquitoes or ticks (Bhatt et al., 2013). Moreover, this genus can also infect a variety of vertebrate species including mammals, birds, reptile and so on, but some are maintained in arthropod- or vertebrate-restricted transmission cycles (Higa et al., 2011; Blitvich and Firth, 2017). Several flaviviruses such as DENV, ZIKV, Yellow fever virus, and West Nile virus have been transmitted by mosquitoes and are the threat to the public health (WHO, 2020).

In Indonesia, many arboviral diseases are prevalent throughout the year. Recently, 59,047 cases of dengue fever, including 444 deaths, were reported in Indonesia during 2016–2017 (Ministry of Health RI, 2018). 326 and 1,702 cases of JEV (family *Flaviviridae*, genus *Flavivirus*) and CHIKV (family *Togaviridae*, genus *Alphavirus*), respectively, were reported in 2016 in Indonesia (Ministry of Health RI, 2018). Moreover, ZIKV (family *Flaviviridae*, genus *Flavivirus*) infection was first reported in Jakarta in 2012 (Kwong et al., 2013). The number of patients with arbovirus infections has tended to increase annually, as well as the population of mosquito vectors that is affected to the distribution of arboviruses in Indonesia (Tosepu et al., 2015).

1.3.1.1. Virus properties of flavivirus

All viruses in the genus *Flavivirus* (family *Flaviviridae*) are single-stranded, positive-sense RNA genome of 10-11 kb encodes a 5’ untranslated region (5’ UTR), with a long open reading frame (ORF) and a 3’ UTR (Brinton and Basu, 2015). The ORF of flavivirus are a large polyprotein encoding 3 structural (C, prM/M, E) and 7 non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) proteins (Guzman et al., 2010; Mackenzie et al., 2004). The

5’ and 3’ UTRs normally consist of approximately 100 and 400–700 nucleotides (nt), respectively (Brinton and Basu, 2015). The UTRs are highly conserved secondary and tertiary structures required for replication and translation (Brinton and Basu, 2015). The virus has relatively icosahedral symmetry, smooth surface, diameter 500 Å and electron-dense core (Miller et al., 2007; Vashist et al., 2011).

The mature or immature of flaviviruses has approximately 50-60 nm that consist of an outer glycoprotein shell and an internal host derived lipid bilayer (Miller et al., 2007; Neyts et al., 1999). In this bilayer an RNA-protein core consisting of genome RNA and capsid proteins (C) are located (Miller et al., 2007). The glycoprotein shell is defined and consists of 180 copies each of an envelope (E) and membrane protein (prM/M). Three structural protein C, prM/M and E were consisted 100 aa, 75 aa and 495 aa, respectively (Chamber et al., 1990; Kuhn et al., 2002; Saxena et al., 2011).

The envelope of virus is reported as a dimer on the surface of the mature viral particle and important in the initial attachment of this particle to the host cells (Kuhn et al., 2002; (Miller et al., 2007)). The prM protein consists of 7 antiparallel β-strands stabilized by 3 disulfide bonds and important in the formation and maturation of the viral particle (Kuhn et al., 2002).

NS1 is a 45 kDa glycoprotein that involve within the viral RNA replication complex. However, NS2A is a ~22 kDa protein that cleaved from NS1 by a membrane bound host protease, cofactor of viral serine protease, correlating with stabilization and substrate recognition of NS3 protease (Miller et al., 2007; Melian et al., 2010; Deng et al., 2011; Yun and Lee, 2014).

NS3 of flavivirus is a multifunctional protein of 619 aa residues, possesses enzymatic activities of serine protease, helicase and nucleoside 5’-triphosphatase, and plays important

roles in the processing of the viral precursor polyprotein and the replication of viral genomic RNA (Yamashita et al., 2008; Deng et al., 2011; Kuhn et al., 2002).

NS4A and NS4B are integral membrane proteins with 16 kDa and 27 kDa, respectively (Zou et al., 2015). NS4 is a high hydrophobicity protein and played a role as a membrane component and this region might be important to adapt each viral growth environment (Cai et al., 2003).

NS5 is the largest, 104 kDa glycoprotein and the most conserved protein of flavivirus (Miller et al., 2007). In addition, NS5 is a key component of the viral RNA replicase complex that presumably includes other viral nonstructural and cellular proteins, carries both methyl transferase and RNA-dependent RNA polymerase (RdRp) domains (Cai et al., 2003; Vashist et al., 2011; Saxena et al., 2011; Sahoo et al., 2008).

1.3.1.1.1. Dengue virus

1.3.1.1.1.1. History

The first record of a case of probable dengue fever is in a Chinese medical encyclopedia from the Jin Dynasty (265–420 AD) which referred to a “water poison” associated with flying insects (Gubler et al., 1998; Christie, 1881). In the previous studies reported two epidemics of dengue-like illness in 1823 and 1870 in Zanzibar and the East African coast (Carey, 1971; Gubler et al., 1998; Gubler and Clarkc., 1995). After that, several outbreaks were reported in 1871 and 1901 in Calcuta, India and Burma (Myanmar), respectively (Carey, 1971).

Several studies reported two outbreaks that possibly dengue, in West Indies and Panama in 1699 (McSherry, 1982). The outbreaks of dengue occurred in 1779 and 1780 in several continents Asia, Africa, and America (Gubler DJ, 1998) and then in 1823 and 1870 in Zanzibar and the East African coast, respectively (Carey, 1971). Since then, the diseases became endemic in many tropical urban centers in several countries including Indonesia, Egypt

and USA (Carey, 1971). The ecological disruption in Southeast Asia, particularly during and after World War II created an ideal condition (i.e. uncontrolled urbanization, population growth, and economic development) for increased transmission of DENV infection (Ooi and Gubler, 2008).

1.3.1.1.1.2. Epidemiology

Distribution of DENV is predominantly in tropical and subtropical areas and more than 2.5 billion people have been exposed to the risk of infection (Guha-Sapir and Schimer, 2005). Recently, the geographic distribution of DENV has expanded and more than 100 countries are endemic particularly in tropical and subtropical regions (Bhatt et al., 2013). The resurgence of the disease appears to be associated with demographic and societal changes particularly in developing countries, there are many socio-economic problems, for instance, substandard housing, crowding, uncontrolled urbanization, poor water and waste management system (Gubler and Clark, 1995).

The DENV has four serotypes (DENV-1, -2, -3, and -4) and may induce long-lived serotype-specific immunity and only confers short-lived cross-immunity (Henchal and Putnak, 1990). The DENV-2 serotype was the predominant serotype in outbreak before in 2000, while DENV-1, -3, -4 were predominant in outbreak after 2009 (Guo et al., 2017). Before isolation techniques enabled the viruses' discovery and characterization DENV has distinct epidemiological patterns associated with the four serotypes of the virus i.e. DENV 1 was first reported in 1943 in French Polynesia and Japan, DENV 2 in 1944 in Papua New Guinea and Indonesia, DENV 3 and DENV 4 both in 1953 in the Philippines and Thailand (Hammon et al., 1960). Recently, these can co-circulate within a region, and indeed many countries are hyper-endemic for all four serotypes (Henchal and Putnak, 1990).

In 2016, a large DENV outbreaks was reported in America and Asia with more than 2.38 million cases, approximately 1.5 million cases with 1,032 deaths in Brazil, 375,000 cases

in Western Pacific Region, 176,411 in Philippine, and 100,028 cases in Malaysia (WHO, 2020). Recently, in 2019, several outbreaks were reported in America with approximately 3.1 million cases and more than 25,000 classified as severe. In Asia, huge number of cases were also reported in Bangladesh (101,000 cases), Malaysia (131,000 cases) Philippines (420,000 cases), and Vietnam (320,000 cases) in 2019 (WHO, 2020).

1.3.1.1.1.3. Transmission cycle

The DENV is transmitted to humans through the bites of infected female *Aedes* mosquitoes, primarily *Ae. aegypti* (Moore et al., 2007), while the other mosquito species including *Ae. albopictus*, *Ae. polynesiensis* and *Ae. scutellaris* are the secondary vectors (Moore et al., 2007; Schaffner et al., 2011).

There are two types of DENV transmissions, sylvatic cycle and endemic/urban cycles. Sylvatic DENV is transmitted in an enzootic cycle, involving non-human primate reservoir hosts and sylvatic *Aedes* spp. mosquito vectors, while the endemic cycle occurs in the urban areas which involve humans and *Ae. aegypti* as primarily vector. Sylvatic DENV is predominant in Africa, while the endemic cycles predominant in Asia, Oceania, the Caribbean, and Latin America (Vasilakis et al., 2008).

In endemic cycle, mosquitoes can be infected from people who are viremic with DENV. After feeding on an DENV-infected person, the virus replicates in the mosquito midgut, before it disseminates to secondary tissues, including the salivary glands (Duong et al., 2015). Moreover, human-to-mosquito transmission can occur up to 2 days before onset of illness (Duong et al., 2015)

Mosquito vector breeding sites to human habitation is a significant risk factors for DENV transmission, particularly in urban areas. *Ae. aegypti* is an endophilic mosquito that prefer living in and around homes in artificial containers such as jars, discarded cans, flower

vases, cement tanks, and plastic buckets around human dwellings (Higa et al., 2011). While, *Ae. albopictus* is more exophilic, living outdoors such as bamboo stumps, tree holes, discarded tires, and flower vases (Ponlawat and Harrington, 2005). The blood-feeding hosts of *Ae. albopictus* includes not only humans but also a wide range of amphibians, reptiles, birds, and mammals (Higa et al., 2011).

1.3.1.1.1.4. Disease and pathogenesis

Recovery from infection of DENV is believed to provide lifelong immunity against the serotype. However, cross-immunity to the other serotypes after recovery is only partial, and temporary. Subsequent infections or secondary infection by other serotypes increase the risk of developing severe dengue (Simmons et al., 2012). The presence of antibodies at sub-neutralizing concentrations, from previous infection, facilitate the infection of macrophages and other cells bearing Fcγ receptors (FcγRs), promoting viral replication (ADE; antibody-dependent enhancement) (Kliks et al., 1988).

Infection of this virus has a wide range of clinical manifestation from asymptomatic, mild dengue fever, to more severe dengue hemorrhagic fever (DHF), and can lead to being potentially fatal dengue shock syndrome which are characterized by coagulopathy, increased vascular fragility, and permeability (Carlos et al., 2005; Simmons et al., 2012; Endy et al., 2011).

1.3.1.1.1.5. Diagnosis

Diagnosis of DENV infection is typically based on clinical presentation, laboratory evaluation, and rapid diagnostic tests, but inaccuracy diagnostic sometimes occurred (Utama et al., 2019). Laboratory diagnosis methods for confirming dengue virus infection may involve detection of the virus, viral nucleic acid, viral proteins or antibodies, or a combination of these

techniques (Shu and Huang, 2004). During the early stages of the disease, virus isolation, nucleic acid or viral protein detection can be used to diagnose the infection (WHO, 2007). At the end of the acute phase of infection, serology is the method of choice for diagnosis. Several methods for detecting nucleic acid of dengue such as, RT-PCR, qRT-PCR, and isothermal amplification methods was used. While, detection of NS1 protein, which was induced a very strong humoral response, was commonly used (WHO, 2007).

1.3.1.1.1.6. Prevention and control

There is no specific medicine to treat the DENV infection. Abundant epidemiological, experimental, and clinical evidence that points to the ADE are the major risk factor for developing DHF. Therefore, there is a difficulty to develop vaccine against the DENV (Kliks et al., 1988). Recently, there is an available vaccine that can induce simultaneous tetravalent immunity against all four DENV genotypes (Thomas and Yoon, 2019), but is not completely protective (Low et al., 2017). Moreover, integrated vector control is also important to break the life cycle of mosquito vectors and virus transmission such as application of insecticides, sterile insect technique, para-transgenesis and production of genetically modified vectors (Rather et al., 2017; Tang et al., 2019).

1.3.1.1.1.7. Situation in Indonesia

In Indonesia, dengue cases tended at a high level every year and widely distributed in all of the provinces in Indonesia (Harapan et al., 2019a). All of dengue serotypes (DENV-1, -2, -3, and -4) are circulating in Indonesia and this disease occurred frequently and it has a high fatal potential (Lestari et al., 2017). The case was first reported in Jakarta and Surabaya in 1968, and then expanded to the other areas such as Bandung, Yogyakarta, Palembang, and Jayapura (Setiati, 2006). Major dengue outbreaks have been reported in 1973, 1988, 1998,

2007, and 2010 with the highest at 86 cases per 100,000 population (Karyanti et al., 2014). Currently, Indonesia reports the highest average number of dengue cases every year among Asia with 0.05 per 100,000 population in 1968 to 86 per 100,000 population in 2010 and decrease to 24.74 per 100,000 population in 2018 (Harapan et al., 2019a, Ministry of Health 2019). Nevertheless, the numbers of dengue cases in 2018 reached 65,602 cases with 467 patients died (Ministry of Health 2019). As a tropical country, Indonesia has a dry and wet season that provides an optimum habitat to support the breeding sites of *Aedes* mosquitoes. The disease incidence fluctuated according to the seasonality patterns, increasing of cases usually occur at the end of the rainy season (December to March) and then decrease in the dry season (June to September) (Tang et al., 2019). The other factors that impact to increasing the dengue cases are urbanization, increased global travel, and lack of effective mosquito control (Tang et al., 2019). In addition, travelers from non-endemic areas to an endemic area pose a health threat to non-endemic areas where competent mosquito vectors are present (Smith, 2012). Several studies relating to molecular epidemiology have been conducted in many cities of Indonesia including Bali, Jakarta, Jambi, and Makasar (Megawati et al., 2017; Lestari et al., 2017; Sasmono et al., 2015; Haryanto et al., 2016). Dengue disease investigation will be particularly useful for understanding the etiology of the disease and to develop control programs.

1.3.1.1.2. Japanese Encephalitis virus

1.3.1.1.2.1. History

Epidemics of encephalitis have been described in Japan in 1870s and reported about every 10 years (Solomon et al., 2000). The disease was called “Yoshiwara cold” in 1904 and was followed by encephalitis epidemic in 1924 with more than 6,000 cases (Miyake, 1964; Solomon et al., 2000). In 1933, the virus was infected to the brain of monkeys and caused fatal

encephalitis (Miyake, 1964). Then, prototype Nakayama strain of Japanese encephalitis virus was successfully isolated from monkeys and was classified in the genus *Flavivirus* (family *Flaviviridae*) (Solomon et al., 2000).

1.3.1.1.2.2. Epidemiology

The first confirmed JE case was reported in Japan in 1924 followed in Korea (1933), China (1940), Philippine (1950), India (1955), and other Asian countries including Indonesia (Chuang and Chen, 2009, van den Hurk et al., 2009; Endy and Nisalak, 2002; Mackenzie et al., 2004; Erlanger et al; 2009; Kuwata et al., 2013; Solomon et al., 2003).

Sequencing analysis divides JEV into five genotypes (GI–V) rising from ancestor viruses from Indonesia–Malaysia region (Zhang et al., 2011a). Genotypes I-III are the most recent genotypes which have spread across Asia (Schuh et al., 2011). GI has been isolated in Cambodia, Thailand, and China, while GII were isolated from southern Thailand, Malaysia, Indonesia, and Northern Australia (Zhang et al., 2011a). GIII was reported mostly from temperate zone of Asia, including Japan, China, Taiwan, the Philippines and the Asian subcontinent (Nabeshima et al., 2009; Uchil and Satchidanandam, 2001; Wang and Liang, 2015).) and genotype IV was reported from Indonesia (Kuwata et al., 2020). In the mid 1990's genetic shift had occurred in Japan, Korea and Vietnam that lead to disappearance of GIII and then progressively GI supplanted it (Zhang et al., 2011a; Nabeshima et al., 2009).

In 2017, JEV outbreaks were reported in several countries such as, India (2,043 cases), China (1,147 cases), Myanmar (442 cases), Cambodia (5 cases), Vietnam (313 cases) and Bangladesh (19 cases) (WHO, 2019a). While in 2018, several countries still reported in high cases, India (1,707 cases), China (1,800 cases), Myanmar (126), and Vietnam (200 cases) (WHO, 2019a).

1.3.1.1.2.3. Transmission cycle

The widespread expansion of JEV cannot be separated from the growth in the human population, land use for irrigated rice agricultural activities, and swine farming (Erlanger et al., 2009). This virus is transmitted mainly by *Culex* spp., *Cx. tritaeniorhynchus* is the primarily vector that breeds in paddy fields (Chen et al., 2000). Other mosquito species such as *Armigeres* spp*., Aedes* spp*., Anopheles* spp*.,* and *Mansonia* spp. have also been reported to be potential vectors (Chen et al., 2000; Oliveira et al., 2018). In the transmission cycle of JEV, swine mainly act as primarily amplifier host and other animals such as bats, wild birds, and other vertebrates may also be the natural host (Schuh et al., 2011). Humans and cattle are considered to be dead-end hosts and do not transmit the virus to mosquitoes due to insufficient infective titer and short duration of viremia (Schuh et al., 2011). In northern temperate areas JE occurs in summer epidemics, whereas in southern tropical areas the disease is endemic and occurs year-round (Vaughn and Hoke, 1992).

1.3.1.1.2.4. Disease and pathogenesis

The majority of human patients are asymptomatic or a mild febrile illness (Saxena et al., 2011). Sometime the infection of JE in humans to be serious in health consequences with encephalitis (Schuh et al., 2011). After the virus was injected via infected mosquito saliva, the virus enters into the reticulo-endothelial system and invades to the central nervous system after the transient period of viremia. After that, the virus distributes in hypothalamus, hippocampus, substantia nigra, and medulla oblongata regions of brain (Das et al., 2010). The virus replicates in neurons and matures in the neuronal secretary system. Manifestation of clinical symptoms of infection are fever, headache, nausea, vomiting, and mental changes such as lethargy, drowsiness, consciousness disorders, and convulsions (Yin et al., 2010). These symptoms are

followed the appearance of nuchal rigidity, photophobia, hyperexcitability, and virous objective neurological signs (Dickerson et al.,1952)

1.3.1.1.2.5. Diagnosis

The virus neutralizing test is currently as the gold standard for the differential serodiagnosis of flaviviruses (Musso and Despres, 2020). The other laboratory diagnosis of JE is generally accomplished by testing serum or CSF to detect virus-specific IgM antibodies (Saxena et al., 2011). Although, the conventional RT-PCR has proved to be high specificity in the diagnosis of JE in both blood and CSF, but it has poor sensitivity as the virus is cleared from the peripheral circulation/CSF. Chavez et al., (2010) stated that the advent of monoclonal antibodies as potential diagnostic tool, therefore the rapid detection of JE antigen in CSF has become possible. However, the most rapid and potential diagnostic tool for JE have been shown to be IgM antibody capture enzyme-linked immunosorbent assays (MAC-ELISAs) and indirect fluorescent antibody (Robinson et al., 2010). The JEV MAC-ELISA is recommended to diagnose acute JEV infections and has been used since 2006 for laboratory-based surveillance of JE (WHO, 2007).

1.3.1.1.2.6. Prevention and control

There are still no specific drugs available to treat JEV infection (Sampath and Padmanabhan, 2009). Prevention methods are very important for minimizing JE infection (Yamanaka et al., 2010; Saxena et al., 2000). The prevention of JEV is based essentially on 4 strategies, i.e., mosquito control, avoiding mosquito bites, pig immunization and human immunization (Mackenzie et al., 2004; van der Hurk et al., 2009). Recently, there are 4 different types of JE vaccines available for humans such as, mouse brain-derived killed-inactivated, cell culture-derived live-attenuated, cell culture-derived killed-inactivated and genetically

engineered live-attenuated chimeric vaccines (Beasley et al., 2008; Wilder and Halstead, 2010). The prevention of vector-human contact is the best preventive method by eliminating potential mosquito breeding areas, environmental sanitation, waste water management by treating the water with larvicide either by fish, drying and wetting of rice fields (Wilke et al., 2019).

1.3.1.1.2.7. Situation in Indonesia

In Indonesia, the existence of four JEV serotypes (GI-IV) has been documented in several areas (Schuh et al., 2013, Kuwata et al., 2020). JE infection was first reported when sero-surveillance was conducted in various localities such as Bali, Nusa Tenggara, Kalimantan, and West Papua in early 1970s (Wijaya, 2007). Although there is no annually JE survey in Indonesia thereafter, sporadic research works and various surveillance studies have been conducted in humans, for instance, surveillance in 2001 resulted 2% (59/2962) of human serum collected from Jakarta and Surabaya showed in high antibody titer (Kari et al., 2006) and in 2016 resulted 13% (43/326) of human serum samples collected from 11 provinces were confirmed positive by IgM ELISA (Burni, 2017). Surveillance of JE have also been conducted in animals such as, 51% (64/126) of cattle, 27% (23/84) of goats, 43% (47/110) of chickens, 44% (14/32) of ducks, 14% (2/14) of horses, and 12% (2/16) of dogs that collected from several areas in Indonesia during 1996 to 1997 were found positive by ELISA (Sendow et al., 2000). Recently the isolation of JEV genotype IV from serum of pigs was reported in Bali in 2018 (Kuwata et al., 2020). Isolation from mosquitoes have also been reported from genus of *Culex* such as, *Cx*. *tritaeniorhynchus*, *Cx. gelidus*, and *Cx. fuscocephala* collected in a pig farm from 1972 to 1974 in Bogor, West Java (Peenen et al., 1975). Moreover, the other isolation of JEV was also reported from several mosquito species such as, *Cx. fuscocephalus, Cx.*

bitaeniorhynchus, Cx. quinquefasciatus, An. vagus, An. kochi and *Ar. subalbatus* collected from cowshed in Central Java during 1986-1988 (Tan et al., 1993).

Although there is indication that JE has been endemic naturally and spread in almost all provinces in Indonesia, but the annual data is not established yet and Bali seems to be the potential area with high cases every year (Garjito et al., 2018). Recently, children vaccination program in Bali province was conducted to reduce the number of JE infection (Im et al., 2018). Until now, the burden of JE in Indonesia is still not established because there is no routine surveillance data generated at the national, provincial, and district levels.

1.3.1.1.3. Zika virus

1.3.1.1.3.1. History

The ZIKV was first isolated from serum of pyrexial rhesus monkey aged in the canopy of Zika forest, Uganda in 1947. After that the virus was isolated from *Aedes africanus* in 1948 in the same forest. (Dick et al., 1952). Since then the virus was reported in several areas such as French Polinesia, Brazil, Micronesia, Africa, and Asian countries (Marchette et al., 1969; WHO, 2018). There are two major lineages of ZIKV; African lineage, reported in Africa, and the Asian lineage, reported in Asia, the Western Pacific Region, the Americas, and Cabo Verde (WHO, 2018).

1.3.1.1.3.2. Epidemiology

ZIKV is a single-stranded RNA virus member of genus *Flavivirus* (family: *Flaviviridae*) and the structure of this virus is similar to DENV and JEV (Marchette et al., 1969). Since the ZIKV was discovered in 1947 from monkeys in Uganda, then, human cases were first reported in Nigeria in 1952 (Macnamara, 1954). After that, it was reported outbreak on large scale in urban areas of African, Asian, and Pacific Island populations (1960s to 1980s),

Island of Yap (2007), French Polynesia (2013-2014), Brazil (2015), New Caledonia, Cook Island, Easter Island, Solomon Island, Vanuatu, and Fiji (2015-2016) (Musso and Gubler, 2016; WHO, 2018; Cao and Musso, 2014; Musso et al., 2015; Macnamara, 1954). The virus was also reported in South, Central, and North America (Musso and Gubler, 2016; Cao and Musso, 2014).

Since ZIKV was isolated from *Ae. africanus* mosquitoes in the Zika Forest, after that it was also isolated from *Ae. apicoargenteus, Ae. luteocephalus, Ae vitattus,* and *Ae. furcifer mosquitoes* in several areas in Africa (Fagbami, 1977; McCrae and Kirya, 1982). In Asia, ZIKV was first isolated from *Ae. aegypti* in Malaysia in 1966 (Marchette et al., 1969). Moreover, the virus was also isolated from *Ae. furcifer* mosquito, suggesting possible vertical transmission (Diallo et al., 2011). The ZIKV lineage circulating in Asia has been described as distinct from the African lineage, suggesting the different sylvatic cycle in Asia and Africa continents (Haddow et al., 2012).

Recent studies have provided new information on the incidence, prevalence, and patterns of ZIKV transmission worldwide. In 2019, a total 87 countries and territories have had evidence of ZIKV infection, Africa, Americas, South-East Asia, and Western Pacific Region. Incidence of ZIKV infection in the Americas peaked in 2016 and declined substantially throughout 2017 and 2018. In Lao People's Republic, nearly 10% had evidence of prior ZIKV infection (Pastorino et al., 2019), India reported a ZIKV outbreak in Rajasthan State in 2018 (WHO, 2018), and 3,473 out of 31,587 (11%) were laboratory confirmed of ZIKV infections in Americas regions (WHO, 2018).

1.3.1.1.3.3. Transmission cycle

The ZIKV is transmitted to human primarily through the bite of an infected *Ae. aegypti* and *Ae. albopictus* (Musso and Gubler, 2016). It was also reported that human-human

transmission by maternal-fetal transmission, sexual transmission, and others, e.g. blood or organ transfusion (Musso and Gubler, 2016).

1.3.1.1.3.4. Disease and pathogenesis

Majority of people infected with ZIKV do not present any symptoms, however, the infection generally induces fever, rash, conjunctivitis, muscle and joint pain, malaise, and headache (Musso and Gubler, 2016). ZIKV may also trigger Guillain Barré syndrome (GBS). GBS is a rare syndrome in which the immune system attacks the peripheral nervous system and can be quite serious, causing muscle weakness, sensory problems, pain, and paralysis (WHO, 2016).

Although mild clinical symptoms, ZIKV infection is associated with fetal death, placental insufficiency, fetal growth restriction, and fetal central nervous system (CNS) injury (Brasil et al., 2016). Moreover, experimental animal infection such as, rats, guinea pigs, and rabbits did not show any symptoms after inoculated intracerebrally (Dick et al., 1952).

1.3.1.1.3.5. Diagnosis

Diagnosis of ZIKV usually relies on the detection of virus RNA in blood during the first few days after symptom onset. It was also detactable in other body fluids such as urine, saliva, and semen (Musso et al., 2015; Kutsuna et al., 2014). Immunohistochemistry analysis with monoclonal antibodies and RT-PCR analysis can be used to detect ZIKV antigen in tissues (Buckley and Gould, 1988). Serology is also usually performed by ELISA with confirmation testing by PRNT according to standard protocols (Johnson et al., 2000). Moreover, isolation of ZIKV can be performed using chicken embryo yolk sacs, allantoic sacs, and chorioallantoic membrane, and cell cultures (Taylor, 1952).

1.3.1.1.3.6. Prevention and control

To date, there is no vaccine for ZIKV, although several are in the development phase with dengue vaccine technology (Abbink et al., 2018). Prevention measures are therefore the same as DENV, JEV and the other mosquito-borne viruses including individual protection from mosquito bites and vector control as mentioned above.

1.3.1.1.3.7. Situation in Indonesia

In Indonesia, sero-surveillance was conducted in 1977 to 1978 resulted 7.8% (17/219) human serum samples collected in Klaten, Central Java were antibody positive against ZIKV (Olson et al., 1981). The other sero-surveillance was conducted in human and animals in 1983 in several areas in Lombok island resulted 13% (9/71) of human sera, 20% (3/15) of horses, 10% (4/41) of cows, 8% (1/13) of carabao, 20% (7/35) of goat, 4% (2/52) of ducks, and 8% (6/71) of bats were found antibody positive against ZIKV (Olson et al., 1983). After that, ZIKV infection case was reported from Australian travelers returning from Jakarta in 2012 and Bali in 2013 to 2015 (Leung et al., 2015; Kwong et al., 2013; Perkasa et al., 2016). A retrospective population-based sero-survey found approximately 9% of children in Indonesia had evidence of prior ZIKV infection by the age of 5 years in 2014 (Sasmono et al., 2018). After that, there is no reported of ZIKV infection.

The mosquito-borne diseases are commonly associated with specific environmental, socioeconomic conditions, and ecological changing such as climate, deforestation, and urbanization, leading to an increase in the incidence of the diseases (Gratz et al., 1999). In Indonesia mosquito-borne viral diseases still received serious attention and has significant implications in economic burden.

1.3.2. Pathogenic Alphaviruses endemic in Indonesia

1.3.2.1. Chikungunya virus

1.3.2.1.1. History

The name chikungunya is derived from the Swahili, meaning as a disease that bends up the joint. The disease was first described in 1952 by Robinson (1955) and was first isolated from mosquitoes in Tanzania (Ross, 1956). After that, several outbreaks have been reported in Transvaal, Zambia, south-eastern Rhodesia, and Zaire (Brighton et al., 1983; Rodger, 1961).

1.3.2.1.2. Virus properties

Chikungunya virus (CHIKV) is a member of the genus *Alphavirus* (Family: *Togaviridae*), single-stranded positive-sense RNA, and has an approximately 12 kb genome (Burt et al., 2017). The virus is spherical and enveloped particle about 70 nm in diameter (Cheng et al., 1995). The genome of alphaviruses encodes 10 different proteins, non-structural proteins (nsP1-nsP4) which are important for replicating the viral genome and the structural proteins (C, E3, E2, 6K/TF, and E1) function in virus assembly (Lee et al., 1996; Cheng et al., 1995).

The nucleocapsid core comprises 240 copies of capsid protein that surround the viral genome. The capsid protein consists of two domains, a highly-charged, N-terminal domain that interacts with the viral RNA in the interior of the nucleocapsid core and a C-terminal domain that has a chymotrypsin-like fold (Choi et al., 1991).

The virus has 8 trimeric glycoprotein spikes cover the surface of and consisted three E1-E2 heterodimers in each spike (Cheng et al., 1995, Zhang et al., 2011b). However, in some alphaviruses, the E3 glycoprotein remains non-covalently associated to these spikes. The spikes are formed by E2 and E1 that responsible for viral entry (Kielian, 2014).

E1 has a short cytoplasmic tail that has been shown to be dispensable, while E2 has a long (> 30 aa) cytoplasmic domain that interacts with a hydrophobic pocket in the capsid protein (Lee et al., 1996). The E2 protein binds to the host-cell receptor and interacts with the capsid protein. The E1 protein is a class II fusion protein that mediates fusion between the virus membrane and the host cell membrane in the endosome (Kielian, 2014).

The roles 6K has been involved in viroporin activity, while TF has been shown to be a virulence factor (Snyder et al., 2013).

1.3.2.1.3. Epidemiology

CHIKV and its outbreaks had been identified in several countries in Africa, Asia, Europe, America, Indian, and Pacific Ocean (Gonzales-Sanchez and Ramirez-Arroyo, 2018). This virus is divided into three genotypes e.i the West African, East/Central/South African (ECSA), and Asian genotype (Caglioti et al., 2013). After CHIKV was identified in 1952 in Tanzania, it was reported outbreak in India, Indian Ocean in 2004, and island in Carribean in 2013 (CDC, 2019). Recently several outbreaks have been reported in several countries, Congo in 2019 (6,149 cases), Sudan in 2018 (13,978 cases), Italy in 2017 (366 cases), France in 2017 (13 cases), Kenya in 2016 (1,792 cases), and Senegal in 2015 (14 cases) (WHO, 2019b).

1.3.2.1.4. Transmission cycle

There are two distinct transmission cycles of CHKV, enzootic sylvatic cycle and an endemic/epidemic urban cycle (Peyrefitte et al., 2007). In the African enzootic sylvatic cycle involves several *Aedes* mosquito species as vectors such as *Ae. furcifer, Ae. vittatus, Ae. fulgens, Ae. luteocephalus, Ae. dalzieli, Ae. vigilax, Ae. camptorhynchites,* and non-human primates as reservoir hosts (Peyrefitte et al., 2007). The virus periodically emerges from enzootic or sylvatic African transmission cycles to initiate urban transmission and epidemics

(Ross, 1956; Leparch-Goffart et al., 2014). The epidemic/endemic transmission cycle was introduced in Asia in 1950 (Peyrefitte et al., 2007; Tsetsarkin et al., 2011). Previous reports revealed that since 2005, large scale outbreaks of CHIKV occurred in several areas in south-western Indian Ocean and Southeast Asia (WHO, 2007; Weaver and Lecuit, 2015). *Ae. aegypti* is the major vector of CHIKV in human and African primates (Jupp et al., 1990; Jupp et al., 1981), while, *Ae. albopictus* can also as act as a secondary vector of the disease (Pialoux et al., 2007).

1.3.2.1.5. Disease and pathogenesis

Generally, CHIKV infection typically presents with a sudden onset of high fever, severe paint in peripheral joints, and sometimes followed by a maculopapular rash (Borgherini et al., 2007). The articular symptoms, often debilitating, usually resolve within in few days to a few weeks, but in some cases in several months (Brighton et al., 1983).

The disease course is divided into three stages, an acute stage, lasting approximately one week, and a chronic stage, which can last from months to years. In acute phase, fever and polyarthralgia are highly indicative of symptoms. The joint pain is often bilateral, symmetric, and debilitating. In the chronic phase, rheumatism and fatigue are the prominent symptoms (Ganesan et al., 2017).

1.3.2.1.6. Diagnosis

Laboratory diagnosis is generally accomplished by testing serum or plasma to detect virus, viral nucleic acid, or virus-specific IgM, and neutralizing antibodies (Johnson et al., 2016). Detection of viral RNA is the primary laboratory test used to diagnose infection in serum collected <6 days after onset of illness (Johson et al., 2016).

CHIKV virus antibodies normally develop toward the end of the first week of illness. Therefore, detection of CHIKV-specific IgM antibody becomes a sensitive test for samples collected approximately >5 days of illness. Recently, commercially are available of MAC-ELISA, and IFA for CHIKV diagnosis (Litzba et al., 2008; Prat et al., 2014).

1.3.2.1.7. Prevention and control

Specific treatment is not available and there is no vaccine to prevent CHIKV. Integrated vector control is the only public health strategy to prevent and control the outbreaks as mentioned above.

1.3.2.1.8. Situation in Indonesia

In Indonesia, the first outbreak reported in Samarinda, Borneo island in 1973 (Kanamitsu et al., 1979), since then in 1983 the disease had an outbreak in Central Java, South Sumatra, and West Kalimantan (Mackenzie et al., 1994). Sero-surveillance was conducted in 1975 in various localities such as, Samarinda, Balikpapan, Surabaya, Bali, Lombok, Kupang, Ujung Pandang, Pomalaa, Ambon, and Jayapura resulted 15.5% (273/1762) of human sera were confirmed seropositive by neutralization test (Kanamitsu et al., 1979). After that, an outbreak of CHIKV was occurred in Yogyakarta in 1999 resulted 30.5% (97/317) human sera were seropositive by ELISA (Porter, 2004). Then, multiple outbreaks have been reported in South Sumatra, Aceh, and West Java in 2001 (Harapan et al., 2019b). The lowest incidence of CHIKV during 2004 to 2015 was 0.16 cases per 100.000 population in 2005, while the highest incidence was 36.2 cases per 100.000 population in 2009 (Ministry of Health, 2007; Laras et al., 2005; Harapan et al., 2019b). However, there has been no deaths cases related to CHIKV infection in Indonesia. Although there is a high number of CHIKV cases in Indonesia, comprehensive surveillance data are still limited.

1.3.3. Pathogenic Reoviruses endemic in Indonesia

1.3.3.1. Banna virus

1.3.3.1.1. History

Banna virus (BAV) was initially isolated from patient with encephalitis and fever in Xishuangbanna, Yunnan Province, China, in 1987. Since then, BAV have been isolated from pigs, cattle, and ticks in China (Xu et al., 1990; Li and Xe, 1992; Liu et al., 2010) and from mosquitoes in Indonesia, China, Vietnam, South Korea and Europe (Brown et al., 1993; Chen and Tao, 1996; Nabeshima et al., 2008).

1.3.3.1.2. Virus properties

BAV belonged to genus *Seadornavirus,* family *Reoviridae,* a large family of viruses, containing 10, 11, or 12 segments of dsRNA genomes (Attoui et al., 2000). The genus was established in the family *Reoviridae* that contains three members; BAV, Liaoning virus, and Kadipiro virus (Attoui et al., 2000). Reovirus particles have icosahedral symmetry with a diameter of approximately 60–85 nm, non-enveloped, although several of viruses can acquire a transient membrane envelope during morphogenesis or cell exit (Mertens, 2004). The reoviruses can be subdivided into two groups; the spiked or turreted viruses with have 12 icosahedral arranged projections situated on the surface of the icosahedral core particle, one at each of the fivefold axes (Baker et al., 1999).

BAV particles contain seven structural proteins; the core (VP1, VP2, VP3, VP8 or VP10), outer capsid (VP9 and VP4), and non-structural protein (VP5, VP6, VP7, VP11 and VP12) (Jaafar et al., 2005a).

BAV VP1 is a highly conserved and an important marker for species identification within the family *Reoviridae* and as the viral RNA polymerase. VP2 is the protein that forms

the innermost sub core layer and resembles several other nucleotide-binding proteins, including dynein, actin, a number of helicases, ATPases, and methyltransferases (Attoui et al., 2000).

VP3 is the least abundant of the BAV core structural proteins, with an estimated seven copies per particle (Jaafar et al., 2005a). VP3 may also provides one or more of the other enzyme functions that are usually associated with the reovirus core, e.g. transmethylase, guanylyl- transferase capping enzyme helicase or nucleoside triphosphatase (NTPase).

The capping enzyme, VP4, is a functional dimer (Ramadevi et al., 1998). The two outer capsid proteins, VP2, and VP5, are responsible for virus entry primarily responsible for membrane penetration activity (Bhattacharya and Roy, 2008).

VP6 is an essential structural protein of 36 kDa with RNA binding and ATP binding activity. VP7 is found to exhibit similarities to certain protein kinases (Sung et al., 2019).

VP8 is smaller, more abundant, as the core-surface T13 protein and forming the core-surface layer. VP9 has been shown to be responsible for virus attachment to the host-cell surface and may be involved in internalization (Attoui et al., 2000). Moreover, VP9, and VP10 may have a collective role similar to that of outer-capsid protein VP4 involvement in cell attachment and penetration (Jaafar et al., 2005a). VP12 of BAV contains dsRNA-binding domains that the possible function of these domains in virus replication (Attoui et al., 2000).

1.3.3.1.3. Epidemiology

In the previous studies, 10 BAV strains was isolated from patients with fever and encephalitis in China (Xu et al., 1990). Several surveillance studies in China showed that 11.4% (130/1141) serum samples of suspected JE cases collected from hospital were BAV IgM antibody positive by ELISA (Tao and Chen, 2005), 17.5% (11/63) serum samples of patients were diagnosed with viral encephalitis were positive for BAV IgM antibody, 58.7% (37/63) were positive for JE, and 11.1% (7/63) were positive for both of IgM antibodies by ELISA

(Song et al., 2017). Moreover, IgM and IgG against BAV were detected by ELISA in serum sample patients with fever and encephalitis in China-Myanmar and Laos border (Wang et al., 2011).

BAV could be divided into 2 phylogenetically different groups, group A is consisted of isolates from China and Vietnam and group B is the strains from Indonesia. Moreover, BAV isolates from China cluster in group A and separated into subgroups mainly according to the geographic origin of the isolate; subgroup A1 is found in the north and subgroup A2 in the south. However, 2 isolates from northern China grouped in sub- group A2 (south), and 3 isolates from Vietnam grouped in subgroup A1 (north) (Liu et al., 2010; Song et al., 2017).

1.3.3.1.4. Transmission cycle

BAV is transmitted to mammals by mosquito blood feeding activities. Wide range of mosquito species have been reported as vectors *Cx. tritaeniorhynchus, Cx. pipiens pallens, Cx. annulus, Cx. pseudovishnui, Cx. modestus, An. sinensis, Ae. vagus, Ae. albopictus, Ae. vexans,* and *Ae. dorsalis* from China, Vietnam, Indonesia, South Korea, and Hungary (Liu et al., 2010; Brown et al., 1993; Chen and Tao, 1996; Nabeshima et al., 2008). Moreover, BAV have also been isolated from *Culicoides* midges (Liu et al., 2016), pigs, cattle, and ticks in China (Li and Xe, 1992).

1.3.3.1.5. Disease and pathogenesis

BAV replicates within insect cells and mice but not in cultured mammalian cells. BAV-infected mice producing a viremia lasting for 5 days was followed by viral clearance. BAV in mice immunized against BAV did not lead to productive infection (Attoui et al., 2006).

The clinical symptoms of disease caused by BAV may be undetected during a JE outbreak. As mentioned above, several serum sample from patients clinically diagnosed JE

cases were BAV IgM positive indicating that BAV epidemics may have occurred but have been clinically misdiagnosed as JE. BAV was considered to responsible of encephalitis in humans (Song et al., 2017; Wang et al., 2011).

1.3.3.1.6. Diagnosis

Persons infected with BAV showed symptoms such as, flulike symptoms, myalgia, arthralgia, fever, and encephalitis (Xu et al., 1990). A serologic diagnostic assay was developed based on VP9, the outer coat protein responsible for cell attachment and neutralization (Jaafar et al., 2005b). Patients infected with BAV have shown a 4-fold increase in anti- BAV antibody titers in paired serum specimens tested by ELISA, showing an immune response to the virus infection (Jaafar et al., 2005b). BAV is a good candidate for the differential diagnosis of viral encephalitis and meningitis cases (Nabeshima et al., 2008).

1.3.3.1.7. Prevention and control

Because there is still unclear the pathogenesis of BAV, specific treatment seems is not available yet. Integrated vector control as mentioned above is strategy to prevent and control the disease.

1.3.3.1.8. Situation in Indonesia

In Indonesia, BAV has been isolated from several mosquito species *Cx. vishnui*, *Cx. fuscocephala, An. vagus, An. subpictus,* and *An. aconitus* collected from Klaten, Cilacap, (Central Java), and Bantul (Yogyakarta) during 1980-1981 (Brown et al, 1993). Since then, there is no report of BAV in Indonesia anymore.

1.3.4. Mosquito-specific viruses

In addition to pathogenic viruses, mosquitoes also harbor a diverse range of RNA viruses which are not pathogenic to vertebrate and known as insect-specific viruses (ISVs) (Calzolari et al., 2012). ISVs are reported to only replicate in mosquito cells but does not in mammalian cells (Bolling et al. 2015a). Because of the host-restriction, there is no vertebrate amplifying host that reported involve in ISVs life cycle (Calzolari et al., 2012). These viruses have been identified in several viral taxa such as *Flaviviridae*, *Phenuiviridae*, *Mesoniviridae*, negeviruses, *Reoviridae*, *Nodaviridae*, *Rhabdoviridae*, and *Togaviridae* (Bolling et al. 2015a; Nga et al., 2011; Nouri et al., 2018). Several ISVs have been reported in different mosquito genera and distributed worldwide, i.e. Cell fusing agent virus (CFAV) (Stollar and Thomas, 1975), Culex flavivirus (CxFV) (Hoshino et al., 2007), Aedes flavivirus (AEFV) (Hoshino et al., 2007), Kamiti River virus (KRV) (Crabtree et al., 2003), Quang Binh virus (Crabtree et al., 2009), and Nakiwogo virus (Cook et al., 2009).

Previous reports revealed that several ISVs have been found in immature stages of mosquitoes (Bolling et al., 2012; Lutomiah et al., 2007; Saiyasombat et al., 2011), they have been probably maintained by vertical transmission from infected female mosquitoes (Blitvich and Firth, 2015). The study of these various ISVs in vector mosquitoes would contribute to the understanding of virus biodiversity and the complex vector-borne viral disease system in nature because ISVs have the potential to disrupt arbovirus transmission or reduce vector abundance (Bolling et al., 2015b).

1.3.4.1. Aedes flavivirus

1.3.4.1.1. History and epidemiology

AEFV belongs to genus *flavivirus,* family *Flaviviridae* and was first isolated from *Ae. albopictus* and *Ae. flavopictus* mosquitoes collected in Japan during 2003-2004 (Hoshino et

al., 2007). The virus has been reported only replicates in mosquito cells and induced weak or moderate cytopathic effects (CPEs) from 5–7 days post-infection (Roiz et al., 2012). Genetic and phylogenetic analyses classified AEFV with the insect flavivirus, but distinct from the other insect-specific flavivirus such as CFA, KRV, and CxFV (Hoshino et al., 2009). The virus might survive in the mosquito population due to several mechanisms such as vertical and/or sexual transmission (Lutomiah et al., 2007). The presence of this virus could be related to the survival of eggs during diapause through vertical transmission and may be related to horizontal transmission by venereal transmission (Lutomiah et al., 2007).

Since the virus was first isolated, several studies reported the detection and isolation of the virus in several localities. In 2007, the AEFV was detected in *Ae. albopictus* collected from Trentino and Padova provinces, Northern Italy with high prevalence (86.6%). After that the virus was successfully isolated in 2008 from C6/36 cell culture and from *Ae. albopictus* in 2010-2011 (Roiz et al., 2012; Rizzo et al., 2014; Calzolari et al., 2012). In 2011, AEFV was isolated from male of *Ae. albopictus* mosquitoes collected in Missouri, USA (Haddow et al., 2013) and Turkey in 2016 (Oncu et al., 2018). Moreover, the virus was also detected in Asia, Thailand in 2015 from *Ae. albopictus* colony established from eggs (Bolling et al., 2015a) and in 2016 in Shanghai, China (Fang et al., 2018).

1.3.4.1.2. Virus properties

AEFV is enveloped virus with icosahedral and spherical geometries and enveloped virions were approximately 50–60 nm in diameter (Roiz et al., 2012). The virus consisted of 11,064 nucleotides in length and contained a single ORF encoding a polyprotein of 3,341 amino acids with 5′ and 3′ untranslated regions (UTRs) of 96 and 945 nucleotides (Hoshino et al., 2009). Polyprotein was divided into 3 structural proteins (C, prM and E) and 7 nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) (Hoshino et al., 2009). The AEFV

3′ UTR contained 64 nucleotides, highly conserved, direct repeat motif in nucleotides 10,499–10,562 and 10,637–10,700, designated repeat 2 and repeat 1, respectively (Roiz et al., 2012). The genome of AEFV had an A at the 5′-terminus, but a C at the 3′-terminus compare with the other flavivirus which has A at its 5′-terminus and a U at its 3′-terminus (Hoshino et al., 2009; Roiz et al., 2012). Moreover, the AEFV 3′ UTR contains two tandem repeats, with significant sequence similarities to the 3′ UTR repeats in CFA and KRV. Therefore, AEFV is closely related to KRV and CFA with respect to its 3′ UTR sequence features (Hoshino et al., 2009; Roiz et al., 2012).

1.3.4.2. Culex flavivirus

1.3.4.2.1. History and epidemiology

CxFV was isolated from *Cx. quiquefasciatus* and *Cx. tritaeniorhyncus* collected in Japan and Indonesia in 2003-2004 (Hoshino et al., 2007). The virus was isolated from both female and male mosquitoes and induced moderate CPE in C6/36 cells, but not in Vero and BHK cells (Blitvich et al., 2009). The virus produced marked syncytia in C6/36 cells after 6–7 days (Kim et al., 2009). After that, CxFV was reported have been isolated from several mosquito species, for instance, *Cx. tritaeniorhyncus, Cx. pipiens, Cx. quiquefasciatus, Cx. restuans, Cx. interfor, Cx. interrogator, Ae. vexans, An. sinensis, Cx. maxi, Cx. usquatus, Cx. coronator* and *Cx. nigripalpus* in several areas in Guatemala (Morales-Betoulle et al., 2008), Mexico (Farfan-Ale et al., 2009; Saiyasombat et al., 2011), Uganda (Cook et al., 2009), Iowa (Blitvich et al., 2009), Chicago (Newman et al., 2011), Trinidad and Texas (Kim et al., 2009), Colorado (Bolling et al., 2015a), China (Huanyu et al., 2012), Taiwan (Chen et al., 2013), Brazil (Machado et al., 2012), Colombia (Miranda et al., 2019) and Argentina (Goenaga et al., 2014).

1.3.4.2.2. Virus properties

CxFV is consisted of 3 structural protein C, prM, and E with length of amino acid were 139 aa, 143 aa, and 427 aa, respectively (Kim et al., 2009). Non-structural protein is consisted of NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 with length of amino acid were 369 aa, 230 aa, 127 aa, 592aa, 189aa, 257 aa, and 889 aa, respectively (Kim et al., 2009). Based on transmission electron microscopy was conducted on C6/36 cells infected by CxFV showed giant multinucleated cells with virions 37–42 nm in diameter within the endo-plastic reticulum of infected cells (Kim et al., 2009). CxFV encodes a polyprotein from a single-strand positive RNA ORF, flanked by 3′ and 5′-UTR (Hoshino et al., 2007). The polyprotein of 3,364 aa is cleaved during and after translation into structural and non-structural proteins in the following order: C-prM(M)-E-NS1-NS2A-NS2B-NS3-NS4A-2K-NS4B-NS5 (Hoshino et al., 2007). Phylogenetic analyses of the envelope protein genes have shown that CxFV can be separated into two distinct clades. One clade contains CxFV isolates from Texas and Asia, while the second clade contains CxFV isolates from Latin America and the Caribbean (Kim et al. 2009). Clades 1 and 2 consist of strong groups with robust branch support, which are 10.4% genetically distant (Bittar et al., 2016). The ORF mean distance among CxFV reported sequences was 0.046. Also, the NS2A region presented the lowest genetic distance and NS4B is the highest (Bittar et al., 2016).

1.3.4.3.3. Situation in Indonesia

CxFV has been isolated from *Cx. quinquefasciatus* mosquitoes collected in Surabaya, East Java, Indonesia during 2003-2004 (Hoshino et al., 2009). Since then, there is no report of CxFV in Indonesia anymore.

1.3.4.3. Cell fusing agent virus

1.3.4.3.1. History and epidemiology

CFAV was first isolated from *Ae. aegypti* cell derived by Peleg in 1974 (Stollar and Thomas, 1975). The virus was recognized by its ability to cause fusion, massive syncytium formation and infected persistently of *Ae. albopictus* cells, but did not replicate or cause fusion of BHK and Vero cells (Stollar and Thomas, 1975). After that, the virus was characterized by Cammisa-Park et al., (1992) and represented the only known insect-only flavivirus. The virus was first isolated from a natural mosquito population including both male and female in Puerto Rico (Cook et al., 2006). Since then, the virus had been isolated from *Ae. aegypti* in several countries, Mexico (Espinoza-Gomez et al., 2011), Indonesia (Hoshino et al., 2009), and Thailand (Kihara et al., 2007). The CFAV was reported sensitive to ether and to deoxycholate (Stollar and Thomas, 1975).

1.3.4.3.2. Virus properties

The CFAV is a single-stranded, positive-sense RNA virus and belongs to genus *Flavivirus* (family *Flaviviridae*). The CFAV genome comprises a single long ORF that encodes three structural and seven non-structural proteins (Cook et al., 2006). The length of structural protein (C, prM, E) were 136 aa, 142 aa, and 427 aa, respectively. While non-structural protein (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) were 390 aa, 232 aa, 124 aa, 577 aa, 168 aa, 258 aa, and 887 aa, respectively (Crabtree et al., 2003). The capsid protein contains 136 aa and

highly basic (26 lysine plus arginine residues) which are 20 of these basic residues are in the N-terminal half of the protein (Cammisa-Park et al., 1992). The E protein contains 427 aa, 5 potential linked glycosylation sites and 14 Cys residues, which was different to the E protein of DEN-4 that has 13 Cys residues (Cammisa-Park et al., 1992).

The virus has similar genome size, structure and gene order to flavivirus. Amino acid sequence similarity between CFAV proteins and those of other flaviviruses is highest for the non-structural proteins NS5 and NS3 and lowest for the structural proteins (Cook et al., 2006). The larger 49 and 16.5 kDa proteins were glycosylated and associated with the envelope and it is much larger than the other flavivirus, while the smaller 13 kDa protein was associated with the viral RNA (Igarashi et al., 1976; Stollar and Thomas, 1975). There is 45% identity of AEFV NS5 and NS3 (34%) protein to other flavivirus (Yamanaka et al., 2013; Cammisa-Park et al., 1992). Besides that, the segments of the CFAV genome have integrated into *A. aegypti* and *Ae. albopictus* genome suggested that the virus has been persistently infecting the mosquitoes for a long time (Crochu et al., 2004).

Moreover, polyprotein cleavage of pre-membrane/membrane was similar to that of KRV (Crabtree et al., 2003). In addition, two neutralizing anti-CFAV (50% plaque reduction test had titers between 1:2000 and 1:4000) had no hemagglutination inhibition activity when tested against several flavivirus antigens including antigens chosen for their high sensitivity to all known flavivirus cross-reacting antibodies (Igarashi et al., 1976). Similarly, when tested by the fluorescent antibody technique, anti-CFA serum failed to react with cells infected with dengue type 2 (DEN-2), JEV, or bovine viral diarrhea viruses (Igarashi et al., 1976).

1.3.4.3.3. Situation Indonesia

CFAV has been isolated from *Ae. aegypti* mosquitoes collected in Surabaya, East Java, Indonesia during 2003-2004 (Hoshino et al., 2009). Since then, there is no report of CFAV in Indonesia anymore.

In Indonesia the burden of vector-borne pathogens is still limited mostly because there is a lack of nation-wide systematic routine surveys, standardize diagnostic approaches and spatio-temporal surveillance of the diseases. Therefore, the vector-borne pathogens data including human cases, animals, and tick vector status are still not clear. Despite these limitations, this study may aid to generate the data of vector-borne pathogens in Indonesia.

2. CHAPTER 1 Detection and isolation of tick-borne bacteria (*Anaplasma* spp., *Rickettsia* spp. and *Borrelia* spp.) in *Amblyomma varanense* ticks on lizard (*Varanus salvator*)

2.1. Abstract

Ticks are one of the arthropods that play an important role in the transmission of numerous pathogens to livestock and humans. I investigated the presence of tick-borne bacteria in 23 *Amblyomma varanense* that fed on a water monitor (*Varanus salvator*) in Indonesia. *Anaplasmataceae* and borreliae were detected by PCR in 17.4% and 95.7% of ticks, respectively. "*Candidatus* Rickettsia sepangensis", spotted fever group of Rickettsia, was detected in 21.7% of ticks. The water monitor is a common reptile that is widely encountered in city areas in Asian countries. Our results suggested that *Am. varanense* on water monitor in Indonesia harbored several kinds of bacteria.

2.2. Introduction

As mentioned in GENERAL INTRODUCTION, there is still limited studies have reported presence of bacteria in ticks associated with animals including reptiles in Indonesia. Several studies reported, *Anaplasma* sp., *Borrelia lusitaniae* and *Rickettsia* sp. have been detected in *Dermacentor marginatus* and *I. ricinus* ticks that feed on green lizards (*Lacerta viridis*) found in Slovakia (Vaclav et al., 2013). The *I. pacificus,* which fed on western fence lizard (*Sceloporus occidentalis*) in USA, and the *Amblyomma flavomaculatum*, that fed on savannah monitor (*Varanus exanthematicus*) in Poland, were positive for *A. phagocytophilum*, the causative agent of human granulocytic anaplasmosis (Nieto et al., 2009; Nowak et al., 2010).

Several reptile-associated (REP) borreliae have been reported in the *Hyalomma*, *Bothriocroton*, *Amblyomma* and/or *Aponomma* genera ticks found in Asia, Australia, Africa and the Middle-East (Takano *et al*., 2010; Takano et al., 2011; Panetta et al., 2017). In addition, the causative agent of African tick-bite fever, *Rickettsia africae*, and that of tick-borne lymphadenopathy, *Rickettsia raoultii*, has been detected in reptile-associated ticks (Andoh et al*.,* 2015). Some reptile-associated ticks infested humans and/or livestock (Burridge, 2001) as well as reptiles, including the water monitor (*Varanus salvator*), which inhabit cities and may be in close relation with human habitat.

In this chapter, I conducted surveillance of tick-borne bacteria carried by *Am. varanense* (synonym, *Aponomma lucasi*) ticks, which feed on *V. salvator* found in Indonesia. In addition, *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp., and *Borrelia* spp. were surveyed and phylogenetically analyzed.

2.3. Material and methods

2.3.1. Collection of ticks

One *V. salvator* was captured by hands on January 2017 in Bogor, West Java Province, Indonesia (6º35'48" S, 106º47'50" E) and ticks were collected from the skin using forceps. After collection of ticks, *V. salvator* was released. The lizard was handled in accordance with good animal practice required by the Animal Ethics Procedures of Indonesia. The *V. salvator* is not an endangered or protected species in Indonesia and permission for collection of ticks on lizard in this area was not required. Tick species, stage and sex were identified based on morphologic features following taxonomic keys and molecular analysis.

2.3.2. Borrelia cultivation and DNA extraction

Eighteen live ticks were dissected under the stereomicroscopy. The salivary glands and midguts were provided for cultivation of borreliae using Barbour-Stoenner-Kelly medium and miscellaneous tissues without salivary gland and midgut were subjected to DNA preparation. DNA extraction and cultivation of borreliae were carried out as described (Takano et al., 2010).

2.3.3. PCR and sequencing analysis

PCR, targeting tick mitochondrial 16S rRNA gene (mt-*rrs*) for both confirmation of tick identification and verification of the quality of DNA, was performed on all DNAs from ticks. The PCRs targeting 16S rRNA gene (*16S rDNA*) and 60-kDa heat shock protein gene (*groEL*) for *Anaplasmataceae*, 17k-Da antigen gene and citrate synthase gene (*gltA*) for *Rickettsia* spp., and flagellin gene (*flaB*) for *Borrelia* spp. were performed as described previously (Supporting Table 1) (Takano et al., 2010; Andoh et al*.,* 2015; Pancholi et al*.,* 1995). The characterization of isolated *Borrelia* sp. was performed by *16S rDNA* of borreliae. PCR amplicons were purified

and directly sequenced (Takano et al., 2010). All sequences were deposited in DDBJ/EMBL/GenBank (accession numbers LC428370-LC428386) (Supporting Table 2).

2.3.4. Phylogenetic analysis

Phylogenetic analysis was performed using MEGA7 software (www.megasoftware.net) (Tamura et al., 2007). The phylogenetic trees were constructed by the neighbor-joining method. Bootstrap analysis (1000 replicates) was carried out according to the Kimura 2-parameter model. All positions containing alignment gaps and missing data were eliminated during the pairwise sequence comparison (pairwise deletion).

2.4. Results

A total of 23 ticks (21 males, a female and a nymph) were collected from one *V. salvator* captured in the central area of Bogor, Indonesia. According to the morphological features and molecular analysis based on mt-*rrs*, all ticks were identified as *Am. varanense.*

Out of 23 ticks, four ticks (17%, 3 males and a female) were PCRs positive for *Anaplasmataceae*. In the present study, two different types of sequences were detected: one from a male (RT4), and the other from 2 males and a female (RT20, 21 and 23). By the phylogenetic analysis of *16S rDNA,* the sequence of RT4 was found to be closely related to that of the *Anaplasma* sp. HN680 (KX505298; 99.5% similarity), whereas the other sequences (RT20, 21 and 23) were closely related to that of uncultured *Anaplasma* sp. (MG346222; 98% similarity) (Fig. 2-1 and 2-2). The *Anaplasma* sp. HN680 was detected in blood of sheep in China (Zhang et al., 2016) and phylogenetically related to *A. marginale* and *A. bovis*, which are causative agents of fatal tick-borne disease in cattle. Although *Am. varanense* is commonly infested on reptiles, it has been reported to feed on various mammals (Burridge, 2001). Since *Anaplasma* sp. detected in present study was found from an adult male, it may be suggested

that *Am. varanense* ticks might acquire the bacteria during a previous blood feeding opportunities.

The other three sequences detected (RT20, 21 and 23) formed an independent monophyletic group and same branch with *Anaplasma* spp. detected in Taiwan, South Korea, Morocco, Tunisia and Poland (Kuo et al., 2017; Sarih et al., 2005; Ekner et al., 2011) (Fig.2-1). Although these *Anaplasma* spp. were detected in worldwide, the sequence similarity was over 98% in *16S rDNA*. Because the ticks were harboring bacterial endosymbionts in spite of geographical origin (Niebylski et al., 1997), there was the possibility that these bacteria might present as the endosymbionts in ticks and distribute worldwide. On the other hand, the endosymbionts were transmitted with trans-ovarially, conferred fitness advantage to their hosts, and adapted to the internal environment of the ticks (Noda et al., 1997). The present study detected *Anaplasma* sp. from only 3 ticks (13%). Moreover, *Anaplasma* sp. MT1216, phylogenetically related with RT20, 21, 23, were detected reportedly from several individuals of lizards (Ekner et al., 2011). Therefore, it was speculated that the bacteria may infect lizards and transmitted through the tick.

In the present study, 22 ticks were positive for *Borrelia* sp. (96%, 20 males, one female and one nymph) by PCR using genomic DNA prepared from miscellaneous tissues of ticks. Moreover, thirty strains were successfully isolated from the midgut and/or salivary glands of 17 live ticks (Supporting Table 2). The similarity of sequence of *16S rDNA* of isolates was 98% to 99% when compared with *Borrelia* spp. detected from *Bothriocroton undatum* (MG004679 or MG004684 in Fig. 2-3). The ticks were collected from wild lace lizard (*Varanus varius*) found in Australia (Panetta et al., 2017). Phylogenetic analysis based on *16S rDNA* and *flaB* demonstrated that isolates in the present study formed a monophyletic group and belonged to REP borreliae (Fig. 2-3 and 2-4). This is the first to isolate and perform molecular characterization of a novel species of REP borreliae from *Am. varanense* ticks fed

on lizard in Indonesia. Phylogenetic analysis of *16S rDNA* sequences of novel isolated *Borrelia* sp. and previously reported *Borrelia* spp. indicated that the REP borreliae co-evolved with host reptiles (Fig. 2-3).

In the present study, the variability of isolated *Borrelia* sp. were found by sequencing of *flaB*. The similarity sequences of *flaB* among *B. andersoni,* '*Candidatus* Borrelia tachyglossi' and *B. turcica* ranged between 98.0-100%, 99.6-99.9% and 99.2-100%, respectively (Clark et al*.,* 2005; Loh et al*.,* 2017; Guner et al*.,* 2003). In the present study, although I analyzed ticks collected from single lizard, the sequences variability of *flaB* among strains ranged from 97.3-99.7%. Because I examined 22 adults and a nymph, I speculated that the tick and also lizard might be infected with several strains of *Borrelia* sp..

In the present study, 5 males (22%, RT2, 5, 19, 20, and 21) were PCRs positive for *Rickettsiae*. Phylogenetic analysis of both genes revealed that, the detected *Rickettsia* sp. belonged to the spotted fever group (SFG). The detected species was identical to '*Candidatus* Rickettsia sepangensis' by *gltA* sequences, which was detected in *Am. varanense* collected from Indian python (*Python molurus*) in Malaysia (Kho et al., 2015) (Fig. 2-5 and 2-6). One of the related species of *Rickettsiae*, *R. tamurae* was detected in blood and skin of human patients in Japan and caused flu-like illness (Imaoka et al*.,* 2011). A serological study conducted in Laos speculated *R. tamurae* AT-1 infection of a patient with fever, headache and jaundice (Phongmany et al*.,* 2006). Although the pathogenicity and reservoir of *Candidatus* R. sepangensis detected in the present study remains unclear, the result in accordance with that of previous reports indicated that the *Rickettsiae* carried by reptile-associated ticks might potentially cause uncharacterized diseases.

2.5. Discussion

The present study is the first report of detection of *Anaplasma* spp., *Rickettsia* sp. and *Borrelia* sp. in *Am. varanense* associated with a reptile found in Indonesia. Because of the regulation of setting the traps, I could not capture several individuals of lizards. Although ticks were collected from a lizard, variety of bacteria was detected from ticks. Moreover, some ticks were co-infected with multiple bacteria; two males (RT20 and 21) were infected with three different bacteria, *Anaplasma* sp., *Candidatus* R. sepangensis and *Borrelia* sp., three males (RT2, 5 and 19) with *Candidatus* R. sepangensis and *Borrelia* sp., and two (one male and one female, RT4 and 23) were co-infected with *Anaplasma* spp. and *Borrelia* sp.. The water monitor is a common reptile and is easily found in city areas in Indonesia and other Asian countries. Therefore, the present study revealed that the reptiles close to humans and its related ticks maintained variety of unknown bacteria. Although the pathogenicity or biology of these detected bacteria is unclear, garnering information of these novel bacteria is essential to device diagnostic strategies for unknown infectious diseases.

Recently, many species of reptiles are currently being traded as pet animals in several countries globally. Tick-borne pathogens pose potential health threats to humans; therefore, knowledge of the dynamics of the circulation of tick-borne bacteria in the environment is essential to assess the risk of infection to both livestock and humans. Although the direct evidences remained to be determined, the observations of the present study provide clues for a possibility that *Am. varanense* ticks can play a role in transmission of pathogenic bacteria from heterothermic to homothermic animals. Accumulation of these epidemiological information from multiple individuals and/or species of reptiles in future study should be informative for this goal.

2.6. Figure legends

Fig. 2-1 Phylogenetic analysis of *16S rDNA* of *Anaplasma* spp. The phylogenetic branches showed support of >70% by tree, which was constructed using neighbor-joining method and bootstrap tests (1000 replicates) were carried out according to the Kimura 2-parameter model. The bar indicates the percentage of sequence divergence. Bold letters indicate the samples analyzed in the present study.

Fig. 2-2 Phylogenetic analysis of *groEL* of Anaplasma spp. The phylogenetic branches showed support of >70% by tree, which was constructed using neighbor-joining method and bootstrap tests (1000 replicates) were carried out according to the Kimura 2-parameter model. The bar indicates the percentage of sequence divergence. Bold letters indicate the samples analyzed in the present study.

Fig. 2-3 Phylogenetic analysis of *16S rDNA* of isolated *Borrelia* sp. The phylogenetic branches showed support of >70% by tree, which was constructed using neighbor-joining method and bootstrap tests (1000 replicates) were carried out according to the Kimura 2-parameter model. The bar indicates the percentage of sequence divergence. *Spirochaeta americana* (AF373921), *Treponema pallidum* (NC000919), *Cristispira* sp. (U42638) were used as outgroup. Bold letters indicate the samples analyzed in the present study.

Fig. 2-4 Phylogenetic analysis of *flaB* of isolated *Borrelia* sp. The phylogenetic branches showed support of >70% by tree, which was constructed using neighbor-joining method and bootstrap tests (1000 replicates) were carried out according to the Kimura 2-parameter model.

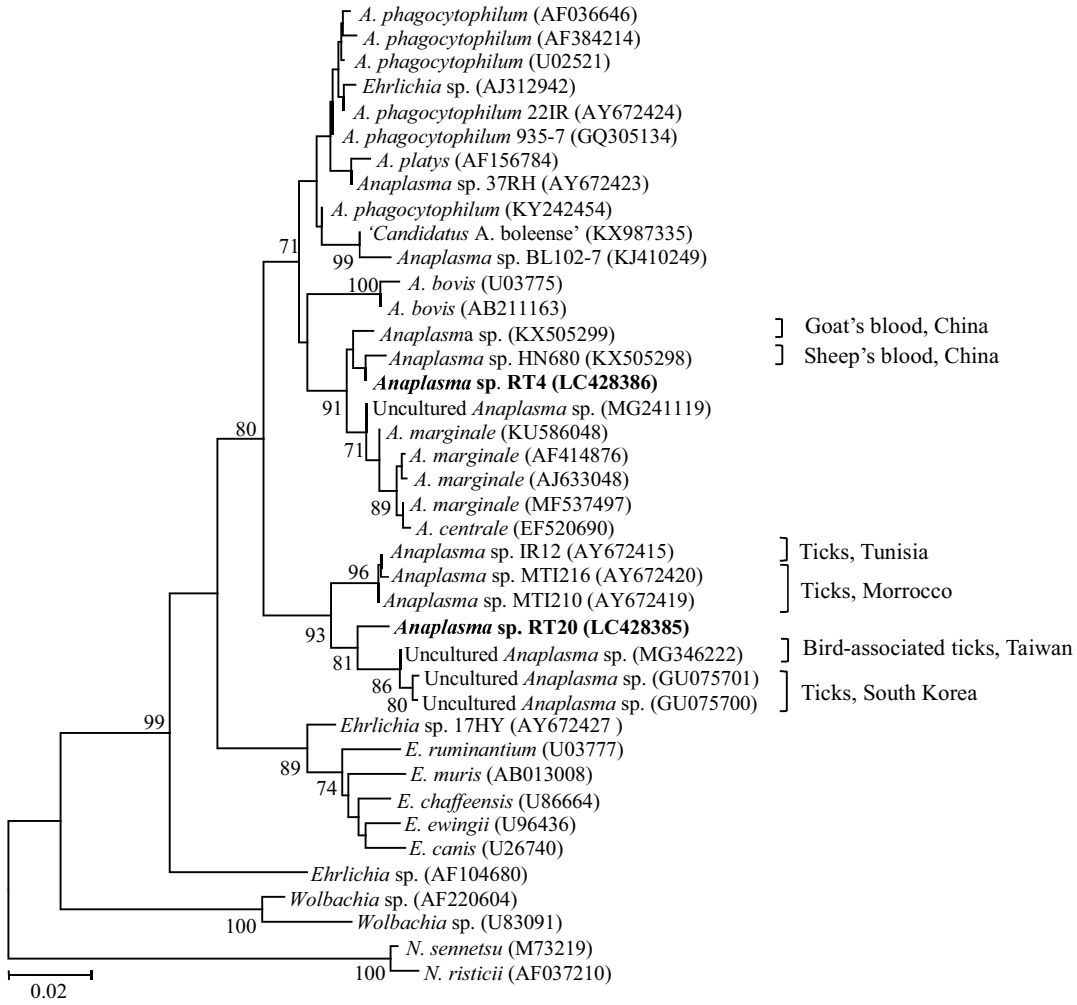
The bar indicates the percentage of sequence divergence. Bold letters indicate the samples analyzed in the present study.

Fig. 2-5 Phylogenetic analysis of *gltA* of *Rickettsia* spp.

The phylogenetic branches showed support of >70% by tree, which was constructed using neighbor-joining method and bootstrap tests (1000 replicates) were carried out according to the Kimura 2-parameter model. The bar indicates the percentage of sequence divergence. Bold letters indicate the samples analyzed in the present study.

Fig. 2-6 Phylogenetic analysis of 17 kDa antigen of *Rickettsia* spp.. The phylogenetic branches showed support of >70% by tree, which was constructed using neighbor-joining method and bootstrap tests (1000 replicates) were carried out according to the Kimura 2-parameter model. The bar indicates the percentage of sequence divergence. Bold letters indicate the samples analyzed in the present study.

Fig. 2-1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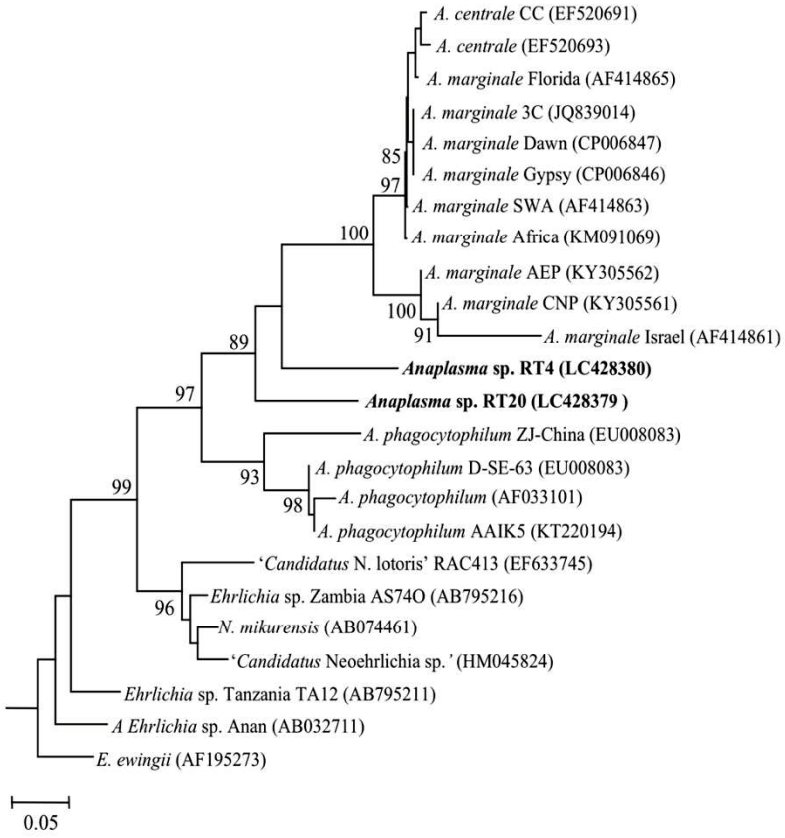
A. phagocytophilum (AF384214)
A. phagocytophilum (U02521)
Ehrlichia sp. (AJ312942)
A. phagocytophilum 22IR (AY672424)
A. phagocytophilum 935-7 (GQ305134)
A. platys (AF156784)
Anaplasma sp. 37RH (AY672423)
A. phagocytophilum (KY242454)
'Candidatus A. boleense' (KX987335)
Anaplasma sp. BL102-7 (KJ410249)
A. bovis (U03775)
A. bovis (AB211163)
Anaplasma sp. (KX505299)
Anaplasma sp. HN680 (KX505298)
Anaplasma sp. RT4 (LC428386)
Uncultured Anaplasma sp. (MG241119)
A. marginale (KU586048)
A. marginale (AF414876)
A. marginale (AJ633048)
A. marginale (MF537497)
A. centrale (EF520690)
Anaplasma sp. IR12 (AY672415)
Anaplasma sp. MTI216 (AY672420)
Anaplasma sp. MTI210 (AY672419)
Anaplasma sp. RT20 (LC428385)
Uncultured Anaplasma sp. (MG346222)
Uncultured Anaplasma sp. (GU075701)
Uncultured Anaplasma sp. (GU075700)
Ehrlichia sp. 17HY (AY672427)
E. ruminantium (U03777)
E. muris (AB013008)
E. chaffeensis (U86664)
E. ewingii (U96436)
E. canis (U26740)
Ehrlichia sp. (AF104680)
Wolbachia sp. (AF220604)
Wolbachia sp. (U83091)
N. sennetsu (M73219)
N. risticii (AF037210)
71
99
100
80
91
71
89
96
93
81
86
80
99
89
74
100
100
0.02
Goat's blood, China
Sheep's blood, China
Ticks, Tunisia
Ticks, Morrocco
Bird-associated ticks, Taiwan
Ticks, South Korea

Fig. 2-2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A. centrale (EF520693)
A. marginale Florida (AF414865)
A. marginale 3C (JQ839014)
A. marginale Dawn (CP006847)
A. marginale Gypsy (CP006846)
A. marginale SWA (AF414863)
A. marginale Africa (KM091069)
A. marginale AEP (KY305562)
A. marginale CNP (KY305561)
A. marginale Israel (AF414861)
Anaplasma sp. RT4 (LC428380)
Anaplasma sp. RT20 (LC428379)
A. phagocytophilum ZJ-China (EU008083)
A. phagocytophilum D-SE-63 (EU008083)
A. phagocytophilum (AF033101)
A. phagocytophilum AAIK5 (KT220194)
'Candidatus N. lotoris' RAC413 (EF633745)
Ehrlichia sp. Zambia AS74O (AB795216)
N. mikurensis (AB074461)
'Candidatus Neoehrlichia sp.' (HM045824)
Ehrlichia sp. Tanzania TA12 (AB795211)
A Ehrlichia sp. Anan (AB032711)
E. ewingii (AF195273)
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97
100
100
91
89
97
93
98
99
96
0.05

Fig. 2-3

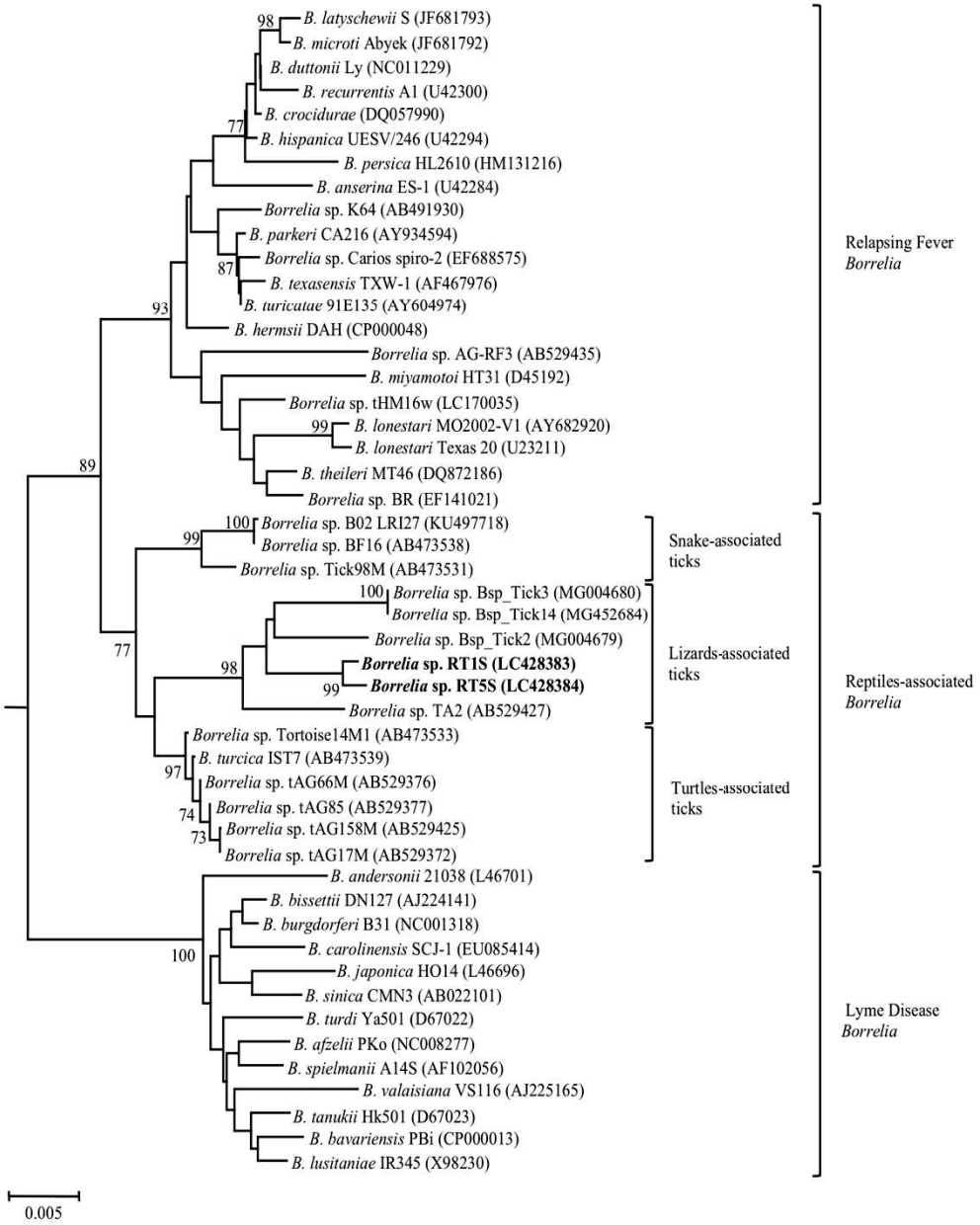
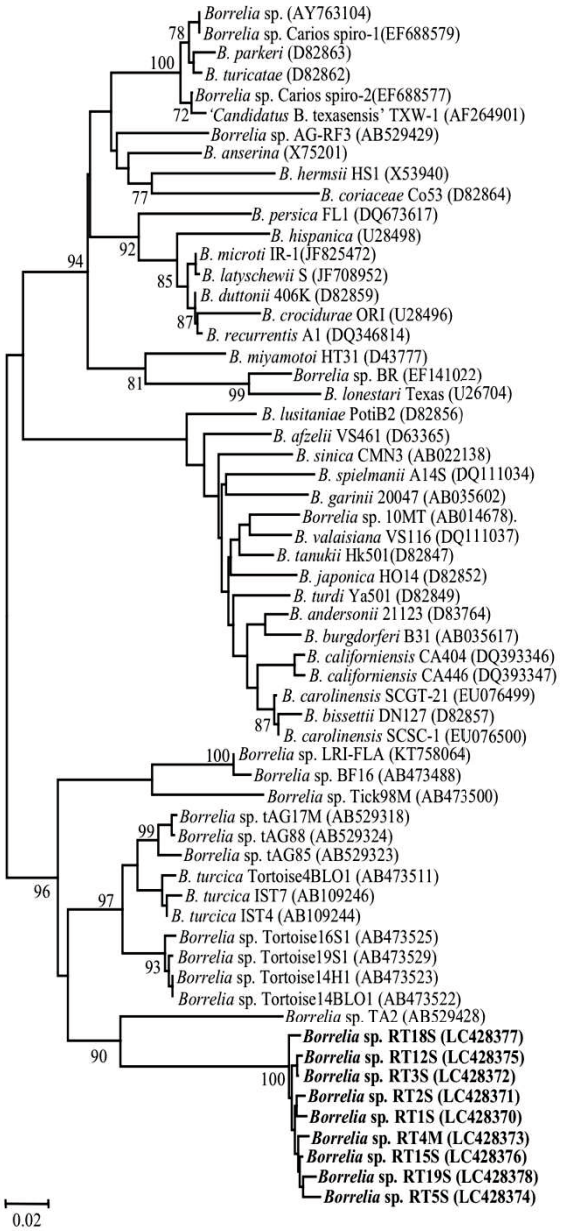

98
B. latyschewii S (JF681793)
B. microti Abyek (JF681792)
B. duttonii Ly (NC011229)
B. recurrentis A1 (U42300)
77
B. crocidurae (DQ057990)
B. hispanica UESV/246 (U42294)
B. persica HL2610 (HM131216)
B. anserina ES-1 (U42284)
Borrelia sp. K64 (AB491930)
B. parkeri CA216 (AY934594)
87
Borrelia sp. Carios spiro-2 (EF688575)
B. texasensis TXW-1 (AF467976)
93
B. turicatae 91E135 (AY604974)
B. hermsii DAH (CP000048)
Borrelia sp. AG-RF3 (AB529435)
B. miyamotoi HT31 (D45192)
Borrelia sp. tHM16w (LC170035)
99
B. lonestari MO2002-V1 (AY682920)
B. lonestari Texas 20 (U23211)
89
B. theileri MT46 (DQ872186)
Borrelia sp. BR (EF141021)
Relapsing Fever
Borrelia
100
Borrelia sp. B02 LRI27 (KU497718)
99
Borrelia sp. BF16 (AB473538)
Borrelia sp. Tick98M (AB473531)
Snake-associated
ticks
100
Borrelia sp. Bsp_Tick3 (MG004680)
Borrelia sp. Bsp_Tick14 (MG452684)
Borrelia sp. Bsp_Tick2 (MG004679)
77
98
Borrelia sp. RT1S (LC428383)
99
Borrelia sp. RT5S (LC428384)
Borrelia sp. TA2 (AB529427)
Lizards-associated
ticks
Reptiles-associated
Borrelia
Borrelia sp. Tortoise14M1 (AB473533)
B. turcica IST7 (AB473539)
97
Borrelia sp. tAG66M (AB529376)
74
Borrelia sp. tAG85 (AB529377)
73
Borrelia sp. tAG158M (AB529425)
Borrelia sp. tAG17M (AB529372)
Turtles-associated
ticks
B. andersonii 21038 (L46701)
B. bissettii DN127 (AJ224141)
B. burgdorferi B31 (NC001318)
100
B. carolinensis SCJ-1 (EU085414)
B. japonica HO14 (L46696)
B. sinica CMN3 (AB022101)
B. turdi Ya501 (D67022)
B. afzelii PKo (NC008277)
B. spielmanii A14S (AF102056)
B. valaisiana VS116 (AJ225165)
B. tanukii Hk501 (D67023)
B. bavariensis PBi (CP000013)
B. lusitaniae IR345 (X98230)
Lyme Disease
Borrelia
0.005

Fig. 2-4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Borrelia sp. AG-RF3 (AB529429)
B. anserina (X75201)
B. hermsii HS1 (X53940)
B. coriaceae Co53 (D82864)
B. persica FL1 (DQ673617)
B. hispanica (U28498)
B. microti IR-1(JF825472)
B. latyschewii S (JF708952)
B. duttonii 406K (D82859)
B. crocidurae ORI (U28496)
B. recurrentis A1 (DQ346814)
B. miyamotoi HT31 (D43777)
Borrelia sp. BR (EF141022)
B. lonestari Texas (U26704)
B. lusitaniae PotiB2 (D82856)
B. afzelii VS461 (D63365)
B. sinica CMN3 (AB022138)
B. spielmanii A14S (DQ111034)
B. garinii 20047 (AB035602)
Borrelia sp. 10MT (AB014678).
B. valaisiana VS116 (DQ111037)
B. tanukii Hk501(D82847)
B. japonica HO14 (D82852)
B. turdi Ya501 (D82849)
B. andersonii 21123 (D83764)
B. burgdorferi B31 (AB035617)
B. californiensis CA404 (DQ393346)
B. californiensis CA446 (DQ393347)
B. carolinensis SCGT-21 (EU076499)
B. bissettii DN127 (D82857)
B. carolinensis SCSC-1 (EU076500)
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Borrelia sp. BF16 (AB473488)
Borrelia sp. Tick98M (AB473500)
Borrelia sp. tAG17M (AB529318)
Borrelia sp. tAG88 (AB529324)
Borrelia sp. tAG85 (AB529323)
B. turcica Tortoise4BLO1 (AB473511)
B. turcica IST7 (AB109246)
B. turcica IST4 (AB109244)
Borrelia sp. Tortoise16S1 (AB473525)
Borrelia sp. Tortoise19S1 (AB473529)
Borrelia sp. Tortoise14H1 (AB473523)
Borrelia sp. Tortoise14BLO1 (AB473522)
Borrelia sp. TA2 (AB529428)
Borrelia sp. RT18S (LC428377)
Borrelia sp. RT12S (LC428375)
Borrelia sp. RT3S (LC428372)
Borrelia sp. RT2S (LC428371)
Borrelia sp. RT1S (LC428370)
Borrelia sp. RT4M (LC428373)
Borrelia sp. RT15S (LC428376)
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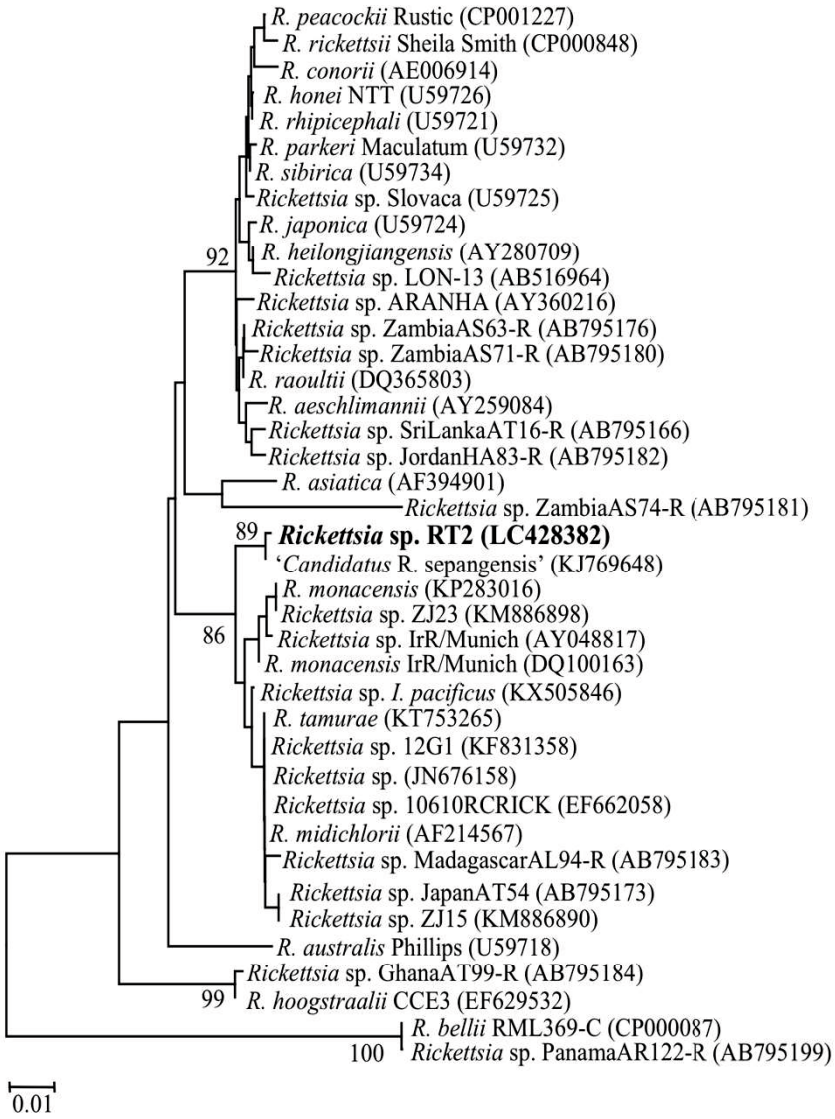
Borrelia sp. RT5S (LC428374)
0.02

Fig. 2-5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R. rickettsii Sheila Smith (CP000848)
R. conorii (AE006914)
R. honei NTT (U59726)
R. rhipicephali (U59721)
R. parkeri Maculatum (U59732)
R. sibirica (U59734)
Rickettsia sp. Slovaca (U59725)
R. japonica (U59724)
92
R. heilongjiangensis (AY280709)
Rickettsia sp. LON-13 (AB516964)
Rickettsia sp. ARANHA (AY360216)
Rickettsia sp. ZambiaAS63-R (AB795176)
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R. aeschlimannii (AY259084)
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R. asiatica (AF394901)
Rickettsia sp. ZambiaAS74-R (AB795181)
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R. monacensis (KP283016)
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86
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R. monacensis IrR/Munich (DQ100163)
Rickettsia sp. I. pacificus (KX505846)
R. tamurae (KT753265)
Rickettsia sp. 12G1 (KF831358)
Rickettsia sp. (JN676158)
Rickettsia sp. 10610RCRICK (EF662058)
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Rickettsia sp. JapanAT54 (AB795173)
Rickettsia sp. ZJ15 (KM886890)
R. australis Phillips (U59718)
Rickettsia sp. GhanaAT99-R (AB795184)
99
R. hoogstraalii CCE3 (EF629532)
R. bellii RML369-C (CP000087)
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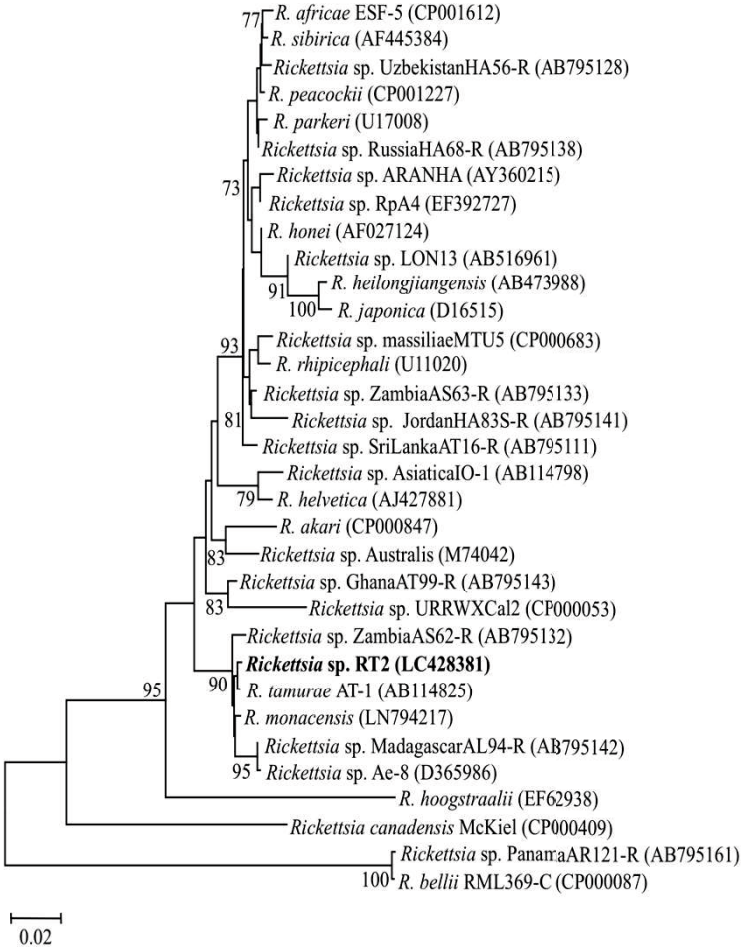
0.01

Fig. 2-6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R. tamurae AT-1 (AB114825)
R. monacensis (LN794217)
Rickettsia sp. MadagascarAL94-R (AB795142)
Rickettsia sp. Ae-8 (D365986)
R. hoogstraalii (EF62938)
Rickettsia canadensis McKiel (CP000409)
Rickettsia sp. PanamaAR121-R (AB795161)
R. bellii RML369-C (CP000087)
77
73
91
100
93
81
79
83
83
95
90
95
100
0.02

3. CHAPTER 2 Mosquito-borne viruses, insect-specific flaviviruses (family *Flaviviridae*, genus *Flavivirus*), Banna virus (family *Reoviridae*, genus *Seadornavirus*), Bogor virus (unassigned member of family *Permutotetraviridae*), and alphamesoniviruses 2 and 3 (family *Mesoniviridae*, genus *Alphamesonivirus*) isolated from Indonesian mosquitoes

3.1. Abstract

Mosquitoes transmit many kinds of arboviruses (arthropod-borne viruses), and numerous arboviral diseases have become serious problems in Indonesia. In this study, I conducted surveillance of mosquito-borne viruses at several sites in Indonesia during 2016–2018 for risk assessment of arbovirus infection and analysis of virus biodiversity in mosquito populations. I collected 10,015 mosquitoes comprising at least 11 species from 4 genera. Major collected mosquito species were *Cx. quinquefasciatus, Ae. albopictus, Cx. tritaeniorhynchus, Ae. aegypti*, and *Ar. subalbatus*. The collected mosquitoes were divided into 285 pools and used for virus isolation using two mammalian cell lines, Vero and BHK-21, and one mosquito cell line, C6/36. Seventy-two pools showed clear CPEs only in C6/36 cells. Using RT-PCR and next-generation sequencing approaches, these isolates were identified as insect flaviviruses (family *Flaviviridae*, genus *Flavivirus*), Banna virus (family *Reoviridae*, genus *Seadornavirus*), new permutotetravirus (designed as Bogor virus) (family *Permutotetraviridae*, genus *Alphapermutotetravirus*), and alphamesoniviruses 2 and 3 (family *Mesoniviridae*, genus *Alphamesonivirus*). I believed that this large surveillance of mosquitoes and mosquito-borne viruses provides basic information for the prevention and control of emerging and re-emerging arboviral diseases.

3.2. Introduction

As mentioned in GENERAL INTRODUCTION, mosquitoes transmit many kinds of arboviruses that cause endemics or epidemics in tropical/subtropical regions worldwide, depending on the distribution and activities of vector mosquitoes (Weaver and Reisen, 2010). Mosquitoes also harbor a diverse range of RNA viruses that do not seem to infect human and other vertebrates (Nouri et al., 2018). These viruses are known as ISVs and can infect and replicate only in invertebrates (Nouri et al., 2018).

Although there are many factors for global emergence and re-emergence of arboviral diseases, one of them is change in the environment surrounding human societies, such as urbanization of rural areas by human migration, global tourism, and expansion of agriculture areas. In addition, trade and transportation have contributed to the introduction of both arboviruses and vector arthropod species. For instance, DENV (family *Flaviviridae*, genus *Flavivirus*) is one of the most common mosquito-borne arboviruses and more than 2.5 billion people are at risk of this viral disease worldwide (Guha-Sapir and Schimmer, 2005). DENV has been transmitted by *Aedes* mosquito species, such as *Ae. aegypti* (Linnaeus) and *Ae. albopictus* (Skuse), and distributions of these mosquito species have been strongly influenced by urbanization factors due to their preference of artificial containers as its larval habitat and internationalization (Guha-Sapir and Schimmer, 2005). Climate change is also associated with emergence and re-emergence of arbovirus diseases because the distribution of arboviruses is dependent on their arthropod vectors (Mordecai et al., 2019).

Since entomological approaches may aid in risk assessment of arbovirus infections, I conducted surveillance of mosquito species and mosquito-borne viruses in Indonesia during 2016–2018 in CHAPTER 1.

3.3. Materials and methods

3.3.1. Mosquito collection

Mosquito collection was conducted from seven provinces of four islands in the eastern part of Indonesia (Bengkulu province in Sumatra) (3°48'1.58"S, 102°15'55.94"E), Banten (-6° 02' 32.82" S, 106° 09' 39.35" E), Jakarta (6°12'52.63"S, 106°50'42.47"E), West Java (6° 54' 53.0784" S, 107° 36' 35.3160" E), and Central Java provinces in Java (6° 58' 0.0012" S, 110° 24' 59.9904" E); Bali province in Bali (8° 24' 34.2648" S, 115° 11' 20.1084" E); and Central Kalimantan province in Borneo (2° 12' 30" S, 113° 55' 0" E) during 2016–2018 (Fig. 3-1). Mosquitoes were collected in animal houses, such as swinery (Bengkulu and Denpasar), cowsheds (Bogor), and non-human primate sanctuaries (Palangka Raya and Bogor), and both indoor and outdoor human residential areas (Bogor, Tangerang, Bengkulu, Solo, Bekasi, and Pasar Minggu). Adult mosquitoes were collected in residential areas at daytime and around animal houses after sunset by using sweep nets and aspirators. Blood-feeding mosquitoes were kept in cages for a few days to let them digest the blood in their guts. The collected mosquitoes were identified based on morphology (Rampa, 1982). After identification, mosquitoes were sorted into pools with a maximum of 96 adults per pool and kept at −80°C for virus isolation.

3.3.2. Cell cultures

Two mammalian cell lines, Vero [Japanese Collection of Research Bioresources (JCRB), Osaka, Japan] and BHK-21 (JCRB), and one mosquito cell line, C6/36 (Health Science Research Resources Bank, Osaka, Japan), were used for virus isolation. Vero and BHK-21 cells were cultured in Dulbecco's modified eagle's medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) containing heat-inactivated 5% fetal bovine serum (FBS; Hyclone Laboratories, South Logan, UT, USA) and 1% penicillin and streptomycin (Pen-Strep; Life Technologies, Carlsbad, CA, USA), and C6/36 cells were cultured in Eagle's minimum

essential medium (Sigma-Aldrich) containing heat-inactivated 10% FBS and 1% Pen-Strep. Cell lines were maintained at 37°C for mammalian cells and 28°C for mosquito cells under 5% CO_2.

3.3.3. Virus isolation

Virus isolation was performed as previously described (Kuwata et al., 2015; Kuwata et al., 2020). Each pool of mosquitoes was homogenized in 500 μl of ice-cold DMEM containing 2% heat-inactivated FBS and 1% Pen-Strep using the Multi-beads shocker (Yasui Kikai Inc., Osaka, Japan). Homogenates were clarified by centrifugation (21,500 x g for 5 min at 4°C), and supernatants were passed through sterile 0.45-μm filters (Corning Inc., Corning, NY, USA). Filtrates were inoculated onto a monolayer of Vero, BHK-21, or C6/36 cells in 24-well culture plates (Sumitomo Bakelite Inc., Tokyo, Japan), and plates were incubated for 2 h to allow virus adsorption. After adding fresh medium, cell cultures were incubated at cell culture conditions. CPEs were observed daily, and supernatants were collected from cells with CPEs. If no CPE was observed, cells were blind-passaged, and after five blind passages, culture supernatants were collected even if no CPEs were observed. These culture supernatants were stored at −80°C until use.

3.3.4. Detection of viral genome

RNA was extracted from culture supernatants using the QIAamp Viral RNA Mini Kit (QIAGEN, Inc., Valencia, CA, USA). RNA was extracted from each culture supernatant from cells with CPEs individually. By contrast, for cells without any CPEs, culture supernatants from 5–8 wells of Vero and BHK-21 cells or 3–4 wells of C6/36 cells were pooled for RNA extraction, which was subsequently used in RT-PCR analyses. If RNA from mixed pools yielded positive RT-PCR results, then RNA was again extracted from each supernatant in the

pool and analyzed individually. RT-PCR was conducted using the QIAGEN OneStep RT-PCR kit (QIAGEN) to detect viral genes. Five universal primer sets were used for detection of flaviviruses (Kuno et al., 1998; Scaramozzino et al., 2001), alphaviruses (Eshoo et al., 2007), phleboviruses, and rhabdoviruses (Kuzmin et al., 2006) (Table 3-1). The RT-PCR condition for each primer sets were 50°C for 30 min (reverse transcription), 95°C for 15 min, followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min (amplification), and final extension at 72°C for 10 min. PCR products were separated by electrophoresis on 2% agarose gels, and intensive bands were purified by using the MinElute Gel Extraction Kit (QIAGEN). Purified PCR products were directly cycle-sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Kit v.3.1 (Thermo Fisher Scientific, Waltham, MA, USA) and ABI PRISM 3130 Genetic Analyzer (Thermo Fisher Scientific). Minimum infection rate (MIR) was calculated as the ratio of the number of positive pools to the total number of mosquitoes tested. For samples from cells that showed clear CPEs but yielded negative RT-PCR results for viral genes, high-throughput analyses were applied to identify viral genomes as described below.

3.3.5. High-throughput analyses

Three selected culture supernatants of C6/36 cells showing different forms of CPEs (pool nos. 2, 34, and 69) were subjected to next-generation sequencing (NGS) analyses (Fig. 3-6). Approximately 50 mL of each culture supernatant were concentrated to approximately 0.5 mL by using Amicon Ultra 50kDa (Merck Millipore, Billerica, MA, USA). Concentrated supernatants were ultra-centrifuged at 60,000 × *g* for 3 h at 4°C through a 25% sucrose cushion. Then, supernatants were carefully removed and pellets were resuspended in 200 μL of sterile PBS on ice. Total RNAs were extracted from samples by using the QIAamp Viral RNA Mini Kit (QIAGEN) according to the manufacturer's instructions. I applied three methods for NGS

analyses: Ion Torrent PGM system (Life Technologies), Illumina MiSeq system (Illumina, San Diego, CA, USA), and MinION nanopore sequencing device (Oxford Nanopore Technologies, Oxford, England, UK).

For the Ion Torrent PGM analysis, I used RNA obtained from the culture supernatant of C6/36 cells inoculated with mosquito pool no. 2 (*Ae. albopictus* collected in Bogor, 2016) as previously described (Itokawa et al., 2015; Nakayama et al., 2019). Briefly, RNA was reverse-transcribed into cDNA, which was then amplified with the SeqPlex RNA Amplification Kit (Sigma-Aldrich). The product was end-repaired and ligated with a barcoded adapter using the Ion Xpress Barcode Adapter Kit (Life Technologies) by T4 ligase (New England Biolabs, Ipswich, MA, USA). Barcoded fragments were subjected to emulsion PCR using the Ion OneTouch 2 system with the Ion PGM Template OT2 200 Kit (Life Technologies). Amplified library beads were subjected to the Ion Torrent PGM sequencer (Life Technologies) with the Ion 318 Chip (Life Technologies). Reads obtained by the Ion Torrent PGM were assembled with CLC Genomics Workbench 7.0 software (CLC bio/QIAGEN, Aarhus, Denmark). BWA-MEM (Li and Durbin, 2009) was used to map the NGS reads on reference of the 12 genome segments (NC_004198, NC_004200, NC_004201-4, NC_004211, NC_004217-21). SAMtools (Li et al., 2009) was used to calculate coverage.

For the Illumina MiSeq analysis, I used RNA obtained from culture supernatant of C6/36 cells inoculated with mosquito pool no. 34 (*Ae. albopictus* collected in Bogor, 2017). The DNA library was prepared by using the NEB Next Ultra RNA Library Prep Kit for Illumina (New England Biolabs). Adaptor ligation was conducted by TruSeq 96 dual-index adapters (Illumina) instead of adapters supplied in the kit. Sequencing of paired ends was performed using MiSeq Reagent Kit v2 (300 cycles) (Illumina). Reads were assembled and large contigs were constructed with CLC Genomics Workbench 7.0 software (CLC Bio/QIAGEN).

For nanopore MinION sequencing, I used RNA obtained from the culture supernatant of C6/36 cells inoculated with mosquito pool no. 69 (*Cx. tritaeniorhynchus* collected in Bogor, 2017). RNA was reverse-transcribed into cDNA, which was amplified with the SeqPlex RNA Amplification Kit (Sigma-Aldrich). After purification using the QIAquick PCR Purification Kit (QIAGEN), synthesized DNAs were directly sequenced by MinION (Oxford Nanopore Technologies) with the Rapid Sequencing Kit (Oxford Nanopore Technologies) according to the manufacturer's instructions. Reads obtained by MinION were subjected to homology search (BLASTX analysis) using the National Center for Biotechnology Information (NCBI) database.

3.3.6. Phylogenetic analyses

To analyze phylogenetic relationships of isolated viruses, available sequences of related virus species or strains were obtained from the NCBI database. Analysis of the aligned matrix data and the construction of phylogenetic trees were performed using MEGA7 (Kumar et al., 2016) under maximum likelihood algorithms. The best-fit model of nucleotide sequences was found by the model selection analysis by jModelTest 2 (Darriba et al., 2012). The statistical significance of resulting trees was evaluated by a bootstrap test with 100 replications (Efron et al., 1996). Sequence data obtained in this study were deposited in the DNA Data Base of Japan under accession numbers LC536082–LC536146.

3.4. Results

3.4.1. Mosquito collections

A total of 10,015 mosquitoes comprising at least 11 species of 4 genera were collected at several sites in Indonesia in this study (Table 3-2). The major collected mosquito species were *Cx. quinquefasciatus* (46.4%), *Ae. albopictus* (20.4%), *Cx. tritaeniorhynchus* (15.8%),

Ae. aegypti (10.0%), and *Ar. subalbatus* (3.6%). Other minor species, such as *Cx. hutchinsoni*, *Cx. gelidus*, *Cx. vishnui*, *Cx. fuscocephala*, *An. vagus*, and *An. barbirostris*, were also collected (Table 3- 2).

I compared the species abundance of collected mosquitoes between residential areas and animal houses (Table 3-2). Although female mosquitoes of *Ae. aegypti* and *Ae. albopictus* were collected in some animal houses, they were mainly captured in residential areas, whereas male mosquitoes of *Ae. aegypti* and *Ae. albopictus* were captured only in residential areas. For *Ar. subalbatus*, only female mosquitoes were collected in this study, and they also tended to be primarily captured in residential areas than in animal houses. *Cx. quinquefasciatus* is one of the common mosquito species in Asian countries, and female mosquitoes of this species were captured in both residential areas and animal houses. By contrast, male mosquitos of *Cx. quinquefasciatus* were primarily captured in residential areas compared with animal houses. More than 1,500 *Cx. tritaeniorhynchus* mosquitoes, known as the main vector for JEV, were collected in this study, and approximately 80% of them were collected in one event (Table 3-2; Bogor, July 2018). Furthermore, female mosquitoes were collected at about 2-fold higher rates than male mosquitoes. Other mosquito species, except for *An. vagus*, were collected only in residential areas.

3.4.2. Virus isolation

The collected mosquitoes were divided into 285 pools and used for virus isolation (Table 3-3). Seventy-two pools showed clear CPEs in C6/36 cells (25.3%), but not in mammalian Vero and BHK-21 cells. The minimum infection rate (MIR) in C6/36 cells was 0.72 (Table 3-3). The main manifestation of CPEs in C6/36 cells was cell degeneration, which ranged from swelling, shrinking, and rounded cells to clustering, syncytium formation, and destruction of monolayer cells (Fig. 3-6, represented by pool nos. 2 and 34). Manifestations of

CPEs included slower growth rate and increasing gaps of cells. Some CPEs in C6/36 cells formed grape-like clusters 4–6 days postinfection or cells were smaller 3–6 days postinfection (Fig. 3-6, represented by pool no. 69).

3.4.3. Detection of viral genome

RNA was extracted from 72 culture supernatants that showed CPEs in C6/36 cells individually, and the other 3–4 supernatants (213 pools, without CPEs) were mixed into one tube for RNA extraction (total of 132 pools, 72 plus 60 samples for C6/36). Because culture supernatants of Vero and BHK-21 cells did not show any CPEs in this study, 5–8 supernatants were mixed in one tube for RNA extraction (40 samples for Vero and 43 samples for BHK-21). These RNAs (total of 215 RNA samples) were analyzed by RT-PCR using five universal primer sets to detect viruses (Table 3-1). Only eight samples yielded positive results, which were shown only for the flavivirus primer set; all other samples were negative for all RT-PCR analyses (Table 3-3). Among the eight flavivirus-positive samples, seven samples were obtained from samples that showed CPEs in C6/36 cells and the other sample was obtained from the mixed Ae. albopictus pool that did not show clear CPEs (Table 3-3). RNA was then extracted from individual supernatants in the mixed pool and RT-PCR was repeated to determine the virus-positive pool.

Sequencing of these eight RT-PCR products revealed that they showed high homology with sequences of insect-specific flaviviruses in the NCBI database. Six *Ae. aegypti* (both male and female) showed 92.4%–98.0% nucleotide identity to that of CFAV strain Culebra (GenBank accession no. AH015271.2) (Cook et al., 2006), one *Ae. albopictus* (female) had 92.4% nucleotide identity to that of AEFV strain 21TN (GenBank accession no. KM871198.1) (Grisenti et al., 2015), and one *Cx. quinquefasciatus* (female) demonstrated 96.2% nucleotide identity to that of CxFV strain Surabaya-2 (GenBank accession no. AB639348.1) (Hoshino et

al., 2012). Among these eight RT-PCR-positive samples, CFAV and CxFV showed CPEs in C6/36 cells, but AEFV did not. All insect-specific flaviviruses were obtained from mosquito pools collected in Bogor, West Java province (Fig. 3-1 and Table 3-3).

Only 7 of 72 samples were determined as insect flaviviruses (CFAV and CxFV), and the other 65 CPE-positive samples were not, by RT-PCR analysis with virus-specific primers. To identify CPE-causative agents that were negative for RT-PCR using consensus primers, I utilized the NGS approach.

3.4.4. Detection and isolation of Banna virus

First, I selected one pool (pool no. 2 from *Ae. albopictus* collected in Bogor, 2016) that showed CPEs in C6/36 cells (Fig. 3-6) for NGS using Ion Torrent (Thermo Fisher Scientific), which demonstrated that this sample contained Banna virus (BAV) belonging to the family *Reoviridae* in the genus *Seadornavirus*. BAV has 12 segments of double-stranded viral RNA, and our NGS result detected all 12 segments of BAV, showing 94.3%–98.3% nucleotide identity to BAV genome (Attoui et al., 2000) (Fig. 3-7).

I next designed BAV-specific primer set, BANV-F and BANV-R (Table 3-1), for detection of BAV VP1 gene (Fig. 3-7) and performed RT-PCR. RNA samples from Vero (40 pools), BHK-21 (43 pools), and C6/36 (65 pools with CPEs) cells for BAV were analyzed. Eight samples that showed CPEs in C6/36 cells were positive, but no samples in Vero and BHK-21 cells were positive. BAV was detected from the pools of female *Ae. albopictus* (4 pools), *Ae. aegypti* (2 pools), and *Cx. tritaeniorhynchus* (2 pools) mosquitoes, all of which were captured in Bogor, West Java province (site no. 4 in Fig. 3-1 and Table 3-3).

3.4.5. Detection and isolation of permutotetravirus

Next, I selected the pool that showed CPEs in C6/36 cells (pool no. 34 from *Ae. albopictus* collected in Bogor, 2017) (Fig. 3-6) and was negative by RT-PCR including BAV-specific primers for NGS by MiSeq (Illumina). The sample contained viral RNAs with high homology to Sarawak virus (SWKV) (Sadeghi et al., 2017), unassigned member of tetravirus. SWKV possesses approximately 5.3 kb of single-stranded positive-sense RNA genome containing two large open reading frames (ORFs (Sadeghi et al., 2017). I detected nearly complete genome sequences (5319 nt, without RACE analysis), showing 88.9% nucleotide identity to that of SWKV strain SWK-M26 (GenBank accession no. NC_040540.1, Fig. 3-8). I tentatively named this isolate Bogor virus (BGV) strain BGV/MQ/5/Bogor/2017.

I designed the BGV-specific primer set, BGV-F and BGV-R (Table 3-1), and performed RT-PCR for detection of BGV (Fig. 3-8). Because members of the family *Permutotetraviridae* are known ISVs (Dorrington et al., 2019), supernatant from C6/36 cells (65 pools that showed CPEs) were analyzed by RT-PCR. Thirty-four samples were positive for BGV, which were obtained from the pools of *Ae. aegypti* (5 pools of female), *Ae. albopictus* (7 pools of female and 9 pools of male), *Ar. subalbatus* (6 pools of female), *Cx. quinquefasciatus* (6 pools of female), and *Cx. fuscocephala* (1 pool of female) mosquitoes collected in West Java (Bogor), Banten, and Central Kalimantan provinces (site nos. 2, 4, and 7 in Fig. 3-1 and Table 3-3).

3.4.6. Detection and isolation of alphamesoniviruses

Lastly, I analyzed one pool that showed CPE in C6/36 cells (pool no. 69 from *Cx. tritaeniorhynchus* collected in Bogor, 2017) (Fig. 3-6) and was negative by RT-PCR for flaviviruses, BAV, and BGV, by using MinION. I found that 4 of 69 reads obtained by MinION showed high homology to Dak Nong virus (Kuwata et al., 2013) (one strain of the viral species

Alphamesonivirus 3, genus *Alphamesonivirus*, family *Mesoniviridae*; GenBank accession no. NC_038297.1) (Fig. 3-9).

I designed alphamesonivirus-specific primers, AMSV-F and AMSV-R (Table 3-1), and performed RT-PCR for detection of viral genome (Fig. 3-9). Because viruses of the family *Mesoniviridae* are also known to infect only insect cells (Vasilakis et al., 2014), RNA samples from C6/36 cells (65 pools with CPEs) were analyzed. Twenty-three samples were positive and originated from *Ae. albopictus* (2 pools of female), *Ar. subalbatus* (1 pool of female), *Cx. quinquefasciatus* (2 pools of female and 1 pool of male), and *Cx. tritaeniorhynchus* (17 pools of female) mosquitoes in West Java and Banten provinces (site nos. 1, 2, and 4 in Fig. 3-1 and Table 3-3). Notably, flaviviruses, BAV, BGV, and alphamesoniviruses were not detected from the same samples.

3.4.7. Phylogenetic analyses

I successfully isolated 8 insect flaviviruses, 8 BAV, 34 BGV, and 23 alphamesoniviruses from mosquitoes in Indonesia (Table 3-3). Next, I analyzed phylogenetic relationships of each viral isolate with related viruses.

To date, more than 25 species of insect flaviviruses have been reported globally (Blitvich and Firth, 2015; Bolling et al., 2015b). I conducted phylogenetic analysis of our eight insect flaviviruses to infer the relationship with the reported insect flaviviruses based on partial NS5 gene sequences (203 nt). As mentioned above, these eight isolates were classified into three species of insect flaviviruses: six CFAV, one AEFV, and one CxFV strain. The phylogenetic tree showed that our isolated flaviviruses formed clusters with each viral species (Fig. 3-2). Among our six CFAV strains, there were only two synonymous substitutions in the 203 bases, and they formed one subcluster differing from another CFAV from Thailand, Australia, USA, Puerto Rico, and Mexico (Fig. 3-2). AEFV has been reported from Japan,

Italy, USA, Thailand (Blitvich and Firth, 2015), Turkey (Oncu et al., 2018) and China (Fang et al., 2018). The result of phylogenetic analysis indicated that our Indonesian AEFV strain AEFV/MQ/29/Bogor/2017 was located as a root of the other AEFV strains (Fig. 3-2). CxFV was first isolated from Japan (Hoshino et al., 2009) and has been reported from many countries, including China, Taiwan, Indonesia, Guatemala, USA, Mexico, Trinidad, Uganda, Argentina, and Brazil, indicating that CxFV is ubiquitous in the world (Blitvich and Firth, 2015). The phylogenetic analysis indicated that our CxFV strain CxFV/MQ/174/Bogor/2017 was most closely related to CxFV strain Surabaya-2 isolated in 2004 from mosquitoes in Java, Indonesia (Hoshino et al., 2012) (Fig. 3-2).

Because AEFV was first isolated in Indonesia, I further analyzed complete genome sequences of AEFV/MQ/29/Bogor/2017 by NGS using MiSeq (Illumina) in the same method for BGV/MQ/5/Bogor/2017 genome. I successfully determined almost complete genome sequences for AEFV/MQ/29/Bogor/2017 (11,072 nt, without RACE analysis) (GenBank accession no. LC536088). To date, four AEFV strains from Japan (strain Narita-21; NC_012932.1) (Hoshino et al., 2009), Thailand (strain Bangkok; KJ741266.1) (Bolling et al., 2015a), Turkey (strain KRD32; MK251047.1) (Oncu et al., 2018), and USA (strain SPFLD-MO-2011-MP6; KC181923.1) (Haddow et al., 2013) have been deposited in the NCBI database with full or nearly-full genome sequences (as of December 2019). Nucleotide sequence identity for the polyprotein region (10,026 nt) between AEFV/MQ/29/Bogor/2017 and the other AEFV strains ranged from 89.5% with strain Narita-21 to 88.2% with strain Bangkok. I conducted phylogenetic analysis of the five AEFV strains based on nucleotide sequences encoding the polyprotein region, which indicated that the five AEFV strains divided into three subbranches: Indonesian strain, Thailand strain, and the other strains (Fig.3-10).

BAV was first isolated from the cerebrospinal fluid of encephalitis patients in China (Xu et al., 1990). Subsequently, the virus has been isolated from mosquito specimens collected

in Indonesia (Brown et al., 1993), Vietnam (Nabeshima et al., 2008), and several provinces of China (Liu et al., 2010) and also *Culicoides* midge in China (Liu et al., 2016; Song et al., 2017). In this study, I determined the sequence of RT-PCR products for eight BAV isolates and then analyzed phylogenetic relationships with other BAV strains based on the partial VP1 gene (736 nt, Fig. 3-7). BAV were divided into some subclusters, and all our eight BAV strains belonged to the same cluster with Indonesian BAV isolated during 1980–1981 (Fig. 3-3). There was no nucleotide substitution among the eight BAV isolates obtained in this study.

On the website of the International Committee on Taxonomy of Viruses (ICTV; https://talk.ictvonline.org/), two viral species, Euprosterna elaeasa virus and Thosea asigna virus, are members of the genus *Alphapermutotetravirus* in the family *Permutotetraviridae* (assigned one genus for this family) as lepidopteran insect viruses. In addition, several related viruses, such as Newfield virus (Webster et al., 2015), SWKV (Sadeghi et al., 2017), Shinobi tetravirus (SHTV) (Fujita et al., 2018), and Egaro virus (no article available, GenBank accession no. NC_030845.1), have been reported from dipteran specimens recently. In this study, I selected 20 BGV isolates from 34 RT-PCR samples positive for BGV and determined their sequences (393 nt, Fig. 3-8). The analysis revealed 23 substitutions causing two amino acid differences. Our isolates showed 87.2%–88.5% nt and 95.4%–96.1% aa sequence identity to that of SWKV strain SWK-MS26 (NC_040540.1), and 84.2%–85.4% nt and 93.1%–93.8% aa sequence identity to that of SHTV strain Shinobi (LC270813.1). Phylogenetic analysis for permutotetraviruses based on the 131 aa sequences of the putative RdRp region indicated that our 20 BGV isolates formed one cluster with SWKV and STHV (Fig. 3-4).

On the ICTV website, members of the family *Mesoniviridae* (order *Nidovirales*, suborder *Mesonidovirineae*) currently consist of nine species (*Alphamesonivirus 1–9*). I carried out sequence analysis of 23 RT-PCR products by using the AMSV-specific primer set. I found that 7 of 23 RT-PCR products were overlapped sequences, possibly due to superinfection of 2

or more alphamesoniviruses. Therefore, I used 16 mesonivirus sequences for phylogenetic analysis (336 nt, Fig. 3-5). The result revealed that they were divided into 2 alphamesonivirus species, 14 isolates of *Alphamesonivirus 2* and 2 isolates of *Alphamesonivirus 3* (Fig. 3-5). *Alphamesonivirus 2* was obtained from pools of *Ae. albopictus*, *Ar. subalbatus*, *Cx. quinquefasciatus*, and *Cx. tritaeniorhynchus* mosquitoes, and *Alphamesonivirus 3* was obtained from pools of *Cx. tritaeniorhynchus* mosquitoes (Table 3-3). There were no substitutions among the 14 isolates of *Alphamesonivirus 2* and 2 isolates of *Alphamesonivirus 3*. I concluded that at least two alphamesonivirus species (*Alphamesonivirus 2* and *Alphamesonivirus 3*), including the possibility for superinfection with another alphamesonivirus species, were distributed in the wild mosquito population in Indonesia.

3.5. Discussion

In this study, I conducted surveillance of mosquito-borne viruses in four islands of Indonesia, Sumatra, Java, Bali, and Borneo, during 2016–2018. A total of 10,015 mosquitoes were collected during the study and used for virus isolation, resulting in isolation of many mosquito-borne viruses belonging to families *Flaviviridae* (three insect flaviviruses: CFAV, AEFV, and CxFV), *Reoviridae* (BAV), *Permutotetraviridae* (tentatively named Bogor virus), and *Mesoniviridae* (*Alphamesoniviruses 2* and *3*). These findings are valuable for quick identification and diagnosis of viruses in captured mosquitoes in Indonesia and contribute to the understanding of the biodiversity of mosquito-virus relationships and virus taxonomy in tropical regions.

Many viruses in this study were predominantly ISVs except for BAV, and no arboviruses, such as JEV and DENV, were isolated. Previous studies reported that DENV genomes were detected by RT-PCR in 0.12% (36/29,252) (Rahayu et al., 2019) and 0.66% (110/16,605) (Mulyatno et al., 2018) in mosquito population. In a DENV-endemic area in Brazil, the MIR

of DENV by RT-PCR in wild *Aedes* mosquitoes was 19.8 (Medeiros et al., 2018). In this study, I collected approximately 3,000 *Aedes* mosquitoes (both male and female of *Ae. aegypti* and *Ae. albopictus*) (Table 3-2) and used them for virus isolation, but not for RT-PCR analysis. Therefore, the number of our collected *Aedes* mosquitoes may be insufficient for detection of *Aedes*-borne arboviruses by virus isolation. Survey of JEV in *Culex* mosquitoes revealed that the MIR of JEV in Indonesia was 0.06 (1/1,485) (Garjito et al., 2019). Recently in China, 10 of 511 pools of *Culex* mosquitoes were positive by RT-PCR for detection of JEV genome, and the MIR of JEV was estimated at 0.91 (Fang et al., 2019). Thenmozhi *et al*. reported that JEV-antigen was detected in 4 of 580 mosquitoes in India by ELISA and the MIR was estimated at 6.9 (Thenmozhi et al., 2013). Because of the dominant religion in Indonesia, rearing of domestic pigs has been restricted except for in some provinces such as Bali (Im et al., 2018). In this study, I collected 1,030 adult female *Cx. tritaeniorhynchus* mosquitoes, known as the main vector for JEV, mainly in Bogor, West Java province (Table 3-2), where domestic pigs are rare (Ministry of Health 2018). Therefore, the number of collected *Cx. tritaeniorhynchus* mosquitoes may not be adequate for isolation of JEV from mosquitoes. I should improve the method for efficient collection of arbovirus-infected mosquitoes for further entomological surveillance and risk assessment of arboviral diseases.

Three insect flaviviruses, CFAV, AEFV, and CxFV, were isolated from *Ae. aegypti, Ae. albopictus*, and *Cx. quinquefasciatus*, respectively (Fig. 3-2 and Table 3-3). Although CFAV and CxFV have been reported from Surabaya city, Java, in Indonesia (Hoshino et al., 2007; Hoshino et al., 2009), the isolation of AEFV is the first report in Indonesia. In the phylogenetic analysis, our CxFV isolate CxFV/MQ/174/Bogor/2017 formed a subcluster with CxFV strain Surabaya-2 (Fig. 3-2). Partial genome sequences encoding NS5 gene of Indonesian CFAV strain Surabaya-10 (Hoshino et al., 2009) was deposited in GenBank (accession no. AB488428.1) and showed overlap of only 91 bp with sequences of our CFAV

strain. Additionally, the highest homology between Surabaya-10 and our CFAV strain was observed in this 91-bp region (data not shown). These results implied that insect flaviviruses have been maintained and circulate in Java, Indonesia.

BAVs were isolated from several species of female mosquitoes, *Ae. aegypti*, *Ae. albopictus*, and *Cx. tritaeniorhynchus* (Table 3-3). A previous study reported that BAV was also isolated from *Cx. pseudovishnui*, *Ae. vagus*, and *Cx. pipiens* at several sites in Indonesia during 1980–1981 (Brown et al., 1993). Other mosquito species, such as *Anopheles sinensis* and *Ae. vexans*, and biting midge (*Culicoides* sp.) have been also reported as potential vectors for BAV in China (Liu et al., 2010; Song et al., 2017; Xia et al., 2018). BAV was first isolated from cerebral fluid of human patients with encephalitis (Xu et al., 1990), and several seroepidemiological studies on BAV infection in human and animals have suggested the potential of BAV to cause arboviral diseases (Song et al., 2017). These findings suggested that BAV has been maintained among several mosquito species in various Asian countries and probably circulate between mosquitoes and vertebrate hosts in nature (Liu et al., 2016). However, I did not detect any BAV isolates from mosquito pools inoculated on both mammalian Vero and BHK-21 cell lines. Further investigation of transmission dynamics and pathogenicity of BAV strains in vertebrates is required.

On ICTV taxonomy, the viral family *Permutotetraviridae* currently consists of one genus *Alphapermutotetravirus* and two species, Euprosterna elaeasa virus and Thosea asigna virus, are assigned. They are known as lepidopteran insect viruses. In 2010, the viral genome of permutotetra virus-like virus, named Newfield virus, was discovered from wild-collected *Drosophila* flies (Webster et al., 2015). In 2017, SWKV was isolated from a pool of male *Ae. albopictus* mosquitoes in Sarawak State (northwest Borneo) of Malaysia as a new member of tetravirus (Sadeghi et al., 2017). Our colleagues reported the isolation of SHTV strain Shinobi from the *Ae. albopictus* mosquito cell line, JCRB, in 2018 as a new member of the family

Permutotetraviridae (Fujita et al., 2018). Overall, three new permutotetraviruses have been reported as a new group of probable dipteran insect tetraviruses. In this study, I successfully determined the almost full genome sequences of BGV strain BGV/MQ/34/Bogor/2017 (5319 nt without terminal regions), which contains two putative ORFs (Fig. 3-8, GenBank accession no. LC536112). The putative amino acid sequences of BGV ORFs showed high homologies to those of SWKV strain SWK-M26 (GenBank accession no. NC_040540.1; 96.5% for ORF1 and 91.3% for ORF2) and SHTV strain Shinobi (GenBank accession no. YP_009553485.1; 94.8% for ORF1 and 88.5% for ORF2).

I obtained 34 BGV isolates from various mosquito species, *Ae. aegypti*, *Ae. albopictus*, *Ar. subalbatus*, *Cx. quinquefasciatus*, and *Cx. fuscocephala*, at several sites in Indonesia (Java and Borneo), suggesting that this virus may be widely distributed in tropical areas and mosquito populations. However, it is unknown whether BGV can transmit among different mosquito species.

Twenty-three alphamesoniviruses were isolated from various mosquito species (Table 3-3), and phylogenetic analysis of these isolates revealed that they were classified as at least two viral species, *Alphamesonivirus 2* and *Alphamesonivirus 3* (Fig. 3-5). *Alphamesonivirus 2,* alias Karang Sari virus, was isolated from specimens of a pool of *Cx. vishnui* mosquitoes in 1981 at Karang Sari, Banten province, Indonesia (Vasilakis et al., 2014). I isolated *Alphamesonivirus 2* from mosquito pools of *Ae. albopictus*, *Ar. subalbatus*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, in Java in 2017. By contrast, *Alphamesonivirus 3,* alias Dak Nong virus, was discovered from a pool of *Cx. tritaeniorhynchus* mosquitoes in Vietnam (Kuwata et al., 2013). Another related strain of *Alphamesonivirus 3*, Kamphaeng Phet virus, was found in Thailand, but no information on mosquitoes was available (Vasilakis et al., 2014). In this study, I obtained *Alphamesonivirus 3* only from pools of *Cx. tritaeniorhynchus* mosquitoes in Java, suggesting that alphamesoniviruses might possess host-specificity to

mosquito species. I found the mixed growth of two or more alphamesonivirus species in the same C6/36 cultures (7 samples), although mixed growth has not been observed among flaviviruses, BAV, and BGV. I hypothesize that no competition occurred among alphamesoniviruses or genetically related viruses in C6/36 cells. These interactions among viruses in mosquitoes should be analyzed to clarify virus-vector interaction.

Mosquito-borne viruses are considered a significant cause of major health problems in Indonesia. Although I did not isolate human-related arboviruses in this study, I believed that this large surveillance of mosquitoes collections and virus isolation provides a more accurate view of the prevalence of arboviruses. Furthermore, the presence and distribution of ISVs in mosquito populations raise interesting issues about their possibility to interact with pathogenic viruses and to be the next emerging infectious pathogen. Monitoring and long-term surveillance of mosquito-borne viruses are critical to prevent and control emerging and re-emerging arboviral diseases in Indonesia.

3.6. Figure legends

Fig. 3-1 Mosquito collection sites in this study. Black points show Bengkulu province (1) in Sumatra; Banten (2), Jakarta (3), West Java (4), and Central Java (5) provinces in Java; Bali province (6) in Bali; and Central Kalimantan province (7) in Borneo.

Fig. 3-2 Phylogenetic tree of representative insect-specific flaviviruses based on the partial NS5 gene (203 nt) by the maximum likelihood algorithm. Each branch was assessed by the bootstrap method with 100 replicates under the generalized time reversible model. Sequence data for reference isolates were obtained from the NCBI database, and the country and year of isolation are provided in parentheses. Bold and underlined letters show isolates detected in this study. DDBJ accession numbers are shown in brackets.

Fig. 3-3 Phylogenetic tree of Banna viruses based on the partial VP1 gene (736 nt) by the maximum likelihood algorithm. Each branch was assessed by the bootstrap method with 100 replicates under the generalized time reversible model. Sequence data for reference isolates were obtained from the NCBI database, and the country and year of isolation are provided in parentheses. Bold and underlined letters show isolates detected in this study. DDBJ accession numbers are shown in brackets.

Fig. 3-4 Phylogenetic tree of Bogor viruses with the related permutotetraviruses based on putative amino acid sequences (131 aa) from partial ORF1 sequences by the maximum likelihood algorithm. Each branch was assessed by the bootstrap method with 100 replicates under the JTT model. Sequence data for reference isolates were obtained from the NCBI database, and the country and year of isolation are provided in parentheses. Bold and

underlined letters show isolates detected in this study. DDBJ accession numbers are shown in brackets.

Fig. 3-5 Phylogenetic tree for alphamesoniviruses based on the partial ORF1 gene (312 nt) by the maximum likelihood algorithm. Each branch was assessed by the bootstrap method with 100 replicates under the generalized time reversible model. Sequence data for reference isolates were obtained from the NCBI database, and the country and year of isolation are provided in parentheses. Bold and underlined letters show isolates detected in this study. DDBJ accession numbers are shown in brackets.

Fig. 3-6 Manifestation of the CPEs by the infection of each pools on mosquito cell line C6/36. These cells were inoculated with the previous supernatants of passage after 5 days post inoculations. Scale bars indicates 100 μm.

Fig. 3-7 Supplemental Figure 2 Schematic diagram of the genome organization of Banna virus (BAV). All segments of BAV (VP1-12) and their expected sizes are presented. Open boxes indicate BAV segments from references (BAV strain JKT-7043, GenBank accession no. AF134519.1), and the sequence fragments obtained in this study are shown as grey boxes. Nucleotide and amino acid identities of BAV segments to reference sequences were shown as Table.

Fig. 3-8 Supplemental Figure 3. Schematic diagram of the genome organization of Bogor virus (BGV). The black boxs indicate the reference sequence of Sawarak virus (SWKV, GenBank accession no. NC_040540.1), and the gray box indicates the sequences obtained in this study

(5319 nt, GenBank accession no. LC536112). Nucleotide identity between BGV and SWKV of the gray region were 88.9 %.

Fig. 3-9 Schematic diagram of the genome organization of *Alphamesonivirus 3* (Dak Nong virus, Genbank accession no. NC_038297.1) and the sequence fragments obtained by the MinION sequencing in this study as gray boxes. Four sequence fragments shown as gray boxes indicate the approximate positon on the *Alphamesonivirus* genome, and the fragment size and nucleotide identities were shown.

Fig. 3-10 Phylogenetic relationships among AEFV 5 strains based on the nucleotide sequences (10026 nt) cording polyprotein region. Bold and underlined letters show our isolates.

Fig. 3-1

Fig. 3-2.

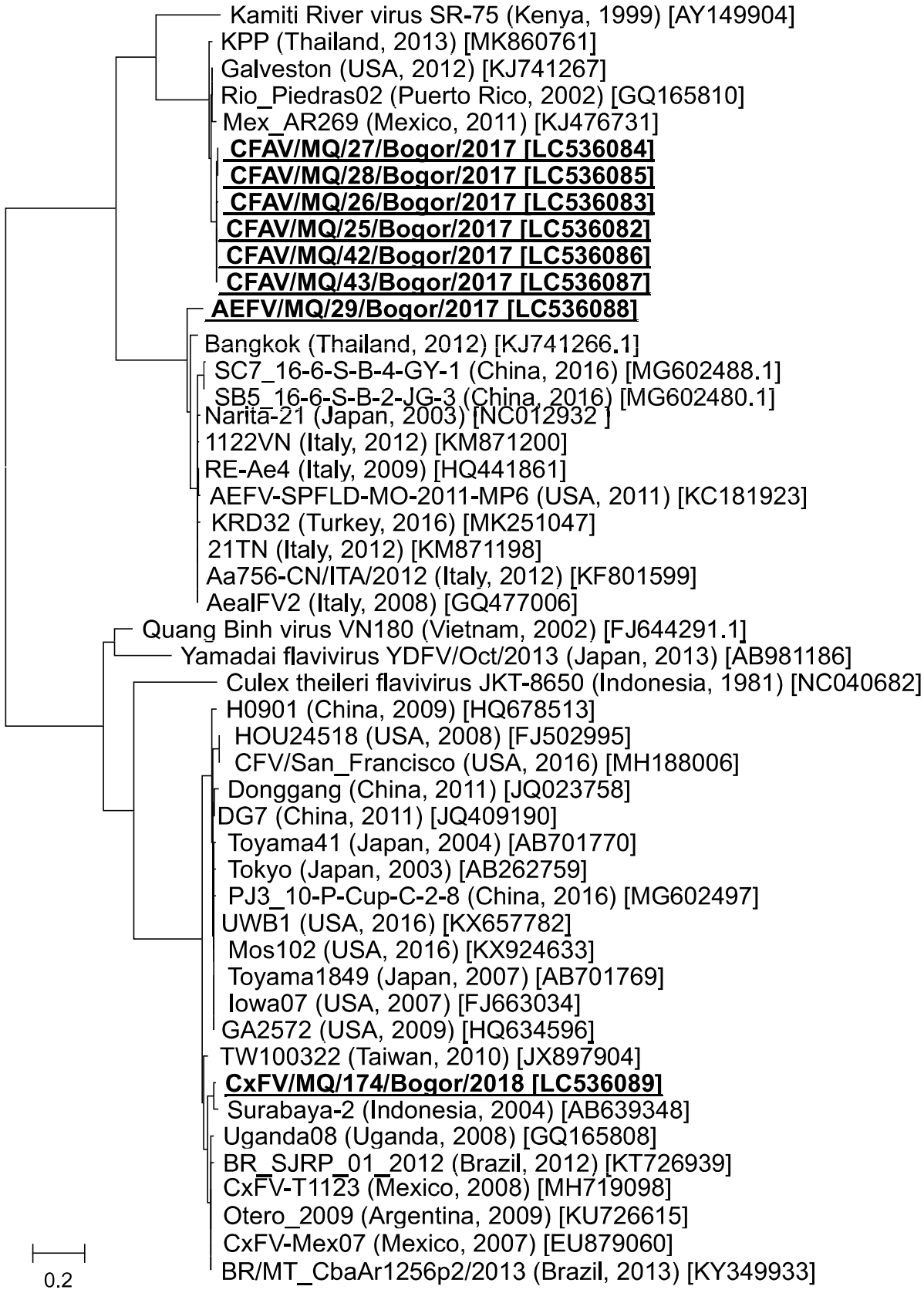
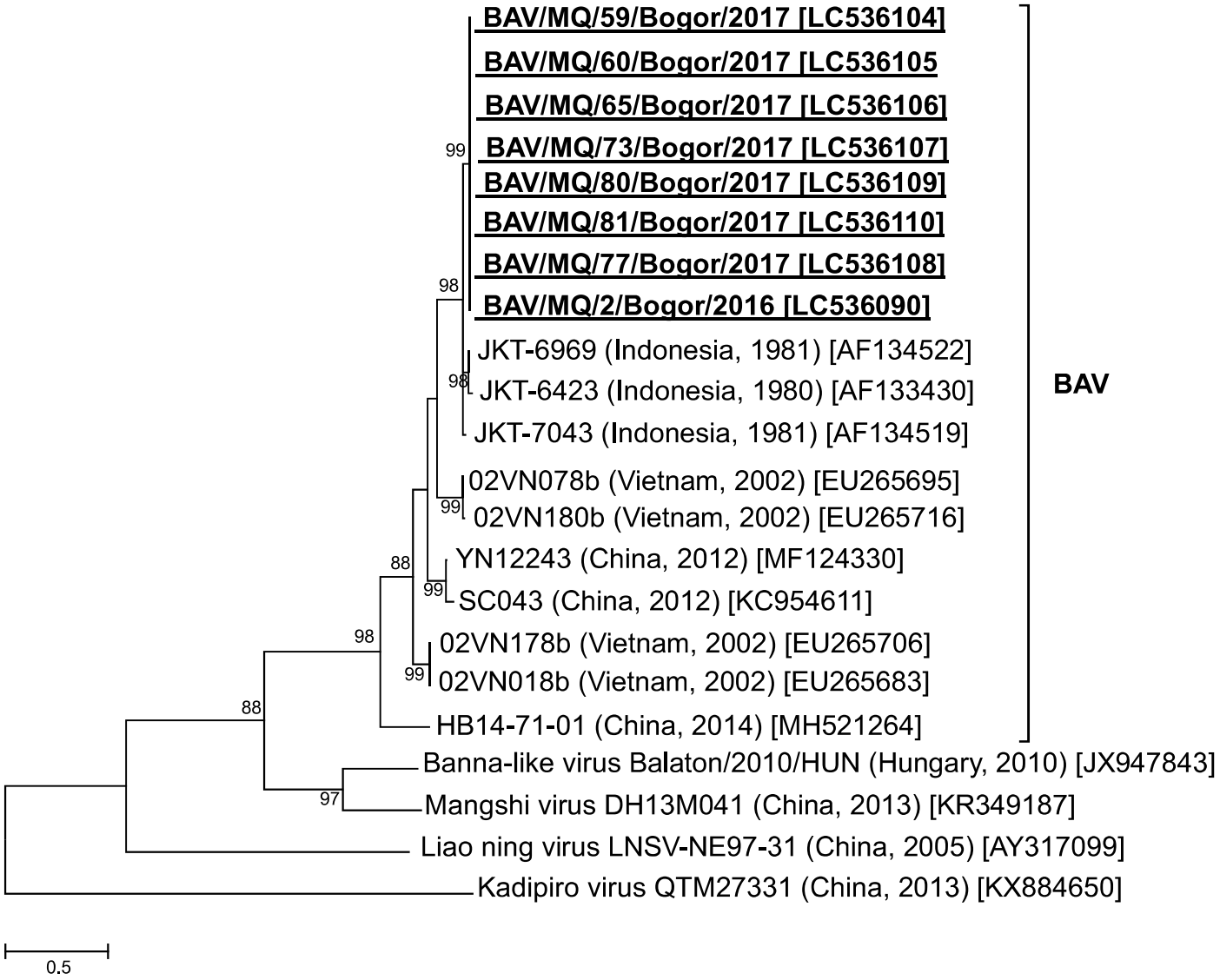

Kamiti River virus SR-75 (Kenya, 1999) [AY149904]
KPP (Thailand, 2013) [MK860761]
Galveston (USA, 2012) [KJ741267]
Rio_Piedras02 (Puerto Rico, 2002) [GQ165810]
Mex_AR269 (Mexico, 2011) [KJ476731]
CFAV/MQ/27/Bogor/2017 [LC536084]
CFAV/MQ/28/Bogor/2017 [LC536085]
CFAV/MQ/26/Bogor/2017 [LC536083]
CFAV/MQ/25/Bogor/2017 [LC536082]
CFAV/MQ/42/Bogor/2017 [LC536086]
CFAV/MQ/43/Bogor/2017 [LC536087]
AEFV/MQ/29/Bogor/2017 [LC536088]
Bangkok (Thailand, 2012) [KJ741266.1]
SC7_16-6-S-B-4-GY-1 (China, 2016) [MG602488.1]
SB5_16-6-S-B-2-JG-3 (China, 2016) [MG602480.1]
Narita-21 (Japan, 2003) [NC012932]
1122VN (Italy, 2012) [KM871200]
RE-Ae4 (Italy, 2009) [HQ441861]
AEFV-SPFLD-MO-2011-MP6 (USA, 2011) [KC181923]
KRD32 (Turkey, 2016) [MK251047]
21TN (Italy, 2012) [KM871198]
Aa756-CN/ITA/2012 (Italy, 2012) [KF801599]
AealFV2 (Italy, 2008) [GQ477006]
Quang Binh virus VN180 (Vietnam, 2002) [FJ644291.1]
Yamadai flavivirus YDFV/Oct/2013 (Japan, 2013) [AB981186]
Culex theileri flavivirus JKT-8650 (Indonesia, 1981) [NC040682]
H0901 (China, 2009) [HQ678513]
HOU24518 (USA, 2008) [FJ502995]
CFV/San_Francisco (USA, 2016) [MH188006]
Donggang (China, 2011) [JQ023758]
DG7 (China, 2011) [JQ409190]
Toyama41 (Japan, 2004) [AB701770]
Tokyo (Japan, 2003) [AB262759]
PJ3_10-P-Cup-C-2-8 (China, 2016) [MG602497]
UWB1 (USA, 2016) [KX657782]
Mos102 (USA, 2016) [KX924633]
Toyama1849 (Japan, 2007) [AB701769]
Iowa07 (USA, 2007) [FJ663034]
GA2572 (USA, 2009) [HQ634596]
TW100322 (Taiwan, 2010) [JX897904]
CxFV/MQ/174/Bogor/2018 [LC536089]
Surabaya-2 (Indonesia, 2004) [AB639348]
Uganda08 (Uganda, 2008) [GQ165808]
BR_SJRP_01_2012 (Brazil, 2012) [KT726939]
CxFV-T1123 (Mexico, 2008) [MH719098]
Otero_2009 (Argentina, 2009) [KU726615]
CxFV-Mex07 (Mexico, 2007) [EU879060]
BR/MT_CbaAr1256p2/2013 (Brazil, 2013) [KY349933]
0.2

Fig. 3-3.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BAV/MQ/60/Bogor/2017 [LC536105
BAV/MQ/65/Bogor/2017 [LC536106]
BAV/MQ/73/Bogor/2017 [LC536107]
BAV/MQ/80/Bogor/2017 [LC536109]
BAV/MQ/81/Bogor/2017 [LC536110]
BAV/MQ/77/Bogor/2017 [LC536108]
BAV/MQ/2/Bogor/2016 [LC536090]
JKT-6969 (Indonesia, 1981) [AF134522]
JKT-6423 (Indonesia, 1980) [AF133430]
JKT-7043 (Indonesia, 1981) [AF134519]
02VN078b (Vietnam, 2002) [EU265695]
02VN180b (Vietnam, 2002) [EU265716]
YN12243 (China, 2012) [MF124330]
SC043 (China, 2012) [KC954611]
02VN178b (Vietnam, 2002) [EU265706]
02VN018b (Vietnam, 2002) [EU265683]
HB14-71-01 (China, 2014) [MH521264]
BAV
Banna-like virus Balaton/2010/HUN (Hungary, 2010) [JX947843]
Mangshi virus DH13M041 (China, 2013) [KR349187]
Liao ning virus LNSV-NE97-31 (China, 2005) [AY317099]
Kadipiro virus QTM27331 (China, 2013) [KX884650]
99
98
98
99
88
99
98
99
88
97
0.5

Fig. 3-4.

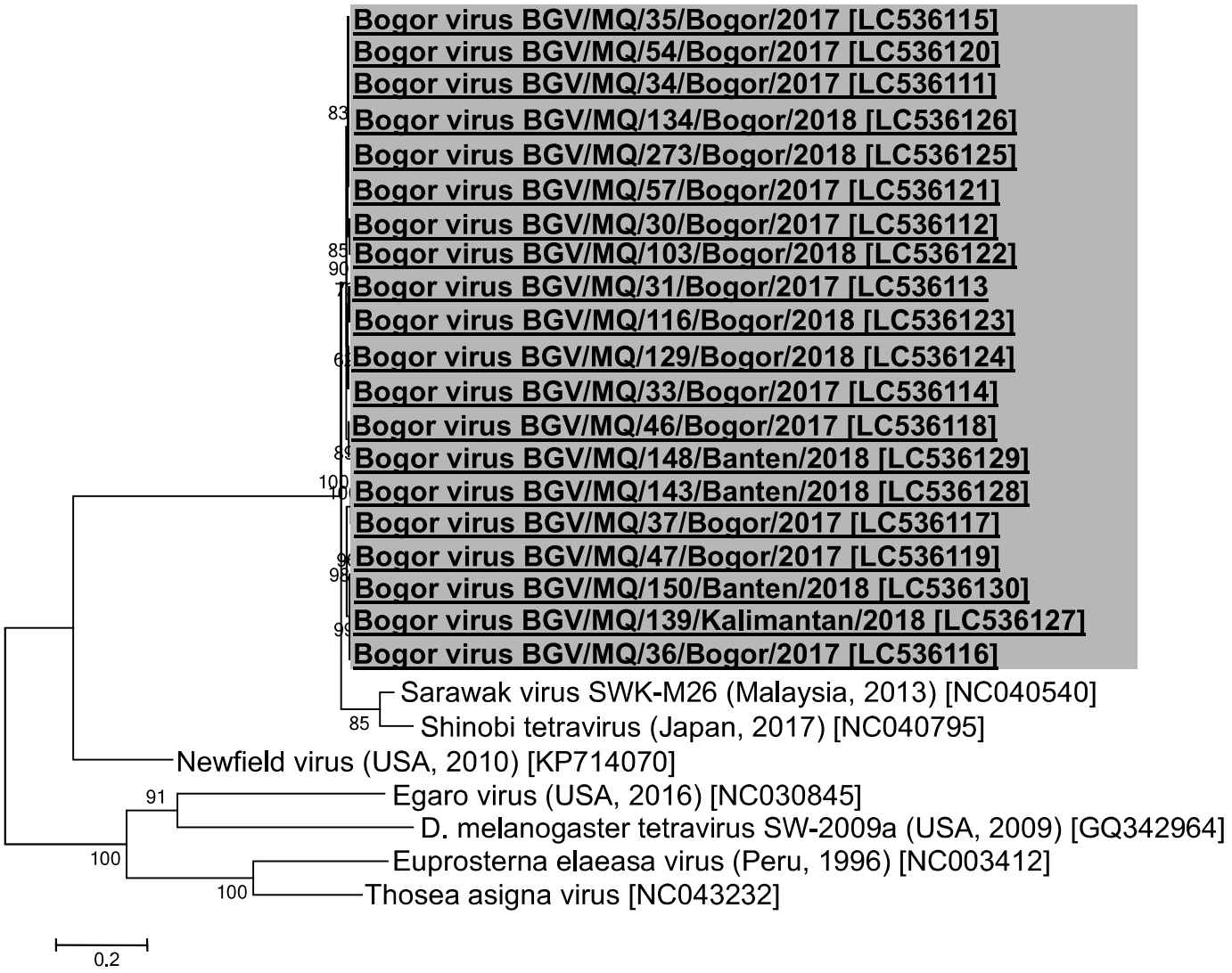
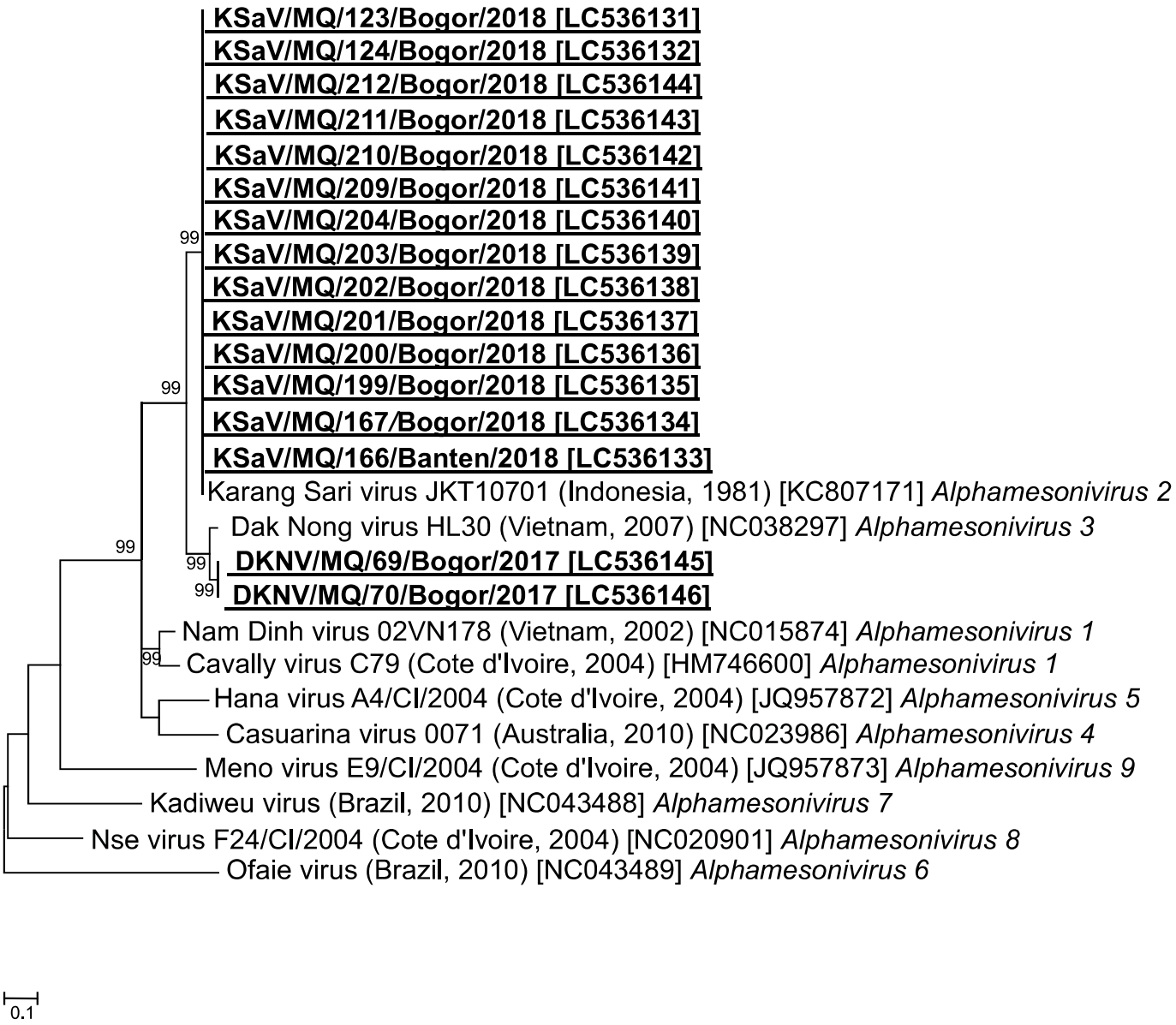

Bogor virus BGV/MQ/35/Bogor/2017 [LC536115]
Bogor virus BGV/MQ/54/Bogor/2017 [LC536120]
Bogor virus BGV/MQ/34/Bogor/2017 [LC536111]
Bogor virus BGV/MQ/134/Bogor/2018 [LC536126]
Bogor virus BGV/MQ/273/Bogor/2018 [LC536125]
Bogor virus BGV/MQ/57/Bogor/2017 [LC536121]
Bogor virus BGV/MQ/30/Bogor/2017 [LC536112]
Bogor virus BGV/MQ/103/Bogor/2018 [LC536122]
Bogor virus BGV/MQ/31/Bogor/2017 [LC536113
Bogor virus BGV/MQ/116/Bogor/2018 [LC536123]
Bogor virus BGV/MQ/129/Bogor/2018 [LC536124]
Bogor virus BGV/MQ/33/Bogor/2017 [LC536114]
Bogor virus BGV/MQ/46/Bogor/2017 [LC536118]
Bogor virus BGV/MQ/148/Banten/2018 [LC536129]
Bogor virus BGV/MQ/143/Banten/2018 [LC536128]
Bogor virus BGV/MQ/37/Bogor/2017 [LC536117]
Bogor virus BGV/MQ/47/Bogor/2017 [LC536119]
Bogor virus BGV/MQ/150/Banten/2018 [LC536130]
Bogor virus BGV/MQ/139/Kalimantan/2018 [LC536127]
Bogor virus BGV/MQ/36/Bogor/2017 [LC536116]
Sarawak virus SWK-M26 (Malaysia, 2013) [NC040540]
Shinobi tetravirus (Japan, 2017) [NC040795]
Newfield virus (USA, 2010) [KP714070]
Egaro virus (USA, 2016) [NC030845]
D. melanogaster tetravirus SW-2009a (USA, 2009) [GQ342964]
Euprosterna elaeasa virus (Peru, 1996) [NC003412]
Thosea asigna virus [NC043232]
0.2

Fig. 3-5.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KSaV/MQ/124/Bogor/2018 [LC536132]
KSaV/MQ/212/Bogor/2018 [LC536144]
KSaV/MQ/211/Bogor/2018 [LC536143]
KSaV/MQ/210/Bogor/2018 [LC536142]
KSaV/MQ/209/Bogor/2018 [LC536141]
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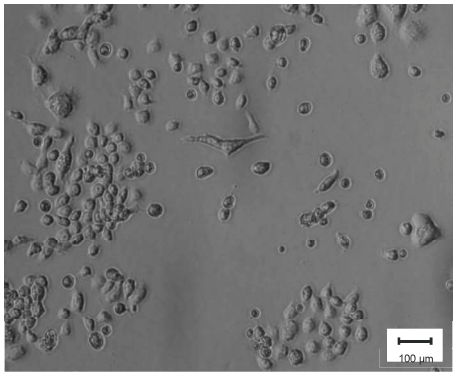
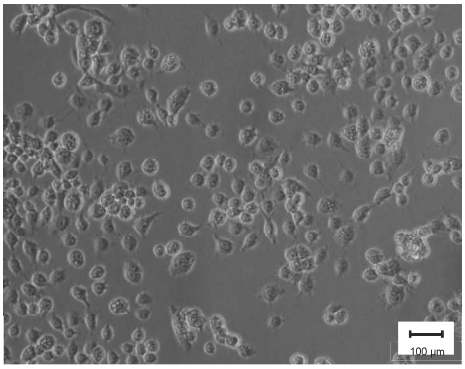
KSaV/MQ/201/Bogor/2018 [LC536137]
KSaV/MQ/200/Bogor/2018 [LC536136]
KSaV/MQ/199/Bogor/2018 [LC536135]
KSaV/MQ/167/Bogor/2018 [LC536134]
KSaV/MQ/166/Banten/2018 [LC536133]
Karang Sari virus JKT10701 (Indonesia, 1981) [KC807171] Alphamesonivirus 2
Dak Nong virus HL30 (Vietnam, 2007) [NC038297] Alphamesonivirus 3
DKNV/MQ/69/Bogor/2017 [LC536145]
DKNV/MQ/70/Bogor/2017 [LC536146]
Nam Dinh virus 02VN178 (Vietnam, 2002) [NC015874] Alphamesonivirus 1
Cavally virus C79 (Cote d'Ivoire, 2004) [HM746600] Alphamesonivirus 1
Hana virus A4/CI/2004 (Cote d'Ivoire, 2004) [JQ957872] Alphamesonivirus 5
Casuarina virus 0071 (Australia, 2010) [NC023986] Alphamesonivirus 4
Meno virus E9/CI/2004 (Cote d'Ivoire, 2004) [JQ957873] Alphamesonivirus 9
Kadiweu virus (Brazil, 2010) [NC043488] Alphamesonivirus 7
Nse virus F24/CI/2004 (Cote d'Ivoire, 2004) [NC020901] Alphamesonivirus 8
Ofaie virus (Brazil, 2010) [NC043489] Alphamesonivirus 6
99
99
99
99
99
99
0.1

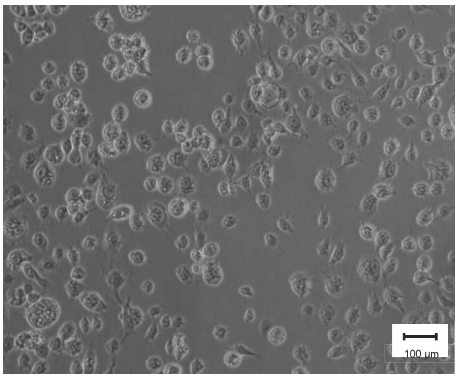
Fig. 3-6



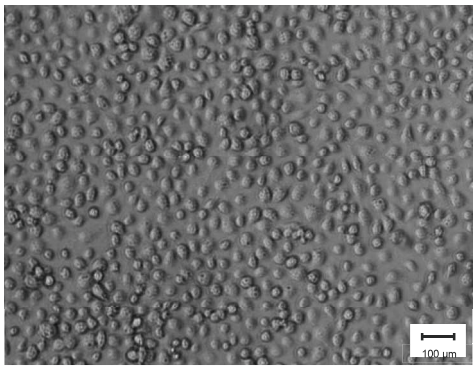
Pool no. 2



Pool no. 34

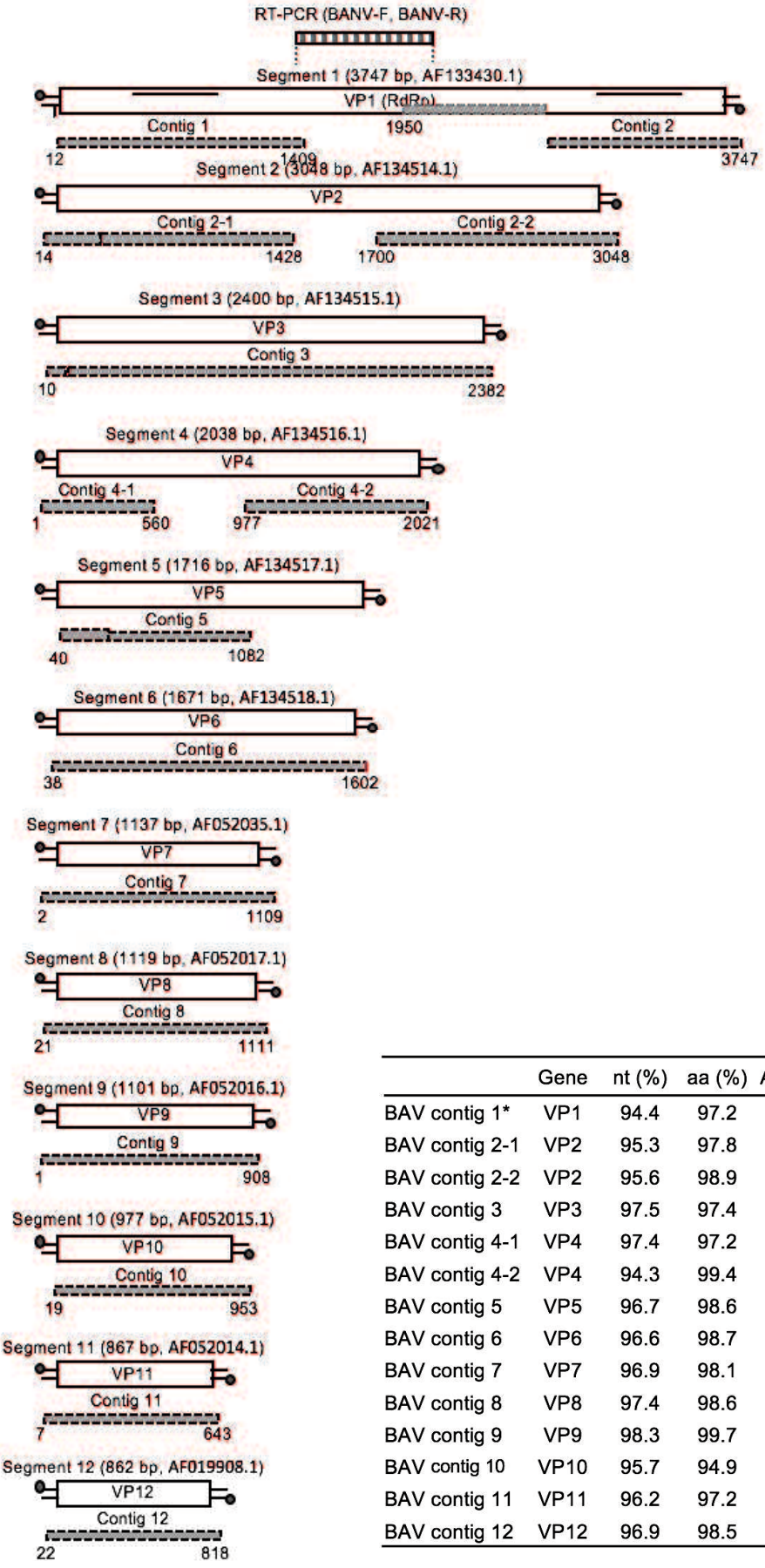


Pool no. 69



Mock

Fig. 3-7



	Gene	nt (%)	aa (%)	Accession no.	Coverage	Read number
BAV contig 1*	VP1	94.4	97.2	LC536090	—	—
BAV contig 2-1	VP2	95.3	97.8	LC536091	69	1215
BAV contig 2-2	VP2	95.6	98.9	LC536092		
BAV contig 3	VP3	97.5	97.4	LC536093	459	6500
BAV contig 4-1	VP4	97.4	97.2	LC536094	310	3459
BAV contig 4-2	VP4	94.3	99.4	LC536095		
BAV contig 5	VP5	96.7	98.6	LC536096	532	5034
BAV contig 6	VP6	96.6	98.7	LC536097	54	524
BAV contig 7	VP7	96.9	98.1	LC536098	691	4955
BAV contig 8	VP8	97.4	98.6	LC536099	52	352
BAV contig 9	VP9	98.3	99.7	LC536100	663	3802
BAV contig 10	VP10	95.7	94.9	LC536101	757	4225
BAV contig 11	VP11	96.2	97.2	LC536102	528	2549
BAV contig 12	VP12	96.9	98.5	LC536103	339	1707

Fig. 3-8

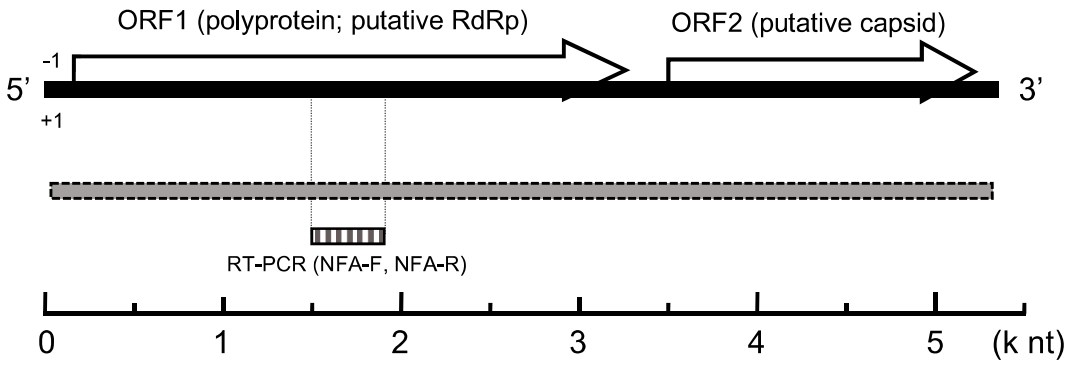

ORF1 (polyprotein; putative RdRp)
ORF2 (putative capsid)
-1
+1
5'
3'
RT-PCR (NFA-F, NFA-R)
0
1
2
3
4
5
(k nt)

Fig. 3-9

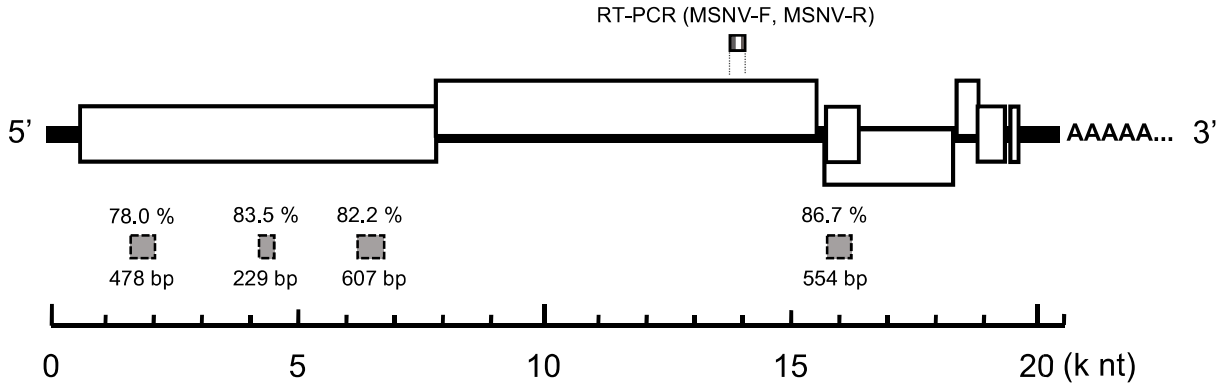

RT-PCR (MSNV-F, MSNV-R)
5'
AAAAA...
3'
78.0 %
478 bp
83.5 %
229 bp
82.2 %
607 bp
86.7 %
554 bp
0
5
10
15
20 (k nt)

Fig. 3-10

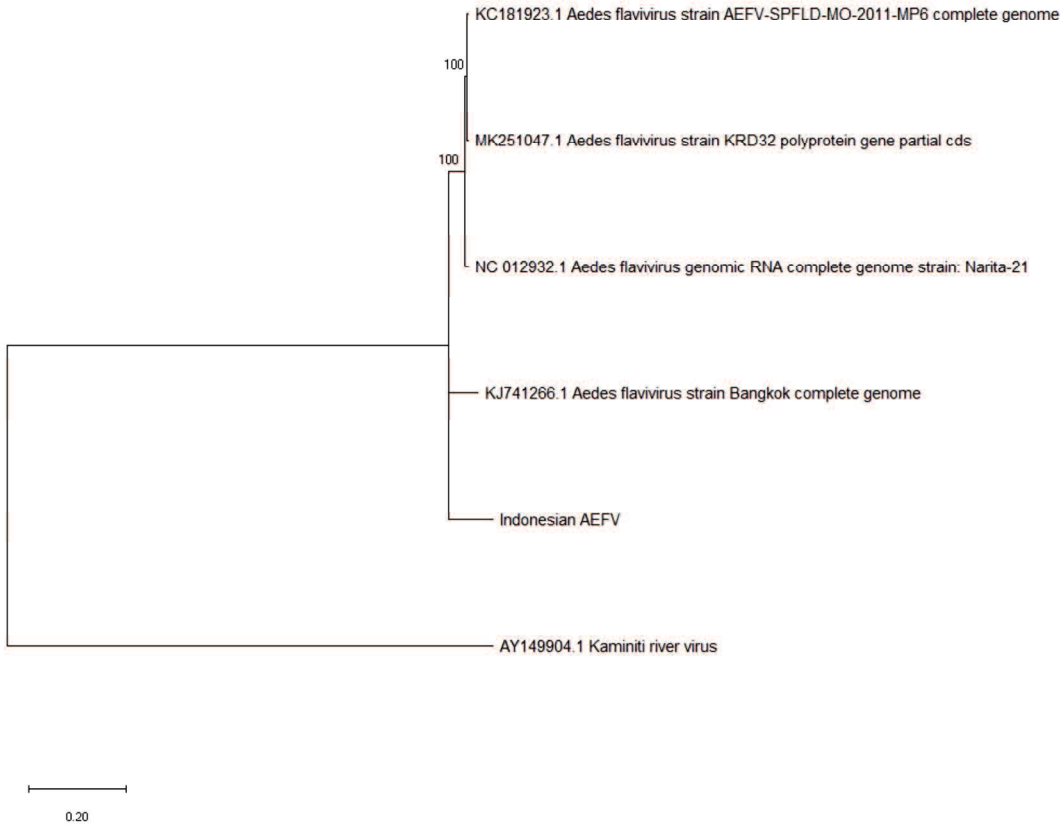

KC181923.1 Aedes flavivirus strain AEFV-SPFLD-MO-2011-MP6 complete genome
100
MK251047.1 Aedes flavivirus strain KRD32 polyprotein gene partial cds
100
NC 012932.1 Aedes flavivirus genomic RNA complete genome strain: Narita-21
KJ741266.1 Aedes flavivirus strain Bangkok complete genome
Indonesian AEFV
AY149904.1 Kaminiti river virus
0.20

Table 3-1 List of primer sets used in this study

Primer name	Direction	Sequence	Size (bp)	Target virus	Target gene	Ref.
MAMD	Sense	5′ -AACATGATGGGRAARAGRGARAA- 3’	260	Flavivirus	NS5	Scaramozzino et al. (2001)
cFD2	Antisense	5′ -GTGTCCCAGCCGGCGGTGTCATCAGC- 3′				Kuno et al. (1998)
VIR2052F	Sense	5′-TGGCGCTATGATGAAATCTGGAATGTT-3’	144	Alphavirus	nsP4	Eshoo et al. (2007)
VIR2052R	Antisense	5′-TAC GAT GTT GTC GTC GCC GAT GAA-3′				
MBPL3100F	Sense	5'-AGTCTCYTCTGCCATYTC-3'	890	Phlebovirus	L	this study
MBPL3287R	Antisense	5'-AGGATCTRGARGGGAACTTRT-3'				
RHNB1520F	Sense	5'-ACIAAIAARTWIATGATGATGAA-3'	188	Rhabdovirus	N	Kuzmin et al. (2006)
RHNB2134R	Antisense	5'-TGIARDATICCYTGCATCAT-3'				
BANV-F	Sense	5’-AGATCCTAACTGTGACCCAATGTT-3’	770	Banna virus	VP1	this study
BANV-R	Antisense	5’-TGTAACTTCTAACAAATCCGCAAA-3’				
BGV-F	Sense	5’-GTAGACGAATGCATGTTCGATAAG-3’	441	Bogor virus	putative RdRp	this study
BGV-R	Antisense	5’-CCGTCTAACTGTGTGGATAACAAG-3’				
AMSV-F	Sense	5’-TATGGCAAACGACGTATAGCAG-3’	371	mesoniviruses	putative RdRp	this study
AMSV-R	Antisense	5’-AAGCATARAYTGGTTGTGACG-3’				

Table 3-2 Number of mosquitoes used for virus isolation in this study

Site	Date	No.	*Aedes aegypti* F	*Aedes aegypti* M	*Aedes albopictus* F	*Aedes albopictus* M	*Aedes* sp. M	*Armigeres subalbatus* F	*Culex quinquefasciatus* F	*Culex quinquefasciatus* M	*Culex tritaeniorhynchus* F	*Culex tritaeniorhynchus* M	*Culex hutchinsoni* F	*Culex hutchinsoni* M	*Culex vishnui* F	*Culex gelidus* F	*Culex fuscocephala* F	*Culex* sp. F	*Anopheles vagus* F	*Anopheles barbirostris* F	*Anopheles* sp. F	Total
Daytime, residence																						
Bogor, West Java	June 2016	1	11	0	66	0	0	6	0	0	24	0	18	0	29	2	0	53	96	0	2	307
Bogor, West Java	Nov 2016	2	22	20	11	12	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	74
Bogor, West Java	May 2017	3	101	54	117	193	0	4	111	64	0	0	0	3	0	0	0	0	0	0	0	647
Tangerang, Banten	May 2017	4	26	0	0	0	0	1	75	9	0	0	0	0	0	0	0	0	0	0	0	111
Bogor, West Java	Oct 2017	5	85	10	96	380	0	3	293	25	30	0	0	0	0	0	0		0	0	0	922
Bengkulu, Bengkulu	Oct 2017	6	41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	41
Solo, Central Java	Oct 2017	7	40	0	0	0	0	0	40	0	0	0	0	0	0	0	0	0	0	0	0	80
Bekasi, West Java	Feb 2018	8	4	0	14	0	0	5	840	301	3	0	0	0	0	0	0	0	0	0	0	1167
Pasar Minggu, Jakarta	March 2018	9	163	0	8	0	0	5	12	0	0	0	0	0	0	0	0	0	0	0	0	188
Tangerang, Banten	March 2018	10	87	0	521	0	0	119	13	0	0	0	0	0	0	0	0	0	0	0	0	740
Tangerang, Banten	July 2018	11	0	0	261	135	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	396
Bogor, West Java	July 2018	12	200	96	0	130	0	125	2599	51	0	100	0	0	0	0	25	0	3	115	0	3444
Tangerang, Banten	July 2018	13	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	50
Night, animal houses																						
Bogor, West Java	June 2016	14	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	15
Bogor, West Java	Nov 2016	15	0	0	0	0	0	26	50	45	71	0	0	0	0	0	0	0	23	0	0	215
Bengkulu, Bengkulu	Oct 2017	16	0	0	0	0	0	6	90	0	52	0	0	0	0	0	0	0	0	0	0	148
Denpasar, Bali	Oct 2017	17	0	0	2	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	10
Bogor, West Java	March 2018	18	43	0	25	0	0	7	10	0	0	0	0	0	0	0	0	0	0	0	0	85
Palangka Raya, Central Kalimantan	March 2018	19	0	0	75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75
Bogor, West Java	July 2018	20	0	0	0	0	0	0	0	0	850	450	0	0	0	0	0	0	0	0	0	1300
Total			823	180	1196	850	9	357	4156	495	1030	550	18	3	29	2	25	53	122	115	2	10,015
Female/male			4.6		1.4				8.4		1.9		6.0									

Table 3-3 Summary for the result of virus isolation (CPE and RT-PCR) on C6/36 cells from collected mosquitoes

Mosquito species	Sex	No of pools	CPE on C6/36	RT-PCR [source of sample no. in table 1]						
				Flavivirus	Alphavirus	Phlebovirus	Rhabdovirus	BAV	BGV	MSNV
Aedes aegypti	F	30	11	4 CFAV [3]	0	0	0	2 [5]	5 [3, 5, 10]	0
Aedes aegypti	M	6	2	2 CFAV [3]	0	0	0	0	0	0
Aedes albopictus	F	33	13	1 AEFV* [3]	0	0	0	4 [1, 5]	7 [3, 5, 10, 18]	2 [8]
Aedes albopictus	M	19	9	0	0	0	0	0	9 [3, 5, 10]	0
Aedes sp.	M	1	0	0	0	0	0	0	0	0
Culex quinquefasciatus	F	99	9	1 CxFV [12]	0	0	0	0	6 [3, 8]	2 [5, 8]
Culex quinquefasciatus	M	10	1	0	0	0	0	0	0	1 [8]
Culex tritaeniorhynchus	F	32	19	0	0	0	0	2 [5]	0	17 [5, 8, 12, 13]
Culex tritaeniorhynchus	M	14	0	0	0	0	0	0	0	0
Culex hutchinsoni	F	2	0	0	0	0	0	0	0	0
Culex vishnui	F	1	0	0	0	0	0	0	0	0
Culex gelidus	F	1	0	0	0	0	0	0	0	0
Culex fuscocephala	F	1	1	0	0	0	0	0	1 [12]	0
Culex sp.	F	1	0	0	0	0	0	0	0	0
Armigeres subalbatus	F	26	7	0	0	0	0	0	6 [8, 10]	1 [5]
Anopheles vagus	F	4	0	0	0	0	0	0	0	0
Anopheles barbirostris	F	4	0	0	0	0	0	0	0	0
Anopheles sp.	F	1	0	0	0	0	0	0	0	0
Total		285	72	8	0	0	0	8	34	23

5. GENERAL CONCLUSION

Vector-borne diseases (VBDs) are threats to humans and animals and many microorganisms including pathogens were reported in arthropods. The roles of these microorganisms have not been still unknow. To assess the risk of VDBs, I investigated the prevalence of mosquito- and tick-borne pathogens in ticks and mosquitoes collected in Indonesia.

In Chapter 1, tick-borne bacteria were surveyed in *Am. varanense* ticks fed on lizard, a water monitor (*V. salvator*). The water monitor is a common reptile, found in city areas and bred as pet animals in Indonesia and the other Asian countries. A total of 23 ticks (21 males, a female and a nymph) were collected from one *V. salvator*. According to the morphological features and molecular analysis based on mt-*rrs*, all ticks were identified as *Am. varanense.* DNA fragments of *Anaplasma* spp. were detected in 4 (17.4%) ticks by PCRs targeting both the 16S rRNA and 60-kDa heat shock protein genes. One of four was closely related with *Anaplasma* sp. detected from sheep in China. The others were related with *Anaplasma* sp. detected from ticks infesting birds in Taiwan. Borrelial DNA fragments were detected in 22 (95.7%) ticks by PCR targeting borrelial flagellin gene. Thirty strains of *Borrelia* sp. were isolated from midguts and/or salivary glands. All strains belonged to the reptile-associated borreliae. Rickettsial DNA fragments were detected from 5 (21.7%) ticks by PCRs targeting both the 17-kDa antigen and citrate synthase genes. The detected *Rickettsia* was classified into spotted fever group of *Rickettsia* and identical with Candidatus *Rickettsia sepangensis*.

In Chapter 2, I conducted surveillance of mosquito-borne viruses at several sites in Indonesia during 2016–2018 for risk assessment of arbovirus infection and analysis of virus biodiversity in mosquito populations. I collected 10,015 mosquitoes comprising 11 species from 4 genera. Major mosquito species collected in this study were *Cx. quinquefasciatus*, *Ae.*

albopictus, *Cx. tritaeniorhynchus*, *Ae. aegypti*, and *Ar. subalbatus*. The collected mosquitoes were subjected for virus isolation using two mammalian cell lines, Vero and BHK-21, and one mosquito cell line, C6/36. Seventy-two pools showed clear CPEs in C6/36 cells. Using RT-PCR and next generation sequencing approaches, these isolates were identified as insect flaviviruses (Cell fusing agent virus, Culex flavivirus, Aedes flavivirus) (family *Flaviviridae*, genus *Flavivirus*), Banna virus (family *Reoviridae*, genus *Seadornavirus*), new permutotetravirus (designed as Bogor virus) (family *Permutotetraviridae,* genus *Alphapermutotetravirus*), and alphamesoniviruses 2 and 3 (family *Mesoniviridae*, genus *Alphamesonivirus*).

In conclusion, novel findings were obtained by surveillance of microorganisms in mosquitoes and ticks, in Indonesia. Ticks on water monitor, *Am. Varanense,* harbored several tick-borne bacteria (*Anaplasma* spp., *Borrelia* spp., and *Rickettsia* spp.). This result suggested that the reptiles living close to humans and the infesting ticks possessed variety of unknown bacteria. In addition to potential pathogenic viruses, Banna virus, Indonesian mosquitoes harbored several arthropod-specific viruses such as Cell fusing agent virus, Culex flavivirus, Aedes flavivirus, Bogor virus, alphamesoniviruses 2 and 3. Although the pathogenicity and biology of these detected viruses and bacteria are unclear, it was guessed that these arthropod-specific microorganisms might interact with pathogenic microorganisms inside the vectors. This large surveillance of VBDs provides basic information for the prevention and control of emerging and re-emerging diseases in Indonesia.

6. ACKNOWLEDGMENT

The previous studies were carried out at the Laboratory of Veterinary Microbiology, Joint Faculty of Veterinary Medicine, Yamaguchi University, Japan, from 2016 to 2020.

First of all, the author would like to show the greatest appreciation to his supervisor, **Prof. Ken Maeda** (Laboratory of Veterinary Microbiology, Yamaguchi University) for providing him this precious opportunity to study a lot as a Ph.D student with the invaluable support and advices.

The author is very grateful to all of his-co supervisors, **Dr. Ai Takano** (Laboratory of Veterinary Microbiology, Yamaguchi University), **Dr. Ryusei Kuwata** (Faculty of Veterinary Medicine, Okayama University of Science), **Dr. Hiroshi Shimoda** (Laboratory of Veterinary Microbiology, Yamaguchi University) for giving him encourage, critical advices, and useful discussion on his experiments, **Prof. Daisuke Hayasaka** (Laboratory of Veterinary Microbiology, Yamaguchi University, and **Prof. Tetsuya Tanaka** (Kagoshima University) for giving suggestions and discussions on his study.

The author sincerely thanks to his laboratory members for their supports in his experiments and making him stay comfortable and joyful during his days in laboratory.

The author is very grateful to his laboratory members of Parasitology and Medical Entomology, and the Dean of Faculty Veterinary Medicine, IPB University to allow him to continue his study in Yamaguchi University.

Finally, author would like to show the deepest appreciation to his family who supported him and sacrificed themselves to allow him to continue his study as Ph.D. in Yamaguchi University, Japan.

6. REFERENCES

Abbink P, Stephenson KE, Barouch DH. 2018. Zika virus vaccines. Nat Rev Microbiol. 16(10): 594-600.

Anacker RL, List RH, Mann RE, Wiedbrauk DL. 1986. Antigenic heterogeneity in high- and low-virulence strains of *Rickettsia rickettsii* revealed by monoclonal antibodies. Infect Immun. 51(2): 653-660.

Ando S, Fujita H. 2013. Diversity between spotted fever group rickettsia and ticks as vector. Med Entomol. Zool. 64(1): 5-7.

Andoh M, Sakata A, Takano A, Kawabata H, Fujita H, Une Y, Goka K, Kishimoto T, Ando S. 2015. Detection of *Rickettsia* and *Ehrlichia* spp. in Ticks Associated with Exotic Reptiles and Amphibians Imported into Japan. PLoS One. 10: e0133700.

Arrigo NC, Weaver SC, Calisher CH. 2016. The taxonomy of arbovirues. In: Vasilakis N and Gubler DJ, editors. Arboviruses: Molecular Biology, Evolution and Control. Poole: Caister Academic Press.

Ashuma, Sharma A, Singla LD, Kaur P, Bal MS, Batth BK, Juyal PD. 2013. Prevalence and haemato-biochemical profile of Anaplasma marginale infection in dairy animals of Punjab (India). Asian Pac J Trop Med. 6(2): 139-144.

Atif FA. 2015. *Anaplasma marginale* and *Anaplasma phagocytophilum*: Rickettsiales pathogens of veterinary and public health significance. Parasitol Res. 114: 3941–3957.

Attoui H, Billoir F, Biagini P, de Micco P, de Lamballerie X. 2000. Complete sequence determination and genetic analysis of Banna virus and Kadipiro virus: proposal for assignment to a new genus (*Seadornavirus*) within the family *Reoviridae*. J Gen Virol. 81(Pt 6): 1507–1015.

Attoui H, Jaafar MF, Belhouchet M, Tao S, Chen B, Liang G, Tesh RB, de Micco P, de Lamballerie X. 2006. Liao ning virus, a new Chinese *seadornavirus* that replicates in transformed and embryonic mammalian cells. J Gen Virol. 87(Pt 1): 199–208.

Baker TS, Olson NH, Fuller SD. 1999. Adding the third dimension to virus life cycles: three-dimensional reconstruction of icosahedral viruses from cryo-electron micrographs. Microbiol Mol Biol Rev. 63: 862–922.

Barbara KA, Farzeli A, Ibrahim IN, Antonjaya U, Yunianto A, Winoto I, Ester, Perwitasari D, Widjaya S, Richards Al, Williams M, Blair PJ. 2010. Rickettsial Infections of Fleas Collected from Small Mammals on Four Islands in Indonesia. J Med Entomol. 47(6): 1173-1178.

Barbour AG. 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J Biol Med. 57: 521–525.

Beard CB. 2014. Lyme disease prevention and control the way forward. Can Commun Dis Rep. 40(5): 91-94.

Beasley DW, Lewthwaite P, Solomon T. 2008. Current use and development of vaccines for Japanese encephalitis. Expert Opin Biol Ther. 8: 95-106.

Beati L, Meskini M, Thiers B, Raoult D. 1997. *Rickettsia aeschlimannii* sp. nov.: a new spotted fever group rickettsia associated with *Hyalomma marginatum* ticks. Int J Syst Bacteriol. 47: 548–554.

Belongia EA. 2002. Epidemiology and impact of coinfections acquired from *Ixodes* ticks. Vector Borne Zoonotic Dis. 2: 265–273.

Beyer AR, Carlyon JA. 2015. Of goats and men: rethinking anaplasmoses as zoonotic infections. Lancet Infect Dis. 15: 619–620.

Bhattacharya B, Roy P. 2008. Bluetongue virus outer capsid protein VP5 interacts with membrane lipid rafts via a SNARE domain. J Virol. 82(21): 10600-10612.

Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, Hay SI. 2013. The global distribution and burden of dengue. Nature. 496. 504–07.

Bittar C, Machado DC, Vedovello D, Ullmann LS, Rahal P, Araújo Junior JP, Nogueira ML. 2016. Genome sequencing and genetic characterization of Culex Flavirirus (CxFV) provides new information about its genotypes. Virol J. 13(1): 158.

Blair PJ, Jiang J, Schoeler GB, Moron C, Anaya E, Cespedes M, Cruz C, Felices V, Guevara C, Mendoza L, Villaseca P, Sumner JW, Richards AL, Olson JG. 2004. Characterization of spotted fever group rickettsiae in flea and tick specimens from northern Peru. J Clin Microbiol. 42(11): 4961–4967.

Blitvich BJ, Lin M, Dorman KS, Soto V, Hovav E, Tucker BJ, Staley M, Platt KB, Bartholomay LC. 2009. Genomic sequence and phylogenetic analysis of Culex flavivirus, an insect-specific flavivirus, isolated from *Culex pipiens* (Diptera: *Culicidae*) in Iowa. J Med Entomol. 46(4): 934–941.

Blitvich BJ, Firth AE. 2015. Insect-specific flaviviruses: a systematic review of their discovery, host range, mode of transmission, superinfection exclusion potential and genomic organization. Viruses. 7(4): 1927–1959.

Blitvich BJ, Firth AE. 2017. A Review of Flaviviruses that Have No Known Arthropod Vector. Viruses. 9(6): 154.

Bolling BG, Olea-Popelka FJ, Eisen L, Moore CG, Blair CD. 2012. Transmission dynamics of an insect-specific flavivirus in a naturally infected *Culex pipiens* laboratory colony and effects of co-infection on vector competence for West Nile virus. Virology. 427(2): 90–97.

Bolling BG, Weaver SC, Tesh RB, Vasilakis N. 2015a. Insect-specific virus discovery: significance for the arbovirus community. Viruses. 7(9): 4911–4928.

Bolling BG, Vasilakis N, Guzman H, Widen SG, Wood TG, Popov VL, Thangamani S, Tesh RB. 2015b. Insect-specific viruses detected in laboratory mosquito colonies and their potential implications for experiments evaluating arbovirus vector competence. Am J Trop Med Hyg. 92(2): 422–428.

Borgherini G, Poubeau P, Staikowsky F, Lory M, Le Moullec N, Becquart JP, Wengling C, Michault A, Paganin F. 2007. Outbreak of chikungunya on Reunion Island: early clinical and laboratory features in 157 adult patients. Clin Infect Dis. 44: 1401-1407.

Brasil P, Pereira JP, Jr Moreira ME, Nogueira RM, Damasceno L, Wakimoto M, Rabello RS, Valderramos SG, Halai UA, Salles TS, Zin AA, Horovitz D, Daltro P, Boechat M, Raja Gabaglia C, Carvalho de Sequeira P, Pilotto JH, Medialdea-Carrera R, da Cunha CD, de Carvalho ALM, Nielsen-Saines K. 2016. Zika Virus Infection in Pregnant Women in Rio de Janeiro. N Engl J Med. 375(24): 2321–2334.

Brighton SW, Prozesky OW, de la Harpe AL. 1983. Chikungunya virus infection. A retrospective study of 107 cases. S Afr Med J. 63(9): 313–315.

Brinton MA, Basu M. 2015. Functions of the 30 and 50 genome RNA regions of members of the genus Flavivirus. Virus Res. 206: 108–119.

Brown SE, Gorman BM, Tesh RB, Knudson L. 1993. Coltiviruses isolated from mosquitoes collected in Indonesia. Virology. 196(1): 363–367.

Buckley A, Gould EA. 1988. Detection of virus-specific antigen in the nuclei or nucleoli of cells infected with Zika or Langat virus. J Gen Virol. 69: 1913–1920.

Burgdorfer W, Sexton DJ, Gerloff RK, Anacker RL, Philip RN, Thomas LA. 1975. *Rhipicephalus sanguineus*: vector of a new spotted fever group rickettsia in the United States. Infect Immun. 12: 205–210.

Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. 1982. Lyme Diseasea Tick-Borne Spirochetosis. Science. 216(4552): 1317-1319.

Burni E. 2017. Vector Borne diseases and zoonotic policy in Indonesia (in Bahasa Indonesia). National Seminar on Rikhus Vektora Final Report 2017, 17–19 December 2017. Yogyakarta.

Burridge MJ. 2001. Ticks (Acari: *Ixodidae*) spread by the international trade in reptiles and their potential roles in dissemination of diseases. Bull Entomol Res. 91: 3–23.

Burt FJ, Chen W, Miner JJ, Lenschow DJ, Merits A, Schnettler E, Kohl A, Rudd PA, Taylor A, Herrero LJ, Zaid A, Ng LFP, Mahalingam S. 2017. Chikungunya virus: An update on the biology and pathogenesis of this emerging pathogen. Lancet Infect Dis. 17: e107-17.

Cai CZ, Han LY, Ji ZL, Chen X, Chen YZ. 2003. SVM-Prot: Web-based support vector machine software for functional classification of a protein from its primary sequence. Nucleic Acids Res. 31(13): 3692–3697.

Caglioti C, Lalle E, Castilletti C, Carletti F, Capobianchi MR, Bordi L. 2013. Chikungunya virus infection: an overview. New Microbiol. 36: 211–27.

Calzolari M, Zé-Zé L, Růžek D, Vázquez A, Jeffries C, Defilippo F, Osório HC, Kilian P, Ruíz S, Fooks AR, Maioli G, Amaro F, Tlustý M, Figuerola J, Medlock JM, Bonilauri P, Alves MJ, Šebesta O, Tenorio A, Vaux A, Bellini R, Gelbic I, Seco MPS, Johnson N, Dottori M. 2012. Detection of mosquito-only flaviviruses in Europe. J Gen Virol. 93(Pt 6): 1215–1225.

Cammisa-Park H, Cisar LA, Kane A, Stollar V. 1992. The complete nucleotide sequence of cell fusing agent (CFA): homology between the nonstructural proteins encoded by CFA and the nonstructural proteins encoded by arthropod-borne flaviviruses. Virology. 189: 511–524.

Cao WC, Gao YM, Zhang PH, Zhang XT, Dai QH, Dumler JS, Fang LQ, Yang H. 2000. Identification of *Ehrlichia chaffeensis* by nested PCR in ticks from Southern China. J Clin Microbiol. 38: 2778–80.

Cao-Lormeau VM, Musso D. 2014. Emerging arboviruses in the Pacific. Lancet. 384: 1571–1572.

Carey DE. 1971. Chikungunya and dengue: a case of mistaken identity?. J Hist Med Allied Sci. 26: 243–262.

Carlos CC, Oishi K, Cinco, MT, Mapua CA, Inoue S, Cruz DJ, Pancho MA, Tanig CZ, Matias RR, Morita K, Natividad FF, Igarashi A, Nagatake T. 2005. Comparison of clinical features and hematologic abnormalities between dengue fever and dengue hemorrhagic fever among children in the Philippines. Am J Trop Med Hyg. 73: 435-440.

Center for Diseases Control and Prevention (CDC). 2018a. Ehrlichiosis: Epidemiology and statistic. https://www.cdc.gov/ehrlichiosis/stats/index.html

Center for Diseases Control and Prevention (CDC). 2018b. Data and suervillance of Lyme disease. https://www.cdc.gov/lyme/datasurveillance/index.

Center for Diseases Control and Prevention (CDC). 2019. Chikungunya virus. https://www.cdc.gov/chikungunya/index.html

Chambers TJ, Hahn CS, Galler R, Rice CM. 1990. Flavivirus genome organization, expression, and replication. Annu Rev Microbiol. 44: 649–688.

Chapman AS, Bakken JS, Folk SM, Paddock CD, Bloch KC, Krusell A, Sexton DJ, Buckingham SC, Marshall GS, Storch GA, Dasch GA, McQuiston JH, Swerdlow DL, Dumler SJ, Nicholson WL, Walker DH, Eremeeva ME, Ohl CA. 2006. Diagnosis and management of tick-borne rickettsial diseases: Rocky Mountain spotted fever, ehrlichioses, and anaplasmosis United States: a practical guide for physicians and other health-care and public health professionals. MMWR Recomm Rep. 55 (RR-4): 1–27.

Chávez JH. Silva JR, Amarilla AA, Figueiredo, MLT. 2010. Domain III peptides from flavivirus envelope protein are useful antigens for serologic diagnosis and targets for immunization. Biologicals. 38(6): 613-618.

Chen B, Tao SJ. 1996. Arboviruses survey in China in recent ten years. Chin Med J. 109: 13–5.

Cheng RH, Kuhn RJ, Olson NH, Rossmann MG, Choi HK, Smith TJ, Baker TS. 1995. Nucleocapsid and glycoprotein organization in an enveloped virus. Cell. 80(4): 621–630.

Chen WJ, Dong CF, Chiou LY, Chuang WL. 2000. Potential Role of *Armigeres subalbatus* (Diptera: *Culicidae*) in the Transmission of Japanese Encephalitis Virus in the Absence of Rice Culture on Liu-Chiu Islet, Taiwan. J Med Entomol. 37: 108-113.

Chen YY, Lin JW, Fan YC, Tu WC, Chang GJ, Chiou SS. 2013. First detection of the Africa/Caribbean/Latin American subtype of Culex flavivirus in Asian country, Taiwan. Comp Immunol Microbiol Infect Dis. 36 (4): 387–396.

Christie J. 1881. On epidemics of dengue fever: Their diffusion and etiology. Glasgow Med J. 16:161–176.

Choi HK, Tong L, Minor W, Dumas P, Boege U, Rossmann MG, Wengler G. 1991. Structure of Sindbis virus core protein reveals a chymotrypsin-like serine proteinase and the organization of the virion. Nature. 354(6348): 37–43.

Chuang C, Chen W. 2009. Genetic evolution of Japanese encephalitis virus. In: Růžek AD. (Ed.), Flavivirus Encephalitis. Intech. 384–404.

Clark K, Hendricks A, Burge D. 2005. Molecular identification and analysis of *Borrelia burgdorferi* Sensu Lato in lizards in the Southeastern United States. Appl Environ Microbiol. 71: 2616–25.

Cook S, Bennett SN, Holmes EC, De Chesse R, Moureau G, de Lamballerie X. 2006. Isolation of a new strain of the flavivirus cell fusing agent virus in a natural mosquito population from Puerto Rico. J Gen Virol. 87(Pt 4): 735–748.

Cook S, Moureau G, Harbach RE, Mukwaya L, Goodger K, Ssenfuka F, Gould E, Holmes EC, de Lamballerie X. 2009. Isolation of a novel species of flavivirus and a new strain of Culex flavivirus (Flaviviridae) from a natural mosquito population in Uganda. J Gen Virol. 90: 2669–2678.

Crabtree MB, Sang RC, Stollar V, Dunster LM, Miller BR. 2003. Genetic and phenotypic characterization of the newly described insect flavivirus, Kamiti River virus. Arch Virol. 148: 1095-118.

Crabtree MB, Nga PT, Miller BR. 2009. Isolation and characterization of a new mosquito flavivirus, Quang Binh virus, from Vietnam. Arch Virol. 154: 857–860.

Crochu S, Cook S, Attoui H, Charrel RN, De Chesse R, Belhouchet M, Lemasson JJ, de Micco P, de Lamballerie X. 2004. Sequences of flavivirus-related RNA viruses persist in DNA form integrated in the genome of *Aedes* spp. mosquitoes. J Gen Virol. 85(Pt 7): 1971–1980.

Darlington PB. 1926. Anaplasmosis in cattle (Galziete) found to exist in Kansas. N. Am. Vet. 7, 39–41.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods. 9(8): 772.

Das S, Chakraborty S. Basu A. 2010. Critical role of lipid rafts in virus entry and activation of phosphoinositide 3' kinase/Akt signaling during early stages of Japanese encephalitis virus infection in neural stem/progenitor cells. J Neurochem. 115(2): 537-549.

de la Fuente J, Torina A, Caracappa S, Tumino G, Furla´ R, Almaza´n C, Kocan KM. 2005. Serologic and molecular characterization of *Anaplasma* species infection in farm animals and ticks from Sicily. Vet Parasitol. 133: 357–362.

de la Fuente J, Estrada-Peña A, Cabezas-Cruz A, Kocan KM. 2016. *Anaplasma phagocytophilum* uses common strategies for infection of ticks and vertebrate hosts. Trends Microbiol. 24: 173–180.

de la Fuente J, Antunes S, Bonnet S, Cabezas-Cruz A, Domingos AG, Estrada-Peña A, Johnson N, Kocan KM, Mansfield KL, Nijhof AM, Papa A, Rudenko N, Villar M, Alberdi P, Torina A, Ayllón N, Vancova M, Golovchenko M, Grubhoffer L, Caracappa S, Fooks AR, Gortazar C, Rego ROM. 2017. Tick-Pathogen Interactions and Vector Competence: Identification of Molecular Drivers for Tick-Borne Diseases. Front Cell Infect Microbiol. 7:114.

Deng X, Shi Z, Li S, Wang X, Qiu Y, Shao D, Wei J, Tong G, Ma Z. 2011. Characterization of nonstructural protein 3 of a neurovirulent Japanese encephalitis virus strain isolated from a pig. Virol J. 8: 209.

Diallo D, Sall AA, Diagne CT, Faye O, Faye O, Ba Y, Hanley KA, Buenemann M, Weaver SC, Diallo M. 2014. Zika virus emergence in mosquitoes in southeastern Senegal, 2011. PLoS One. 9: e109442.

Dick GW, Kitchen SF, Haddow AJ. 1952. Zika virus (I) Isolations and serological specificity. Trans R Soc Trop Med Hyg. 46: 509–520.

Dickerson RB, Newton JR, Hansen JE. 1952. Diagnosis and immediate prognosis of Japanese B encephalitis; observation based on more than 200 patients with detailed analysis of 65 serologically confirmed cases. Am J Med. 12: 277-288.

Dorrington RA, Jiwaji M, Awando JA, Bruyn MM. 2019. Advances in Tetravirus Research: New insight into the infectious virus lifecycle and an expanding host range. Curr Issues Mol Biol. 34: 145–162.

Drancourt M, Kelly P, Regnery R, Raoult D. 1992. Identification of spotted fever group rickettsiae using polymerase chain reaction and restriction-endonuclease length polymorphism analysis. Acta Virol. 36: 1–6.

Drancourt M, Raoult D. 1994. Taxonomic position of the rickettsiae: current knowledge. FEMS Microbiol Rev. 13: 13–24.

Dumler JS, Barbet AF, Bekker CPJ, Dasch GA, Palmer GH, Ray SC, Rikihisa Y, Rurangirwa FR. 2001. Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: Unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. Int J Syst Evol Microbiol. 51: 2145–65.

Duong V, Lambrechts L, Paul RE, Ly S, Lay RS, Long KC, Huy R, Tarantola A, Scott TW, Sakuntabhai A, Buchy P. 2015. Asymptomatic humans transmit dengue virus to mosquitoes. Proc Nat Acad Sci USA. 112(47): 14688–14693.

Dyahningrum DM, Mufasirin, Harijani N, Hastutiek P, Koesdiarto S, Yunus M. 2019. Identification of Blood Parasite on Sacrifical Cattle Slaughtered during Idul Adha 1438 H in Surabaya City and Sidoarjo Regency. JoPS. 3(2): 77-82.

Efron B, Halloran E, Holmes S. 1996. Bootstrap confidence levels for phylogenetic trees. Proc Natl Acad Sci USA. 93(23): 13429–13434.

Ekner A, Dudek K, Sajkowska Z, Majlathová V, Majlath I, Tryjanowski P. 2011. *Anaplasmataceae* and *Borrelia burgdorferi sensu lato* in the sand lizard *Lacerta agilis* and co-infection of these bacteria in hosted *Ixodes ricinus* ticks. Parasit Vectors. 4:182.

Elbaum-Garfinkle S. 2011. Close to home: a history of Yale and Lyme disease. Yale J Biol Med. 84(2): 103-108.

Endy TP, Nisalak A. 2002. Japanese encephalitis virus: ecology and epidemiology. Curr Top Microbiol Immunol. 267: 11-48.

Endy TP, Anderson KB, Nisalak A, Yoon IK, Green S, Rothman AL, Thomas SJ, Jarman RG, Libraty DH, Gibbons RV. 2011. Determinants of Inapparent and Symptomatic Dengue Infection in a Prospective Study of Primary School Children in Kamphaeng Phet, Thailand. PLoS Negl Trop Dis. 5: e975.

Eremeeva MX, Yu, Raoult D. 1994. Differentiation among spotted fever group rickettsiae species by analysis of restriction fragment length polymorphism of PCR-amplified DNA. J Clin Microbiol. 32: 803–810.

Erlanger TE, Weiss S, Keiser J, Utsinger J, Wiedenmayer K. 2009. past, present and future of Japanese encephalitis. Emerg Infect Dis. 115: 1–7.

Eshoo MW, Whitehouse CA, Zoll ST, Massire C, Pennella TT, Blyn LB, Sampath R, Hall TA, Ecker JA, Desai A, Wasieloski LP, Li F, Turell MJ, Schink A, Rudnick K, Otero G, Weaver SC, Ludwig GV, Hofstadler SA, Ecker DJ. 2007. Direct broad-range detection of alphaviruses in mosquito extracts. Virology. 368(2): 286–295.

Espinoza-Gomez F, Lopez-Lemus AU, Rodriguez-Sanchez IP, Martinez-Fierro ML, Newton-Sanchez OA, Cha´vez-Flores, E. Delgado-Enciso I. 2011. Detection of sequences from a potentially novel strain of cell fusing agent virus in Mexican Stegomyia (Aedes) aegypti mosquitoes. Arch Virol. 156: 1263–1267.

Fagbami A. 1977. Epidemiological investigations on arbovirus infections at Igbo-Ora, Nigeria. Trop Geogr Med. 29: 187–191.

Faisal MD, Haryanto AT, Tjahajati I. 2019. Diagnosis and molecular characterization of *Anaplasma platys* in dog patients in Yogyakarta area, Indonesia. Indones J Biotechnol. 24(1): 43-50.

Fang Y, Zhang Y, Zhou ZB, Shi WQ, Xia S, Li YY, Wu JT, Liu Q, Lin GY. 2018. Co-circulation of Aedes flavivirus, Culex flavivirus, and Quang Binh virus in Shanghai, China. Infect Dis Poverty. 7(1): 75.

Fang Y, Zhang Y, Zhou ZB, Xia S, Shi WQ, Xue JB, Li YY, Wu JT. 2019. New strains of Japanese encephalitis virus circulating in Shanghai, China after a ten-year hiatus in local mosquito surveillance. Parasit Vectors. 12(1): 22.

Farfan-Ale JA, Lorono-Pino MA, Garcia-Rejon JE, Hovav E, Powers AM, Lin M, Dorman KS, Platt KB, Bartholomay LC, Soto V, Beaty BJ, Lanciotti RS, Blitvich BJ. 2009. Detection of RNA from a Novel West Nile-like virus and high prevalence of an insect-specific flavivirus in mosquitoes in the Yucatan Peninsula of Mexico. Am J Trop Med Hyg. 80: 85-95.

Felsenfeld O. 1965. Borreliae, Human relapsing fever, and parasite-vector-host relationships. Bacteriol Rev. 29: 46–74.

Foggie A. 1951. Studies on the infectious agent of tick-borne fever. J Pathol Bacteriol. 63: 1–15.

Fujita R, Kato F, Kobayashi D, Murota K, Takasaki T, Tajima S, Lim CK, Saijo M, Isawa H, Sawabe K. 2018. Persistent viruses in mosquito cultured cell line suppress multiplication of flaviviruses. Heliyon. 4(8): e00736.

Fukunaga M, Takahashi Y, Tsuruta Y, Matsushita O, Ralph D, McClelland M, Nakao, M. 1995. Geneticand phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. Int J Syst Bacteriol. 45: 804–810.

Gage KL, Burkot TR, Eisen RJ, Hayes EB. 2008: Climate and vector borne diseases. Am J Prev Med. 35(5): 436-450.

Ganesan VK, Duan B, Reid SP. 2017. Chikungunya Virus: Pathophysiology, Mechanism, and Modeling. Viruses. 9(12): 368.

Garjito TA, Widiarti, Anggraeni YM, Alfiah S, Satoto TBT, Farchanny A, Samaan G, Afelt A, Manguin S, Frutos R, Aditama TY. 2018. Japanese encephalitis in Indonesia: An update on epidemiology and transmission ecology. Acta Tropica. 187: 240-247.

Garjito TA, Prihatin MT, Susanti L, Prastowo D, Sa'adah SR, Taviv Y, Satoto TBT, Waluyo J, Manguin S, Frutos R. 2019. First evidence of the presence of genotype-1 of Japanese encephalitis virus in *Culex gelidus* in Indonesia. Parasit Vectors. 12(1): 19.

Gern L, Estrada-Pena A, Frandsen F, Gray JS, Jaenson T, Jongejan F, Kahl O, Korenberg E, Mehl R, Nuttall PA. 1998. European reservoir hosts of *Borrelia burgdorferi* sensu lato. Zentralbl Bakteriol B. 287: 196–204.

Gil H, Barral M, Escudero R, García-Pérez AL, Anda P. 2005. Identification of a new Borrelia species among small mammals in areas of northern Spain where Lyme disease is endemic. Appl Environ Microbiol. 71(3):1336–1345.

Goenaga S, Fabbri CM, García JB, Rondán JC, Gardenal N, Calderón GE, Enria DA, Levis SM. 2014. New strains of Culex flavivirus isolated in Argentina. J Med Entomol. 51(4): 900-906.

Gonzales-Sanchez JA, Ramirez-Arroyo GF. 2018. Chikungunya virus: History, geographic distribution, clinical picture, and treatment. P R Health Sci J. 37(4): 187-194.

Gould E, Pettersson J, Higgs S, Charrel R, de Lamballerie X. 2017. Emerging arboviruses: Why today?. One Health. 4:1-13.

Gratz NG. 1999. Emerging and resurging vector-borne disease. Annu Rev Entomol. 44: 51-75.

Gribble DH. 1969. Equine ehrlichiosis. J Am Vet Med Assoc. 155: 462– 469.

Grisenti M, Vázquez A, Herrero L, Cuevas L, Perez-Pastrana E, Arnoldi D, Rosà R, Capelli G, Tenorio A, Sánchez-Seco MP, Rizzoli A. 2015. Wide detection of Aedes flavivirus in north-eastern Italy-a European hotspot of emerging mosquito-borne diseases. J Gen Virol. 96(Pt 2): 420–430.

Gross L. 1996. How Charles Nicolle of the Pasteur Institute discovered that epidemic typhus is transmitted by lice: reminiscences from my years at the Pasteur Institute in Paris Proc Natl Acad Sci. 93: 10539–10540.

Gubler DJ, Clark GG. 1995. Dengue/dengue hemorrhagic fever: The emergence of a global health problem. Emerg Infect Dis. 1: 55–57.

Gubler DJ. 1998. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev. 11: 480-496.

Guha-Sapir D, Schimmer B. 2005. Dengue fever: new paradigms for a changing epidemiology. Emerg Themes Epidemiol. 2(1): 1.

Guner ES, Hashimoto N, Kadosaka T, Imai Y, Masuzawa T. 2003. A novel, fast-growing *Borrelia* sp. isolated from the hard tick *Hyalomma aegyptium* in Turkey. Microbiology. 149: 2539–44.

Guo C, Zhou Z, Wen Z, Liu Y, Zeng C, Xiao D, Ou M, Han Y, Huang S, Liu D, Ye X, Zou X, Wu J, Wang H, Zeng EY, Jing C, Yang G. 2017. Global epidemiology of dengue outbreaks in 1990-2015: a systematic review and meta-analysis. Front Cell Infect Microbiol. 7: 317.

Guzman MG, Halstead SB, Artsob H, Buchyl P, Farrar J, Gubler DJ, Hunsperger E, Kroeger A, Margolis HS, Martinez E, Nathan MB, Pellegrino JL, Simmons C, Yoksan S, Peeling RW. 2010. Dengue: a continuing global threat. Nat Rev Microbiol. 8: S7–S16.

Haddow AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, Huy R, Guzman H, Tesh RB, Weaver SC. 2012. Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. PLoS Negl Trop Dis. 6: e1477.

Haddow AD, Guzman H, Popov VL, Wood TG, Widen SG, Haddow AD, Tesh RB, Weaver SC. 2013. First isolation of Aedes flavivirus in the Western Hemisphere and evidence of vertical transmission in the mosquito *Aedes (Stegomyia) albopictus* (Diptera: *Culicidae*). Virology. 440(2): 134–139.

Halle S, Dasch GA, Weiss E. 1977. Sensitive enzyme-linked immunosorbent assay for detection of antibodies against typhus rickettsiae, *Rickettsia prowazekii* and *Rickettsia typhi*. J Clin Microbiol. 6: 101–110.

Hammon WM, Rudnick A, Sather GE. 1960. Viruses associated with epidemic hemorrhagic fevers of the Philippines and Thailand. Science. 131(3407):1102–3.

Harapan, Michie A, Mudatsir, Sasmono RT, Imrie A. 2019a. Epidemiology of dengue hemorrhagic fever in Indonesia: analysis of five decades data from the National Disease Surveillance. BMC Research. 12:50.

Harapan H, Michie A, Mudatsir M, Nusa R, Yohan B, Wagner AL, Sasmono RT, Imrie A. 2019b. Chikungunya virus infection in Indonesia: a systematic review and evolutionary analysis. BMC Infect Dis. 19(1): 243.

Harbach RE. 2013. Mosquito taxonomic inventory. http://mosquitotaxonomic-inventory info/.

Haryanto S, Hayati RF, Yohan B, Sijabat L, Sihite IF, Fahri S, Meutiawati F, Halim JA, Halim SN, Soebandrio A, Sasmono RT. 2016. The molecular and clinical features of dengue during outbreak in Jambi, Indonesia in 2015. Pathog Global Health. 110(3): 119–129.

Henchal EA, Putnak JR, 1990. The dengue viruses. Clin Microbiol Rev. 3: 376–96.

Heppner DG, Wongsrichanalai C, Walsh DS, McDaniel P, Eamsila C, Hanson B, Paxton H. 1997. Human ehrlichiosis in Thailand. Lancet. 350: 785–786.

Higa Y. 2011. Dengue Vectors and their Spatial Distribution. Trop Med Health. 39(4 Suppl): 17–27.

Hoshino K, Isawa H, Tsuda Y, Yano K, Sasaki T, Yuda M, Takasaki T, Kobayashi M, Sawabe K. 2007. Genetic characterization of a new insect flavivirus isolated from *Culex pipiens* mosquito in Japan. Virology. 359(2): 405–414.

Hoshino K, Isawa H, Tsuda Y, Sawabe K, Kobayashi M. 2009. Isolation and characterization of a new insect flavivirus from *Aedes albopictus* and *Aedes flavopictus* mosquitoes in Japan. Virology. 391(1): 119–129.

Hoshino K, Takahashi-Nakaguchi A, Isawa H, Sasaki T, Higa Y, Kasai S, Tsuda Y, Sawabe K, Kobayashi M. 2012. Entomological surveillance for flaviviruses at migratory bird stopover sites in Hokkaido, Japan, and a new insect flavivirus detected in *Aedes galloisi* (Diptera: *Culicidae*). J Med Entomol. 49(1): 175–182.

Hove P, Khumalo ZTH, Chaisi ME, Oosthuizen MC, Brayton KA, Collins NE. 2018. Detection and Characterisation of Anaplasma marginale and A. centrale in South Africa. Vet Sci. 5(1): 26.

Huanyu W, Haiyan W, Shihong F, Guifang L, Hong L, Xiaoyan G, Lizhi S, Rayner S, Aiqiang X, Guodong L. 2012. Isolation and identification of a distinct strain of Culex Flavivirus from mosquitoes collected in Mainland China. Virol J. 9: 73.

Hubálek Z, Halouzka J. 1997. Distribution of *Borrelia burgdorferi* sensu lato genomic groups in Europe, a review. Eur J Epidemiol. 13: 951–7.

Hubálek Z. 2009. Epidemiology of lyme borreliosis. Curr Probl Dermatol. 37: 31-50.

Hudson JR. 1950. The recognition of tick-borne fever as a disease of cattle. Brit Vet J. 106: 3–17.

Igarashi A, Harrap KA, Casals J, Stollar V. 1976. Morphological, biochemical, and serological studies on a viral agent (CFA) which replicates in and causes fusion of Aedes albopictus (Singh) cells. Virology. 74(1): 174–187.

Imaoka K, Kaneko S, Tabara K, Kusatake K, Morita E. 2011. The First Human Case of *Rickettsia tamurae* Infection in Japan. Case Rep Dermatol. 3: 68–73.

Im J, Balasubramanian R, Yastini NW, Suwarba IGN, Andayani AR, Bura V, Jeon HJ, Clements JD, Marks F. 2018. Protecting children against Japanese encephalitis in Bali, Indonesia. Lancet. 391(10139): 2500–2501.

Ismail N, Bloch KC, Mc Bridge JW. 2010. Human ehrlichiosis and Anaplasmosis. Clin Lab Med. 30: 261-292.

Itokawa K, Komagata O, Kasai S, Tomita T. 2015. A single nucleotide change in a core promoter is involved in the progressive overexpression of the duplicated CYP9M10 haplotype lineage in *Culex quinquefasciatus.* Insect Biochem Mol Biol. 66: 96–102.

Jaafar MF, Attoui H, Mertens PPC, de Micco P. de Lamballerie X. 2005a. Structural organization of an encephalitic human isolate of Banna virus (genus *Seadornavirus,* family *Reov*iridae). J Gen Virol. 86: 1147–1157.

Jaafar MF, Attoui H, Bahar MW, Siebold C, Sutton G, Mertens PP, De Micco P, Stuart DI, Grimes JM, De Lamballerie X. 2005b. The structure and function of the outer coat protein VP9 of Banna virus. Structure (London, England: 1993). 13(1): 17–28.

Jahfari S, Krawczyk A, Coipan EC, Fonville M, Hovius JW, Sprong H, Takumi K. 2017. Enzootic origins for clinical manifestations of Lyme borreliosis. Infect Genet Evol. 49: 48–54.

Javkhlan G, Enkhtaivan B, Baigal B, Myagmarsuren P, Battur B, Battsetseg B. 2014. Natural *Anaplasma phagocytophilum* infection in ticks from a forest area of Selenge province, Mongolia. Western Pac Surveill Response J. 5(1): 21-24.

Jeffries CL, Mansfield KL, Phipps LP, Wakeley PR, Mearns R, Schock A, Bell S, Breed AC, Fooks AR, Johnson N. 2014. Louping ill virus: an endemic tick-borne disease of Great Britain. J Gen Virol. 95(Pt 5): 1005–1014.

Jiang J, Soeatmadji DW, Henry KM, Ratiwayanto S, Bangs MJ, Richards AL. 2006. *Rickettsia felis* in *Xenopsylla cheopis*, Java, Indonesia. Emerg Infect Dis. 12(8): 1281–1283.

Jiang J, Temenak JJ, Richards AL. 2003. Real-time PCR duplex assay for Rickettsia prowazekii and Borrelia recurrentis. Ann N Y Acad Sci. 990: 302–310.

Johnson AJ, Martin DA, Karabatsos N, Roehrig JT. 2000. Detection of anti-arboviral immunoglobulin G by using a monoclonal antibody-based capture enzyme-linked immunosorbent assay. J Clin Microbiol. 38: 1827–1831.

Johnson BW, Russell BJ, Goodman CH. 2016. Laboratory Diagnosis of Chikungunya Virus Infections and Commercial Sources for Diagnostic Assays. J Infect Dis. 214(suppl 5): S471-S474.

Jongejan F, Uilenberg G. 2004. The global importance of ticks. Parasitology. 129: S3–S14.

Jupp PG, McIntosh BM, Dos Santos I, DeMoor P. 1981. Laboratory vector studies on six mosquito and one tick species with chikungunya virus. Trans R Soc Trop Med Hyg. 75: 15–19.

Jupp PG, McIntosh BM. 1990. *Aedes furcifer* and other mosquitoes as vectors of chikungunya virus at Mica, northeastern Transvall, South Africa. J Am Mosq Control Assoc. 6: 415–420.

Kanamitsu M, Taniguchi K, Urasawa S, Ogata T, Wada Y, Saroso SJ. 1979. Geographic distribution of arbovirus antibodies in indigenous human populations in the IndoAustralian archipelago. Am J Trop Med Hyg. 28: 351—363.

Kari K, Liu W, Gautama K, Mammen MP, Clemens JD, Nisalak A, Subrata K, Kim HK, Xu ZY. 2006. A hospital-based surveillance for Japanese encephalitis in Bali, Indonesia. BMC Med. 4.

Karyanti MR, Uiterwaal CSPM, Kusriastuti R. Hadinegoro SR, Rovers MM, Heesternbeek H, Hoes AW, Brijning-Verhagen P. 2014. The changing incidence of Dengue Haemorrhagic Fever in Indonesia: a 45-year registry-based analysis. BMC Infect Dis. 14: 412.

Kazar J, Brezina R. 1991. Control of rickettsial diseases. Eur J Epidemiol. 7: 282-286.

Kho KL, Koh FX, Tay ST. 2015. Molecular evidence of potential novel spotted fever group rickettsiae, *Anaplasma* and *Ehrlichia* species in *Amblyomma* ticks parasitizing wild snakes. Parasit Vectors. 8: 112.

Kielian M. 2014. Mechanisms of Virus Membrane Fusion Proteins. Annu Rev Virol. 1(1): 171-189.

Kihara Y, Satho T, Eshita Y, Sakai K, Kotaki A, Takasaki T, Rongsriyam Y, Komalamisra N, Srisawat R, Lapcharoen P, Sumroiphon S, Iwanaga S, Ushijima H, Endoh D, Miyata T, Sakata A, Kashige N, Miake F, Fukushi S, Saijo M, Kurane I, Morikawa S, Mizutani, T. 2007. Rapid determination of viral RNA sequences in mosquitoes collected in the field. J Virol Methods. 146: 372–374.

Kim DY, Guzman H, Bueno R, Dennett JA, Auguste AJ, Carrington CVF, Popov VL, Weaver SC, Beasley DWC. 2009. Characterization of Culex flavivirus (Flaviviridae) strains isolated from mosquitoes in the United States and Trinidad. Virology. 386: 154-159.

Kliks SC, Nimmanitya S, Nisalak A, Burke DS. 1988. Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. Am J Trop Med Hyg. 38: 411–419.

Kocan KM, de la Fuente J, Guglielmone AA, Melende´z RD. 2003. Antigens and alternatives for control of *Anaplasma marginale* infectionin cattle. Clin Microbiol Rev. 16: 698–712.

Kocan KM, de la Fuente J, Blouin EF, Coetzee JF, Ewing SA. 2010. The natural history of *Anaplasma marginale.* Vet Parasitol. 167(2-4): 95-107.

Kuhn RJ, Zhang W, Rossmann MG, Pletnev SV, Corver J, Lenches E, Jones CT, Mukhopadhyay S, Chipman PR, Strauss EG, Baker TS, Strauss JH. 2002. Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. Cell, 108(5): 717–725.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for Bigger Datasets. Mol Biol Evol. 33(7): 1870–1874.

Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB. 1998. Phylogeny of the genus Flavivirus. J Virol. 72(1): 73–83.

Kuo CC, Lin YF, Yao CT, Shih HC, Chung LH, Liao HC, Hsu YC, Wang HC. 2017. Tick-borne pathogens in ticks collected from birds in Taiwan. Parasit Vectors. 10: 587.

Kutsuna S, Kato Y, Takasaki T, Moi ML, Kotaki A, Uemeru H, Matono T, Fujiya Y, Mawatari M, Takeshita N, Hayamawa S, Kana- gawa S, Ohmagari N. 2014. Two cases of Zika fever imported from French Polynesia to Japan, December 2013 to January 2014. Euro Surveill. 19: 20683.

Kuwata R, Satho T, Isawa H, Yen NT, Phong TV, Nga PT, Kurashige T, Hiramatsu Y, Fukumitsu Y, Hoshino K, Sasaki T, Kobayashi M, Mizutani T, Sawabe K. 2013. Characterization of Dak Nong virus, an insect nidovirus isolated from *Culex* mosquitoes in Vietnam. Arch Virol. 158(11): 2273–2284.

Kuwata R, Sugiyama H, Yonemitsu K, Van Dung N, Terada Y, Taniguchi M, Shimoda H, Takano A, Maeda K. 2015. Isolation of Japanese encephalitis virus and a novel insect-specific flavivirus from mosquitoes collected in a cowshed in Japan. Arch Virol. 160(9): 2151–2159.

Kuwata R, Torii S, Shimoda H, Supriyono, Phichitraslip T, Prasertsincharoen N, Takemae H, Bautista R, Ebora V, Abella J, Dargantes AP, Hadi UK, Setiyono A, Baltazar ET, Simborio LT, Agungpriyono S, Jittapalapong S, Rerkamnuaychoke W, Hondo E, Maeda

K. 2020. Distribution of Japanese Encephalitis Virus, Japan and Southeast Asia, 2016-2018. Emerg Infect Dis. 26: 125–128.

Kuzmin IV, Hughes GJ, Rupprecht CE. 2006. Phylogenetic relationships of seven previously unclassified viruses within the family *Rhabdoviridae* using partial nucleoprotein gene sequences. J Gen Virol 87 (Pt8): 2323–2331.

Kwong JC, Druce JD, Leder K. 2013. Case report: Zika virus infection acquired during brief travel to Indonesia. Am J Trop Med Hyg. 89(3): 516–517.

Laras K, Sukri NC, Larasati RP, Bangs MJ, Kosim R, Djauzi WT, Master J, Kosasih H, Hartati S, Beckett C, Sedyaningsih ER, Beecham III HJ, Corwin AL. 2005. Tracking the re-emergence of epidemic chkingunya virus in Indonesia. Trans R Soc Trop Med Hyg. 99(2): 128-41.

Lee S, Owen KE, Choi HK, Lee H, Lu G, Wengler G, Brown DT, Rossmann MG, Kuhn RJ. 1996. Identification of a protein binding site on the surface of the alphavirus nucleocapsid and its implication in virus assembly. Structure. 4(5): 531–541.

Leparch-Goffart I, Nougairede A, Cassadou S, de Lamballerie X. 2014. Chikungunya in the Americas. Lancet. 383: 514.

Lestari CSW, Yohan B, Yunita A, Meutiawati F, Hayati RF, Trimarsanto H, Sasmono RT. 2017. Phylogenetic and evolutionary analyses of dengue viruses isolated in Jakarta, Indonesia. Virus Genes. 53 (6): 778-788.

Leung GH, Baird RW, Druce J, Anstey NM. 2015. Zika virus infection in Australia following a monkey bite in Indonesia. Southeast Asian J Trop Med Public Health. 46(3): 460–464.

Li D. 2013. A highly pathogenic new bunyavirus emerged in China. Emerg Microbes Infect. 2(1): e1.

Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler Transform. Bioinformatics 25: 1754-1760.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. and 1000 Genome Project Data Processing Subgroup. 2009. The Sequence alignment/map (SAM) format and SAMtools. Bioinformatics 25: 2078-2079.

Li QP, Xe XC. 1992. First isolation of new orbivirus (Banna) from ticks and infected cattle sera in Xingjiang. Endemic Dis Bull.7: 64–9.

Liu H, Li MH, Zhai YG, Meng WS, Sun XH, Cao YX, Fu SH, Wang HY, Xu LH, Tang Q, Liang GD. 2010. Banna virus, China, 1987–2007. Emerg Infect Dis. 16(3): 514–517.

Liu H, Gao XY, Fu SH, Li MH, Zhai YG, Meng WS, Sun XH, Lv Z, Wang HY, Shen XX, Cao YX, He Y, Liang GD. 2016. Molecular evolution of emerging Banna virus. Infect Genet Evol. 45: 250–255.

Litzba N, Schuffenecker I, Zeller H, Drosten C, Emmerich P, Charrel R, Kreher P, Niedrig M. 2008. Evaluation of the first commercial chikungunya virus indirect immunofluorescence test. J Virol Methods. 149: 175–9.

Loh SM, Gillett A, Ryan U, Irwin P, Oskam C. 2017. Molecular characterization of '*Candidatus* Borrelia tachyglossi' (family *Spirochaetaceae*) in echidna ticks, *Bothriocroton concolor.* Int J Syst Evol Microbiol. 67: 1075–80.

Low JG, Ooi EE, Vasudevan SG. 2017. Current Status of Dengue Therapeutics Research and Development. J Infect Dis. 215: S96-S102.

Lutomiah JJ, Mwandawiro C, Magambo J, Sang RC. 2007. Infection and vertical transmission of Kamiti river virus in laboratory bred *Aedes aegypti* mosquitoes. J Insect Sci. 7: 1–7.

Machado DC, Mondini A, dos Santos Santana V, Yonamine PT, Neto CF, Zanotto PM, Nogueira ML. 2012. First identification of Culex flavivirus (*Flaviviridae*) in Brazil. Intervirology. 55 (6): 475–483.

MacLeod JR, Gordon WS. 1983. Studies in tick-borne fever of sheep. I. Transmission by the tick, *Ixodes ricinus*, with a description of the disease produced. Parasitology. 25: 273–285.

Mackenzie JS, Lindsay MD, Coelen RJ, Broom AK, Hall RA, Smith DW. 1994. Arboviruses causing human disease in the Australasian zoogeographical region. Arch Virol. 136: 447—467.

Mackenzie JS, Gubler DJ, Petersen LR. 2004. Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. Nat Med. 10(12 Suppl): S98–S109.

Macnamara FN. 1954. Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. Trans R Soc Trop Med Hyg. 48: 139 –145.

Maeda K, Markowitz N, Hawley RC, Ristic M, Cox D, McDade JE. 1987. Human infection with *Ehrlichia canis*, a leukocytic rickettsia. N Engl J Med. 316(14): 853-856.

Marchette NJ, Garcia R, Rudnick A. 1969. Isolation of Zika virus from Aedes aegypti mosquitoes in Malaysia. Am J Trop Med Hyg. 18: 411–415.

Mansfield KL, Johnson N, Phipps LP, Stephenson JR, Fooks AR, Solomon T. 2009. Tick-borne encephalitis virus - a review of an emerging zoonosis. J Gen Virol. 90: 1781-94.

Margos G, Castillo-Ramirez S, Cutler S, Dessau RB, Eikeland R, Estrada-Peña A, Gofton A, Graña-Miraglia L, Hunfeld KP, Krause A, Lienhard R, Lindgren PE, Oskam C, Rudolf I, Schwartz I, Sing A, Stevenson B, Wormser GP, Fingerle V. 2020. Rejection of the name Borreliella and all proposed species comb. nov. placed therein. Int J Syst Evol Microbiol. 10.

McCrae AW, Kirya BG. 1982. Yellow fever and Zika virus epizootics and enzootics in Uganda. Trans R Soc Trop Med Hyg. 76: 552–562.

McHugh CP. 1994. Arthropods: Vectors of Disease Agents. Laboratory Medicine. 25(7): 429–437.

McSherry JA. 1982. Some medical aspect of the darien scheme: What is dengue?. Scott Med J. 27: 183-184.

Medeiros AS, Costa DMP, Branco MSD, Sousa DMC, Monteiro JD, Galvão SPM, Azevedo PRM, Fernandes JV, Jeronimo SMB, Araújo JMG. 2018. Dengue virus in *Aedes aegypti* and *Aedes albopictus* in urban areas in the state of Rio Grande do Norte, Brazil: Importance of virological and entomological surveillance. PLoS One. 13(3): e0194108.

Megawati D, Masyeni S, Yohan B, Lestarini A, Hayati RF, Meutiawati F, Suryana, K, Widarsa T, Budiyasa DG, Budiyasa N, Myint K, Sasmono RT. 2017. Dengue in Bali: Clinical characteristics and genetic diversity of circulating dengue viruses. PLoS Negl Trop Dis. 11(5): e0005483.

Melian EB, Hinzman E, Nagasaki T, Firth AE, Wills NM, Nouwens AS, Blitvich BJ, Leung J, Funk A, Atkins JF, Hall R, Khromykh AA. 2010. NS1′ of flaviviruses in the Japanese encephalitis virus serogroup is a product of ribosomal frameshifting and plays a role in viral neuroinvasiveness. J Virol. 84: 1641-1647.

Mendoza-Roldan JA, Colellla V, Lia RP, Nguyen VL, Barros-Battesti DM, Latta R, Dantas-Torres F, Otranto D. 2019. *Borrelia burgdorferi* (sensu lato) in ectoparasites and reptiles in southern Italy. Parasit Vectors. 12: 35.

Mertens P. 2004. The dsRNA viruses. Virus Res. 101: 3–13.

Miller S, Kastner S, Krijnse-Locker J, Bühler S, Bartenschlager R. 2007. The non-structural protein 4A of dengue virus is an integral membrane protein inducing membrane alterations in a 2K-regulated manner. J Biol Chem. 282(12): 8873–8882.

Ministry of Health of the Republic of Indonesia. Kemenkes RI. Profil kesehatan Indonesia tahun 2005. Jakarta: Kementerian Kesehatan RI; 2007.

Ministry of Health of the Republic of Indonesia. 2018. Indonesia health profile 2017. http://www.depkes.go.id/resources/download/pusdatin/profil-kesehatan-indonesia/indonesia-health-profile-2017.pdf.

Ministry of Agricultural of the Republic of Indonesia. 2018. Pig Production 2014-2018 In: Livestock and Animals Health Statistic 2018, p. 88. http://ditjenpkh.pertanian.go.id/userfiles/File/Buku_Statistik_2018__Final_ebook.pdf.

Ministry of Health of the Republic of Indonesia. 2019. INFODATIN. https://www.depkes.go.id/resources/download/pusdatin/infodatin/InfoDatin-Situasi-Demam-Berdarah-Dengue.pdf.

Miranda J, Mattar S, Gonzalez M, Lopez HR, Aleman A, Aponte J. 2019. First report of Culex flavivirus infection from *Culex coronator* (Diptera: *Culicidae*), Colombia. Virol J. 16: 1.

Miyake M. 1964. The pathology of Japanese encephalitis. A review. Bull World Health Organ. 30(2): 153-160.

Moore PR, Johnson PH, Smith GA, Ritchie SA, van der Hurk F. 2007. Infection and dissemination of dengue virus type 2 in *Aedes aegypti, Aedes albopictus*, and *Aedes scutellaris* from the Torres strait, Australia. J Am Mosq Control Assoc. 23: 383–810.

Morales-Betoulle ME, Pineda ML, Sosa SM, Panella N, Lopez MR, Cordon-Rosales C, Komar N, Powers A, Johnson BW. 2008. Culex flavivirus isolates from mosquitoes in Guatemala. J Med Entomol. 45: 1187-1190.

Mordecai EA, Caldwell JM, Grossman MK, Lippi CA, Johnson LR, Neira M, Rohr JR, Ryan SJ, Savage V, Shocket MS, Sippy R, Stewart Ibarra AM, Thomas MB, Villena O. 2019. Thermal biology of mosquito-borne disease. Ecol Lett. 22(10): 1690–1708.

Mulyatno KC, Kotaki T, Yotopranoto S, Rohmah EA, Churotin S, Sucipto TH, Amarullah IH, Wardhani P, Soegijanto S, Kameoka M. 2018. Detection and serotyping of Dengue

viruses in *Aedes aegypti* and *Aedes albopictus* (Diptera: *Culicidae*) collected in Surabaya, Indonesia from 2008 to 2015. Jpn J Infect Dis. 71(1): 58–61.

Musso D, Cao LVM, Gubler DJ. 2015. Zikavirus: following the path of dengue and chikungunya?. Lancet. 386: 243-244.

Musso D, Gubler DJ. 2016. Zika virus. Clin Microbiol Rev. 29: 487-524.

Musso D, Despres P. 2020. Serological Diagnosis of Flavivirus-Associated Human Infections. Diagnostics. 10: 302.

Nabeshima T, Nga PT, Guillermo P, Mdel CP, Yu F, Thuy NT, Trang BM, Hien NT, Nam VS, Inoue S, Hasebe F, Morita K. 2008. Isolation and molecular characterization of Banna virus from mosquitoes, Vietnam. Emerg Infect Dis. 14(8): 1276–1279.

Nabeshima T, Loan H, Inoue S, Sumiyoshi M, Haruta Y, Nga PT, Huoung V, 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. J Gen Virol. 90(Pt 4): 827-832.

Nakayama E, Tajima S, Kotaki A, Shibasaki K, Itokawa K, Kato K, Yamashita A, Sekizuka T, Kuroda M, Tomita T, Saijo M, Takasaki T. 2018. A summary of the imported cases of Chikungunya fever in Japan from 2006 to June 2016. J Travel Med. 25(1).

Ndung'u LW, Aguirre C, Rurangirwa FR, McElwain TF, McGuire TC, Knowles DP, Palmer GH. 1995. Detection of Anaplasma ovis infection in goats by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. J Clin Microbiol. 33(3): 675–679.

Neyts J, Leyssen P, De Clercq E. 1999. Infections with Flaviviridae. Verh K Acad Geneeskd Belg. 61(6): 661-699.

Newman CM, Cerutti F, Anderson TK, Hamer GL, Walker ED, Kitron UD, Ruiz MO, Brawn JD, Goldberg TL. 2011. Culex Flavivirus and West Nile Virus Mosquito Coinfection and

Positive Ecological Association in Chicago, United States. Vector-Borne Zoonotic Dis.11(8): 1099–1105.

Nieto NC, Foley JE, Bettaso J, Lane RS. 2009. Reptile infection with *Anaplasma phagocytophilum*, the causative agent of granulocytic anaplasmosis. J Parasitol. 95: 1165–70.

Niebylski ML, Peacock MG, Fischer ER, Porcella SF, Schwan TG. 1997. Characterization of an endosymbiont infecting wood ticks, *Dermacentor andersoni*, as a member of the genus *Francisella*. Appl Environ Microbiol. 63: 3933–40.

Nilsson K, Jaenson TGT, Uhnoo I, Lindquist O, Pettersson B, Uhlen M, Friman G, Pahlson C. 1997. Characterization of a spotted fever group rickettsia from *Ixodes ricinus* ticks in Sweden. J Clin Microbiol. 35: 243–247.

Nga PT, Mdel CP, Lauber C, Parida M, Nabeshima T, Yu F, Thuy NT, Inoue S, Ito T, Okamoto K, Ichinose A, Snijder EJ, Morita K, Gorbalenya AE. 2011. Discovery of the first insect nidovirus, a missing evolutionary link in the emergence of the largest RNA virus genomes. PLoS Pathog. 7(9): e1002215.

Noda H, Munderloh UG, Kurtti TJ. 1997. Endosymbionts of ticks and their relationship to *Wolbachia* spp. and tick-borne pathogens of humans and animals. Appl Environ Microbiol. 63: 3926–32.

Nouri S, Matsumura EE, Kuo YW, Falk BW. 2018. Insect-specific viruses: from discovery to potential translational applications. Curr Opin Virol. 33: 33–41.

Nowak M, Cieniuch S, Stanczak J, Siuda K. 2010. Detection of *Anaplasma phagocytophilum* in *Amblyomma flavomaculatum* ticks (Acari: *Ixodidae*) collected from lizard *Varanus exanthematicus* imported to Poland. Exp Appl Acarol. 51: 363–71.

Nuti M, Serafini DA, Bassetti D, Ghionni A, Russino A, Rombola P, Macri G, Lillini E. 1998. Ehrlichia infection in Italy. Emerg Infect Dis. 4: 663–665.

Obiegala A, Król N, Oltersdorf C, Nader J, Pfeffer M. 2017. The enzootic life-cycle of *Borrelia burgdorferi* (sensu lato) and tick-borne rickettsiae: an epidemiological study on wild-living small mammals and their ticks from Saxony, Germany. Parasit Vectors. 10: 115.

Ohashi N, Inayoshi M, Kitamura K, Kawamori F, Kawaguchi D, Nishimura Y, Naitou H, Hiroi M, Masuzawa T. 2005. *Anaplasma phagocytophilum*-infected ticks, Japan. Emerg Infect Dis. 11(11): 1780–1783.

Oliveira ARS, Strathe E, Etcheverry L, Cohnstaedt LW, McVey DS, Piaggio J, Cernicchiaro N. 2018. Assessment of data on vector and host competence for Japanese encephalitis virus: a systematic review of the literature. Prev Vet Med. 154: 71–89.

Olson JG, Ksiazek TG, Suhandiman, Triwibowo. 1981. Zika virus, a cause of fever in central Java, Indonesia. Trans R Soc Trop Med Hyg. 75: 389 –393.

Olson JG, Ksiazek TG, Gubler DJ, Lubis SI, Simanjuntak G, Lee VH, Nalim S, Juslis K, See R. 1983. A survey for arboviral antibodies in sera of humans and animals in Lombok, Republic of Indonesia. Ann Trop Med Parasitol. 77: 131–137.

Öncü C, Brinkmann A, Günay F, Kar S, Öter K, Sarıkaya Y, Nitsche A, Linton YM, Alten B, Ergünay K. 2018. West Nile virus, Anopheles flavivirus, a novel flavivirus as well as Merida-like rhabdovirus Turkey in field-collected mosquitoes from Thrace and Anatolia. Infect Genet Evol. 57: 36–45.

Ooi EE, Gubler DJ. 2008. Dengue in Southeast Asia: epidemiological characteristics and strategic challenges in disease prevention. Cad. Saúde Pública, Rio de Janeiro. 25: Sup 1: S115-S124.

Ortigao MR, Gubler DJ. 2020. Human Diseases Associated With Vectors (Arthropods in Disease Transmission), Ed: Edward T. Ryan, David R. Hill, Tom Solomon, Naomi E. Aronson, Timothy P. Endy in Hunter's Tropical Medicine and Emerging Infectious Diseases. 10: 1063-1069.

Paddock CD, Childs JE. 2003. *Ehrlichia chaffeensis*: a prototypical emerging pathogen. Clin Microbiol Rev. 16: 37-64.

Pancholi P, Kolbert CP, Mitchell PD, Reed KDJr, Dumler JS, Bakken JS, Telford SR 3rd, Persing DH. 1995. *Ixodes dammini* as a potential vector of human granulocytic ehrlichiosis. J Infect Dis. 172: 1007–12.

Panetta JL, Sima R, Calvani NED, Hajdusek O, Chandra S, Panuccio J, Slapeta J. 2017. Reptile-associated *Borrelia* species in the goanna tick (*Bothriocroton undatum*) from Sydney, Australia. Parasit Vectors 10: 616.

Parker RR, Kohls GM, Cox GW, Davis GE. 1939. Observations on an infectious agent from *Amblyomma maculatum*. Public Health Rep. 54: 1482–1484.

Parola P, Raoult D. 2001. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. Clin Infect Dis. 32: 897–928.

Parola P, Cornet JP, Sanogo YO, Miller RS, Thien HV, Gonzalez JP, Raoult D, Telford III S. R, Wongsrichanalai C. 2003. Detection of *Ehrlichia* spp., *Anaplasma* spp., *Rickettsia* spp., and other eubacteria in ticks from the Thai-Myanmar border and Vietnam. J Clin Microbiol. 41(4): 1600–1608.

Pastorino B, Sengvilaipaseuth O, Chanthongthip A, Vongsouvath M, Souksakhone C, Mayxay M, Thirion L, Newton PN, de Lamballerie X, Dubot-Pérès A. 2019. Low Zika Virus Seroprevalence in Vientiane, Laos, 2003-2015. Am J Trop Med Hyg. 100(3): 639–642.

Payne RC, Ward DE, Usman M, Rusli A, Djauhari D, Husein A. 1988. Prevalence of bovine haemoparasites in Aceh province of Northern Sumatra: Implications for imported cattle. Prev Vet Med. 6 (4): 275-283.

Peenen PV, Irsiana R, Saroso J, Shope S, Joseph P. 1975. First isolation of Japanese encephalitis virus from mosquitoes near Bogor, West Java, Indonesia. J Med Entomol. 12(5): 573-574.

Perkasa A, Yudhaputri F, Haryanto S, Hayati RF, Ma'roef CN, Antonjaya U, Yohan B, Myint KS, Ledermann JP, Rosenberg R, Powers AM, Sasmono RT. 2016. Isolation of Zika Virus from Febrile Patient, Indonesia. Emerg Infect Dis. 22: 924-5.

Peyrefitte CN, Rousset D, Pastorino BA, Pouillot R, Bessaud M, Tock F, Mansaray H, Merle OL, Pascual AM, Paupy C, Vessiere A, Imbert P, Tchendjou P, Durand JP, Tolou HJ, Grandadam M. 2007. Chikungunya virus, Cameroon, 2006. Emerg Infect Dis. 13(5): 768–771.

Philip RN, Casper EA, Anacker RL, Cory J, Hayes SF, Burgdorfer W, Yunker E. 1983. *Rickettsia bellii* sp. nov.: a tick-borne rickettsia, widely distributed in the United States, that is distinct from the spotted fever and typhus biogroups. Int J Syst Bacteriol. 33: 94–106.

Phongmany S, Rolain JM, Phetsouvanh R, Blacksell SD, Soukkhaseum V, Rasachack B. 2006. Rickettsial Infections and Fever, Vientiane, Laos. Emerg Infect Dis. 12: 256–62.

Pialoux G, Gauzere B-A, Jaureguiberry S, Strobel M. 2007. Chikungunya, an epidemic arbovirosis. Lancet Infect Dis. 7: 319–327.

Piesman J, Eisen L. 2008. Prevention of tick-borne diseases. Annu Rev Entomol. 53: 323–43.

Ponlawat A, Harrington LC. 2005. Blood feeding patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. J Med Entomol. 42: 844–910.

Porter KR, Tan R, Istary Y, Suharyono W, Sutaryo, Widjaja S, Ma'Roef C, Listiyaningsih E, Kosasih H, Hueston L, McArdle J, Juffrie M. 2004. A serological study of Chikungunya virus transmission in Yogyakarta, Indonesia: evidence for the first outbreak since 1982. Southeast Asian J Trop Med Public Health. 35(2): 408–415.

Prat CM, Flusin O, Panella A, Tenebray B, Lanciotti R, Leparc-Goffart I. 2014. Evaluation of commercially available serologic diagnostic tests for chikungunya virus. Emerg Infect Dis. 20: 2129–32.

Putra WG, Widyastuti SK, Batan IW. 2019. Case report: anaplasmosis and ehrlichiosis on Bali local dog. IMV. 8(4): 502-512.

Radolf JD, Caimano MJ, Stevenson B, Hu LT. 2012. Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. Nat Rev Microbiol. 10: 87–99.

Rahayu A, Saraswati U, Supriyati S, Kumalawati DA, Hermantara R, Rovik A, Daniwijaya EW, Fitriana E, Setyawan S, Ahmad RA, Wardana DS, Indriani C, Utarini A, Tantowijoyo W, Arguni E. 2019. Prevalence and distribution of Dengue virus in *Aedes aegypti* in Yogyakarta city before deployment of Wolbachia infected *Aedes aegypti.* Int J Environ Res Public Health. 16(10).

Ramadevi N, Roy P. 1998. Bluetongue virus core protein VP4 has nucleoside triphosphate phosphohydrolase activity. J Gen Virol. 79: 2475–2480.

Rampa R. 1982. A guide to the Genera of Mosquitoes (Diptera: *Culicidae*) of Thailand with Illustrated Keys, Biological Notes and Preservation and Mounting Techniques. Mosq Sys. 14.

Raoult D, Roux V. 1997. Rickettsioses as paradigms of new or emerging infectious diseases. Clin Microbiol Rev. 10: 694–719.

Rather IA, Parray HA, Lone JB, Paek WK, Lim J, Bajpai VK, Park YH. 2017. Prevention and Control Strategies to Counter Dengue Virus Infection. Front Cell Infect Microbiol. 7: 336.

Reboul TE, Boyer PH, Bergström S, Vial L, Boulanger N. 2018. Relapsing Fevers: Neglected Tick-Borne Diseases. Front Cell Infect Microbiol. 8: 98.

Richards AL, Ratiwayanto S, Rahardjo E, Kelly DJ, Dasch GA, Fryauff DJ, Bangs MJ. 2003. Serologic evidence of infection with ehrlichiae and spotted fever group rickettsiae among residents of Gag Island, Indonesia. Am J Trop Med Hyg. 68: 480–484.

Richards AL, Soeatmadji DW, Widodo MA, Sardjono TW, Yanuwiadi B, Hernowati TE, Baskoro AD, Roebiyoso, Hakim L, Soendoro M, Rahardjo E, Putri MP, Saragih JM, Strickman D, Kelly DJ, Dasch GA, Olson JG, Church CJ, Corwin AL. 1997. Seroepidemiologic evidence for murine and scrub typhus in Malang, Indonesia. Am J Trop Med Hyg. 57: 91−95.

Ripoll CM, Remondegui CE, Ordonez G, Arazamendi R, Fusaro H, Hyman MJ, Paddock CD, Zaki SR, Olson JG, Santos-Buch CA. 1999. Evidence of rickettsial spotted fever and ehrlichial infections in a subtropical territory of Jujuy, Argentina. Am J Trop Med Hyg. 61: 350– 354.

Rizzo F, Cerutti F, Ballardini M, Mosca A, Vitale N, Radaelli MC, Desiato R, Prearo M, Pautasso A, Casalone C, Acutis P, Peletto S, Mandola ML. 2014. Molecular characterization of flaviviruses from field-collected mosquitoes in northwestern Italy, 2011-2012. Parasite Vectors. 7: 395.

Robertson RG, Wisseman CL. 1973. Tick-borne rickettsiae of the spotted fever group in west Pakistan. II. Serological classification of isolates from west Pakistan and Thailand: evidence for two new species. Am J Epidemiol. 97: 55–64.

Robinson MC. 1955. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. I. Clinical features. Trans R Soc Trop Med Hyg. 49(1): 28-32.

Robinson JS, Featherstone D, Vasanthapuram R, Biggerstaff BJ, Desai A, Ramamurty N, Chowdhury AH, Sandhu HS, Cavallaro KF, Johnson BW. 2010. Evaluation of three commercially available Japanese encephalitis virus IgM enzyme-linked immunosorbent assays. Am J Trop Med Hyg. 83(5): 1146-1155.

Rodger LM. 1961. An outbreak of suspected Chikungunya fever in Northern Rhodesia. S Air Med. 35: 126-128.

Roiz D, Va´zquez A, Rosso F, Arnoldi D, Girardi M, Cuevas L, Perez-Pastrana E, Sa´nchez-Seco MP, Tenorio A, Rizzoli A. 2012. Detection of a new insect flavivirus and isolation of Aedes flavivirus in northern Italy. Parasit Vectors 5: 223.

Ross RW. 1956. The Newala epidemic. III. The virus: isolation, pathogenic properties and relationship to the epidemic. J Hyg (Lond). 54: 177–191.

Roy P. 1996. Orbivirus structure and assembly. J Virol. 216: 1-11.

Rudenko N, Golovchenko M, Grubhoffer L, Oliver JH. 2011. Updates on Borrelia burgdorferi sensu lato complex with respect to public health. Ticks Tick Borne Dis. 2: 123–8.

Sadeghi M, Popov V, Guzman H, Phan TG, Vasilakis N, Tesh R, Delwart E. 2017. Genomes of viral isolates derived from different mosquitos species. Virus Res. 242: 49–57.

Sahoo GC, Dikhit MR, Das P. 2008. Functional assignment to JEV proteins using SVM. Bioinformation. 3(1): 1-7.

Saiyasombat R, Bolling BG, Brault AC, Bartholomay LC, Blitvich BJ. 2011. Evidence of efficient transovarial transmission of Culex flavivirus by *Culex pipiens* (Diptera: *Culicidae*). J Med Entomol. 48(5): 1031–1038.

Sampath A, Padmanabhan R. 2009. Molecular targets for flavivirus drug discovery. Antiviral Res. 81: 6-15.

Sarih M, M'Ghirbi Y, Bouattour A, Gern L, Baranton G, Postic D. 2005. Detection and Identification of *Ehrlichia* spp. in Ticks Collected in Tunisia and Morocco. J Clin Microbiol. 43: 1127–32.

Sasmono RT, Wahid I, Trimarsanto H, Yohan B, Wahyuni S, Hertanto M, Yusuf I, Mubin H, Ganda IJ, Latief R, Bifani PJ, Shi PY, Schreiber MJ. 2015. Genomic analysis and growth characteristic of dengue viruses from Makassar, Indonesia. Infect Gene Evol. 32: 165-177.

Sasmono RT, Dhenni R, Yohan B, Pronyk P, Hadinegoro SR, Soepardi EJ, Ma'roef CN, Satari HI, Menzies H, Hawley WA, Powers AM, Rosenberg R, Myint K, Soebandrio A. 2018. Zika Virus Seropositivity in 1-4-Year-Old Children, Indonesia, 2014. Emerg Infect Dis. 24(9): 1740–1743.

Saxena SK, Tiwari S, Saxena R, Mathur A, Nair MPN. 2011. Japanese Encephalitis: an Emerging and Spreading Arbovirosis: In Flavivirus Encephalitis (Book), Daniel Ruzek (Ed.), In Tech, Croatia (European Union). 295-316.

Saxena SK, Singh A, Mathur A. 2000. Antiviral effect of nitric oxide during Japanese encephalitis virus infection. Int J Exp Pathol. 81(2): 165-172.

Scaramozzino N, Crance JM, Jouan A, De Briel DA, Stoll F, Garin D. 2001. Comparison of flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. J Clin Microbiol. 39(5): 1922–1927.

Schaffner F, Vazeille M, Kaufmann C, Failloux A-B, Mathis A. 2011. Vector competence of *Aedes japonicus* for chikungunya and dengue viruses. Eur Mosq Bull. 29: 141–2.

Schwan TG, Schrumpf ME, Hinnebusch BJ, Anderson DE, Konkel ME. 1996. GlpQ: an antigen for serological discrimination between relapsing fever and Lyme borreliosis. J Clin Microbiol. 34: 2483–2492.

Schuh AJ, Tesh RB, Barrett ADT. 2011. Genetic characterization of Japanese encephalitis virus genotype II strains isolated from 1951 to 1978. J Gen Virol. 92: 516–527.

Schuh AJ, Guzman H, Tesh RB, Barrett ADT. 2013. Genetic diversity of Japanese encephalitis virus isolates obtained from the Indonesian archipelago between 1974 and 1987. Vector Borne Zoonotic Dis. 13: 479–88.

Scott JD, Lee MK, Fernando K, Durden LA, Jorgensen DR, Mak S, Morshed MG. 2010. Detection of Lyme disease spirochete, *Borrelia burgdorferi* sensu lato, including three

novel genotypes in ticks (Acari: *Ixodidae*) collected from songbirds (*Passeriformes*) across Canada. J Vector Ecol. 35:124–39.

Seki M, Watanabe Y, Kawabata H. 2018. A case of Lyme disease in a Japanese woman. Infect Drug Resist. 11: 625–628.

Sendow I, Bahri S, Sarosa A. 2000. Japanese-B-Encephalitis prevalence in various animal Spesies di Indonesia. Jitv. 5: 276–279.

Setiati, TE, Wagenaar JFP, de Kruil MD, Mairuhu ATA, van Gorp ECM, Soemantri A. 2006. Changing Epidemiology of Dengue Haemorrhagic Fever in Indonesia. Dengue Bulletin. 30: 1-4.

Shu PY, Huang JH. 2004. Current advances in dengue diagnosis. Clin Diagn Lab Immunol. 11(4): 642–650.

Simmons CP, Farrar JJ, van Nguyen VC, Wills B. 2012. Dengue. N Engl J Med. 366: 1423–32.

Smith K, Kilborne FL. 1893. Investigations into the nature, causation and prevention of southern cattle fever. In: 8th and 9th Am. Rep. Bur. An. Ind., pp. 177–1893.

Smith WA. 2012. Dengue infections in travellers. Paediatr Int Child Health. 32: Suppl 1(s1) 28–32.

Snyder JE, Kulcsar KA, Schultz KL, Riley CP, Neary JT, Marr S, Jose J, Griffin DE, Kuhn RJ. 2013. Functional characterization of the alphavirus TF protein. J Virol. 87(15): 8511–8523.

Solomon T, Dung NM, Kneen R, Gainsborough M, Vaughn DW, Khanh VT. 2000. Japanese encephalitis. J Neurol Neurosurg Psychiatry. 68(4): 405-415.

Solomon T, Ni H, Beasley DW, Ekkelenkamp M, Cardosa MJ, Barrett AD. 2003. Origin and evolution of Japanese encephalitis virus in southeast Asia. J Virol. 77(5): 3091-3098.

Song S, Li Y, Fu S, Lei W, Guo X, Feng Y, Gao X, Li X, Yang Z, Xu Z, He Y, Wang H, Zhou H, Wang B, Lu X, Liang G. 2017. Genome sequencing and phylogenetic analysis of Banna virus (genus *Seadornavirus*, family *Reoviridae*) isolated from Culicoides. Sci China Life Sci. 60(12): 1372–1382.

Stanek G, Strle F. 2018. Lyme borreliosis-from tick bite to diagnosis and treatment. FEMS Microbiol Rev. 42: 233–58.

Steere AC, Strle F, Wormser GP, Hu LT, Branda JA, Hovius JW, Li X, Mead PS. 2016. Lyme borreliosis. Nat Rev Dis Primers. 2:16090.

Stollar V, Thomas VL. 1975. An agent in the *Aedes aegypti* cell line (Peleg) which causes fusion of *Aedes albopictus* cells. Virology. 64(2): 367–377.

Strle F, Nadelman RB, Cimperman J, Nowakowski J, Picken RN, Schwartz I, Maraspin V, Aguero-Rosenfeld ME, Varde S, Lotric-Furlan S, Wormser GP. 1999. Comparison of culture-confirmed erythema migrans caused by *Borrelia burgdorferi* sensu stricto in New York State and by *Borrelia afzelii* in Slovenia. Ann Intern Med. 130(1): 32-36.

Sumrandee C, Baimai V, Trinachartvanit W, Ahantarig A. 2016. Molecular detection of *Rickettsia, Anaplasma, Coxiella* and *Francisella* bacteria in ticks collected from Artiodactyla in Thailand. Ticks Tick Borne Dis. 7(5): 678-689.

Sung PY, Vaughan R, Rahman SK, Yi G, Kerviel A, Kao CC, Roy P. 2019. The Interaction of Bluetongue Virus VP6 and Genomic RNA Is Essential for Genome Packaging. J Virol. 93(5): e02023-18.

Sykes RA, Makiello P. 2016. An estimate of Lyme borreliosis incidence in western Europe. J Public Health. 39: 74–81.

Takada N, Fujita H, Yano Y, Tsuboi Y, Mahara F. 1994. First isolation of a rickettsia closely related to Japanese spotted fever pathogen from a tick in Japan. J Med Entomol. 31: 183–185.

Takano A, Goka K, Une Y, Shimada Y, Fujita H, Shiino T, Watanabe H, Kawabata H. 2010. Isolation and characterization of a novel *Borrelia* group of tick-borne borreliae from imported reptiles and their associated ticks. Environ Microbiol. 12: 134–46.

Takano A, Fujita H, Kadosaka T, Konnai S, Tajima T, Watanabe H, Ohnishi M, Kawabata H. 2011. Characterization of reptile-associated *Borrelia* sp. in the vector tick, *Amblyomma geoemydae*, and its association with Lyme disease and relapsing fever *Borrelia* spp.. Environ Microbiol Rep. 3: 632–7.

Tamura A, Ohashi AH. Urakami, Miyamura S. 1995. Classification of *Rickettsia tsutsugamushi* in a new genus, Orientia gen. nov., as *Orientia tsutsugamushi* comb. nov. Int J Syst Bacteriol. 45: 589–591.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol. 24: 1596–9.

Tan R, Nalim S, Suwasono H, Jennings GB. 1993. Japanese encephalitis virus isolated from seven species of mosquitoes collected at Semarang, Regency, Central Java. Bull. Penelit. Kesehat. 21, 1–5.

Tan HP, Dumler JS, Maley WR, Klein AS, Burdick JF, Fred PF, Thuluvath PJ, Markowitz JS. 2001. Human monocytic ehrlichiosis: an emerging pathogen in transplantation. Transplantation. 71: 1678–1680.

Tang SCN, Rusli M, Lestari P. 2019. Climate Variability and Dengue Hemorrhagic Fever in Surabaya, East Java, Indonesia. Preprints. 2019020161.

Tao SJ, Chen BQ. 2005. Studies of Coltivirus in China. Chin Med J. 118: 581-586.

Taylor RM. 1952. Studies on certain viruses isolated in the tropics of Africa and South America; their growth and behavior in the embryo- nated hen egg. J Immunol. 68: 473–494.

Theiler A. 1910. Gall sickness of South Africa (anaplasmosis of cattle). J Comp Pathol Ther. 23: 98-115.

Thenmozhi V, Paramasivan R, Samuel PP, Kamaraj T, Balaji T, Dhananjeyan KJ, Venkatasubramani K, Leo SV, Babu RS, Govindarajan R, Tyagi BK. 2013. Japanese encephalitis virus isolation from mosquitoes during an outbreak in 2011 in Alappuzha district, Kerala. J Vector Borne Dis. 50(3): 229–231.

Thomas SJ, Yoon IK. 2019. A review of Dengvaxia®: development to deployment. Hum Vaccin Immunother.15(10): 2295–2314.

Tosepu R, Effendy DS, Ahmad La OAI, Sakka A, Asfian P, Lestari H. 2015. The role of public health center in Indonesia. PHI. 1(2): 38–43.

Trape JF, Duplantier JM, Bouganali H, Godeluck B, Legros F, Cornet JP, Camicas JL. 1991. Tick-borne borreliosis in West Africa. Lancet. 337:473–5.

Tsetsarkin KA, Chen R, Sherman MB, Weaver SC. 2011. Chikungunya virus: evolution and genetic determinants of emergence. Curr Opin Virol. 1: 310–7.

Tuomi, J. 1967. Experimental studies on bovine tick-borne fever. 1. Clinical and haematological data, some properties of the causative agent, and homologous immunity. Acta Pathol Microbiol Scand. 70: 429–445.

Uchida T. 1993. Rickettsia japonica, the etiologic agent of Oriental spotted fever. Microbiol Immunol. 37: 91–102.

Uchil PD, 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. Am J Trop Med Hyg. 65: 242–251.

Utama I, Lukman N, Sukmawati DD, Alisjahbana B, Alam A, Murniati D, Utama I, Puspitasari D, Kosasih H, Laksono I, Karyana M, Karyanti MR, Hapsari M, Meutia N, Liang CJ,

Wulan WN, Lau CY, Parwati K. 2019. Dengue viral infection in Indonesia: Epidemiology, diagnostic challenges, and mutations from an observational cohort study. PLoS Negl Trop Dis. 13: e0007785.

Vaclav R, Ficova M, Prokop P, Betakova T. 2011. Associations between coinfection prevalence of *Borrelia lusitaniae*, *Anaplasma* sp., and *Rickettsia* sp. in hard ticks feeding on reptile hosts. Microb Ecol. 61: 245–53.

Van der Hurk AF, Richie SA, Mackenzie JS. 2009. Ecology and geographical expansion of Japanese Encephalitis virus. Annu Rev Entomol. 54: 17-35.

Vasilakis N, Durbin A, Rosa AP, Muñoz-Jordán JL, Tesh RB, Weaver SC. 2008. Antigenic relationships between sylvatic and endemic dengue viruses. Am J trop Med Hyg. 79 (1): 128-32.

Vasilakis N, Guzman H, Firth C, Forrester NL, Widen SG, Wood TG, Rossi SL, Ghedin E, Popov V, Blasdell KR, Walker PJ, Tesh RB. 2014. Mesoniviruses are mosquito-specific viruses with extensive geographic distribution and host range. Virol J. 11: 97.

Vashist S, Bhullar D, Vrati S. 2011. La protein can simultaneously bind to both 3'- and 5'-noncoding regions of Japanese encephalitis virus genome. DNA Cell Biol. 30(6): 339-346.

Vaughn DW, Hoke CH. 1992. The epidemiology of Japanese en- cephalitis: prospects for prevention. Epidemiol Rev. 14: 197–221.

Vrijmoeth HD, Ursinus J, Harms MG, Zomer TP, Gauw SA, Tulen AD, Kremer K, Sprong H, Knoop H, Vermeeren YM, van Kooten B, Joosten L, Kullberg BJ, Hovius J, van den Wijngaard CC. 2019. Prevalence and determinants of persistent symptoms after treatment for Lyme borreliosis: study protocol for an observational, prospective cohort study (Lyme Prospect). BMC Infect Dis. 19: 324.

Walker DH, Cain BG, Olmstead PM. 1978. Specific diagnosis of Rocky Mountain spotted fever by immunofluorescence demonstration of *Rickettsia rickettsii* in biopsy of skin. Public Health Lab. 36: 96–100.

Wang J, Zhang H, Sun X, Fu S, Wang H, Feng Y, Wang H, Tang Q, Liang GD. 2011. Distribution of Mosquitoes and Mosquito-Borne Arboviruses in Yunnan Province near the China-Myanmar-Laos Border. Am J Trop Med Hyg. 84:738-46.

Wang H, Liang, G. 2015. Epidemiology of Japanese encephalitis: past, present, and future prospects. Ther Clin Risk Manag. 11: 435-48.

Weaver SC, Reisen WK. 2010. Present and future arboviral threats. Antiviral Res. 85(2): 328–345.

Weaver SC. 2013. Urbanization and geographic expansion of zoonotic arboviral diseases: mechanisms and potential strategies for prevention. Trends Microbiol. 21(8): 360-363.

Weaver SC, Lecuit M. 2015. Chikungunya virus and the global spread of a mosquito-borne disease. N Engl J Med. 372: 1231–1239.

Webster CL, Waldron FM, Robertson S, Crowson D, Ferrari G, Quintana JF, Brouqui JM, Bayne EH, Longdon B, Buck AH, Lazzaro BP, Akorli J, Haddrill PR, Obbard DJ. 2015. The discovery, distribution, and evolution of viruses associated with *Drosophila melanogaster.* PLoS Biol. 13(7): e1002210.

Weiss E, Coolbaugh JC, Williams JC. 1975. Separation of viable *Rickettsia typhi* from yolk sac and L cell host components by renografin density gradient centrifugation. Appl Microbiol. 30: 456–463.

Wijaya A. 2007. Japanese encephalitis (JE) surveillance in Indonesia. Project Activities and Results (February 2007). pp. 1–2.

Wilke ABB, Beier JC, Benelli G. 2019. Complexity of the relationship between global warming and urbanizationan obscure future for predicting increases in vector-borne infectious diseases. Curr Opin Insect Sci. 35: 1-9.

Wilder-Smith A, Halstead SB. 2010. Japanese encephalitis: update on vaccines and vaccine recommendations. Curr Opin Infect Dis. 23: 426-31.

Woldehiwet Z. 2006. Anaplasma phagocytophilum in ruminants in Europe. Ann NY Acad Sci.1078: 446-460.

World Health Organization (WHO). 2007. Dengue: guidelines for diagnosis, treatment, prevention and control.

World Health Organization (WHO). 2016. Zika Virus and Guillain Barré Syndrome http://apps.who.int/iris/bitstream/10665/252650/1/zikasitrep22Dec2016-eng.pdf

World Health Organization (WHO). 2018. Zika virus infection: India, 2 November 2018. https://www.who.int/emergencies/diseases/zika/india-november-2018/en/

World Health Organization (WHO). 2019a. Japanese encephalitis reported cases. https://apps.who.int/immunization_monitoring/globalsummary/timeseries/tsincidencejapenc.html

World Health Organization (WHO). 2019b. https://www.who.int/csr/don/01-may-2019-chikungunya

World Health Organization (WHO). 2020. Vector-borne diseases. https://www.who.int/en/news-room/fact-sheets/detail/vector-borne-diseases.

Xia H, Liu H, Zhao L, Atoni E, Wang Y, Yuan Z. 2018. First isolation and characterization of a group C Banna virus (BAV) from *Anopheles sinensis* mosquitoes in Hubei, China. Viruses. 10(10).

Xu P, Wang Y, Zuo J, Lin J. 1990. New orbiviruses isolated from patients with unknown fever and encephalitis in Yunnan province. Chin J Virol. 6: 27–33.

Yabsley MJ. 2010. Natural history of *Ehrlichia chaffeensis*: vertebrate hosts and tick vectors from the United States and evidence for endemic transmission in other countries. Vet Parasitol. 167(2-4): 136–148.

Yamamoto S, Kawabata N, Tamura A, Urakami H, Ohashi N, Murata M, Yoshida Y. Kawamura A Jr. 1986: Immunological properties of *Rickettsia tsutsugamushi*, Kawasaki strain, isolated from a patient in Kyushu. Microbiol Immunol. 30: 611- 620.

Yamanaka A, Mulyatno KC, Susilowati H, Hendrianto E, Utsumi T, Amin M, Lusida MI, Soegijanto S. Konishi E. 2010. Prevalence of antibodies to Japanese encephalitis virus among pigs in Bali and East Java, Indonesia, 2008." Jpn J Infect Dis. 63(1): 58-60.

Yamanaka A, Tongrungkiat S, Ramasoota P, Konishi E. 2013. Genetic and evolutionary analysis of cell-fusing agent virus based on Thai strains isolated in 2008 and 2012. Infect Genet Evol. 19: 188–194.

Yamashita T, Unno H, Mori Y, Tani H, Moriishi K, Takamizawa A, Agoh M, Tsukihara T, Matsuura Y. 2008. Crystal structure of the catalytic domain of Japanese encephalitis virus NS3 helicase/nucleoside triphosphatase at a resolution of 1.8 A. Virology. 373: 426-436.

Yang J, Han R, Liu Z, Niu Q, Guan G, Liu G, Luo J, Yin H. 2017. Insight into the genetic diversity of Anaplasma marginale in cattle from ten provinces of China. Parasites & vectors. 10(1): 565.

Yin Z, Wang H, Yang J, Luo H, Li Y, Hadler SC, Sandhu HS, Fischer M, Jiang Y, Zhang Z, Liu G, Li L, Johnson BW, Liang X. Acute Meningitis and Encephalitis Syndrome (AMES) Study Group (2010). 2010 Japanese encephalitis disease burden and clinical features of Japanese encephalitis in four cities in the People's Republic of China. Am J Trop Med Hyg. 83(4): 766–773.

Yun SI, Lee YM. 2014. Japanese encephalitis: the virus and vaccines. Hum Vaccin Immunother. 10(2): 263-279.

Zhang JS, Zhao QM, Guo XF, Zuo SQ, Cheng JX, Jia N, Wu C, Dai PF, Zhao JY. 2011a. Isolation and genetic characteristics of human genotype 1 Japanese encephalitis virus, China, 2009. PLoS One. 6(1): e16418.

Zhang R, Hryc CF, Cong Y, Liu X, Jakana J, Gorchakov R, Baker ML, Weaver SC, Chiu W. 2011b. 4.4 Å cryo-EM structure of an enveloped alphavirus Venezuelan equine encephalitis virus. EMBO J. 30(18): 3854–3863.

Zhang Y, Lv Y, Zhang F, Zhang W, Wang J, Cui Y, Wang R, Jian F, Zhang L, Ning C. 2016. Molecular and phylogenetic analysis of *Anaplasma* spp. in sheep and goats from six provinces of China. J Vet Sci. 17: 523–9.

Zintl A, Moutailler S, Stuart P, Paredis L, Dutraive J, Gonzalez E, O'Connor J, Devillers E, Good B, Muireagain C, De Waal T, Morris F, Gray J. 2017. Ticks and Tick-borne diseases in Ireland. Ir Vet J. 70: 4.

Zou J, Xie X, Wang QY, Dong H, Lee MY, Kang C, Yuan Z, Shi PY. 2015. Characterization of dengue virus NS4A and NS4B protein interaction. J Virol. 89(7): 3455–3470.