SUPAPORN
WACHARAPLUESADEE

Laboratory Chief

Faculty of Medicine
Chulalongkorn University
Thailand

Dr. Supaporn Wacharapluesadee is the laboratory chief of Neuroscience Center for Research and Development and associate director of WHO Collaborating Centre for Research and Training on Viral Zoonoses, Faculty of Medicine, Chulalongkorn University, Bangkok Thailand. She received her undergraduate degree in Medical Technology at Chiang Mai University, earned her master's degree in Biochemistry at Mahidol University and her Ph.D. in Biomedical Science at Chulalongkorn University, Bangkok Thailand.

During 1994-1997, she worked in the malaria research laboratory at the Department of Entomology, Armed Forces Research Institute of Medical Sciences. In 1997, she worked at Chulabhorn Research Institute in cancer research areas. During 1998-2000, she worked in HIV research at The HIV/AIDS Collaboration Thai-USA. She started working at Chulalongkorn University in 2000, in the Neurology Division, Department of Medicine.

Dr. Wacharapluesadee's current research focus is in the area of viral encephalitis and zoonoses. She is a molecular biologist and field virologist. Her interests are on diagnostics, pathogenesis, and surveillance researches. She has developed several nucleic acid amplification methods for ante-mortem detection of rabies virus in humans and post-mortem diagnosis in animals. Doctor Wacharapluesadee and her colleagues are interested in the causes and mechanisms that differentiate rabies as furious or paralytic forms. She has developed the PCR protocols for Nipah virus detection from biological samples which is the most sensitive and reliable among others published protocol. Dr. Wacharapluesadee and her colleagues have engaged in wildlife research since 2002. Her group has found that Nipah virus infected bats in Thailand and demonstrated the seasonal prevalence of Nipah virus transmission in Pteropus lylei. This finding correlate with the seasonal outbreaks found in Bangladesh.

Dr. Wacharapluesadee's surveillance team included various scientific disciplines for example medical doctors, veterinarians, wildlife veterinarians, wildlife zoologists and scientists. The one health approach is being used in her current research. The collaboration involved the government sectors, academic sectors and the local community. She believed that the community or village-based initiation is the most powerful tool for the success of disease control and prevention, particularly with zoonoses.

ASSESSING THE RISK OF NIPAH VIRUS EMERGENCE
in Thailand

Supaporn Wacharapluesadee1, Nuttavadee Pamaranon2, Apai Suthisunk2, Noppawan Buamitoop2, Prateep Duengkae3, Patarapol Maneeorn4, Sanipa Suradhat5, Rachod Tantilertcharoen5, Pierre Rollin6, Henry Wilde1, Thiravat Hemachudha1

Since the initial outbreak of Nipah virus pneumonia/encephalitis in Malaysian abattoir pig workers in 1998 (Chua et al., 2000), there have been more than 25 human outbreaks in India and Bangladesh. Outbreaks in Bangladesh have occurred almost every year since 2001 and tended to be seasonal (Luby et al., 2009; Lo et al., 2012). Pteropid bats are the primary natural reservoirs (Yob et al., 2001; Halpin et al. 2011; Yadav et al. 2012). Their distribution ranges across the tropical regions of Asia and Africa. Viral transmission occurs among bats and spills over to other dead end host species including humans. Humans develop disease from having contact with virus contaminated tissues from infected pigs (Malaysia) (Chua, 2003) or otherwise by drinking bat-contaminated fresh date palm sap (Bangladesh) (Luby et al., 2006). Human-to-human transmission poses a serious threat and has been only documented in Bangladesh (Gurley et al., 2007).

To date there has been no report of pig or human cases of Nipah virus (NiV) infection in Thailand. However, there are several factors which could allow NiV emergence in Thailand. Thailand borders Malaysia where NiV outbreaks have been documented in 1998. Thailand harbors at least 20 million bats with 138 species among which several have been reported as NiV reservoirs. There are approximate 9.7 million pigs in this country and this has led to a preparedness initiative program. It consists of cooperation and joint activities among inter-sectoral organizations concerned with surveillance of wildlife, pigs and active testing for NiV infection in patients with encephalitis. Evaluation of health status and determination of risk behaviors as well as serology screening of residents in NiV prevalent zones are being conducted. This has been done in parallel with studies of bat ecology, behavior, population size and relation to seasons.

The Department of Livestock Development (DLD) under the Ministry of Agriculture and Cooperatives of Thailand has conducted sero-surveillance for Nipah infection in pigs since 1998. Regions 8th and 9th adjacent to Malaysia and regions 2nd and 7th

[1] WHO-CC for Research and Training on Viral Zoonoses, Faculty of Medicine, Chulalongkorn University Bangkok,
[2] Department of Livestock Development, Ministry of Agriculture and Cooperatives, Bangkok,
[3] Faculty of Forestry, Kasetsart University, Bangkok,
[4] Department of National Parks, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment,
[5] Center of Excellence in Emerging Infectious Diseases in Animals, Faculty of Veterinary Science, Chulalongkorn University (CU-EIDAs),
[6] Centers for Disease Control and Prevention, Atlanta, Georgia, USA

with high density of pigs were chosen from a total of 9 regions. Serum samples were collected from pigs at both farms and backyards for seroprevalence study. During 1998-2010, 44,658 pig sera were all negative by ELISA testing. In 2011, 3,352 tested pig sera from twenty (of 77) provinces were also negative. Passive surveillance was performed by postmortem examination of pig lung tissues with interstitial pneumonia. A total of 1,996 lung tissue specimens during 1998-2011 were all negative. This surveillance plan has been continually updated and adjusted, based on the previously obtained information. This included risk-based surveillance, according to the geographical location and seasonal preferences. For effective incident management and coordination of emergency responses, DLD has developed a Manual on Emergency Preparedness and Contingency Plan for NiV outbreaks. This manual has been distributed to the relevant DLD officers.

Further surveys of NiV infection in Thai bats is being conducted since 2002. Three species of flying foxes (genus Pteropus); P. lylei, P.hypomelanus, and P.vampyrus, have now been shown to be major reservoirs of NiV in Thailand (Wacharapluesadee et al., 2005). Although NiV infection has been found mainly in 3 species, P. lylei is dominant reservoir due to its population size and distribution. Furthermore, its habitat is closer to humans compared to the other two Pteropus species. Genetic characterization of NiV found in P. lylei in Thailand, based on 357 base pairs of nucleoprotein gene, indicated 2 existing genotypes: Bangladesh- and Malaysian- like (Wacharapluesadee and Hemachudha, 2007). Seasonal prevalence of NiV transmission in P. lylei has been shown during April-May (Wacharapluesadee et al., 2010). Although both strains are co-circulating, the Bangladesh-like genotype dominates in P.lylei bat populations.

Assessing the risk of Nipah virus emergence at the high risk areas, has been conducted in villagers, pigs, and bats at the areas where NiV infection has been demonstrated in bats. Bats were trapped monthly at two pig farms, using mist nets and harp traps. Blood, saliva and urine specimens were collected from each bat for further identification. Numbers of bats that were captured at the pig farms were highest in May, which is the weaning period that juvenile started to fly. Bats that were captured at pig farms were NiV positive only in May (by serology and/or viral RNA). The findings of seasonal preference in May were similar to our previous findings from pooled urine samples at bat roosts (Wacharapluesadee et al., 2010). The finding of NiV infected bats at the pig farms raises concern of possible NiV transmission to pigs. Although there were no evidence of NiV infection in pigs in the same study, measures to prevent bats from coming into contact with pigs and avoidance of feeding pigs with partially eaten fruits must be actively promoted.

To ensure that there is no human transmission of NiV, a community in Chonburi province located near a temple inhabited by NiV infected bats (P.lylei), was chosen for community-level study. After obtaining written consent, a standard questionnaire which included questions related to potential risk behaviors was administered to 418 local residents. Blood samples were assayed for NiV antibody using an indirect ELISA for IgG antibodies against NiV-infected cell lysate. Positive criteria for NiV infection was a dilution ≥1:400. No NiV-specific IgG antibodies were found. The low NiV prevalence in P.lylei (9.3%) and different culture of not drinking fresh date palm sap may have negative impact upon emergence of infection in Thailand. This is in

contrast with the finding in Bangladesh. People living in risk regions are being educated to avoid risk behavior and monitored continuously concerning symptoms possibly related to NiV infection. Febrile patients and those with respiratory or encephalitis symptoms living in or near NiV zones require particular attention. To date, transmission to human has not been identified in Thailand. Cerebrospinal fluid of 232 patients with encephalitis admitted to King Chulalongkorn Memorial Hospital during 2001-2012, were tested negative for NiV RNA.

Inter-sectoral cooperation among wildlife-animal-human departments in preparedness response to emergence of NiV outbreak in Thailand is a successful example of a transboundary "one health" approach. Establishment of alert system and promotion of awareness from the bottom level (village-based) is also the key of the success for this one health initiative. The ultimate goal of the whole process cannot be achieved without public recognition and willingness, otherwise this cannot be sustainable. Policy makers must value this as an opportunity in strengthening public health infrastructure.

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Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, et al. Nipah virus: a recently emergent deadly paramyxovirus. Science. 2000; 288:1432-5.

Chua KB. Nipah virus outbreak in Malaysia. J Clin Virol. 2003; 26:265-75.

Gurley ES, Montgomery JM, Hossain MJ, Bell M, Azad AK, Islam MR, Molla MA, Carroll DS, Ksiazek TG, Rota PA, Lowe L, Comer JA, Rollin P, Czub M, Grolla A, Feldmann H, Luby SP, Woodward JL, Breiman RF. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infect Dis. 2007 Jul;13(7):1031-7.

Halpin K, Hyatt AD, Fogarty R, Middleton D, Bingham J, Epstein JH, et al. Pteropid bats are confirmed as the reservoir hosts of henipaviruses: a comprehensive experimental study of virus transmission. Am J Trop Med Hyg. 2011; 85: 946-51.

Lo MK, Lowe L, Hummel KB, Sazzad HM, Gurley ES, Hossain MJ, et al. Characterization of Nipah virus from outbreaks in Bangladesh, 2008-2010. Emerg Infect Dis. 2012; 18:248-55.

Luby SP, Rahman M, Hossain MJ, Blum LS, Husain MM, Gurley E, et al. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis. 200; 12:1888-94.

Luby SP, Hossain MJ, Gurley ES, Ahmed BN, Banu S, Khan SU, et al. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007. Emerg Infect Dis. 2009; 15:1229-35.

Wacharapluesadee S, Lumlertdacha B, Boongird K, Wanghongsa S, Chanhome L, Rollin P, et al. Bat Nipah virus, Thailand.Emerg Infect Dis. 2005;11:1949-51.

Wacharapluesadee S, Hemachudha T. Duplex nested RT-PCR for detection of Nipah virus RNA from urine specimens of bats. J Virol Methods. 2007; 141:97-101.

Wacharapluesadee S, Boongird K, Wanghongsa S, Ratanasetyuth N, Supavonwong P, Saengsen D, et al. A Longitudinal Study of the Prevalence of Nipah Virus in Pteropus lylei Bats in Thailand: Evidence for Seasonal Preference in Disease Transmission. Vector Borne Zoonotic Dis. 2010; 10:183-90.8.

Yadav PD, Raut CG, Shete AM, Mishra AC, Towner JS, Nichol ST, et al. Detection of Nipah Virus RNA in Fruit Bat (Pteropus giganteus) from India. Am J Trop Med Hyg. 2012; 87:576-8.

Yob JM, Field H, Rashdi AM, Morrissy C, van der Heide B, Rota P, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis. 2001; 7:439-41.