TRACKING EMERGING PATHOGENS IN CAMBODIA WITH "ONE HEALTH" PARADIGM

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FOR RESEARCH, FOR HEALTH,



nniversai

Definition

Emerging infectious disease

Newly identified or previously unknown infectious agents that cause public health problems either locally or internationally

One Health concept

Strategy for expanding interdisciplinary collaborations and communications in all aspects of health care for humans, animals and the environment. More than 60% of emerging diseases are zoonoses More than 70% of emerging epidemics are caused by pathogens which originated in wild animals



Factors favoring emergence and transmission in Southeast Asia

- Close contact between human and livestock populations
 - → provides excellent conditions for the frequent exchange of pathogens between animal and human populations.
- High human and animal density
- Globalization of trade
- Geographical position
- Warm and humid climate
- Richness in wildlife host species
- Socio-economic situation and the socio-cultural practices

Bordier & Roger 2013 Jones et al 2008

Emerging Infectious Diseases 1940 – 2004

Viruses, Bacteria, Rickettsiae, Vivax, Zoonotic, Vector Borne and Resistance

Zoonotic pathogens from wild life

Zoonotic pathogens from non wild life



Drug resistant pathogens

Vector borne pathogens

Jones et al. Nature, 2008

The global threat of infectious diseases



- Chikungunya virus
- H7N9 avian influenza
- Pathogens for tomorrow in SEA (Avian reassortants/recombinants, West Nile virus, SFTS bunyavirus, MERS coronavirus, other zoonotic viruses, resistant micro-organisms ?



Key Tasks in Dealing with Emerging Diseases

- Surveillance at national, regional, global level
 - clinical/epidemiological
 - laboratory
 - ecological
 - anthropological
- Investigation and early control measures
- Implement prevention measures
 - behavioural, political, environmental
- Monitoring, evaluation

A mystery disease in Cambodia

- June 2012, Cambodia
- Health / political



Dr Beat Richner, Khanta Bopha hospital



Emergence of enterovirus EV71 in 2012: a model of transversal collaborative study in the region (Cambodia, China, Hong Kong, Vietnam, France)

- 1st cases probably in April 2012 but confirmation (after testing for >40 pathogens) in June 2012
- EV71: Close phylogenetic relationship with strains sequenced in China 2009-2011, and Vietnam 2012
- Comparative virological, molecular and immunological studies: Patients presenting with encephalitis + pulmonary edema, encephalitis, or mild HFMD
- Assess the CFR; look for the R0

(Pasteur and ADB support)





Tracking emerging pathogens in Southeast Asia





Credit: A. Tarantola

Clinical and epidemiological studies

- Clinicians with expertise on infectious diseases
- Ward and emergency room of ID clinic
- Clinical research unit
- Foci and/or outbreaks
- Human epi and clinical aspects
- Very close collaboration in the field :
 - with Eco-epidemiologists
 - with Health authorities
 - National (MoH)
 - International (WHO, FAO)



Laboratory diagnosis and new pathogens discovery

- Microbiological diagnosis of known pathogens
- Provide the clinicians a laboratory diagnosis in a timely manner for the microorganisms that can be treated or that are the most frequent







General strategy of laboratory diagnosis



 Implement the appropriate diagnostic tests (1st line diagnostics, including commercial RDTs) in order to provide the clinicians with a diagnostic in a timely manner for all the infections that can be treated



General strategy of laboratory diagnosis



• **Detection of unusual/unknown pathogens** using various (combined) approaches: pan-generic PCRs, cell cultures, high throughput sequencing, IGM/IgM ELISA *etc*.



PCR vs HTS



General strategy of laboratory diagnosis

- Develop new diagnostic tools : new sensitive and accessible serological, molecular, virological, etc., methods to detect the new microorganisms
- **Characterize new pathogens** with the involvement of experts from various fields: genomics, proteomics, pathophysiology, immunology, epidemiology, ecology, entomogy, zoology, *etc*.









Vientiane November 6, 2013

Vision of IPC on « One Health » approach

- > Mobilize and coordinate forces present in SEA to :
 - develop our knowledge, particularly in the anticipation, early detection, containment and control of zoonoses, and emerging disease;
 - understand the causes and the mechanisms behind emergences and the crossing of the inter-species barrier;
 - analyze the consequences of the emergences on the ecological, social, political and economic outcomes.



Tracking zoonotic (wild life) viruses

- Identify in high-risk areas the spillover of well-known as well as novel viruses in high-risk animal species (bats, rodents) which may constitute a risk to the human population (PEDICT Project; EU One Health Project)
- Thousands of samples from Laos and Cambodia tested for: flaviviruses, henipaviruses, coronaviruses, astroviruses, lyssaviruses, filoviruses, paramyxoviruses, hantaviruses, arenaviruses, reoviruses, influenza viruses, enteroviruses, ...







Tracking known virus: Nipah virus

- Genus *Henipavirus*
 - Newly discovered virus
 - Related to Hendra virus
 - Transmitted by Pteropterus bats





- Severe, rapidly progressive encephalitis in humans
 - High mortality rate

First isolated in Malaysia in 1998

- Close contact with infected pigs

Endemic diseases in Bengladesh since 2001

- Palm sap drinking
- Human to human transmission





Isolation of Nipah virus in Cambodia



Isolation in 2004

Reynes et al ., EID 2005

Flying foxes ecology and risk of Nipah Emergence



Rural market



Battabang province



Case/Cluster field investigation



















Sample collection Serology PCR Isolation Sequencing Bat population dynamics Population behavior Potential routes of contamination

> Multidisciplinary General database

Credit: J. Cappelle

Ecological and environmental variables

Determinants Indicators

- Water Water Level
- Human Density
- Vegetation
- Associated environmental indicators by remote sensing
- Association of season set of symptoms Landscape indicators



Credit: J. Cappelle

Cappelle et al, EcoHealth 2010

Tracking unknown viruses in bats

More than 70 viruses were characterized in 74 bat species (FAO, 2011)

✤Lyssavirus : rabdoviruses (EBLV, Lagos bat virus, ABLV, ...)

- Henipavirus : Hendra and Nipah viruses
- **Coronavirus :** SARS virus and other Coronaviruses
- **Filovirus** : Ebola and Marburg viruses
- ✤ 7 families of chiroptera are known in Cambodia (50 sp.)

SO/ Microchiropera	F/Pteropodidae**	« Flying foxes »
SO/ Megachiroptera	F/ Emballonuridae	« sheath-tailed bats »
	F/Megadermatidae	« False vampires »
	F/Molossidae**	« Free-tailed bats »
	F/Rhinolophidae	« Horsehoes bats» « Mustached-bats »
	SF/ Hipposideridae	« Old World Leaf-nosed Bats »
	F/Vespertilionidae**	



Pteropus lylei



rhinolophus borneensis



Kerivoul a titania



Detection of viruses in bats by molecular analysis

Samples collected by WCS

→ 3 types of PCR:
→ Nested RT- PCR
→ SYBR Green RT-PCR
→ RT-PCR Taqman

	Bats					
	Virus	Brain	O/R swabs	Organs	Urine	Feces
Alphavirus						
Arenavirus	х		Х	Х	х	Х
Astrovirus	х		Х			Х
Bunyaviridae						
Coronavirus	x		Х	Х		
Filovirus	х	Х	Х	Х	Х	Х
Flavivirus	х	Х	Х	Х	Х	
Hantavirus						
Henipavirus	х		Х	Х	Х	Х
Herpesviridae						
Influenza A	х		Х			
Lyssavirus*	х	Х	Х		Х	
Orthoreovirus						
Paramyxovirus	х	Х	Х	Х	Х	
Poxviridae						
Retrovirus						
Rhabdoviridae	х	Х	Х		60 ème	
seadornavirus			Х	Х	Institut	Pasteur

PREDICT project (USAID)

Viruses detected so far

- Samples collected by WCS
- About 1260 chiropterus in Cambodia





- 3 families of viruses detected so far:
- →Coronavirus
- \rightarrow Astrovirus
- → Paramyxovirus





Viruses detected so far

- ✤ 3 families of viruses detected in all families of chiropterus
 - \rightarrow 70 samples coronavirus + (69 partially sequenced)
 - \rightarrow 75 samples astrovirus + (62 partially sequenced)
 - \rightarrow 3 paramyxovirus + (2 partially sequenced)
- mainly Pteropodidae (17) and Vesperdilionidae (13)



Localisation of positive cases in Cambodia



Sequence characterization

Partial phylogenetic analysis:

→Probable Co-evolution of AstroV with their host species

 \rightarrow Sequences quite distant from those of human astroviruses

→Biais : low representativity of sequences in terms of geographic origins (mainly China), lack of sequences available

 \rightarrow Small portion of the genome sequenced

♦NEXT: High throughput sequencing using Roche 454 (1M sequences /run) after cell cultures concentration



Virus isolation on cell culture

✤ Isolation tested on cell lines: sur lignées VERO6, LLCMK2, BHK, C6/36 (2011)

Cytopathic effect observed but no virus identified

 Culture of chyropterus cells (CSIRO)
 Pteropus alecto (kidney: <u>PaKi</u>, lung: PaLu, spleen: PaSp, Fœtus: <u>NBC</u>)
 Miniopterus schreibersii (MsKi and MsLu)

Some RT-PCR positive samples tested positive on cell supernatant by RT-PCR

 Culture of primary cells from local bat species

Ongoing



Tracking unknown viruses in rodents

+25 species of rodents in Cambodia

Familly	Muridae	Hystricidae	Sciuridés	
Cambodia	18	1	7	



Bandicota indica (Herbreteau)



Mus caroli (Herbreteau)



Porc-épic Hystrix brachyura



Tamiops rodolphii

Hantavirus : Hantavirosis with kidney and pulmonary syndrome

→ <u>Hantavirus</u> detected in Cambodia (*Rattus sp. Bandicota sp., Maxomoys*) (*Reynes et al., 2003; Blasdell et al., 2009*)

◆Arenavirus: Lympho-choriomeningitis virus (LCMV), others?
 → Circulation of LCMV in China and Thailand (serology) (Mus musculus, B. savilei et R. norvegicus) (Juan, 2013).



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Creation of a Regional Platform for a multidisciplinary study of communicable infectious diseases and emerging pathogens

- To develop an competitive technical platform for the surveillance of emerging infectious diseases in Cambodia and in the SEA region
- To develop an integrated approach of new problematics in human and animal health with « One Health » dimension
- To increase the competitivity, efficacy, and innovation by fostering a multidisciplinary and cross-sectoral research (microbiology, clinic, ecology, environment, socio-economic, epidemiology) with external partners, and by offering an attracting scientific environment
- To develop the **training** capacity in all domains of infectious diseases

Creation of a regional platform for research on communicable infectious diseases and emerging pathogens in Southeast Asia







Major partners supporting the creation of the Platform

sviesan

Networking, dynamic, promotion, coordination, harmonisation, valorisation, transdisciplinarity



Mobilisation, animation of brain storming, programmation, training expertise, networking in the South





Institut de recherche pour le développement









