TRACKING EMERGING PATHOGENS IN CAMBODIA WITH “ONE HEALTH” PARADIGM

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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

• 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)
Antimalarial drug resistance
Or...TB, Flu, Typhoid, HIV, EID........
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


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

**PCR**
- Short amplicon
- Conserved region
- Specific amplification

**HTS**
- Whole genome
- Conserved and variable regions
- Random amplification

Picornaviridae
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, entomology, zoology, etc.
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 Bangladesh 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

Credit: J. Cappelle
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
Determinants

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

Indicators

- Water Level

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.)

### Family Table

<table>
<thead>
<tr>
<th>Family</th>
<th>Subfamily</th>
<th>Common Name</th>
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<tbody>
<tr>
<td>SO/Microchiropera</td>
<td>F/Pteropodidae**</td>
<td>« Flying foxes »</td>
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<tr>
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<td>F/ Emballonuridae</td>
<td>« sheath-tailed bats »</td>
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<td>F/Megadermatidae</td>
<td>« False vampires »</td>
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<td></td>
<td>F/Rhinolophidae</td>
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<td>« Mustached-bats »</td>
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<td>SF/ Hipposideridae</td>
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<td>« Old World Leaf-nosed Bats »</td>
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<tr>
<td>F/Vespertilionidae**</td>
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</table>

*Pteropus lylei*  
*rhinolophus borneensis*  
*Kerivoul a titania*  
*myotis muricola*
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

<table>
<thead>
<tr>
<th>Virus</th>
<th>Brain</th>
<th>O/R swabs</th>
<th>Organs</th>
<th>Urine</th>
<th>Feces</th>
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</table>
Viruses detected so far

- Samples collected by WCS
- About 1260 chiropterus in Cambodia

→ 6 families of chiropterus identified

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

- Pteropodidae: 42%
- Rhinolophidae: 7%
- Emballonuridae: 6%
- Hipposideridae: 5%
- Chiropteridae: 36%
- Vespertilionidae: 1%
- Phyllostomidae: 2%
Viruses detected so far

3 families of viruses detected in all families of chiroptera

→ 70 samples coronavirus + (69 partially sequenced)
→ 75 samples astrovirus + (62 partially sequenced)
→ 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
  - Sequences quite distant from those of human astroviruses
  - Biais: low representativity of sequences in terms of geographic origins (mainly China), lack of sequences available
  - 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)
  > 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

<table>
<thead>
<tr>
<th>Family</th>
<th>Muridae</th>
<th>Hystricidae</th>
<th>Sciuridés</th>
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<tbody>
<tr>
<td>Cambodia</td>
<td>18</td>
<td>1</td>
<td>7</td>
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</table>

- **Hantavirus**: Hantavirosis with kidney and pulmonary syndrome
  - Hantavirus detected in Cambodia (*Rattus sp.*, *Bandicota sp.*, *Maxomoyoys*) (Reynes *et al.*, 2003; Blasdell *et al.*, 2009)

- **Arenavirus**: Lympho-choriomeningitis virus (LCMV), others?
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UNION EUROPÉENNE
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 competitiveness, 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

PR-Asia
Major partners supporting the creation of the Platform

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

Mobilisation, animation of brainstorming, programmation, training expertise, networking in the South