Surveillance of Emerging Infectious Diseases in Wildlife Trade to Increase Awareness for Zoonoses Prevention and Wildlife Conservation

conduct by

APEIR Wildlife Research Team

present by: Assist Prof.Witthawat Wiriyarat (Co-PI of Thai team)

Faculty of Veterinary Science, Mahidol University

Member countries and PI

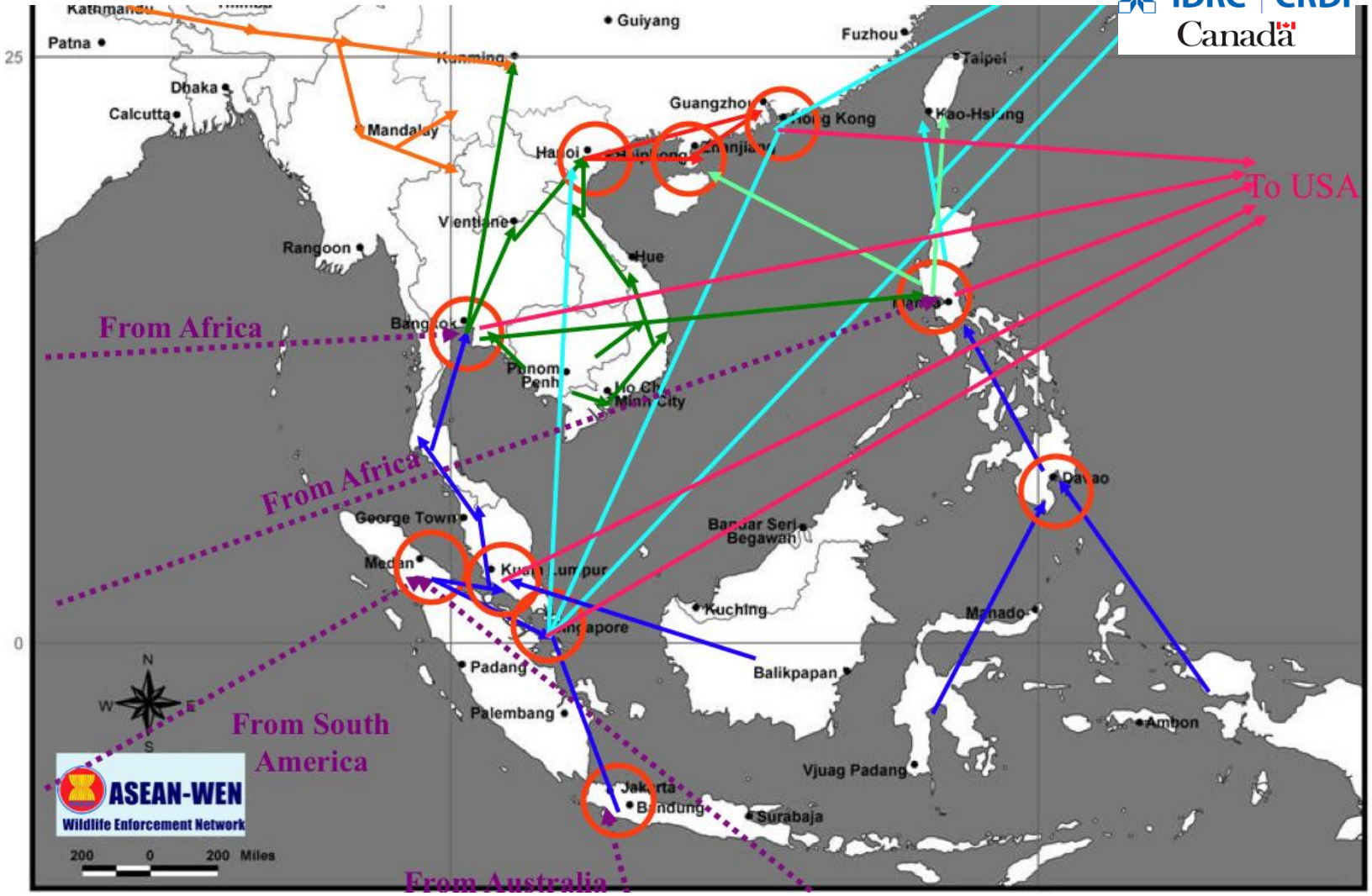
- Cambodia
  - Cheang Dany
- China
  - Prof. Lei Fumin
- Lao PDR
  - Dr. Sithong Phiphakhavong
- Thailand
  - Assoc.Prof. Parntep Ratanakorn
- Vietnam
  - Dr. Le Manh Hung

APEIR Wildlife Research Team

Background and rationale

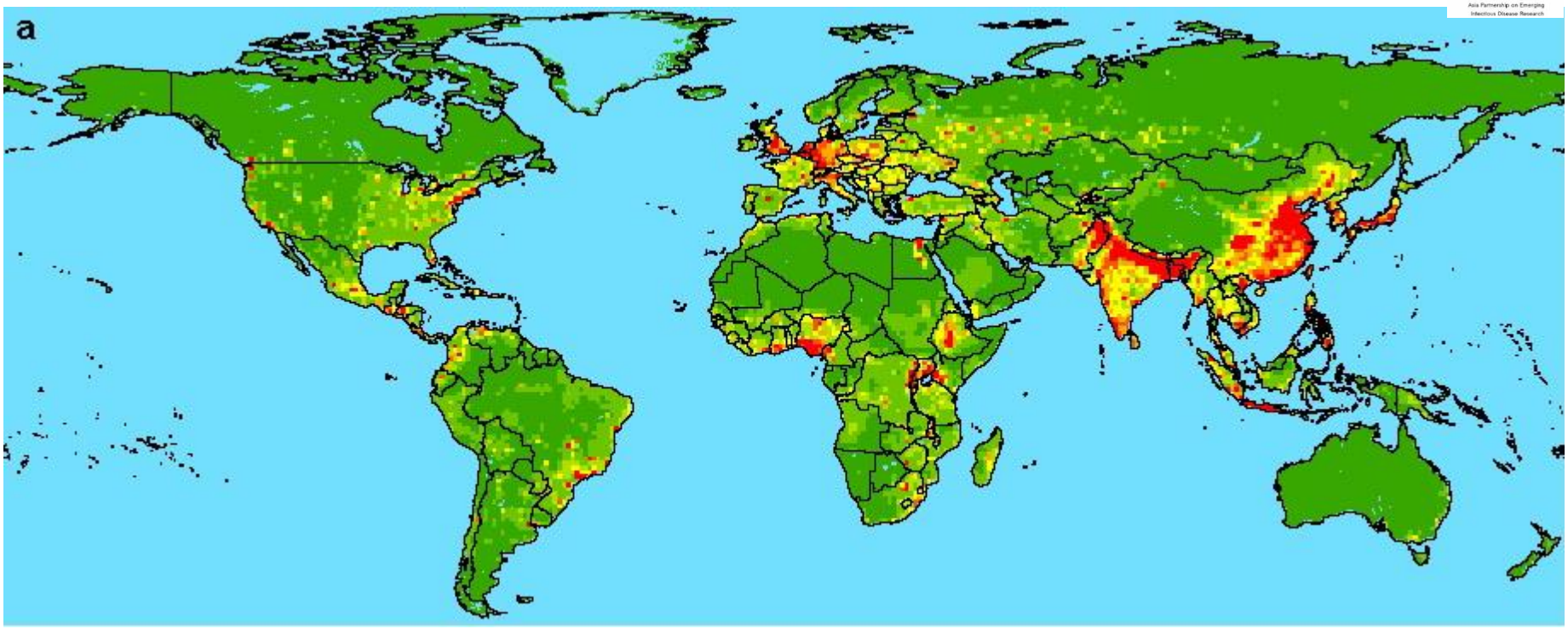
- Wildlife trade may provide mechanisms for disease transmission at levels that not only cause human disease outbreaks but also threaten livestock, international trade, rural livelihoods, native wildlife populations, and ecosystem health.

- China and Southeast Asia are wildlife trade hotspots, functioning as supplier, consumer and a general import-export wildlife.



Common illegal wildlife trade routes in SE Asia based on case studies

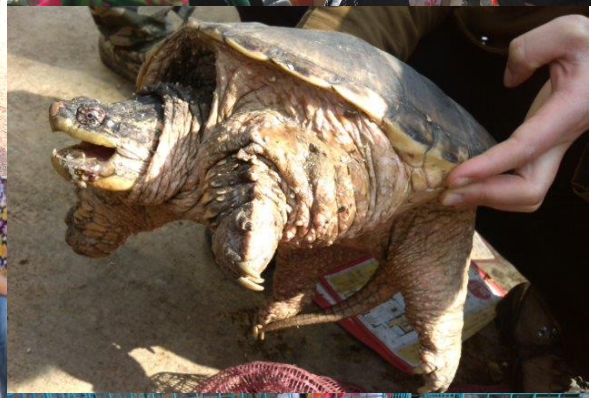
(Source; ASEAN Wildlife Enforcement Network(ASEAN-WEN)(www.asean-wen.org))



Map of zoonotic pathogens from wildlife, shown from lowest occurence (green) to highest (red)

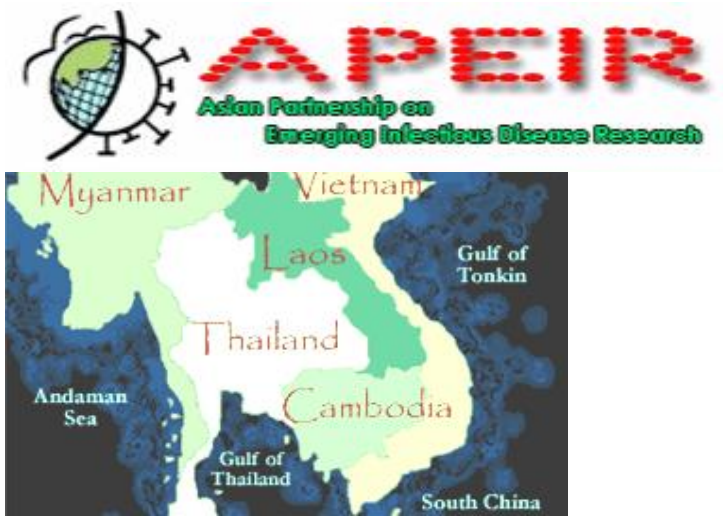
Picture from http://www.columbia.edu/cu/news/08/02/hotspots.html

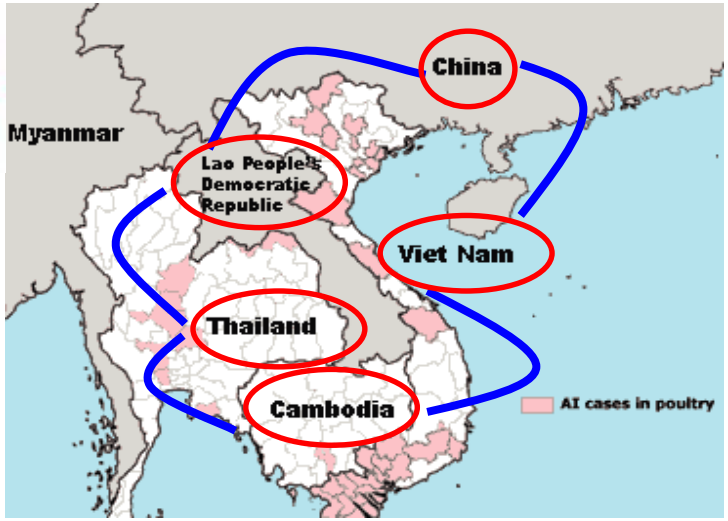
Bush meat and wildlife product



Project goal

- To demonstrate the zoonosis situation in wildlife trade in the region and to raise awareness for zoonosis prevention in wildlife trade in order to improve human and animal health and to contribute to wildlife conservation efforts.





Objectives

1. To estimate the degree of zoonotic pathogens in wildlife trade within the region.

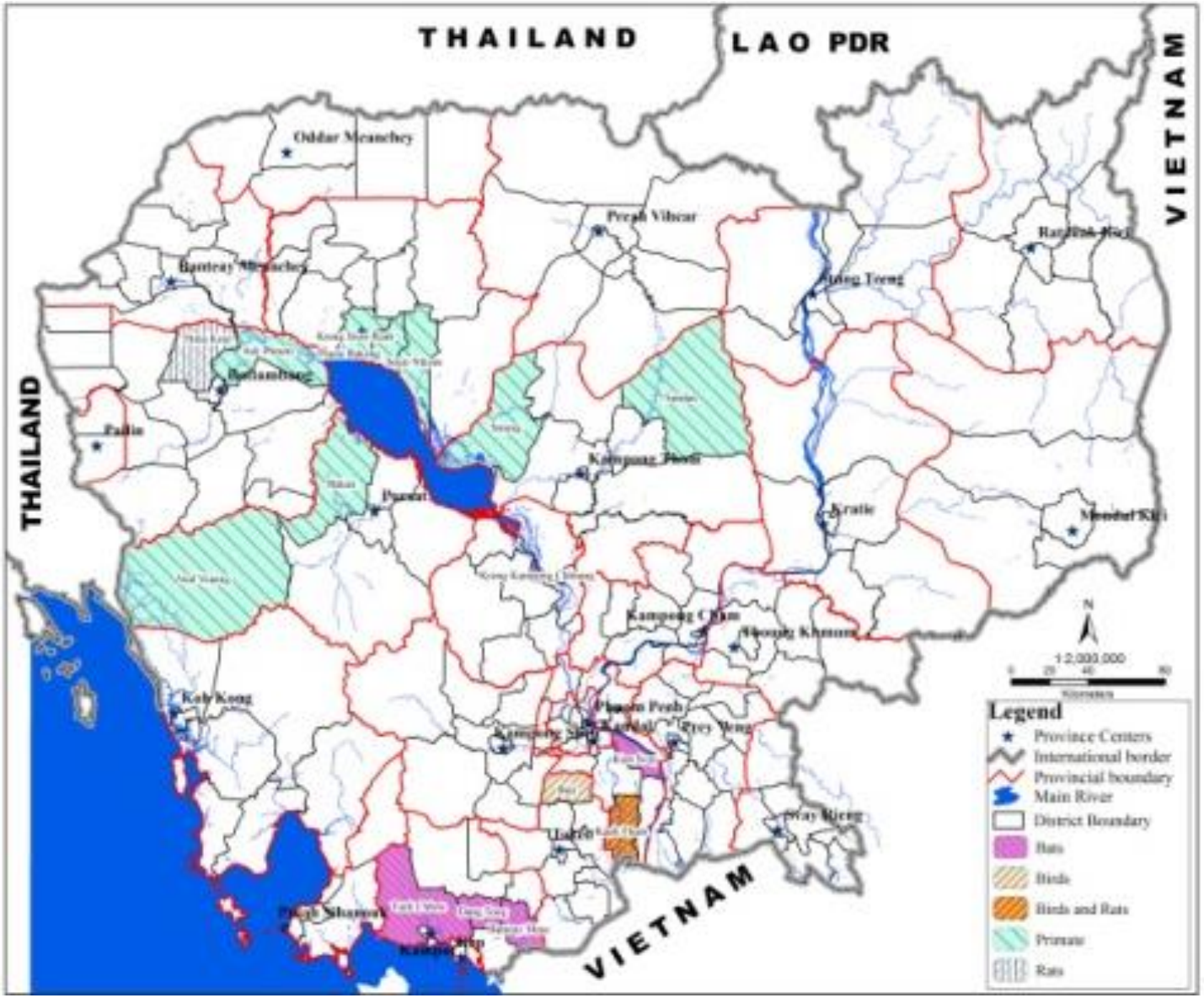
Significant finding

- Totally, 21,267 sample were collected from wildlife and environment in 4 countries for zoonosis detection
  - Cambodia:1,029 sample from 756 sample animals
  - China: 17,403 samples in total from15,356 birds and 2,047 Environmental samples
  - LaoPDR: 1,096 samples from 530 animals
  - Thailand: 1,739 samples were collected from 791 animals

Significant finding

- Cambodia
  - 1,029 sample from 756 sample animals
  - The occurrence of Herpes B virus, Simian T-cell Leukemia virus (STLV), and Simian Retrovirus (SRV) virus-related antibodies and Hepatitis B virus was tested in serum samples
  - 15.4% positive rate for the B virus-related antibodies through 13.7% for the STLV virus-related antibodies to 6.9% for the SRV virus-related antibodies.

Cambodia study sites



Cambodia: sample collection






Significant finding

- China
  - 18% positive with AIV (3174/17403 samples)
  - A H5N1 strain from central China was identified by pathogenic experiments and sequence analyses.
  - Phylogenetic discovered a novel Clade 2.3.2.1c H5N1 reassortant virus caused several outbreaks in wild birds in some regions of China from late 2014 to 2015.

China:Team members

Institute of Zoology, C.A.S
Prof. Fumin Lei*

Institute of Microbiology, C.A.S
Prof. Wenjun Liu

Taishan Medical University
Prof. Weifeng Shi

Shaanxi Normal University
Prof. Hongfeng Zhao

Southwest Forestry University
Prof. Xu Luo

Guangxi University
Prof. Lijiang Yu

*team leader

Fieldworks

Fig. 1 setting up mist net fo

Fig. 2 A sno

wfinch which blood

Fig. 3 Fieldtrip in Badong nature reserve, cent

China: Investigation on wildlife trades

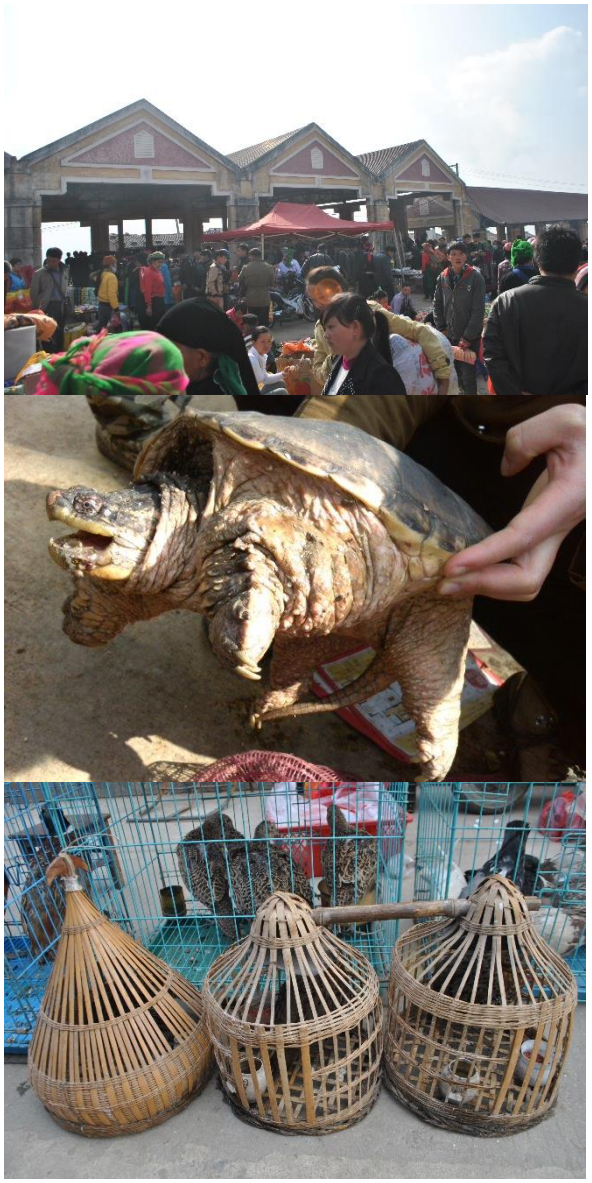


Fig. 4 Photo records of wild trades at border market between China andVietnam

Fig. 5 A porcupine farm in Yunnan, Southwest China

Fig. 6 Scrub sampling in a poultry market, Guangdong, South China

China: Outputs

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Highly Pathogenic Avian Influenza A(H5N1) Virus Struck Migratory Birds in China in 2015

Received: 30 May 2015
Accepted: 01 July 2015
Published: 11 August 2015

Yuhai Bi[1,2,6], Zhenjie Zhang[3], Wenjun Liu[1,6], Yanbo Yin[5], Jianmin Hong[7], Xiangdong Li[8], Haiming Wang[9], Gary Wong[1], Jianjun Chen[6,10], Yunfeng Li[11], Wendong Ru[11], Ruyi Gao[11], Di Liu[1,6], Yingxia Liu[2], Boping Zhou[2], George F. Gao[1,2,6,12], Weifeng Shi[3] & Fumin Lei[4,6]

Approximately 100 migratory birds, including whooper swans and pochards, were found dead in the Sanmenxia Reservoir Area of China during January 2015. The causative agent behind this outbreak was identified as H5N1 highly pathogenic avian influenza virus (HPAIV). Genetic and phylogenetic analyses revealed that this Sanmenxia H5N1 virus was a novel reassortant, possessing a Clade 2.3.2.1c HA gene and a H9N2-derived PB2 gene. Sanmenxia Clade 2.3.2.1c-like H5N1 viruses possess the closest genetic identity to A/Alberta/01/2014 (H5N1), which recently caused a fatal respiratory infection in Canada with signs of meningoencephalitis, a highly unusual symptom with influenza infections in humans. Furthermore, this virus was shown to be highly pathogenic to both birds and mammals, and demonstrate tropism for the nervous system. Due to the geographical location of Sanmenxia, these novel H5N1 viruses also have the potential to be imported to other regions through the migration of wild birds, similar to the H5N1 outbreak amongst migratory birds in Qinghai Lake during 2005. Therefore, further investigation and monitoring is required to prevent this novel reassortant virus from becoming a new threat to public health.

The H5N1 subtype of highly pathogenic avian virus (HPAIV), initially identified during 1996 in China,

VIROLOGICA SINICA
DOI: 10.1007/s12250-016-3750-4

RESEARCH ARTICLE

Highly pathogenic avian influenza H5N1 Clade 2.3.2.1c virus in migratory birds, 2014–2015

Yuhai Bi[1,2,7✉], Jianjun Chen[2,4,7✉], Zhenjie Zhang[3], Mingxin Li[2,4], Tianlong Cai[5], Kirill Sharshov[6], Ivan Susloparov[6], Alexander Shestopalov[6], Gary Wong[1], Yubang He[8], Zhi Xing[9], Jianqing Sun[9], Di Liu[1], Yingxia Liu[1], Lei Liu[1], Wenjun Liu[3,7], Fumin Lei[6,7], Weifeng Shi[5✉], George F. Gao[1,3,7,10]

1. Shenzhen Key Laboratory of Pathogen and Immunity, State Key Discipline of Infectious Disease, Shenzhen Third People's Hospital, Shenzhen 518112, China
2. CAS Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology Chinese Academy of Sciences, Wuhan 430071, China
3. CAS Key Laboratory of Pathogenic Microbiology and Immunology (CASPMI), Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
4. CAS Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology Chinese Academy of Sciences, Wuhan 430071, China
5. Institute of Pathogen Biology, Taishan Medical College, Shandong post code 271000, China
6. CAS Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
7. Center for Influenza Research and Early-warning (CASCIRE), Chinese Academy of Sciences, Beijing 100101, China
8. Research Institute of Experimental and Clinical Medicine, Novosibirsk State University, Novosibirsk 630090, Russia
9. Qinghai Lake National Nature Reserve, State Forestry Administration, Qinghai 810007, China
10. Office of Director-General, Chinese Center for Disease Control and Prevention (China CDC), and Collaborative Innovation Center for Diagnosis and Treatment of Infectious Disease, Zhejiang University, Hangzhou 310058, China

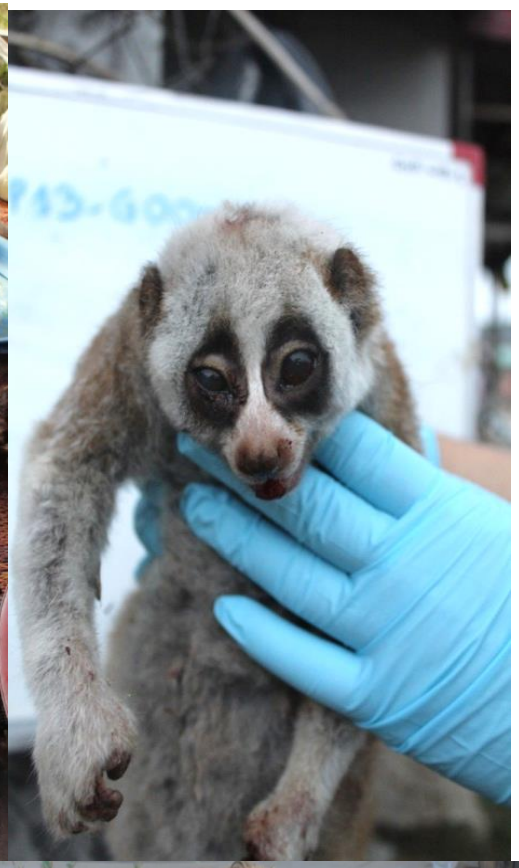
A novel Clade 2.3.2.1c H5N1 reassortant virus caused several outbreaks in wild birds in some regions of China from late 2014 to 2015. Based on the genetic and phylogenetic analyses, the viruses possess a stable gene constellation with a Clade 2.3.2.1c HA, a H9N2-derived PB2 gene and the other six genes of Asian H5N1-origin. The Clade 2.3.2.1c H5N1 reassortants displayed a high genetic relationship to a human H5N1 strain (A/Alberta/01/2014). Further analysis showed that similar viruses have been circulating in wild birds in China, Russia, Dubai (Western Asia), Bulgaria and Romania (Europe), as well as domestic poultry in some regions of Africa. The affected areas include the Central Asian, East Asian-Australasian, West Asian-East African, and Black Sea/Mediterranean flyways. These results show that the novel Clade 2.3.2.1c reassortant viruses are circulating worldwide and may have gained a selective advantage in migratory birds, thus posing a serious threat to wild birds and potentially humans.

Bi YH, Zhang ZJ, Liu WJ, Yin YB, Hong JM, Li XD, Wang HM, Wong G, Chen JJ, , Li YF, Ru WD, Gao RY, Liu D, Liu YX, Zhou BP, Gao GF, Shi WF and Lei FM. 2015. Highly Pathogenic Avian Influenza A (H5N1) Virus Struck Migratory Birds in China in 2015. Scientific Reports5:12986.

Bi YH, Chen JJ, Zhang ZJ, Li MX, Cai TL, Sharshov K, Susloparov I, Shestopalov A, Wong G, He YB, Xing Z, Sun JQ, Liu D, Liu YX, Liu L, Liu WJ, Lei FM, Shi WF and Gao GF.2016. Highly pathogenic avian influenza H5N1 Clade 2.3.2.1c virus in migratory birds, 2014-2015.Virologica Sinica. DOI: 10.1007/s12250-016-3750-4.

Significant finding

- LaoPDR
  - Wildlife trade project : 1, 096 samples from 530 animals were collected from wildlife markets and roadside markets/stalls to test for priority zoonotic pathogens
  - 127 positive samples
    - 73 coronaviruses in bats, rodents and wild viverids
    - 44 *Leptospira* sp in rodents
    - 6 *Rickettsia* sp in rodents
    - 1 *Lactococcus garvieae* in
    - *1 Kurthia* spp
    - 1 *Ehrlichia* spp. TC251-2; 1
    - *1 Anaplasma marginale* in Muntjac

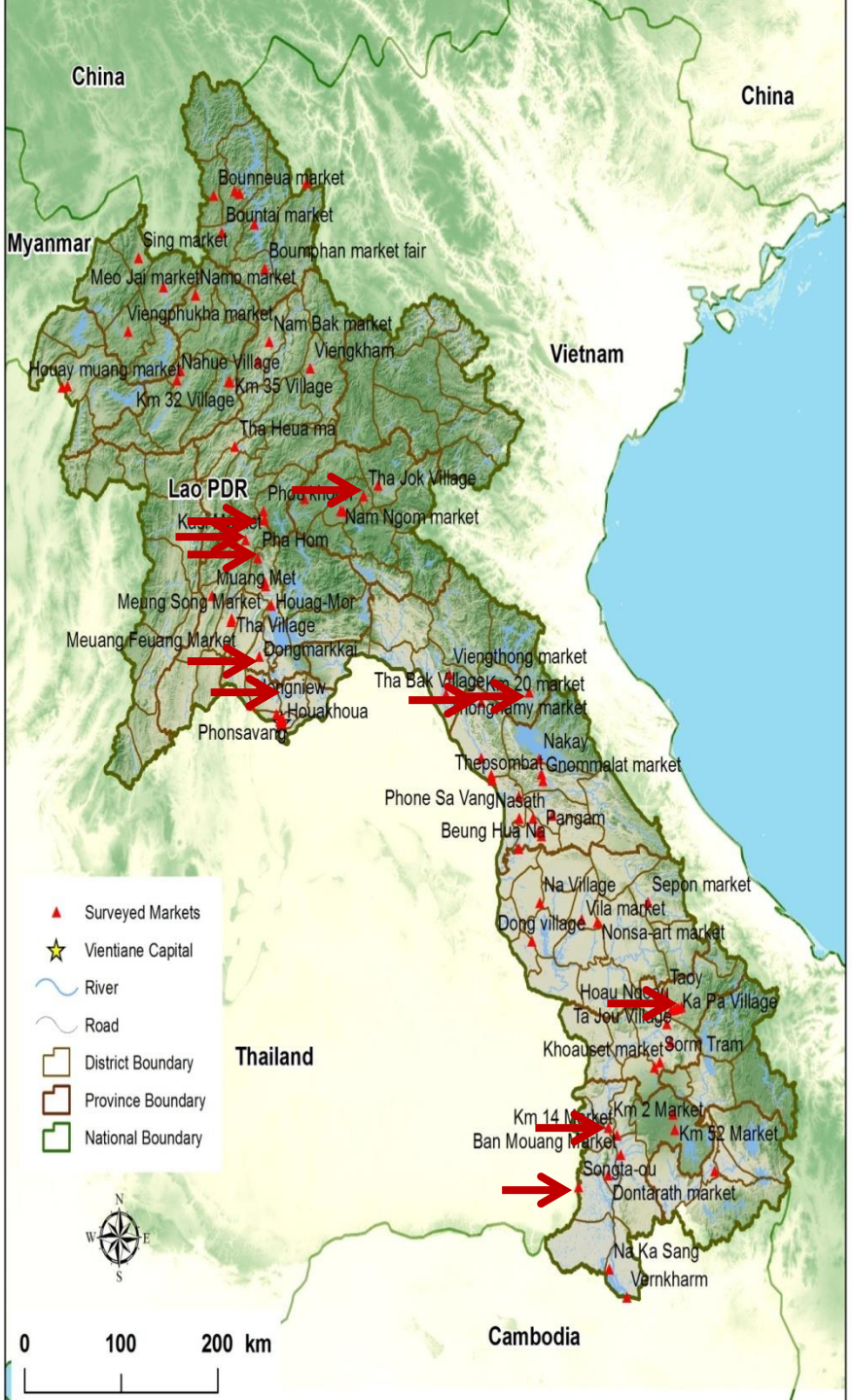


Activities for study Wildlife Trade In Lao PDR

LaoPDR study sites

- From late 2014- early 2016,

Wildlife trade observed at 21 sites, in 14 districts 7 Provinces between 16 markets 5 roadside markets/stalls



LaoPDR: sample collection

Significant finding

- Thailand
  - Totally, 1,739 samples were collected from 791 animals within 22 of 77 provinces of Thailand
  - 29.8% positive rate (45/151) for pathogenic *Leptospira spp*. in wild rat bush meat
  - One slow lories serum was positive for Herpes B virus antibody

Significant finding

- Thailand
  - 16S ribosomal RNA sequencing by using next generation sequencing (NGS) technique shown the surprisingly result when tested for gut pathogen in the fecal sample of turtle, slow loris, bat and rat
  - NGS result showed about 1,761 bacterial species were detected in feces of those animal including commensal and pathogenic bacteria. Interestingly, 43 species of pathogenic bacteria of human and animal were found

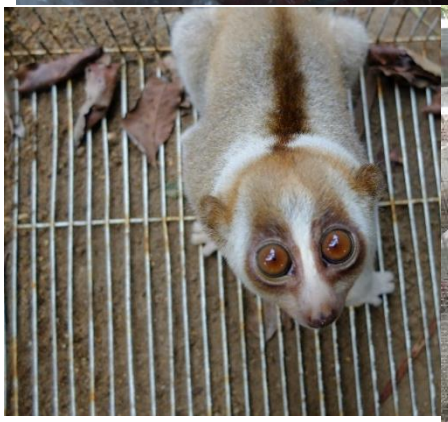
Acinetobacter baumannii
Actinobacillus pleuropneumoniae
Campylobacter fetus
Clostridium perfringens
Corynebacterium cystitidis
Corynebacterium minutissimum
Corynebacterium mastitidis
Corynebacterium tuberculostearicum
Elizabethkingia meningoseptica
Enterococcus avium
Enterococcus casseliflavus
Enterococcus faecalis
Enterococcus faecium
Enterococcus gallinarum
Francisella hispaniensis
Klebsiella pneumoniae
Klebsiella oxytoca
Klebsiella granulomatis
Klebsiella variicola
Leptospira fainei
Leptospira licerasiae
Moraxella lacunata

Mycobacterium lepromatosis
Neisseria gonorrhoeae
Neisseria mucosa
Neorickettsia helminthoeca
Pasteurella multocida
Pasteurella pneumotropica
Propionibacterium acnes
Pseudomonas aeruginosa
Rhodococcus equi
Salmonella enterica
Serratia marcescens
Staphylococcus aureus
Streptococcus bovis
Streptococcus pneumoniae
Streptococcus pseudopneumoniae
Streptococcus suis
Ureaplasma diversum
Yersinia enterocolitica
Yersinia frederiksenii
Yersinia pestis
Yersinia ruckeri

Thailand study sites

Thailand: sample collection



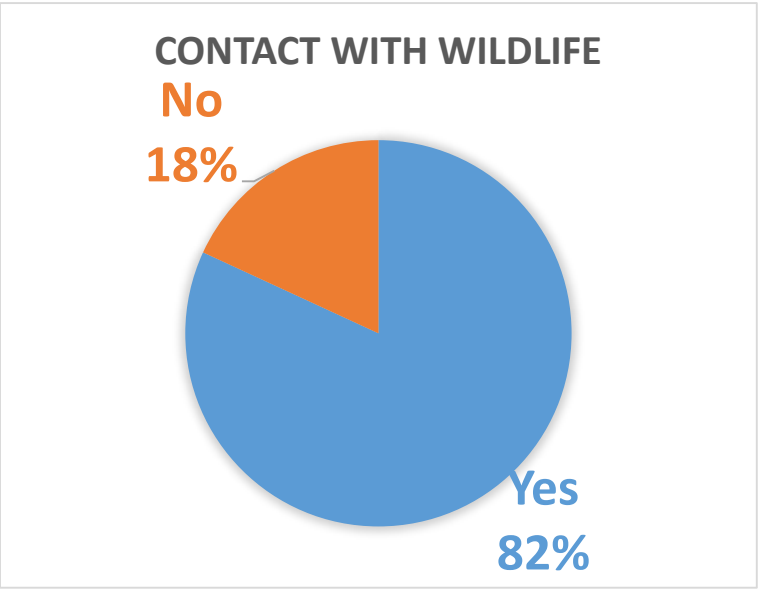



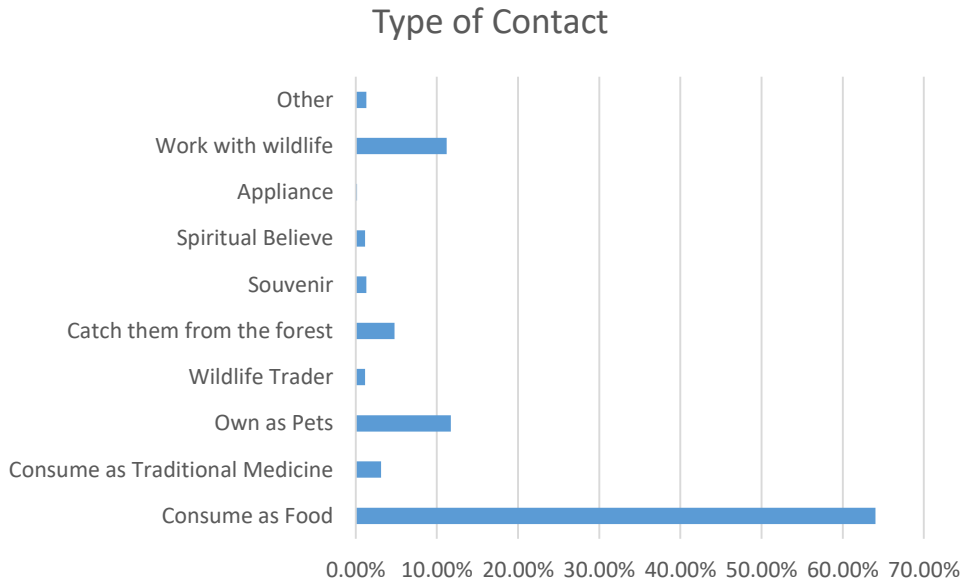

Objective 2.

- **To identify the risk situation and risk factors of disease transmission from wildlife trade to humans including the existence of current zoonotic prevention and outbreak response measures.**

Thailand KAP study result

- A total of 585 questionnaires were collected in 12 sample sites





Thailand KAP study result

- There were still a significant number of participants who do not think that they may get some zoonotic diseases via wildlife consumption
- 78.84% of wildlife products consumed were live animals; whole animals (fresh); as well as meat parts (fresh)

Thailand KAP study result

- For exotic pet owner, mammals were the most common animals owned as pet (59.66%)
- 56.34% of exotic pet owner imply that they did not have any health care program for their pets and 18.18% of the owner will treat their pet by themselves when the pet falling sick.
- There were 23.94% of exotic pet owners reported to have ever released their pets to the environment which might create an invasive alien species problem as well as transmit the diseases to the other animal and human later.

Thailand KAP study result

- For wildlife trader, the primary way to obtain wildlife and its products for trade was that caught them from the nature (68.00%)
- 47.83% of wildlife trader, participating in this study, have inappropriate practices which are throwing the animal containers away anywhere convenient (26.09%) and putting the containers away without cleaning (21.74%) after using

Thailand KAP study result

- There were 74.74% of the participant admitted that they did not wear any protective equipment while having direct contact with wildlife
- 12% of the participants thought that diseases could not be transmitted from wildlife to human.
- These study results imply that a number of people were still lack of proper knowledge, attitude and practices for protect themselves and family from zoonoses infection that may transmit from wildlife

(2) Improved understanding of knowledge, attitudes and practices (KAP) associated with disease prevention of specific stakeholders, local authorities and local communities from pathogens in the wildlife trade.

Map showing sample locations in the KAP survey

IDRC CRDI APEIR

International Development Research Centre (IDRC)

ASIAN Partnership for Emerging Infectious Diseases Research (APEIR)

Technical Report

Knowledge, Attitudes and Practices (KAP) Survey of Zoonoses in the Wildlife Trade in Cambodia

"Surveillance of Emerging Infectious Diseases in the Wildlife Trade to Increase Awareness for Zoonoses Prevention and Wildlife Conservation"- Phase 2 (Cambodia Component)

IDRC Grant Number: 106915-001

Phnom Penh, Cambodia
31 March 2017

There were 1,555 questionnaires administered by interviewers in 98 communes in 73 of 170 districts located in 21 of the country's 24 provinces, as well as in Phnom Penh, between April and October 2015 and April and November 2016.

(2) Improved understanding of knowledge, attitudes and practices associated with disease prevention of specific stakeholders, local authorities and local communities from pathogens in the wildlife trade.

- There was also a substantial number of respondents, however, who are more limited in their knowledge of zoonoses, as well as less aware of the means of protecting themselves and their families from wildlife disease infections.



Objectives 3

- To raise (improve) awareness of specific stakeholders of how to prevent diseases which originate from wildlife and also to raise awareness for wildlife conservation efforts.

Awareness promotion

- TV show
  - Mahidol Channel
  - Thai PBS
- Infographic chip video
- Leaflet for awareness and knowledge promotion

Awareness promotion

providing knowledge about zoonosis to exotic pet owner

Wildlife keeper training for zoonosis and biosafety

Awareness promotion

Training the student in community schools

Lao PDR: Sharing results to the villager

China: Community works

Fig. 7 Visiting in a field station, Badong nature reserve, Central China

Fig. 8 Interview with local forestry protection staff

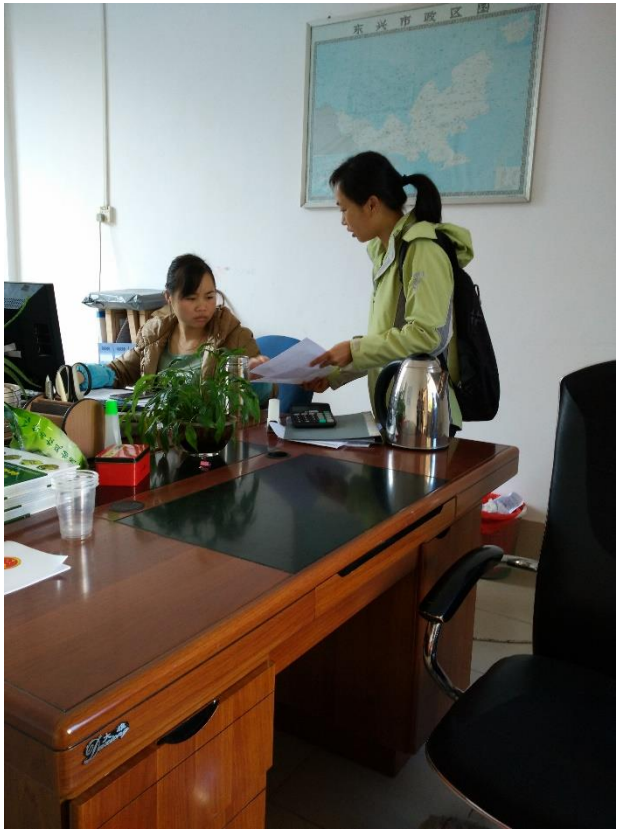


Fig. 8 Visiting in local forestry administration

China: Outputs

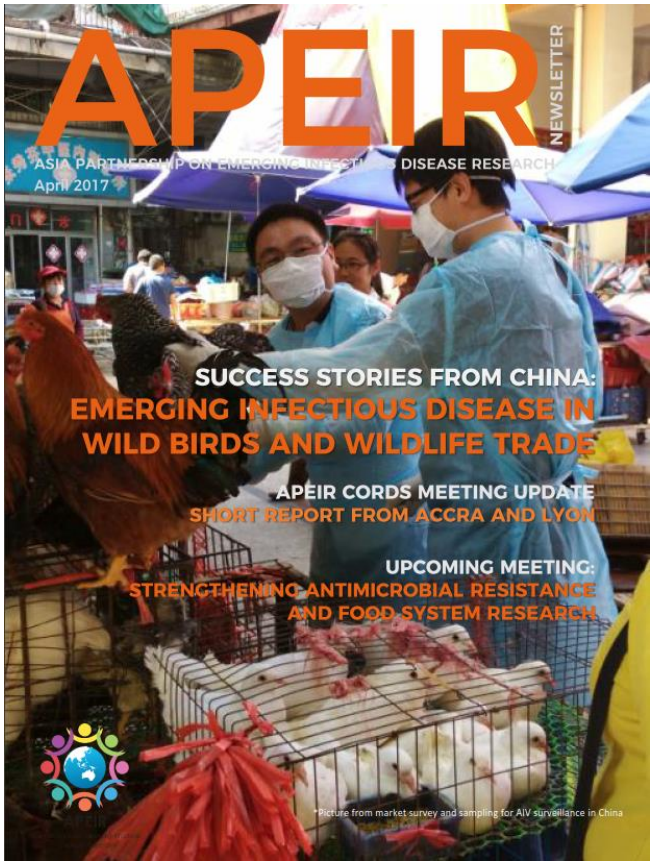


Fig. 9 APEIR newsletter on Chinese team work

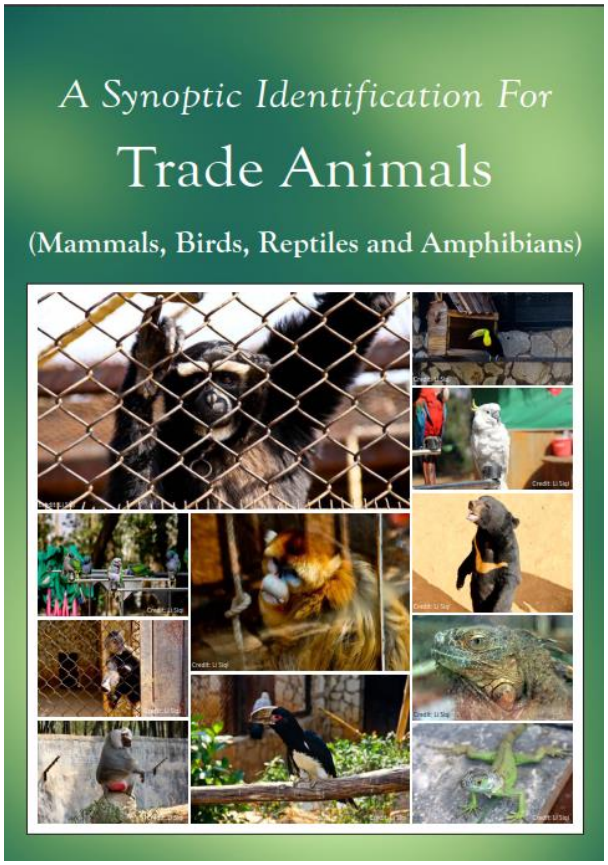


Fig. 10 A synoptic identification for trade animals

Capacity building

Cambodia capacity building

Field training on wildlife diseases surveillance and monitoring techniques

Cambodia capacity building

Monthly Cambodia Zoonotic Working Group Meeting on Research Activities Associated with Human Health, Livestock, and Wildlife One Health Initiative

Lao PDR capacity building

- Opened the inception workshop to introduce the project activities and plan to the provincial discussion maker from Agriculture and Forestry department , Public health department, Natural resources and development from the province of Champasack , Xaysomboun, Borikhamxay, and Savannakhet on 12 May 2014 at Champasack province, Lao PDR

Lao PDR capacity building

- 13-14 May 2014 the training on wildlife disease surveillance and monitoring techniques

- 12-14 July 2014 Lao wildlife trade project organized the training on Biosecurity and BSLIII Laboratory Management

Thailand: capacity building

Laboratory diagnosis and biosafety training, MU-FVS

IDRC | CRDI Canada

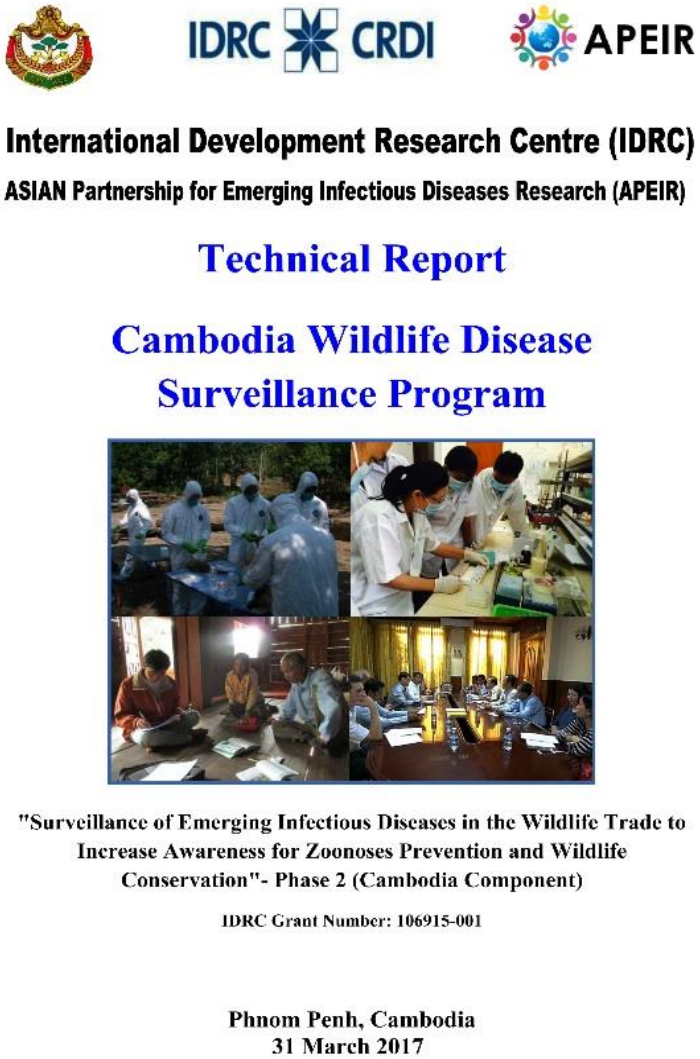
APEIR
Asia Partnership on Emerging Infectious Disease Research

FAO Reference Center for Zoonotic and Wildlife Diseases

The Monitoring and Surveillance Center for Zoonotic Diseases on Wildlife and Exotic Animals

EMPRES WILDLIFE UNIT

Cambodia: outcome



The development of the National Wildlife Disease Surveillance technical report was adopted from materials prepared by the World Organisation for Animal Health (OIE). Its principal purpose was to define a practical roadmap for depicting the means of establishing and maintaining a national wildlife disease surveillance program that would contribute to the enactment of those actions that are required to be undertaken to establish such a program in Cambodia.

China: outcomes

- Social activities could improved awareness of specific stakeholders of the prevention from diseases and the awareness for wildlife conservation.
- A fieldwork in Hubei province reinforced the awareness of wild birds conservation in local communities.

Lao PDR: outcome

- Long term data on wildlife trade provide important insight into zoonotic disease risk
- Building durable wildlife surveillance mechanisms is essential for
  - Lao PDR's capacity to detect priority and emergent zoonosis
  - Strengthening an often neglected part of One Health

Thailand: outcome

- Early warning for zoonosis prevention and control was strengthen
- Thailand national wildlife health center and networking was established
- Wildlife farming standardization was created in Thailand

Regional: outcome

- Strengthen national and regional capacity for zoonosis surveillance in wildlife

- Strengthen national and regional Ecohealth/One health collaboration with Public Health and Livestock, especially in the field of zoonosis surveillance and responses.

Acknowledgements

- APEIR-IDRC
- Health Systems Research Institute (HSRI)
- Department of Wildlife and Biodiversity, Cambodia
- Institute of Zoology, CAS, China
- Department of Livestock and Fisheries, Lao PDR
- FAO Reference Center for Zoonotic and Wildlife Diseases, MU-FVS, Thailand
- Institute of Ecology and Biological Resources, Vietnam

Thank you


APEIR Proposal Development Workshop
28 August - 1 September 2012