





A BioZoonoSEA Workshop

Under the umbrellas of **BioZoonoSEA**, the “Biotechnology Platform for Research and Training on Parasitic Zoonoses in South East Asia”, **NAHIAT**, the “Network on Atypical Human Infections by Animal Trypanosomes”, **GREASE** network “Management of Epidemiological Emerging Risk in South East Asia”, and with the financial support of the **French Ministry of Foreign Affairs**, French Embassy, Bangkok, Thailand, and the **ComAcross** Project “Companion Approach for Cross-sectoral collaboration in health risks management in SEA”, we have implemented a series of Workshop & Training entitled:

“Diagnosis and epidemiology of trypanosomoses and their vectors in South East Asia”

which was held in partnership with **Kasetsart University** (Thailand), **Central Mindanao University** (Philippines), and the French institutes **CIRAD** and **IRD**;

This Workshop / Training aimed at sharing knowledge and experience on trypanosomes and their vectors: diagnosis epidemiology, impact and control of the disease as well as trapping, identification, studies of transmission mechanisms, epidemiological impact as vectors and ways of control. In the future, this program will support the sustainable strengthening of Lao's veterinary services and university to control Surra. It also aims at developing joint research projects amongst French and South East Asian research institutes and universities.

Workshop coordinators:

Pr Sathaporn Jittapalapong, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand; fvetspi@ku.ac.th

Dr Marc Desquesnes, CIRAD, International Center for Cooperation in Agricultural Research for Development, UMR InterTryp/FVM/KU, Thailand; marc.desquesnes@cirad.fr

Dr Vannaphone Putthana, NUOL, National University Of Lao, Nabong, Lao; vannaphoneputthana@yahoo.com

Dr Stéphane Herder, IRD, Institute of Research for the Development, UMR InterTryp/FVM/KU, Thailand; stephane.herder@ird.fr

Speakers and/or supervisors:

The main speakers were Dr Alan Dargantes (CMU, Philippines), Dr Marc Desquesnes (CIRAD, UMR InterTryp/FVM KU), Dr Stéphane Herder (IRD/UMR InterTryp/FMV KU), Dr Rongthip Masmeatathip (FA/KU/KPS), Dr Serge Morand (CIRAD-AGIRs/CICML), and Dr Vannaphone Putthana (NUOL, Lao). Thippakone Lacksiv (NUOL) and Piangjai Chalermwong (FVM/KU) assisted training and training translation.

Results and outputs knowledges were:

- an overview of the problem of trypanosomes in Asia;
- How to implement and interpret specific diagnosis of trypanosomoses in mammals, using parasitological, serological and DNA based methods;
- How to implement trapping and identification of the main genera/species of biting insects acting as mechanical vectors of trypanosomes in livestock;
- discussions on a draft of common research project for the control of biting insects;
- discussions to draft common strategy for epidemio-surveillance and impact evaluation of surra at the regional level, under the GREASE Network and **BioZoonoSEA** umbrella.

General schedule

This Workshop & training program was split into 3 parts: a one day *Open Workshop One Health* in Vientiane (*Centre d'Infectiologie Christophe Mérieux du Laos*), a 4-days *Workshop/Training* in Nabong (NUOL), and a 5 days training on the **BioZoonoSEA** platform (Kasetsart university, Bangkok, Thailand) for 12 young scientists or technicians from Lao, Philippines and Cambodia. All these objectives were made possible thanks to the financial support of the French Ministry of Foreign Affairs and the ComAcross project.

Partners:

- Faculty of Agriculture, National University of Laos, Nabong ; infrastructures are available in Nabong
- supporting institute: Veterinaries without borders (Vétérinaires sans frontières, Canada VWB/VSF) (implementing a development project to support livestock breeding in Lao).
- *Centre d'Infectiologie Christophe Mérieux du Laos (CICML)*, Ministry of Health PO. Box 3888; Vientiane.



Group photo of the participants to the open Workshop on
“Trypanosomes, hosts, reservoirs and vectors in a One-Health concept, in
South East Asia” CICML, 5th October 2015

Open Workshop

October 5th, 2015; Seminar at CICML, Vientiane

This “One Health Open workshop” was held under the umbrellas of the BioZoonoSEA Platform, the *NAHIAT* and *GREASE* networks, and the *ComAcross* project, in order to strengthen cross-sectorial and multidisciplinary exchanges about trypanosomosis survey and management among professional from different sectors (Animal health, human health, environment, rural development, NGO, private partners). Lectures and open discussions were held to develop future collaborations in the OneHealth (OH) conceptual framework, on trypanosomes and vectors, including livestock, wildlife, environment (both social and natural), veterinary and human medicine ; it was open to a large audience, including various scientific fields and geographical areas of Lao.

The open workshop gathered 52 participants from 5 countries (Lao, Philippines, Italy, Thailand and France); it was opened in the presence of Mr Jérôme Dubois-Mercent, representative of the French Embassy in Lao, Dr Philippe Girard, representative of CIRAD, Dr Vannaphone, Dr Phimpa and Dr Manolin, respectively representatives of NUOL and CICML.



Dr Ot Manolin Dir CICML; Jérôme Dubois-Mercent, SCAC, French Embassy Vientiane; Dr Vannaphone Putthana NUOL



P Girard, SEA-CIRAD delegate P Paboriboun, scient Dir CICML; Marc Desquesnes, CIRAD; Alan Dargantes CMU Philippines

11 lectures were given, covering the generalities on Trypanosomes and their vectors, overviewing and updating the situation of *Trypanosoma evansi* (surra) in Philippines, Thailand and Lao, the rodent's trypanosomes (*T. lewisi*, *T. conorhini*) and *T. cruzi* (Chagas). It provided generalities and updates on the situation of haematophagous biting insects acting as mechanical vectors in South East Asia.



Stéphane Herder, IRD; Serge Morad, CIRAD AGIR's/CICML; R. Masmeatathip, Fac Agr. Kasetsart U; M Camoin, VWB Lao



Participants

Discussions

Dr Phimpa P Paboriboun

Participants

During the discussions the Lao participants emphasized on information and the needs on trypanosomes as follows:

Trypanosomes:

- it was emphasized that situations are diverse from a place to another, and from a host species to another, which makes the impact of disease difficult to evaluate ; the situation in Lao is not known since there was no recent studies on surra. However, the parasite is present and some outbreaks have been identified, especially in horses, in the past.
- Support and training on diagnosis of surra are requested, but the disease is not a priority for sanitary authorities;
- Medical and economic impacts cannot be evaluated since the diagnosis methods are rarely applied;
- Use of chemicals is large and not really under control, Diminazen aceturate is mostly employed
- It was suggested to use Diminazene aceturate (7mg/kg) in bovines, and to use Quynapiramine in horses only;
- In Lao, as in the other S-E Asian countries, evaluation of the economic impact of surra would be a priority; unless it is made, the disease will ever remain neglected.
- Impact of trypanosomes in humans is not known, although 2 *Trypanosoma* species largely distributed in SEA (*T. evansi* & *T. lewisi*) may be responsible of atypical human infections.
- The priority and first step would be to implement a serological survey in bovines to evaluate the prevalence of the infection.



Lunch time break at CICML

During the discussions the Lao participants emphasized on information and the needs on biting insects as follows:

Biting insects :

- Tabanids and stomoxes are known to be an important pest in livestock, but they have been very few studied, and the means of control are mostly limited to the use of sprayed insecticides, with more or less success.
- Support and training to investigate the hematophagous insects as vectors of surra is highly requested since there are almost no data available in Lao.
- There is need to identify a taxonomist in Lao, to refer to for biting insect studies;
- The use of insecticide in bovine farms would represent a high risk for human health due to residues in meat, milk and environment.
- All means of control should be developed, especially ecological ones and non-, or low-chemical ones, aiming at reducing the spread of insecticide through livestock industry.
- Training in insects as vectors may take time, so it was suggested to identify students (master ?) to work on that topic for several years in order to have sustainable skills in this field of investigation.
- Specific training of NUOL staff to the trapping and identification of insects is much expected, both during the registered workshop and possibly in a further specific training to be held in Bangkok early November 2015.

Closing ceremony

At the end of the day, Dr Desquesnes, workshop coordinator (CIRAD/Kasetsart University), Dr Ot (CICML) and Dr Vannaphone (NUOL) made a brief summary of the exchanges before group picture and closing ceremony.



List of participants : see at the end of the document.

Open Workshop schedule

October 5th, 2015; Seminar at CICML, Vientiane

Time	Programme	Speaker	Duration
International seminar on “Trypanosomes, hosts, reservoirs and vectors in a One-Health concept, in SEA”			
08.45 – 08.50	Opening Speech (OS) from the hosting CICML	Dr Ot Manolin & Dr Phimpha Paboriboune	5 min
08.50 – 08.55	OS the organizing institute NUOL	Vannaphone Putthana	5 min
08.55 – 09.00	OS French Embassy Vientiane	Jérôme Dubois-Mercent	5 min
09.00 – 09.05	OS CIRAD-ASEC	Phillippe Girard	5 min
09.05 – 09.15	OS a coordinator of <i>BioZoonoSEA</i>	Marc Desquesnes	10 min
09.15 – 09.30	Coffee break 15 min		
09.30 – 09.50	L1 : One Heath / EcoHealth conceptual framework GREASE	Stéphane Herder IRD/KU for Aurélie Binot CIRAD-AGIRs/KU	20 min
09.50 – 10.30	L2 : ComAcross project	S Herder for A Binot CIRAD-AGIRs/KU	40 min
10.30 – 11.00	L3 : Generalities on kinetoplastidae	S Herder IRD/KU	30 min
11.00 – 11.30	L4 : Generalities on trypanosomes and the NAHIAT :	M Desquesnes CIRAD/KU	30 min
11.30 – 12.00	L5 : Rodents as reservoir of zoonotic pathogens	Serge Morand CIRAD-AGIRs/IM	30 min
12.00 – 12.30	L6 : Surra and vectors in the Philippines	Alan Dargantes CMU	30 min
12.30 – 13.45	Lunch Time 75 min		
13.45 – 14.00	L 7 : Tabanids as vectors of trypanosomes	M Desquesnes CIRAD/KU	15 min
14.00 – 14.15	L 8 : Stomoxes as biting insects and vectors	Roungthip Masmeatathip, FA/KU	15 min
14.15 – 14.30	L 9 : NUOL presentation and priorities, and Surra in Lao	V Putthana	15 min
14.30 – 15.00	L 10 : VSF projects in Lao	Margot Camoin	30 min
15.00 – 15.15	L 11 : Surra in Thailand	M Desquesnes CIRAD/KU	15 min
15.15 – 15.30	Coffee break 15 min		
15.30 – 16.55	Open Discussion on One Health Concepts: Biting flies and Trypanosomes	Stéphane Herder , M Desquesnes, V Putthana, P Paboriboune	85 min
16.55 – 17.00	Closing remarks	V Putthana & Ot Manolin	5 min

Registered Workshop

6th-9th October 2015

NUOL, Nabong



From 6th to 9th October 2015 the “Registered Workshop” limited to 26 participants was held at Nabong. This workshop / Training allowed giving lectures and practices on: (1) trypanosomes both in humans and animal reservoir in Asia, diagnosis methods and potential control methods, and (2) their transmission mechanisms and vectors, including trapping, identification and control. It included lectures, field work, practices and laboratory activities.



On 6th October an overview was given by Dr R Masmeatathip and M Desquesnes on biting insects, biology of stomoxine flies and tabanids, the direct effect (nuisance) of the biting flies, including annoyance, stress, pain, toxic effects, skin lesions and blood spoliation, and the indirect effects of these mechanical vectors: pathogen transmission. The phenology of biting insects, including nycthemeral and seasonal activity. A field picture was given with the example of the situation in the Philippines. Trapping technologies were presented with a special emphasize on Vavoua, Malaise and Nzi traps that can be used in SEA to trap tabanids and stomoxes. Finally, a practical session was implemented to set up insect traps for biting flies in the Field: Malaise, Nzi and Vavoua traps.



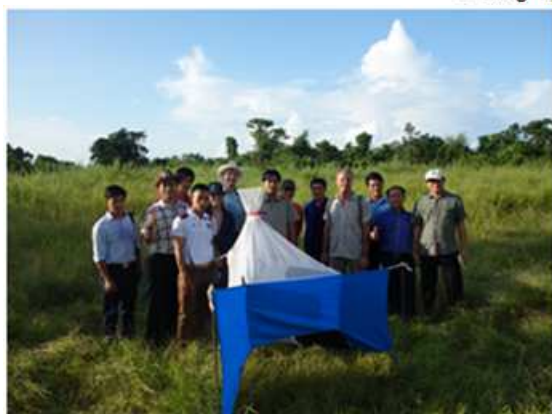
Clearing is required before setting up the traps



NZI trap properly set up



Setting up of the Vavoua trap



Setting up of the NZI trap



Setting up of the Malaise Trap



and final snap of successful cleaning !

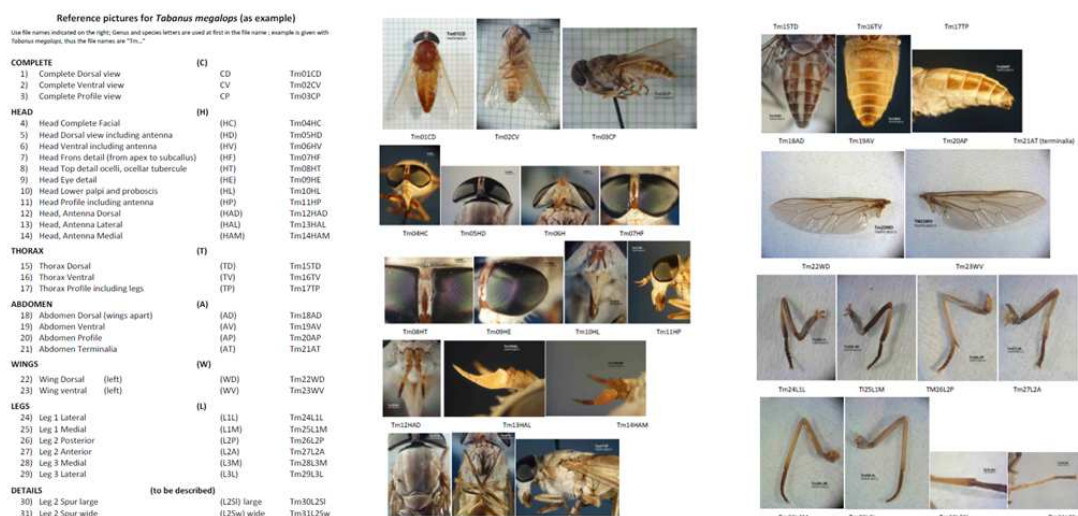
On 7th of October, the morning session was devoted to identification of biting flies: generalities, Tabanids and stomoxiine flies identification, including genera and species *Stomoxys*, *Hematobia* and *Musca crassirostris*, were presented and further on carried out on the insects freshly trapped in the field.



In the afternoon, lectures were given by Dr S Herder on Trypanosomes genetic, evolution and classification. Methods for the preparation of DNA for molecular characterization of trypanosomes were presented. A practical session allowed all participants to proceed Hematocrit Centrifuge Technique, blood smear preparation, fixation, staining and reading of the HCT and smears.



On 8th of October, the morning session was devoted to the guidelines to take pictures for the biting flies's description; biting flies trapped in NUOL were identified and pinned by Dr R Masmeatathip and M Desquesnes, in order to leave identified reference samples available at NUOL, for further studies.



In the afternoon, lectures were given on molecular characterization of Trypanosomes, PCR procedures and blood meal analysis in insect vectors. Lectures were given on serological diagnosis of trypanosomoses using CATT and ELISA techniques; a practical demonstration of the CATT was proceeded with and by the participants.



On the 9th of October, the morning session was devoted to the control of biting flies using traps and screens, as well as other methods of protection, repellent etc. In the afternoon, lecture and practical cases were presented on the control of trypanosome using trypanocides both in Thailand and the Philippines.



A discussion session was then opened in which participant drafted the after-workshop. Two mini-projects were proposed to evaluate the prevalence of surra in bovines in 3 provinces of Lao, and to study the biting fly activity (species identification and seasonal variation) in 3 provinces of Lao, to generate basic knowledge on their potential impact.

Certificates of attendance were given to all participants and lecturers of the workshop





Left to right: Dr Rounthip, Desquesnes & Manolin



Dr Khao and Desquesnes

Closing ceremony was followed by a last group picture.



Registered Workshop schedule

Tuesday, October 6th, 2015:

Biting insects biology & trapping

Time	Programme	Lecture/Speaker	Remark
09.00 – 09.30 30 min	Lecture 1 : An overview on biting insects	Marc Desquesnes, CIRAD./KU	T
09.30 – 09.50 20 min	L2 : Biology of stomoxiine flies	R Masmeatathip, FA/KPS/KU	T
09.50 – 10.20 30 min	L3 : Biology and direct effects of Tabanids	M Desquesnes, CIRAD/KU	T
10.20 – 10.45 25 min	L4 : Biting flies of veterinary importance in the Philippines	A Dargantes	T
10.45– 11.00	Coffee break 15min		
11.00 – 11.30 30 min	L5 : Seasonality of stomoxes and tabanids in Thailand	R Masmeatathip	T
11.30 – 12.30 60 min	L6 : Trapping technologies for biting flies	M. Desquesnes, CIRAD/KU	T
12.30 – 13.30	Lunch Time 60 min		
13.30 – 16.30 3 hours	Field trip 1 (F1): Setting up of insect traps for biting flies in the Field: Malaise, Nzi and Vavoua traps	Dr M Desquesnes Dr R Masmeatathip Dr A Dargantes Piangjai &Thippakone and all participants	FT1

Wednesday, October 7th, 2015:
Biting flies trapping & identification
Trypanosome DNA based identification

Time	Programme	Lecture/Speaker	Remark
9.00 – 10.00 60 min	L7: Identification of biting flies: generalities & Tabanids	M Desquesnes CIRAD/KU	T
10.00 – 10.30 30 min	L8: Identification of <i>Stomoxys</i> , <i>Hematobia</i> and <i>Musca crassirostris</i>	R Masmeatathip, FA/KPS/KU	T
10.30 – 10.45	Coffee break 15 min		
10.45 – 11.15	Field trip 2: collection of biting flies trapped in the field	M Desquesnes, R Masmeatathip, A Darantes Piangjai & Tippakone and all participants	FT2
11.15 – 12.30 75 min	PW1: Genus identification of the biting insects captured	R Masmeatathip, FA/KU M Desquesnes CIRAD/KU	PW1
12.30 – 13.15	Lunch Time 45 min		
13.15 – 13.45 30 min	L9: Biting flies control	Marc Desquesnes	T
13.45 – 14.15 30 min	L10: Trypanosomes genetic, evolution and classification	S Herder IRD/KU	T
14.15 – 14.45 30 min	L11: Preparation of DNA for molecular characterization of trypanosomes	S Herder IRD/KU	T
14.45 – 16.15 75 min	PW2: Preparation of samples and DNA for PCR: filter paper and Chelex	S Herder IRD/KU	PW2
16.15 – 17.30	Field trip 3: collect of biting flies trapped in the field and removal of the traps; freezing of the insects	M Desquesnes R Masmeatathip A Dargantes, Piangjai & Tippakone and all participants	FT3

Thursday, October 8th, 2015:
Biting flies & Trypanosomes: identification

One team is going to collect blood from bovines

Time	Programme	Lecture/Speaker	Remarks
8.30 – 9.00 30 min	PW3: Picture chart for biting flies's description	M Desquesnes	PW3
09.00 – 10.30 1.30 hours	PW4: Biting flies identification and pinning	M Desquesnes R Masmeatathip, A Dargantes	PW4
10.30 – 10.45	Coffee break	15 min	
10.45 – 11.15	L12 : Parasitological diagnosis of Trypanosomes	M Desquesnes	T
11.15 – 12.15	PW5 : Parasitological diagnosis of Trypanosomes (HCT and GSBS +/- MI)	M Desquesnes & Piangjai, Tippakone & A Dargantes	PW5
12.15 – 13.00	Lunch Time	45 min	
13.00 – 13.30	L13 : Molecular characterization of Trypanosomes	S Herder	T
13.30 – 15.00 90 min	PW6: Practice of PCR for trypanosome identification: master mixed, thermocycling and gel preparation	S Herder, M Desquesnes,	PW6
15.00 – 15.30	L14 : Serological diagnosis of Trypanosomes	M Desquesnes	T
15.30 – 16.00	PW7: CATT test & ELISA coating	M Desquesnes, Piangjai & Tippakone	PW7
16.00 – 16.15	Coffee break	15 min	
16.15 – 16.45 30 min	PW8: Practice of PCR for trypanosome identification: gel loading and electrophoretic migration	S Herder, M Desquesnes,	PW8
16.45– 17.00	L15 : Blood meal analysis in insect vectors	S Herder,	T
17.00 – 17.30 30 min	PW9: Practice of PCR for trypanosome identification: lecture of the gel	S Herder, M Desquesnes,	PW9

Friday, October 9th, 2015:

Trypanosomes: parasitological, serological and molecular diagnosis

Time	Programme	Lecture/Speaker	Remarks
09.00 – 11.00 3hours	Practical work 10: serological diagnostic using ELISA	M Desquesnes, Piangjai & Tippakone	PW10
11.00 – 11.45	L16 : Trypanosome diagnosis: interpretation of results	M Desquesnes	T
11.45 – 12.30	L17 : Generalities on the control of surra	Dr. Marc Desquesnes	T
12.30 – 13.30	Lunch Time 60 min		
14.00 – 15.00	L18 : Impact and Control of surra in the Philippines	A Dargantes	T
15.15 – 16.15 60 min	Questions and discussions	All participants	
16.15 – 16.30	Closing ceremony Distribution of the certificates of attendance	All participants	

List of the participants

No	Name, title & Institution	Origin (Province)	email
1	Vannaphone Putthana, NUOL	Vientiane, Lao	vannaphoneputthana@yahoo.com
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3	Dr Phimpha Paboriboune, scientific director, CICML	Vientiane, Lao	phimpha@ccm-laos.org
4	Serge Morand, CIRAD AGIRs	Vientiane, Lao	serge.morand@univ-montp2.fr
5	Margot CAMOIN, VWB	Vientiane, Lao	margot@vetswithoutborders.ca
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20	Khampasert	from section of Animal - fishery PAFO of Borlikhamxay	
21	Vatsana Chanthavong	Lao Elephant Care & Management	vatsana.chanthavong@yahoo.com

		program/Elefantasia/DLF	
22	representative	from Section of Animal - Fishery PAFO of Savanhnakhet	
23	Sophie Bosh	Laos Wildlife Rescue	sophiebosch@gmail.com
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27	Dr SisengKhoungsy	FMD project Luang Prabang	
28	Khamphay	from Animals Health Center PAFO of Champasuk	Jo.pakse@gmail.com
29	representative	Other SDC funded project?	
30	Dean of FAG	Faculty of Agriculture, NUOL	
31	Kham Phommachanh	Head of Department of livestock and fishery, FAG, NUOL.	
32	Assoc. prof. Thonglom phoumavong	Department of livestock and fishery, FAG, NUOL.	
33	Chantha Chanda	Department of livestock and fishery, FAG, NUOL.	
34	Saivisen	from Department of Economical and food safety FAG, NUOL.	
35	Dr. Phachone	from Veterinary Division DLF, MAF	
36	Souk Phomehuksa	from Animals Health Laboratory center MAF	
37	Dr. Wattana theppanya	from National Animals health Laboratory DLF, MAF	
38	representative Epidemiology center	Epidemiology center	
39	Dr. Soulapharp	from Sethathilath Hospital MOH	
40	representative	from Mahosrth Hospital MOH	
41	representative from DAFO of Hartsaiphong district	from DAFO of Hartsaiphong district Vientiane Capital	
42	representative	from Mittapharp Hospital Vientiane Capital	
43	representative from DAFO of Parkngeum district	DAFO of Parkngeum district Vientiane Capital	
44	representative University of Health	University of Health MOH	
45	Phout Inthavong	from DLF MAF	

46	Chintana	from DLF MAF	
47	representative from DAFO of Nasaithong district	DAFO of Nasaithong district	
48	representative from DAFO of Saithany district	DAFO of Saithany district	
49	representative from Veterinary Unit	Section of Animals and Fisery, PAFO, VTE Capital	
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