

Regional Hands-on Laboratory Training on Rabies Diagnosis

OIE Reference Laboratory for Rabies in Changchun Veterinary Research Institute
(CVRI), P.R. China

14-18 August 2017



Under the support of the government of the People's Republic of China, OIE RRAP continues its efforts to enhance the capacity of rabies diagnosis in the region.

The aims of the training were to:

- 1) Promote understanding of the global rabies situation as well as the OIE Standards and activities on rabies.
- 2) Enhance rabies laboratory skills focusing on antigen detection (FAT, dRIT, RT-nested PCR, real-time RT-PCR and sample collection).
- 3) Exchange experiences of the current rabies situation and control strategy, and facilitate cooperation between participating countries.

A total of 15 participants from 13 Members* joined the training program at the Reference Laboratory. All trainees were able to learn about the OIE Standards and activities on rabies as well as the rabies control plan and experiences in China. Representatives from each country also outlined the epidemiology, diagnostics and control policy/strategy for rabies in their own country, discussed the problems they faced and stressed the need to strengthen cooperation among Asian countries. Two Member Countries hadn't developed national rabies prevention and control plans, while five planned to eliminate rabies in the near future. The main constraints for rabies control and diagnosis were identified as: 1) lack of public awareness, 2) insufficient vaccines, 3) stray animal populations, 4) lack of disease reporting system, 5) lack of PCR reagents for molecular diagnosis and 6) insufficiently trained staff for rabies diagnosis.

The five-day training course provided participants with both didactic and practical hands-on training experience on the OIE internationally standardized rabies diagnostic techniques and how these can be used, as the region moves towards rabies elimination. The course provided practical laboratory sessions on: animal brain sample collection using the drinking straw method, the fluorescent antibody test (FAT), the direct, rapid immunohistochemical test (dRIT), RT-nested PCR, one step real-time PCR, gene amplification and phylogenetic analysis. Participants took an enthusiastic part in both the working sessions and discussion. Some participants had limited prior practice in their laboratory, especially for dRIT, RT-nPCR, and phylogenetic analysis. However, under the instruction of the CVRI trainers, all tests were smoothly implemented in compliance with the SOP.

A plenary discussion in the final afternoon covered several topics, including the regional rabies laboratory network, samples and information sharing among OIE Members, diagnostic reagents for RT-PCR and FAT, receiving and shipping samples. Several participants showed strong interest in the OIE vaccine bank and the regional rabies lab-network.

Taking advantage of the training, two participants from Indonesia were also invited to extend their stay for an extra week to learn more serological test techniques in the laboratory as a follow-up activity of Dr Tu's earlier visit to Indonesia. The two participants agreed that this targeted training addressed their needs well and would be the technical foundation for the establishment of serological test for rabies in their laboratory.

Participants appreciated that the course was a real hands-on training with real samples and reagents, and agreed that it will help them better conduct national rabies control strategies in their own countries.

**Participants joined the training from: Bangladesh, Bhutan, Cambodia, India, Indonesia, Korea D.P.R., Malaysia, Mongolia, Myanmar, Nepal, Sri Lanka, Thailand and Vietnam.*

Regional Hands-on Laboratory Training on Rabies Diagnosis

Changchun Veterinary Research Institute
Chinese Academy of Agricultural Sciences

Changchun, China, 14-18 August 2017

DAY 1	Monday , 14 August 2017	
Opening Ceremony		
08:30-09:00	Registration	Dr Yan Liu
09:00-09:40	Opening session	Dr Changchun Tu
09:00-09:10	Opening remarks by China	Dr Gongmin Wang Deputy Director General of the Veterinary Bureau of the Ministry of Agriculture of PR China
09:10-09:20	Remarks by OIE RRAP	Dr Noriyoshi Ojima OIE Regional Representative for Asia and the Pacific (OIE RRAP)
09:20-09:35	Remarks by CVRI	Dr Wensen Liu Head of Department of science and technology, Changchun Veterinary Research Institute (CVRI)
09:35-10:00	Group photo and Tea break	
Session 1: Introductory Session and group discussion		Dr Jing Wang OIE RRAP
10:00-10:15	OIE standards and activities on rabies	Dr Noriyoshi Ojima
10:15-10:30	Rabies Control in China	Dr Shichun Ma Director of Division of Zoonotic Disease Control, China Animal Disease Control Center
10:30-12:00	Country report on rabies Each country has five minutes to: 1. Brief the national rabies diagnostic systems, rabies situation and control experience; 2. Indicate the current gaps and main constraints of rabies laboratory diagnosis	Dr Jing Wang
12:00-13:00	Lunch	

Session 2: Sample Collection		
13:00–14:00	<p>Lecture: Overview of OIE Manual of Diagnostic tests and Vaccines for Terrestrial Animals</p> <p>Sample collection and transport, rabies fluorescent antibody test (FAT), direct rapid immunohistochemical test (dRIT)</p>	Dr Ye Feng
14:00–14:30	Tea break, Questions and Answers (Q&A)	
14:30–17:00	<p>Operation: Collection of brain tissue using straw technique and preparation of brain tissue smears</p>	Dr Ye Feng, Dr Wenjie Gong, Dr Yan Zhang, Dr Hualei Wang
18:00	Welcome Dinner (18: 30—20: 30 Changbaishan Hotel)	

DAY 2	Tuesday , 15 August 2017	
Session 3: Identification of the agent		
Part I - Immunochemical identification of rabies virus antigen		Dr Ye Feng
09:00–12:00	Operation: dRIT (Slides preparation, Staining)	Dr Ye Feng, Dr Wenjie Gong, Dr Yan Zhang, Dr Hualei Wang
12:00–13:00	Lunch	
13:00–14:00	Operation: dRIT (Read the result under Light Microscope)	
14:00–14:30	Tea break ; Q&A	
14:30–17:30	Operation: FAT (Slides preparation, Staining, Reading)	

DAY 3	Wednesday, 16 August 2017	
Session 3: Session 3: Identification of the agent		
Part II – Molecular Techniques		Dr Yan Liu
09:00–09:30	Lecture: Nucleic acid-based diagnostics including RT-nested PCR and real-time PCR	Dr Yan Liu
09:30–10:30	Operation: RNA extraction	Dr Yan Liu, Dr Ye Feng, Dr Wenjie Gong, Dr Hualei Wang
10:30–11:00	Tea Break; Q&A	
11:00–12:00	Operation: Reverse Transcription	
12:00–13:00	Lunch	
13:00–14:30	Operation: 1- round and 2- round PCR	
14:30–15:00	Tea Break; Q&A	
15:00–17:00	Operation: One-step real-time PCR	

DAY 4		Thursday, 17 August 2017
Session 3: Identification of the agent		
Part II – Molecular Techniques (continuation)		Dr Yan Liu
09:00–10:00	<i>Operation:</i> Gel Electrophoresis of PCR products (Pouring the gel) Read the result of real-time PCR	Dr Yan Liu, Dr Ye Feng, Dr Wenjie Gong, Dr Hualei Wang
10:00–10:30	Tea Break; Q&A	
10:30–12:00	<i>Operation:</i> Gel Electrophoresis of PCR products (Running the gel)	
12:00–13:00	Lunch	
Session 4: Phylogenetic analysis		Dr Biao He
13:00–14:00	<i>Lecture:</i> Phylogenetic analysis of rabies virus	Dr Biao He
14:00–14:30	Tea Break; Q&A	Dr Biao He, Dr Ye Feng, Dr Wenjie Gong, Dr Hualei Wang
14:30–16:00	<i>Operation:</i> G and N gene amplification	

DAY 5		Friday, 18 August 2017
Session 4: Phylogenetic analysis (continuation)		Dr Biao He
09:00–10:00	<i>Operation:</i> Gel Electrophoresis of PCR products	Dr Biao He
10:00–10:30	Tea Break; Q&A	Dr Biao He, Dr Ye Feng, Dr Wenjie Gong
10:30–12:00	<i>Operation:</i> Phylogenetic analysis of rabies viruses	
12:00–13:00	Lunch	
Session 5: Ways forward and Closing Ceremony		Dr Jing Wang
13:00–13:30	<i>Lecture:</i> Procurement of materials and setting up of basic rabies laboratory	Dr Changchun Tu
13:30–14:00	Tea Break; Q&A	
14:00–15:30	Round table discussion	Dr Changchun Tu
15:30–16:00	Concluding remarks and closing ceremony	OIE RRAP

Training on Rabies Serology (FAVN) for Indonesia

Changchun Veterinary Research Institute
 Chinese Academy of Agricultural Sciences
 Changchun, China
 21-25 August 2017
 AGENDA (Tentative)

DAY 1	21 August(Monday)	
09:00 - 10:00	<i>Lecture:</i> Serological test accredited by OIE Cell culture, Preparation of CVS to 100TCID ₅₀ , Fluorescent Antibody Virus Neutralization (FAVN)	Dr. Ye Feng
10:00 –11:30	<i>Demonstration:</i> Cell culture (BHK-21)	Ye Feng, Weidi Xu
13:30 –16:30	<i>Demonstration:</i> TCID ₅₀ determination of CVS-11 stock virus	Ye Feng, Weidi Xu
DAY 2	22 August(Tuesday)	
09:00–11:30	<i>Operation:</i> Cell culture, TCID ₅₀ determination	Ye Feng, Weidi Xu
13:30 –16:30	<i>Demonstration:</i> FAVN (Serum inactivation and titration)	Ye Feng, Weidi Xu
DAY 3	23 August(Wednesday)	
09:00–11:30	<i>Demonstration:</i> FAVN (Fixation and staining of microtitre plates)	Ye Feng, Weidi Xu
13:30 –16:30	<i>Operation:</i> FAVN (Serum inactivation and titration)	Ye Feng, Weidi Xu
DAY 4	24 August(Thursday)	
09:00–11:30	<i>Operation:</i> FAVN (Fixation and staining of microtiter plates)	Ye Feng, Weidi Xu
13:30 –16:30	<i>Assessment:</i> Cell culture, TCID ₅₀ determination	Dr. Ye Feng
DAY 5	25 August(Friday)	
09:00–11:30	<i>Assessment:</i> FAVN (Serum inactivation and titration)	Dr. Ye Feng
13:30 –16:30	<i>Assessment:</i> FAVN (Fixation and staining of microtitre plates)	Dr. Ye Feng