OIE Collaborating Centre
"Diagnosis and Control of Animal Diseases
and Related Veterinary Product Assessment in Asia"

Symposium on Prevention and Control of Foot and Mouth Disease in East Asia

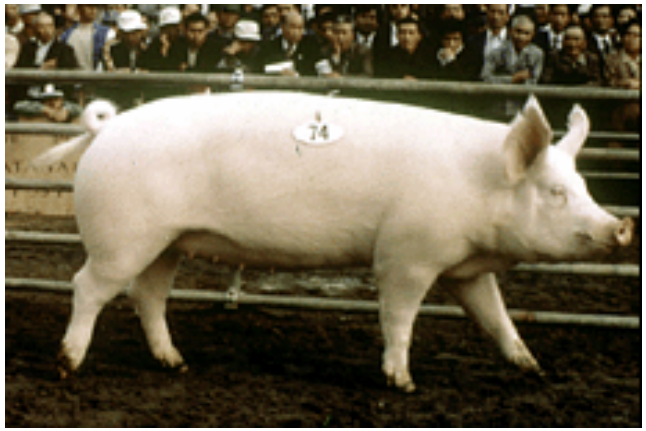
Challenges to inhibit Pandemic FMD Outbreaks in East Asia

- To reduce FMD viruses in the POOL 1 region -

National Institute of Animal Health (NIAH), NARO *Kenichi Sakamoto*

November 13, 2014

Roles of susceptible Animals in FMD

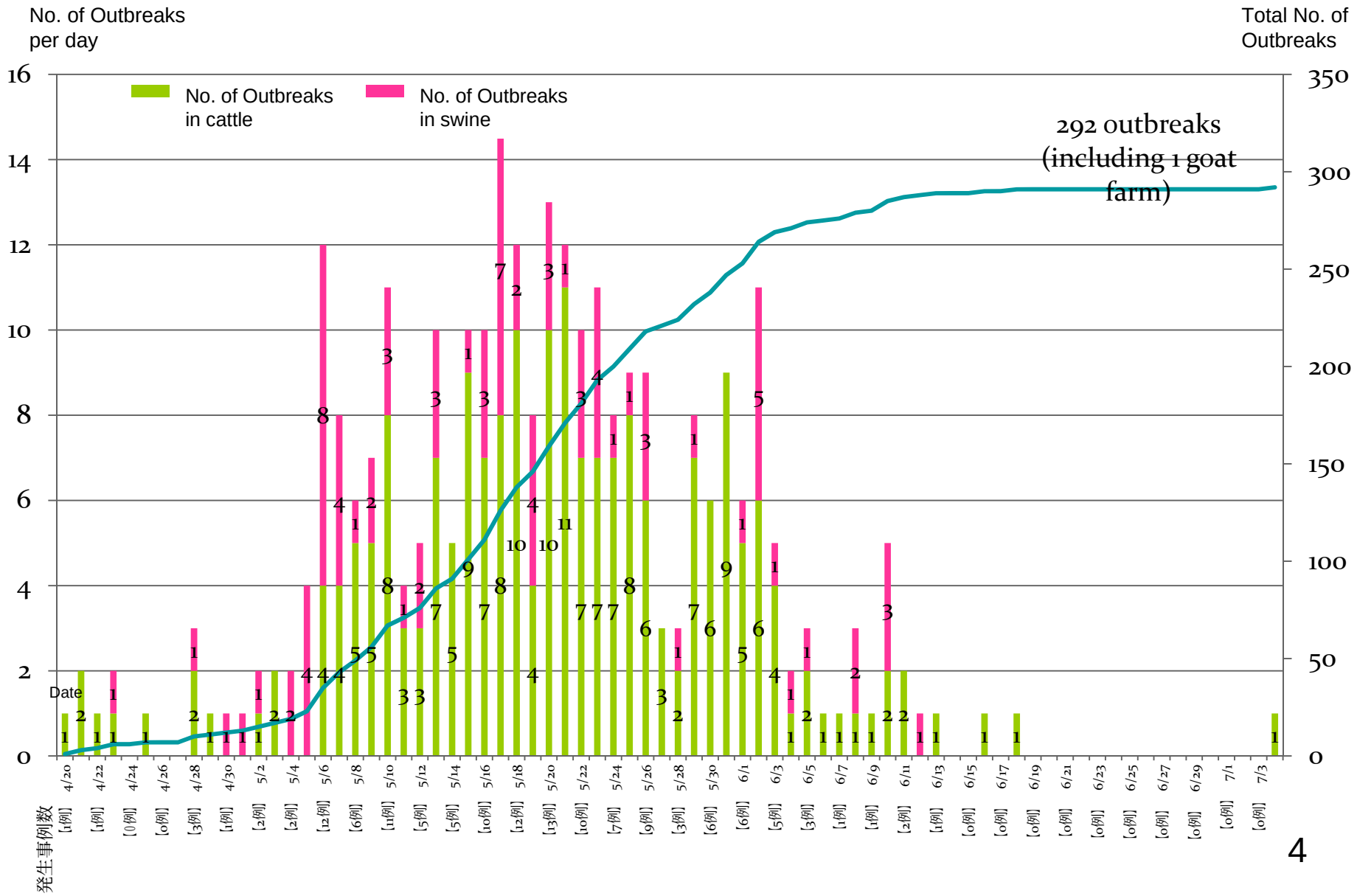


Minimal effective infecting dose	**$10^{1.0}$ ID_{50}**	**$10^{2.6}$ ID_{50}**
Excretion of virus	**10^{5} ID_{50}**	**10^{8} ID_{50}**
Epidemiological role	**Detector**	**Amplifier**

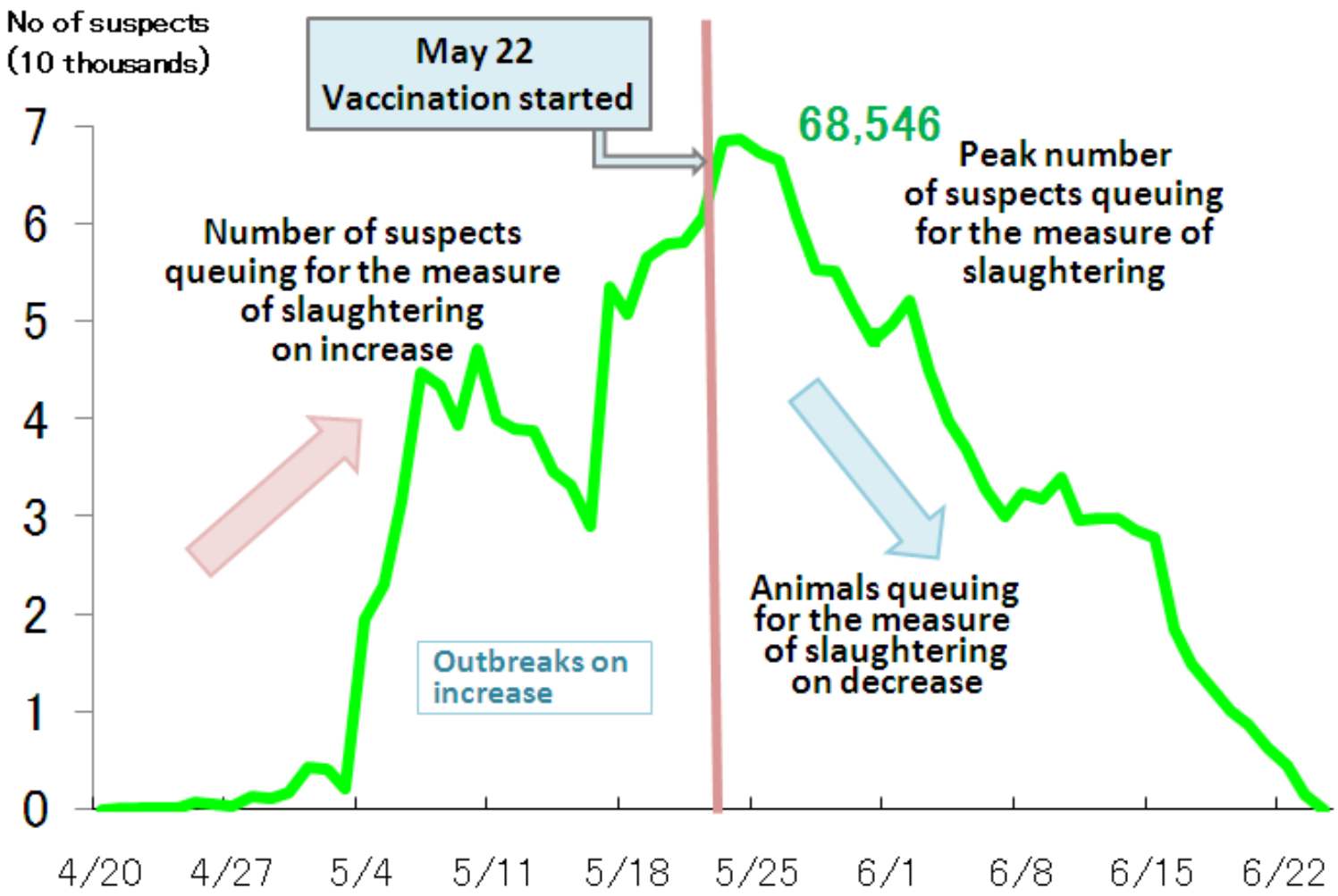
Economic Impact in Recent FMD Outbreaks

Year	Country	No. of Animal Destroy	Economic Damage
1997	Taipei China	Swine 4 million	3.6 Billion US$ in first year
2000	Japan	Bovine 740	72.7 Million US$
2000	S. Korea	Bovine 2200 (FMD Vaccine to 850,000 Bovine)	273 Million US$
2002	S. Korea	Swine 160,000	225 Million US$
2001	UK	Susceptible Animals 6 million	14.4 Billion US$
2010	Japan	Swine & Bovine 290,000	3 Billion US$ for complete recovery
2010-	S. Korea	Swine & Bovine 3.5 Million	3 Billion US$

Number of outbreaks (Cattle and Swine)



Number of queuing to be slaughtered



A Mobile Rendering Machine

Astronomical Titers of FMDV in Vesicular Epithelial tissue (水疱上皮中の口蹄疫ウイルス量)

According to **Hyslop NStG**. *Journal of Comparative Pathology* 75, 111–7, 1965

$10^{9.6}TCID_{50}$**/1g of Vesicular Epithelial tissue**
in the climax clinical stage of FMDV infected Cattle

Supposing that it is approximately $10^{10}TCID_{50}$, since the minimal infection dose is about $10^{1.0}TCID_{50}$, viruses in 1g of the tissue can infect

one billion cattle.

(Weak points of FMDV: Following conditions make its infectivity 1/10.) Bachrach HL. Proceedings of the Society for Exp. Biology & Medicine. 95, 147-52, 1975

61℃	30sec	(pH7.5 neutral zone)	pH5.0	1sec (Temp. 4℃)
20℃	11days		pH6.0	1min
4℃	18weeks		pH7.0-7.5	more than 5 weeks

Under the conditions of 61℃ for 5 min (neutral zone) or pH5.0 for 10sec (4℃) the viruses in the 1g epithelial tissue lose the infectivity to Cattle.

Foot and mouth disease

- Cattle, Pigs, Sheep and Goats are mostly susceptible
- Acute febrile contagious disease in cloven-hoofed animals
- Vesicular forming in mouth, nose, mamma and foot
- Strong infectiousness
- 7 distinct serotype
- Direct economic damage by nutrition disorder and dyskinesia
- Huge impact on international trade of animals and animal produc

Distribution of the livestock population in the world during the period 2005-2011

世界における家畜頭数

OIE GS80 2012

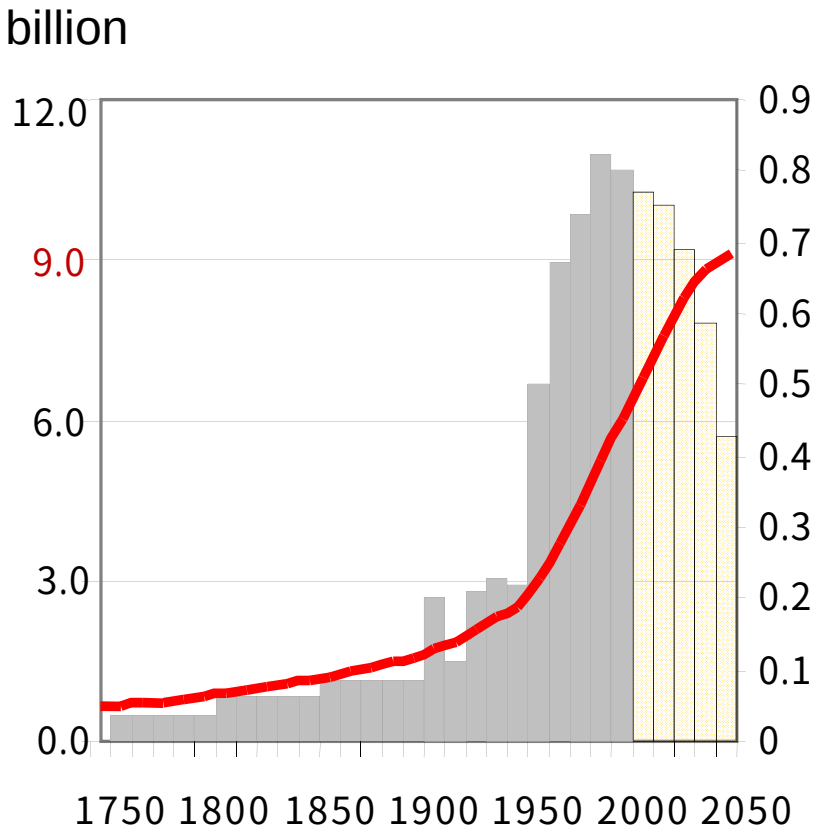
Species	2005	2006	2007	2008	2009	2010	2011	Average
Birds	33,271,448,687	32,391,345,243	33,696,549,515	34,912,310,631	36,042,555,371	37,299,983,433	37,142,651,637	***34,965,263,502***
Sheep/ Goats	2,203,939,767	2,306,546,698	2,169,820,661	2,136,203,987	2,165,490,141	2,228,510,854	2,220,054,529	***2 ,204,366,662***
Swine	1,630,554,884	1,654,988,867	1,494,927,400	1,559,759,472	1,609,835,913	1,610,175,564	1,621,376,365	***1,597,374,066***
Cattle	1,591,943,729	1,590,417,881	1,554,092,649	1,556,764,311	1,580,804,694	1,614,498,875	1,603,347,457	***1,584,552,799***

General Information of Asia

1. Human Population

World population 7.0 billions (2011.11)

More than 60% 4.2 billions in Asia



Red line: Total population

Bar: increasing population year by year

2. Populations of domestic animals

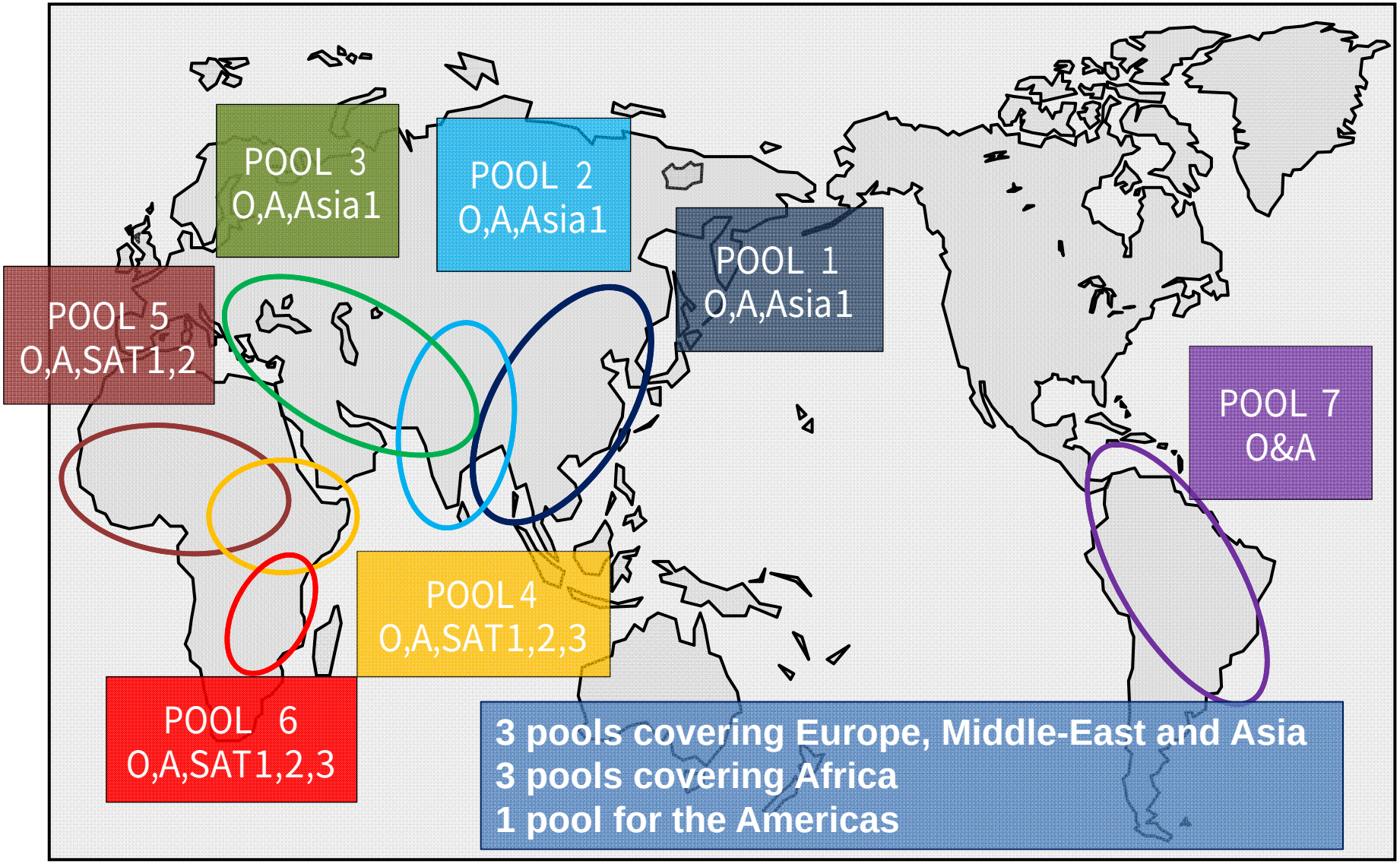
(2007 FAO)

	World Population	Ratio of Asia
Cattle	1.38 billion	33.3%
Pigs	0.92 billion	58.5%
Sheep	1.09 billion	35.5%

3. Rapid Increase of economic activities in Asia Region

Active movements of people, animals, animal commodities

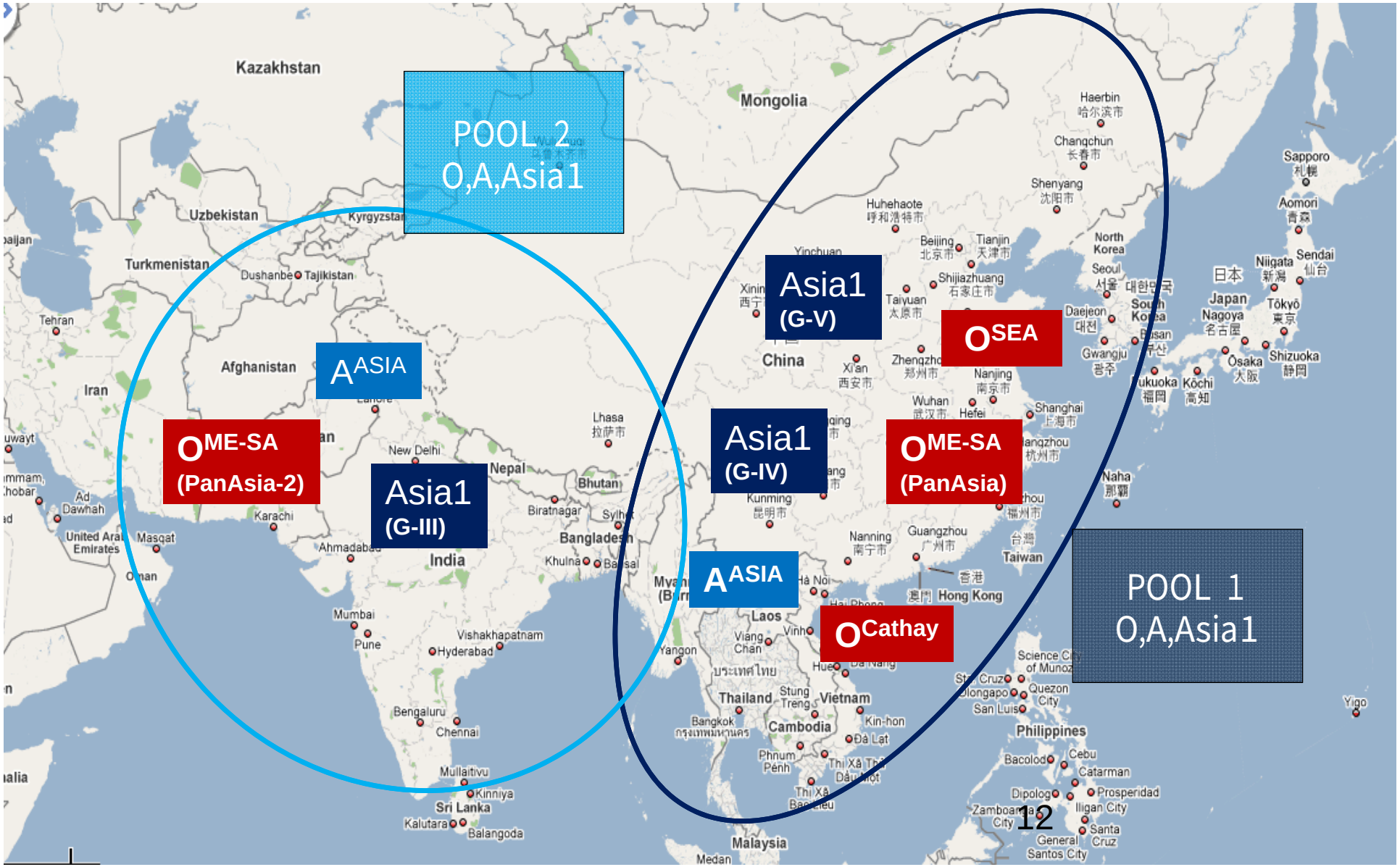
Model of related FMDVs Distribution



(OIE/FAO_WRLFMD、;OIE/FAO Global Conf. on FMD, Paraguay, 2009)

FMD virus in Asia

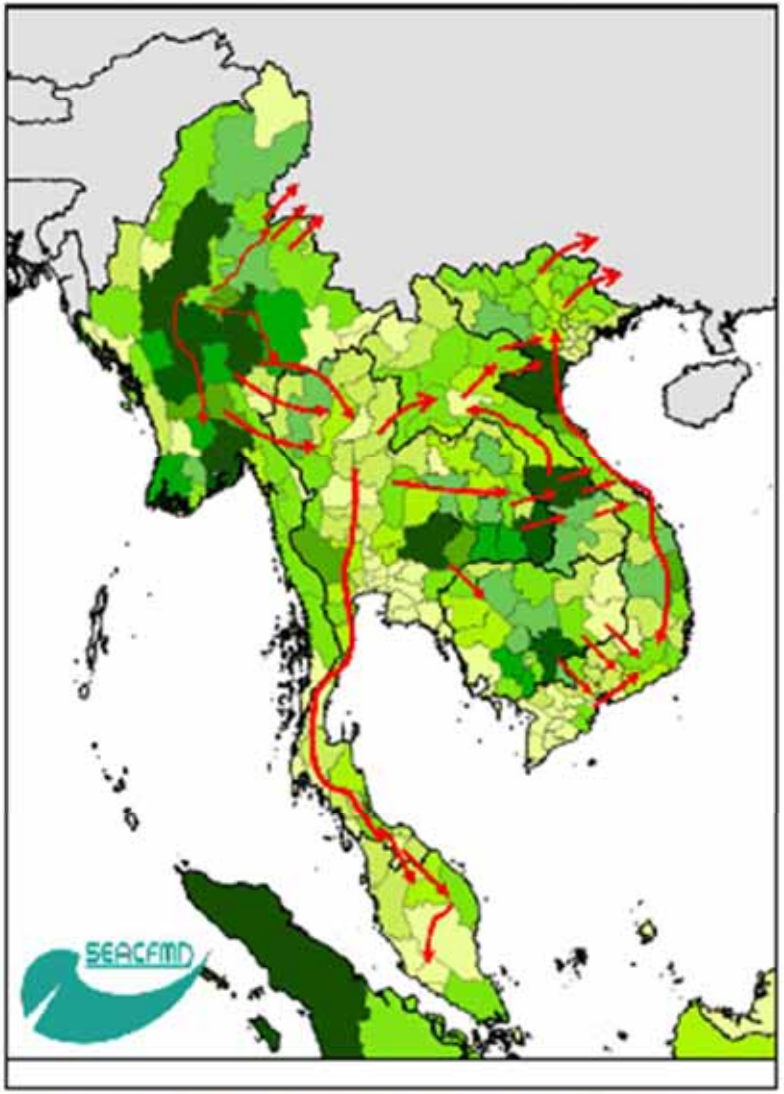
(Hammond et al. http://web.oie.int/eng/A_FMD2009/FMD_presentation/Session%202_1/2_1_1_Hammond.pdf)

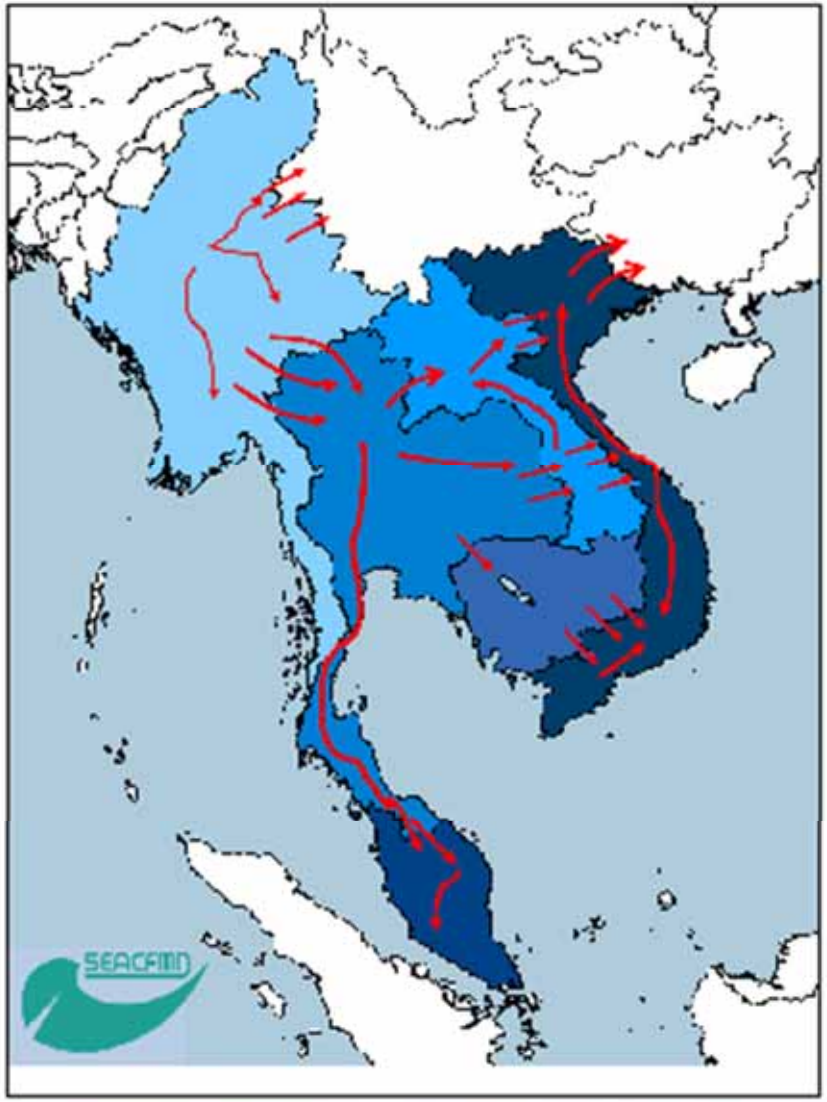


The recent characteristic of FMD outbreaks in East Asia

- The FMD spreads more quickly than before.
- *FMD outbreaks are predominantly caused by FMDV serotype O.*
- *The two main topotypes involved are South-East Asia (SEA) and Middle East – South Asia (ME-SA).*
- *FMDV of the SEA topotype (Mya-98 lineage) is widespread in South-East Asia and East Asia (POOL 1).*
- *The economic impact of FMD in East Asia (People's Republic of China, Japan and S. Korea) has been severe in 2010–2011.*
- *FMD outbreaks due to serotype A have been sporadically observed in recent years.*
- *Serotype Asia 1 newly appeared in Pakistan from 2010 and Bahrain and Iran in 2011.*

Cattle movement pattern vis-à-vis population and price





Cattle/Buffalo Trade vs. population and price

	Cattle (millions)	Buffalo (millions)	Import	Export	Comparative Unit Price Ranking	
Cambodia	3.34	0.72	-	+++	**	Moderate Low
Lao PDR	1.35	1.16	-	+++	**	Moderate Low
Malaysia	0.8	0.14	+++	-	*****	High
Myanmar	12.63	2.84	-	++++	*	Low
Thailand	9.34	1.58	++	++	***	Moderate
Vietnam	6.88	3	+++	-	****	Moderate High

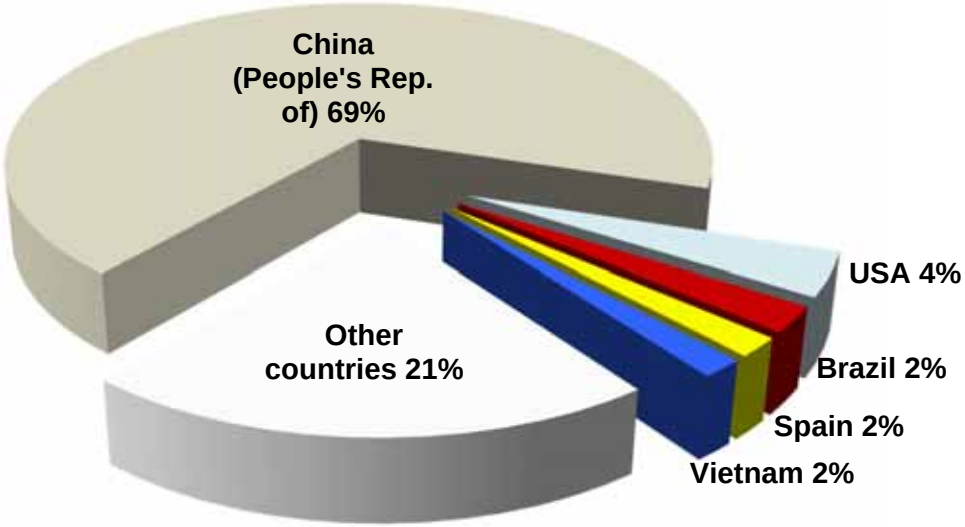
Border between Malaysia and Thailand (Malaysia side)

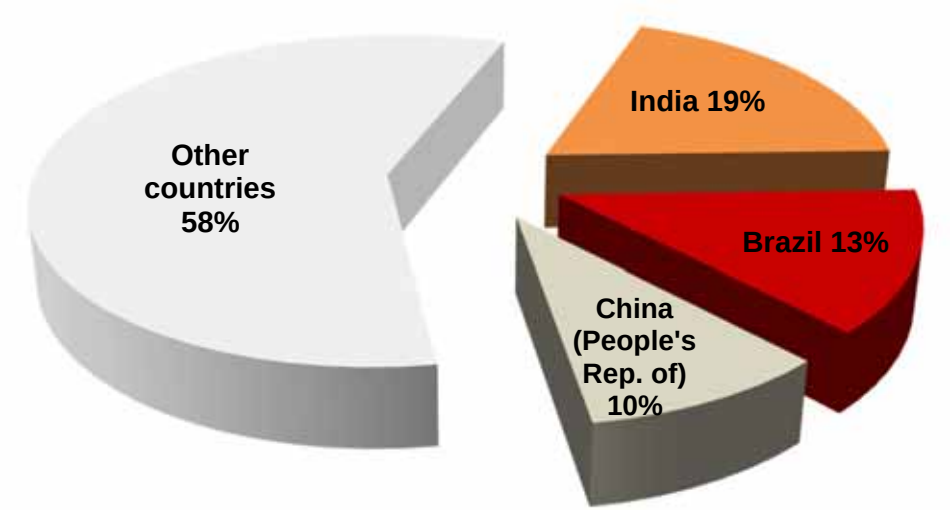
A only shallow river exists in dry season there. The cattle does not wear any ear tags or any brand. They can cross this border without any inspections by animal quarantine services.

Distribution of the livestock population in 2011

OIE GS80 2012

Swine production
1,6 billion



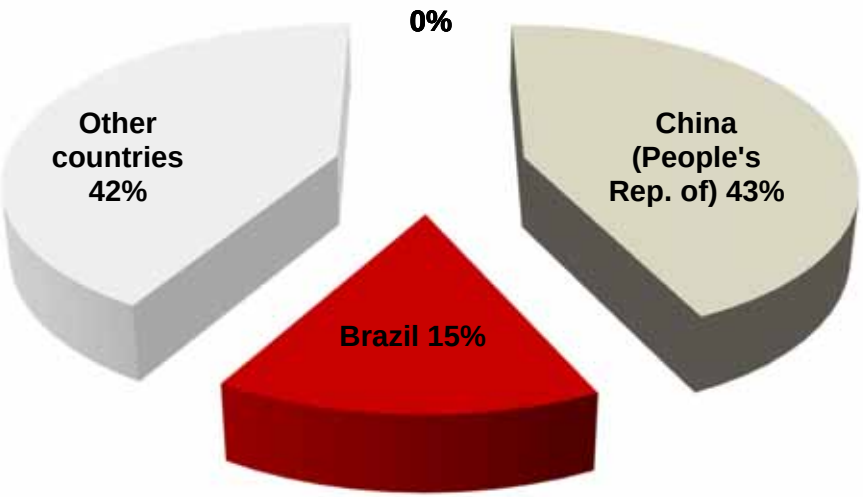


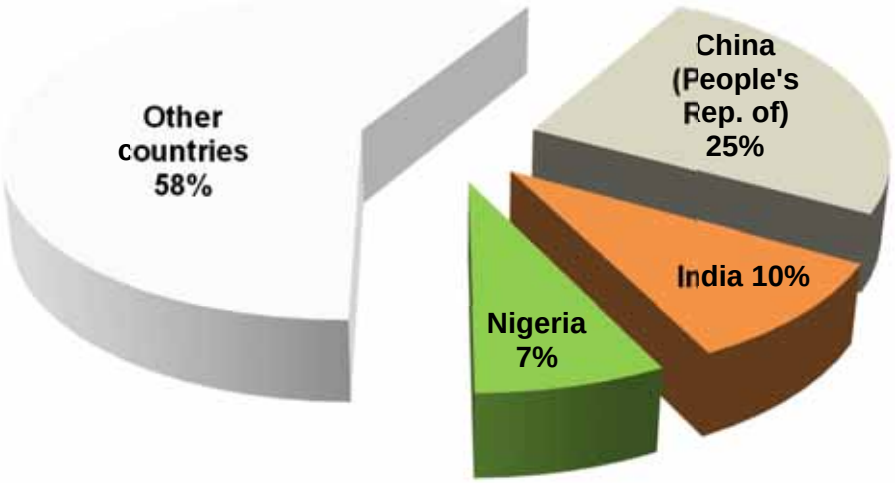
Cattle production
1,6 billion

Distribution of the livestock population in 2011

OIE GS80 2012

Birds production
37,1 billion





Sheep and goats production
2,2 billion

Mechanism of FMD spread in East Asia

China has the important role of the FMD outbreaks in East Asia

1) Long border with FMD epidemic countries in South East and Central Asia
2) Large numbers of susceptible animals (pigs, sheep, cattle)
3) Rapid increase of economic activities (A raising nation)
4) Active cross border movement of people and animal commodities

FMD (Epidemic countries) → China → East Asia countries

The Scheme of FMD virus spread in East Asia

Recent FMD outbreaks in East Asia

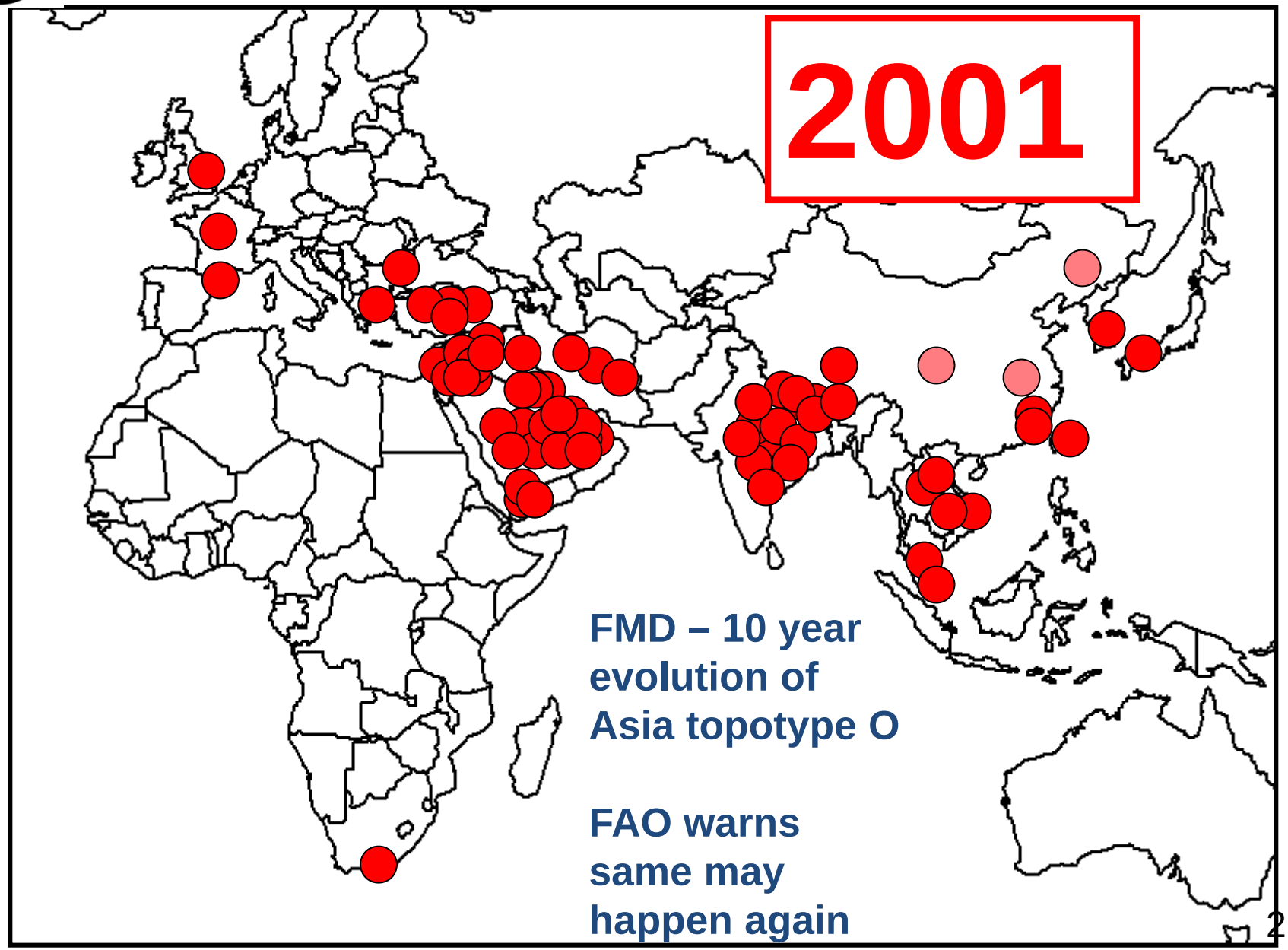
1)1997- O: Cathay topotype:
Taiwan (Nationwide) Illegal Movement of pigs from China

2) 1999-2002 O: ME-SA topotype, PanAsia lineage:
China, Taiwan, Japan, Korea, Amur region of Russia, Mongolia

3) 2005- Asia 1:
Hong Kong, China, Amur region of Russia, Mongolia, North Korea

4) 2009- A: ASIA topotype, China, South Korea

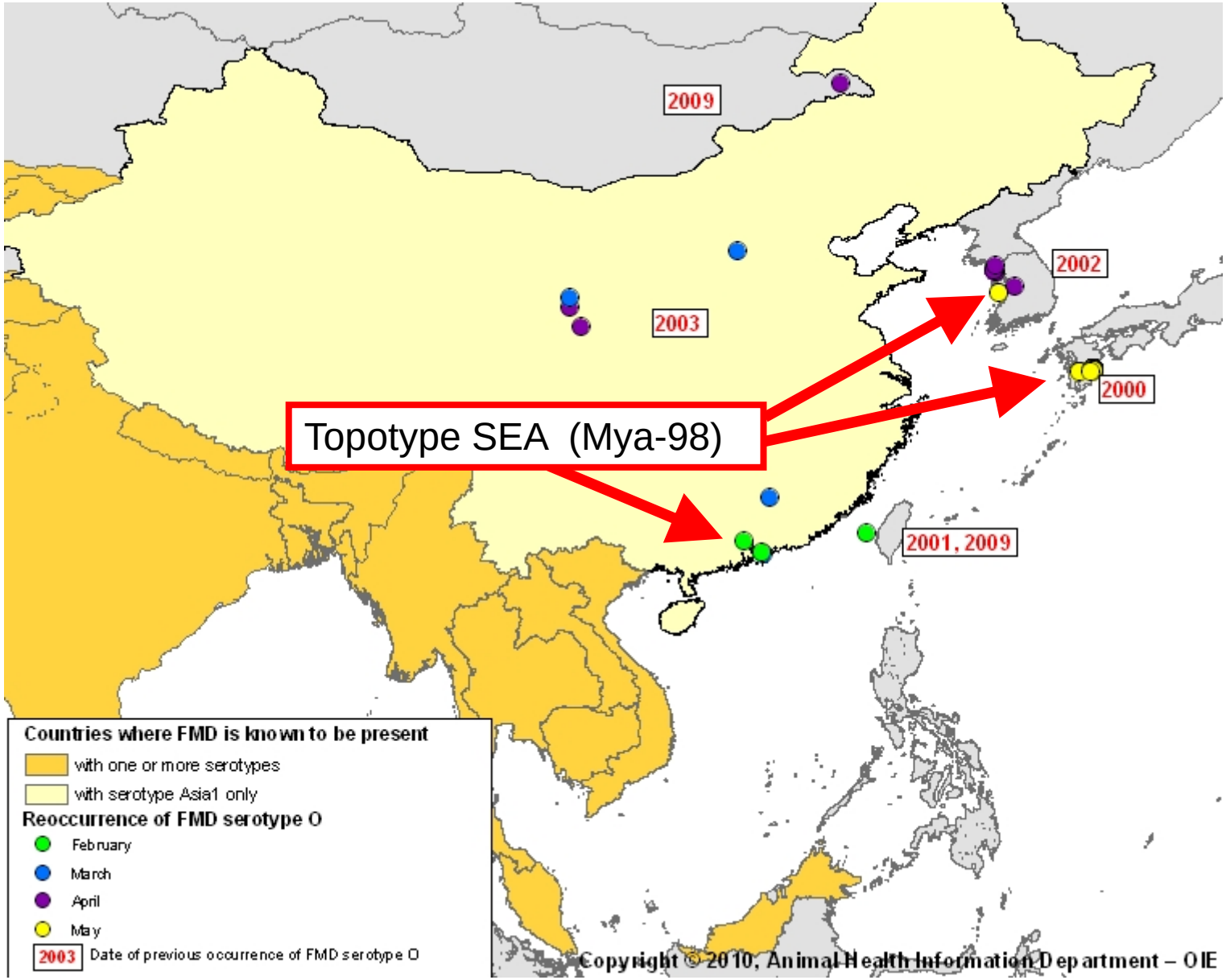
5) 2010-2011 O,SEA topotype, Mya-98 lineage:
China, South Korea, Japan, Amur region of Russia, Mongolia, North Korea



FMD – 10 year evolution of Asia topotype O

FAO warns same may happen again near future

Recent serotype O Outbreaks



To reduce FMD outbreaks in the region

1. Sharing Disease Information in Asian Region
   (South East, Central and East Asia)
2. Early notification of the FMD to Member countries in the region and OIE
3. Strengthen the border control to prevent FMD virus entry
4. Scientific research collaborations of the FMD laboratories to control the disease among China, Japan, Korea and Taiwan
5. Technical support to South East Asian countries for diagnosis of FMD
6. Financial and economic supports to provide good matching FMD vaccines to SEA countries

Cooperation and collaboration to control FMD outbreaks in the region

Activities and Challenges
To reduce FMD outbreaks in East Asia

1. Disease Information Sharing

- Symposium on Prevention and Control of Foot and Mouth Disease in East Asia

  Japan (2011) - S. Korea (2012) - China (2013) - Japan (2014) -
- Southeast Asia & China FMD Meeting (SEACFMD)
- OIE/JTF Meetings
- OIE/FAO GF-TAD

Technical Transfer in NIAH Japan on FMD Diagnosis (2012-2014)

- FMD diagnosis Training for Mongolia
- Advance FMD Diagnostic Training for RRF-FMD
- FMD Diagnostic Training for Myanmar

FMD Research Network & Collaboration in East Asia

1. OIE/JTF FMD Scientific Meetings

FMD Research Laboratory Network in East Asia:

(2013 Mongolia, 2015 Japan)

2. FMD Research Collaboration

NIAH (Japan) + AHRI (Taiwan) + QIA (S. Korea)

Subject: In vivo & In vitro Efficacy of the Anti-FMDV agent

OIE/JTF Project on FMD Control in Asia

The 1st FMD Scientific Meeting for East Asia

October 8, 2013

Wild Horse Center, Hustai National Park, Mongolia

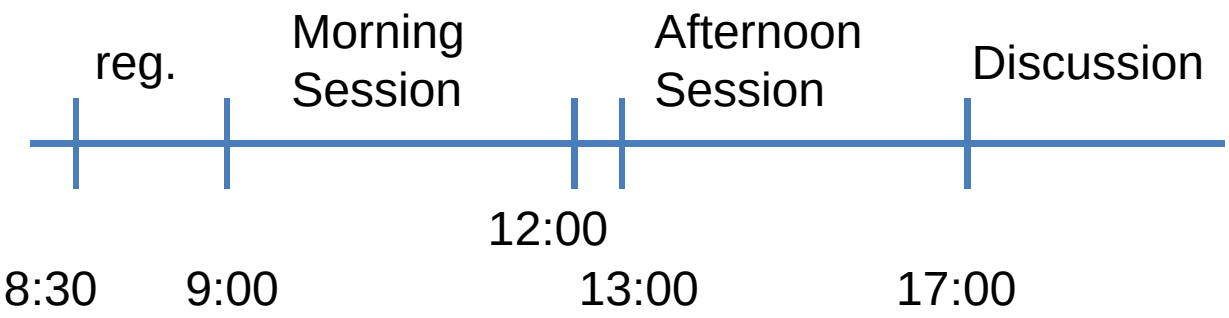
	Starting	Ending	Speaker	Title
Introduction	8:30	8:40	Dr. Kenichi Sakamoto	
SESSION 1: Key Note Lecture Chair: Dr. Gideon Bruckner	8:40	9:10		Phylogenic Study of FMD Virus Isolates Recovered in the Territory of Russia in 2000-2013
Session2 : Current Scinetific Achievement in the Members				
SESSION 2-1: China Chair:	9:10	9:30	Dr. Xinwen Bai	Molecular Variability confers Phenotypic Differences to Biological Properties of PanAsia-1 Strains of FMDV Serotype O in China
	9:30	9:50	Dr. Huichen Guo	In vitro assembly of virus-like particle and its application
	9:50	10:10	Dr. Haixue Zheng	Engineering Foot-and-Mouth Disease Virus with Improved Properties for the Development of Effective Vaccine
SESSION 2-2: Japan Chair: Dr. Kenichi Sakamoto	10:10	10:40	Dr. Katuhiko Fukai	Experimental infections in animals using a foot-and-mouth disease virus isolated from the 2010 epidemic in Japan
	10:40	11:10	Dr. Manabu Yamada	Characters of FMD DIVA tests and Verification of Efficacy of Expired FMD O Type Vaccines
SESSION 2-3: DPR Korea Chair: Dr Prof Kyong Gun Ri	11:10	11:40	Dr. Prof. Sung Chil Jong	Typing for FMDV occurred in DPRK
	11:40	12:10	Dr. Tae Sik Hong	Vaccination for Control FMD in DPRK
Lunch 12:10-13:10				
SESSION 2-4: RO Korea Chair: Dr. Jing-Hyeon Park	13:10	13:40	Dr. Kwang-Nyeong Lee	
	13:40	14:10	Dr. Hyang-Sim Lee	
SESSION 2-5: Chinese Taipei (Taiwan) Chair: Yeou-Liang Lin	14:10	14:40	Dr. Ming-Chung Deng	Study on the induced antibodies duration of single dose FMD vaccination in commercial pigs in Taiwan
	14:40	15:10	Dr. Shu-Chia Hu	Comparison of the efficiency between single and double doses FMD vaccination in pigs in Taiwan
	15:10	15:40	Dr. Yeou-Liang Lin	Repeatedly vaccination does not induce detectable antibodies to non-structural protein of foot-and-mouth disease virus in pigs
SESSION 2-6: Mongolia Chair: Dr Ganzorig Khuukhenbaatar	15:40	16:10	Dr. Ts. Purevkhuu	
	16:10	16:40	Dr. Gerelmaa Ulziibat	
	16:40	17:10	Dr. Dashzeveg Bold	
General Discussion	17:10	17:40	Dr. Gideon Bruckner & Dr. Kenichi Sakamoto	
Conclution and Closing	17:40	18:00	Dr. Hirofumi Kugita & Dr. Baatar Togoonyam	

Scientific Meeting of FMD 2015

Purpose and Goal:
Sharing the information of FMD research works by the Laboratories in the members and to develop future research collaborations between the laboratories

Design of the Meeting:
(1) Oral (20-30 min) presentation on any resent or on-going FMD research works
(2) At least two topics from each member or group
(3) One hour for each country or group
(4) One day meeting (ex. Starting 8:30 - Finishing18:00)
(5) By young researchers
(6) Chaired by Senior researchers of own country or region
(7) Select one best presentation

Time and Place:
(1) July 2015 ?
(2) Japan ?
(3) Tokyo Uni.?



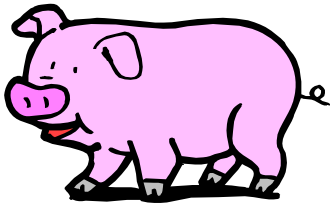
Time Course

Budget: Supported by OIE /JTF

T-1105 antiviral agent efficacy test with pigs (2012)

FMDV donor： O/Taw/1997 Cathay strain infected pigs cohabitating for 24hours

Sample: T-1105 200 mg/kg/day Twice a day with food (Total amount 1170g)



Body Weight：30kg

No. of Animal ：25 head s (donor 2 x 4 group:8 heads)

Tested group

Group 1. Administration of T-1105 one day before cohabitation

No. of administration 15 times until day 6 th morning ：5 heads

Group 2. Administration of T-1105 same day with cohabitation

No. of administration 13 times until day 6 th morning ：5 heads

Group 3. Administration of T-1105 one day after cohabitation

No. of administration 11 times until day 6 th morning ：5 heads

Group 4. Control group without administration: 2 heads

Difference of appearance of clinical sings in Groups
抗ウイルス剤の投与時間の異なる群における症状の出現

Days after Cohabitation	Vesicular forming		Difficulty of standing	Milder clinical signs
	1	2	12	8
Group 1	4 / 5	5 / 5	0 / 5	2 / 5
Group 2	4 / 5	5 / 5	0 / 5	2 / 5
Group 3	3 / 5	5 / 5	1 / 5	3 / 5
Group 4	2 / 2	2 / 2	1 / 2	0 / 2

・同居後2日目には**すべての群で全頭に水疱**が確認された。
・対照群に比べて**症状の軽減や起立困難の低減**が認められた。

Support for providing good matching FMD vaccines to SEA countries

The Characters of the donated FMD vaccines

- **Inactivate vaccine**
- **6 PD_{50} O Manisa (Double potency)**
- **R1 value 0.35 (VNT) with O/JPN/2010, SEA topotype (Mya/98) by NIAH**
- **Selected in the FMD vaccine meeting every year by MAFF**
- **Purchased from foreign vaccine makers**
- **Valid for 1 – 1.5 year**
- **Discarded after storage**
- **Donated after storage**
- **Verified the efficacy of the vaccines by single shot in Myanmar before in small scale**

FMD Vaccine Donation from Japan

Japanese Ministry of Agriculture and Fishery and Forestry (MAFF) donated FMD vaccines in 2012:

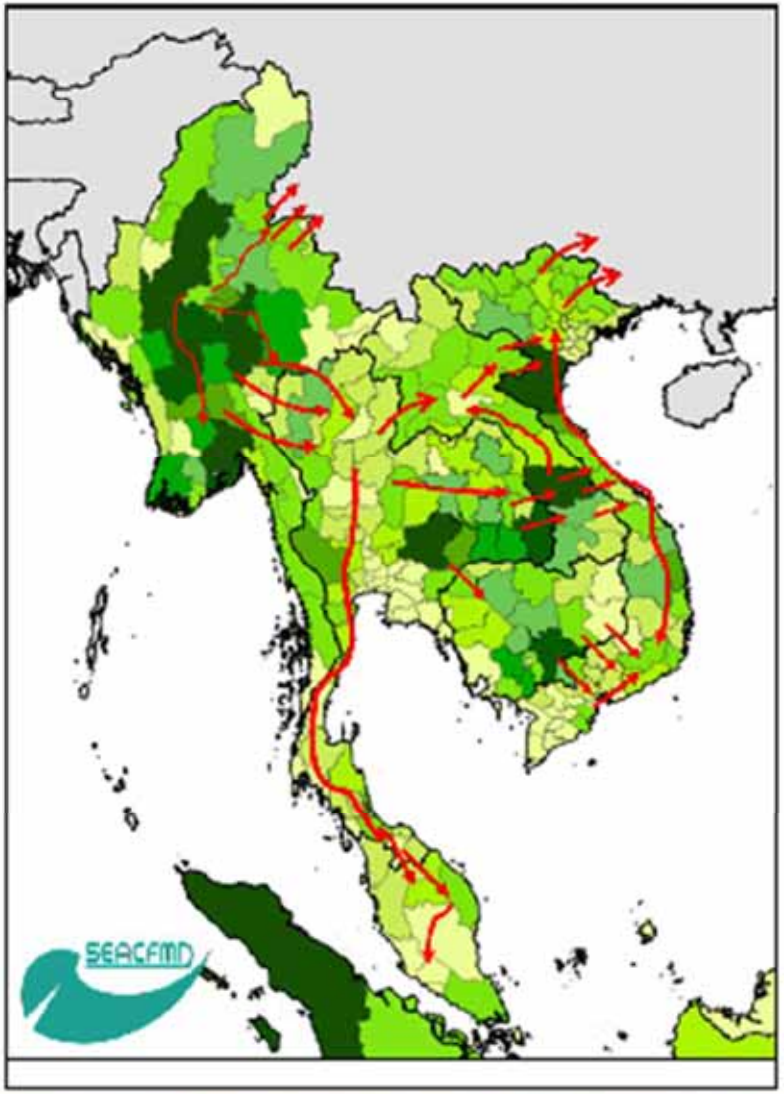
200,000 doses O Manisa (6PD50)- Laos

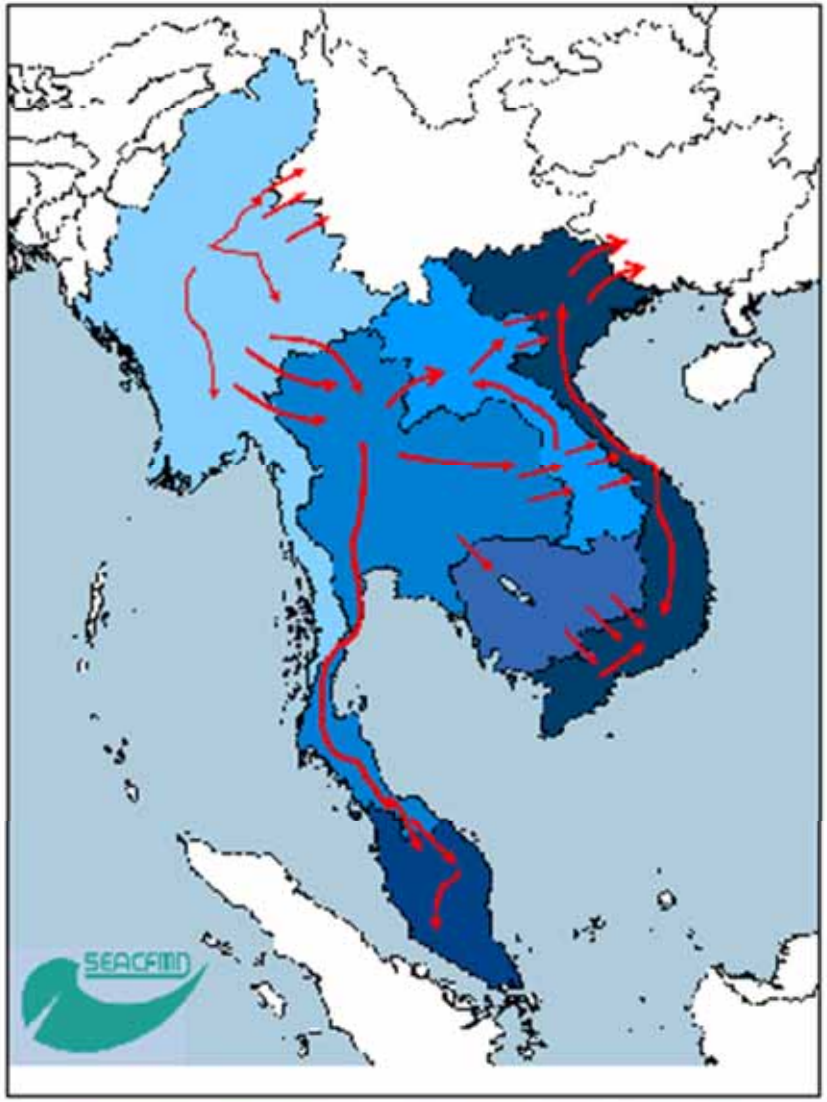
Japanese Ministry of Agriculture and Fishery and Forestry (MAFF) donated FMD vaccines in 2014:

100,000 doses O Manisa (6PD50) - Myanmar

100,000 doses O Manisa (6PD50)- Laos
100,000 doses A Malaysia 97 (6PD50) - Laos

Cattle movement pattern vis-à-vis population and price





Introduction

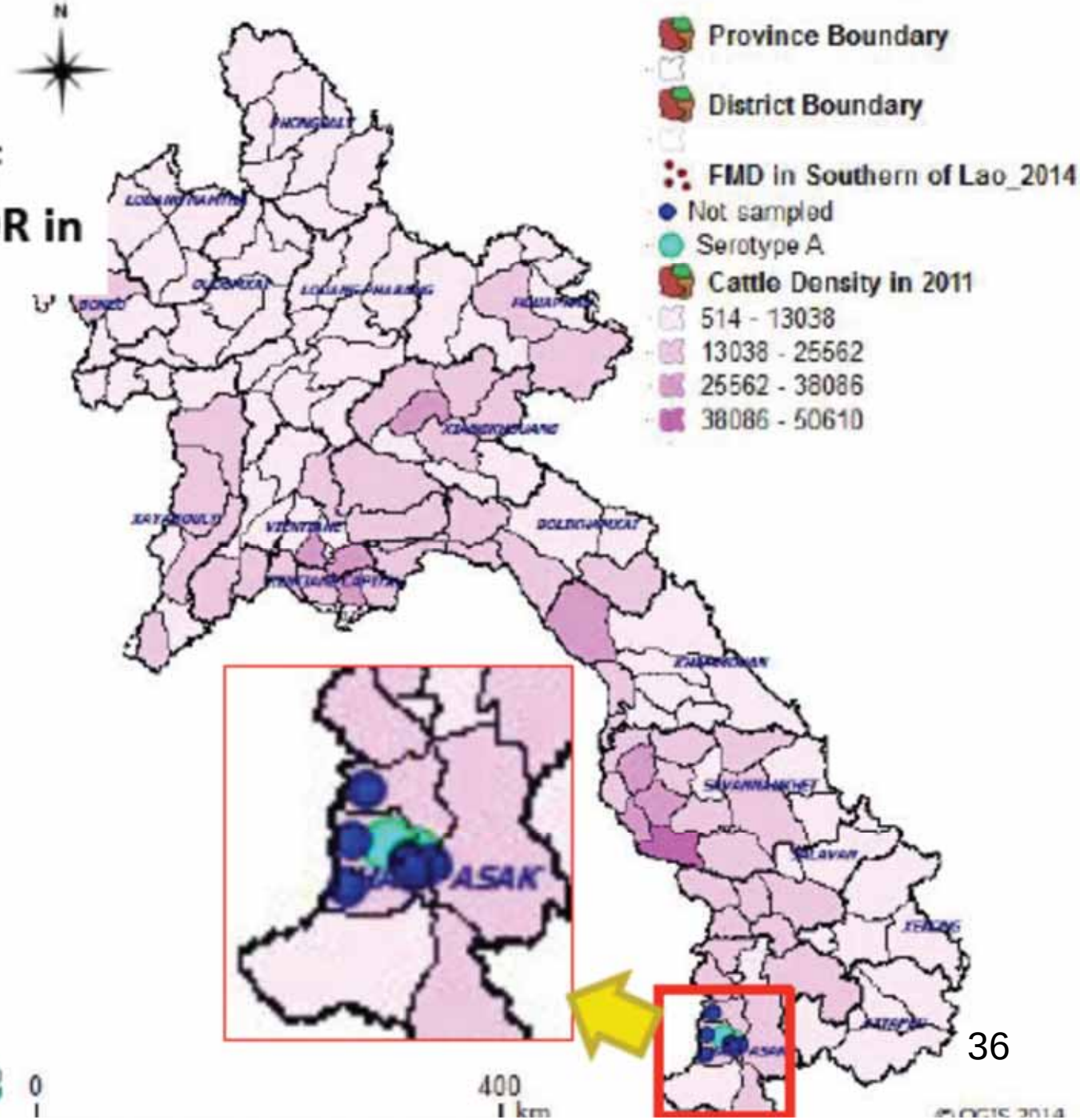
•Laos requested Japan 200,000 doses of O MANISA which had been stored for emergency use in Japan In 2012 and another 100, 000 doses of O Manisa and type A vaccines were donated in 2014 early.

•About 100,000 heads of cattle and buffalos were vaccinated twice in one month interval in Xiengkhouag Province, north-east of Laos in 2012 and once in 2014.

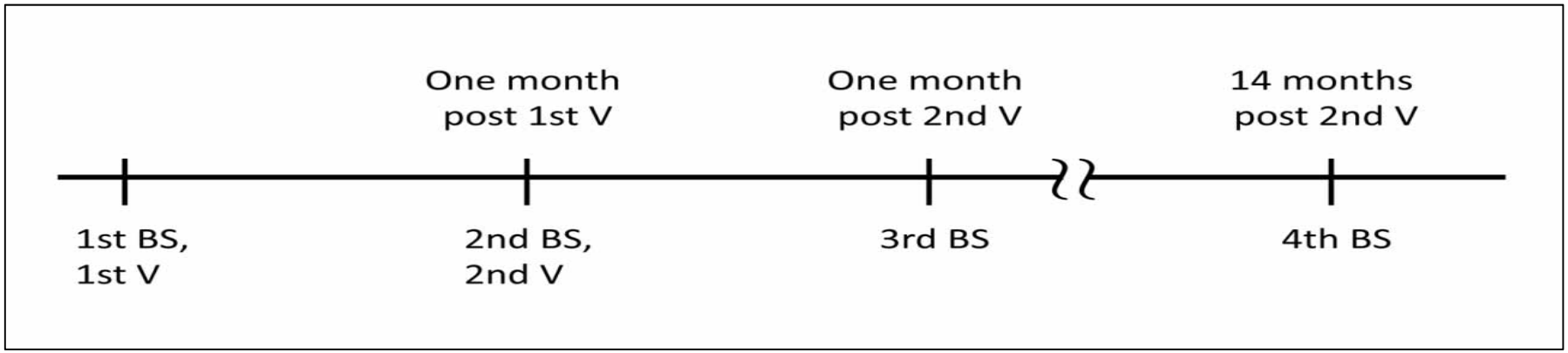
•To verify the efficacy of the vaccines, the blood samples were collected from about 200 heads of cattle and buffalos before, after the 1st and 2nd vaccination in one month intervals and14 months after the last vaccination.

•The sera were isolated and kept each time in National Animal Disease Diagnostic Laboratory (NADDL) in Vientiane and they were tested by LPB-ELISA

Distribution of FMD in Lao PDR in 2014



Blood Sampling & Antibody test for verifying the efficacy of the vaccines



2012 Sep. 29 – Oct. 4	Ear-tag for identification Pre blood sampling (BS 1) & serum isolation the 1st vaccination
Nov. 3 – 8	BS 2 (one month after the 1st vaccination) & serum isolation the 2nd vaccination
Dec. 3 – 8	BS 3 (one month after the 2nd vaccination) & serum isolation
2013 Jan. 14 - 18	Antibody assay by LPB-ELISA in Vientiane Lab (NADDL)
2014 Early Jan.	BS 4 (14 months after last vaccination)
April	Tested by LPB-ELISA in NADDL

Blood sampling & Vaccination
(Perfect combination of Laos and Japan Teams)

(**Beer Lao Team**) Ear-tags, Keeping cattle & buffalos, Vaccination
(**Oishii Team**) Blood sampling, Serum Isolation, Record of blood sampling, Antibody test & technical transfer of the assay

Sampling population and the tested numbers

(No. of BS 1 : pre-vaccination)
Cattle 139 Buffalos 50

(No. of BS 2 : one month after 1st vaccination)

	Sampling No.	Sampling ratio
Cattle	106	76.3% (106/139)
Buffalos	38	76.0% (38/50)

(No. of BS 3 : one month after 2nd vaccination)

	No. of sampling	Sampling ratio	Ratio of sampling 3 times
Cattle	107	77.0% (107/139)	64.7% (90/139)
Buffalos	34	68.0% (34/50)	62.0% (31/50)

90 cattle and 31 buffalos were successful in collecting samples in all three times in this period and used this study (About 60% of the cattle and buffalos).

(No. of BS4: In 2014) Cattle 57 41.0% (57/139)
Buffalos 13 26% (13/50)

The several green ear tags of the buffalos were dropped off. It means that about 40% of the cattle and buffalos were still kept in the two villages.

Antibody Detection Assay

(Method)
The antibody assay against type O was tested by LPB-ELISA kit (developed by WRL for FMD) in both screening and titration tests.

(No. tested)
(1) For the screening test (BS collected 3 times)
Cattle 90 Buffalos 31
(2) For the titration test (pre-vaccination serum samples of the animals whose titers were not more than x32 **)** Cattle 19 Buffalos 6

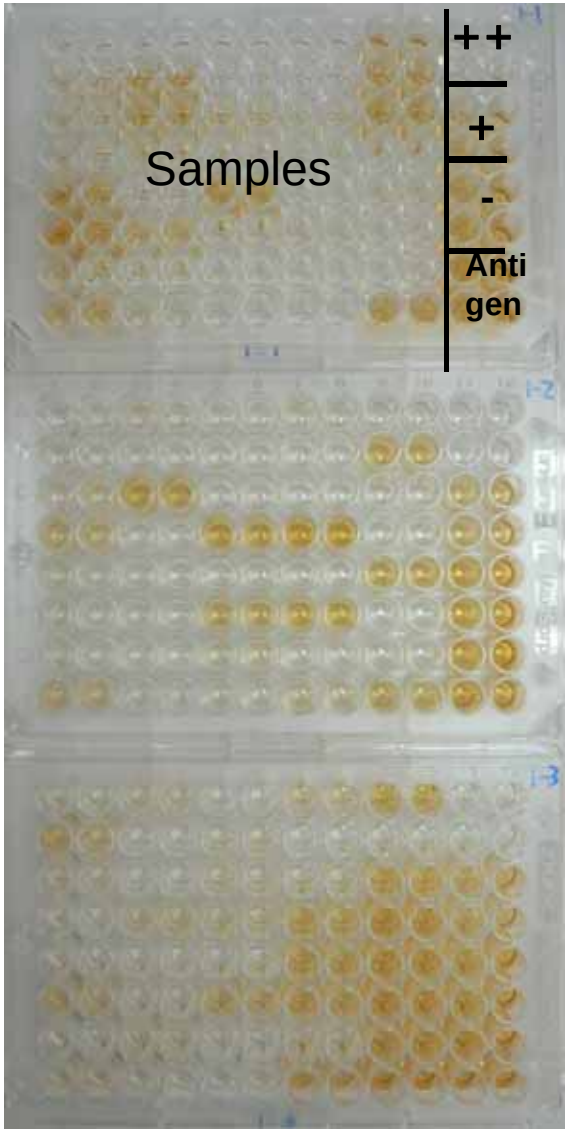
From the screening ELISA test ;
・More than 80% of the cattle and buffalos were revealed to be sero-positive against type O before the vaccination.

・By the vaccination, antibody titre of all the animals (cattle 90 & buffalos 31) turned to be higher and by the 2nd vaccination their immunity levels became more higher.

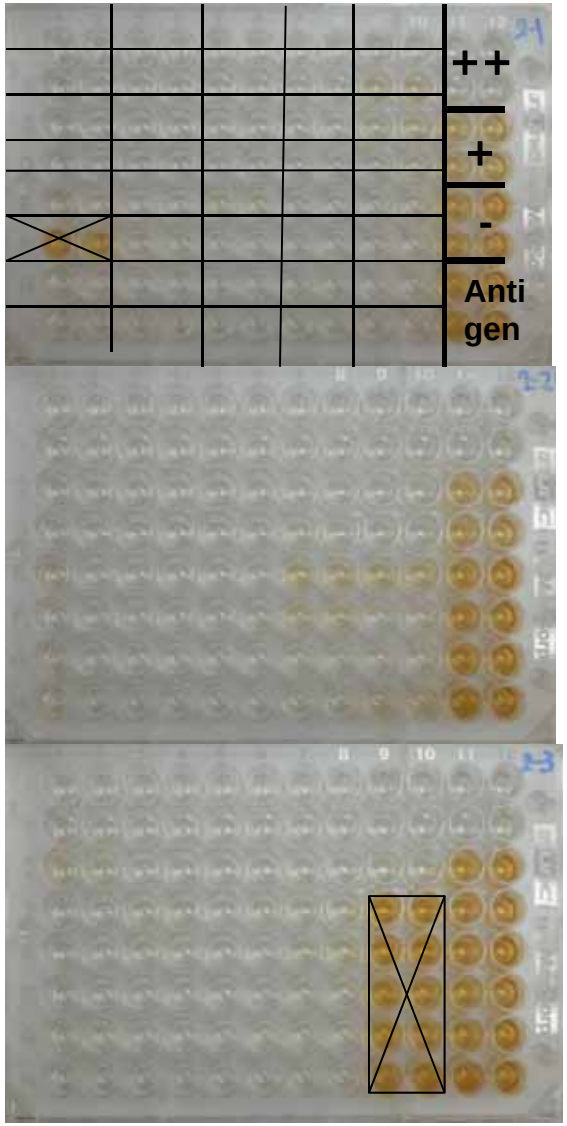
90% of the animals vaccinated even after 14 months still kept enough antibody titers.

Screening Test of Before and After Vaccination by LPB-ELISA

Before Vaccination



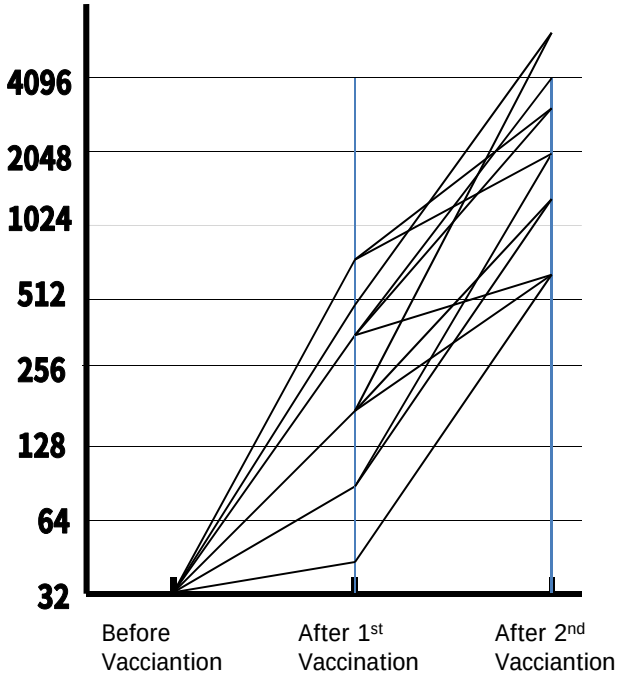
After 1st Vaccination



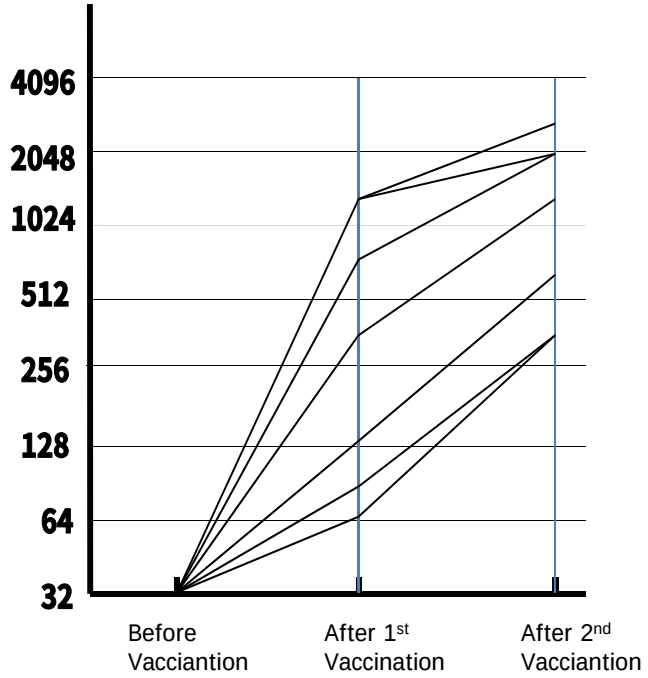
After 2nd Vacciantion

Antibody titers of the cattle and buffalos whose tiers were less than x 32 before the vaccination

Numbers of samples : 19 cattle and 6 buffalos whose titer were less than x 32 before the vaccination by the screening test of EPB-ELISA



Antibody titers of Cattle

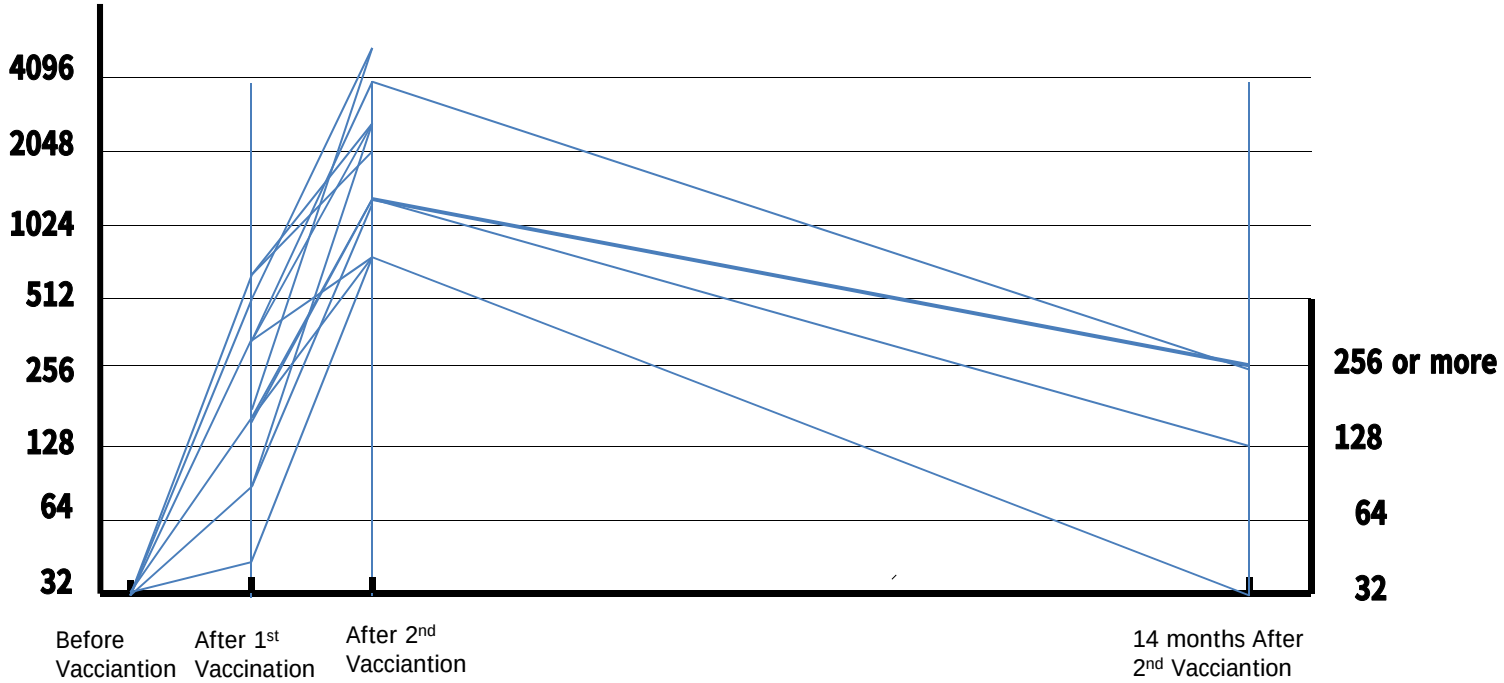


Antibody titers of Buffalos

After 1st vaccination: 1:45-1:1448, After 2nd vaccination: 1:362-1:5792

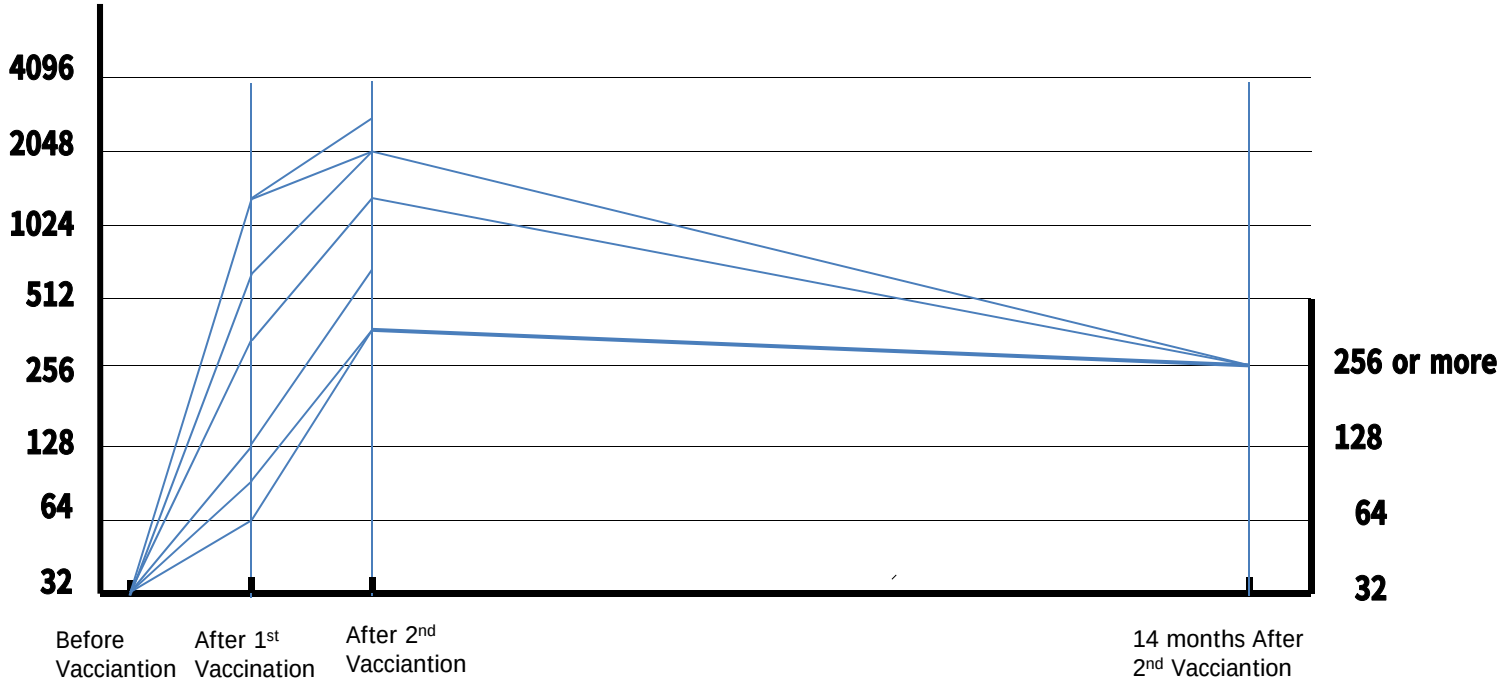
Antibody titers are to be higher and higher after 1st and 2nd vaccination.

Antibody Titers of the Cattle of14months after the last FMD vaccination



Antibody titers of Cattle

Antibody Titers of the Buffalos of14months after the last FMD vaccination

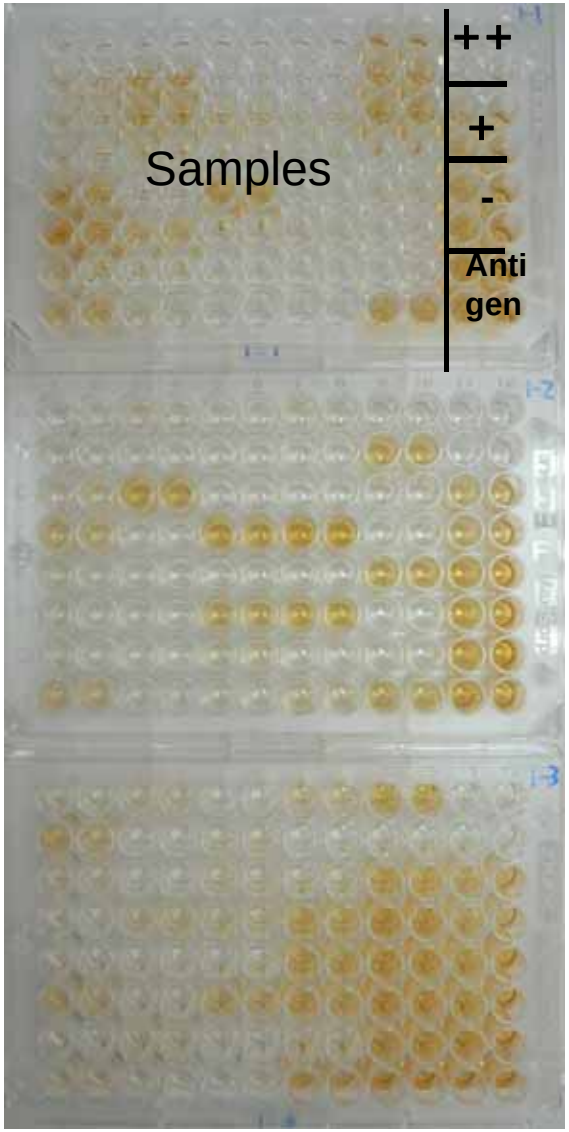


Antibody titers of Buffalos

90% of the animals vaccinated even after 14 months still kept enough antibody titers.

Screening Test of samples of “Before” Vaccination by LPB-ELISA

Before Vaccination



In the screening test, Many samples of before vaccination have high titer.

(45 <, 71 cattle and 25 buffalos)

Those animals were

Infected animals? or Vaccinated animals?

Differentiation between FMD-infected animals and non-infected, vaccinated animals

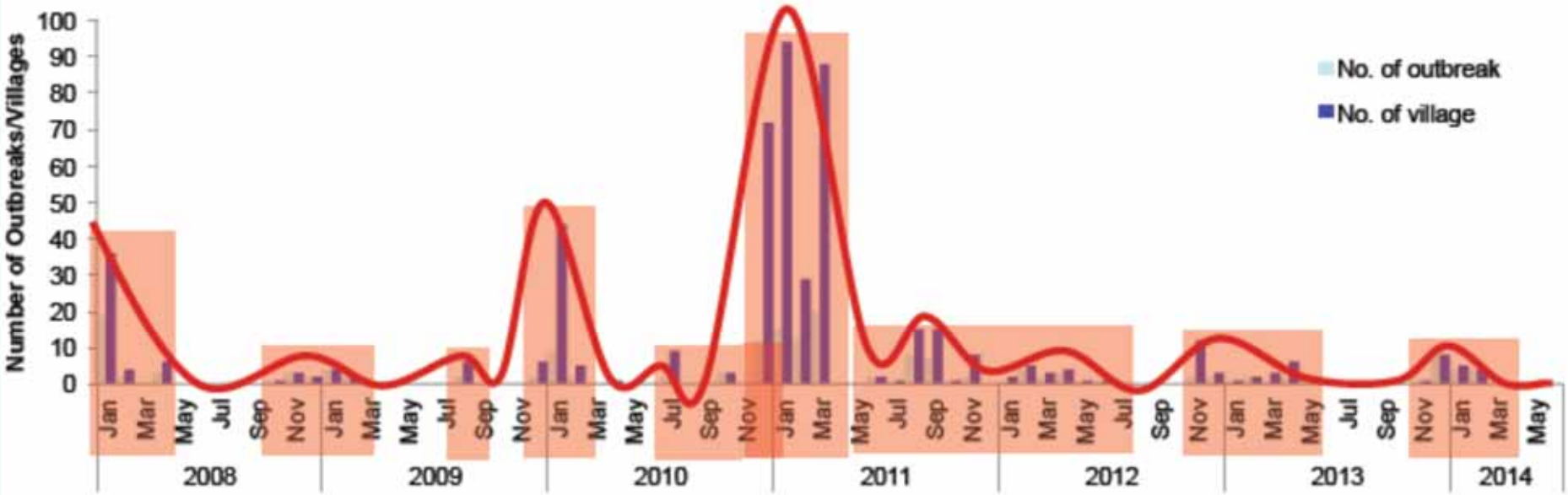
Kits： PrioCHECK FMD NS

Samples：Animals that had high LPBE titer
(45 <, 71 cattle and 25 buffalos)

Examined animals of 76.1% in cattle and 88% in buffalos that had high LPBE titer before vaccinateion were judged as FMD-infected animals.

This result suggests that
there was no report of FMD outbreaks in this area,
but FMD outbreak had occurred in this area in Laos before.

Temporal Distribution of FMD in Lao PDR: 2008-June 2014



- ✓ Most of FMD outbreak occurred in dry season (Nov. March)
- ✓ More seriously were in late 2010 to early 2011 with the highest peak found in January 2011 with 15 outbreaks (OB) in 94 villages.
- ✓ FMD outbreaks in 2014 is 2 times decreased compared to 2013.

Conclusions

- Even three months passed after expired date, the vaccine was found to have enough efficacies by the screening and titration tests with LPB-ELISA.

- There was no report of any side-effects by this 200,000 doses vaccination.

- The vaccines were shipped to Laos just after finishing storage periods from Japan. They were kept under suitable condition and transferred to the vaccination site by good cold chain until used.

- FMD DIVA tests (NSP ELISA kits) for detection of NSPs have characteristics of high specificity and low sensitivity.

- About 80% of the animals were already sero-positive against FMDV type O. (No report of FMD outbreak in the two villages for several years and no FMD clinical signs observed within 2 months of this field study, however, FMD DIVA test showed about 80% of examined animals were judged as FMD-infected animals.)

- About 90% of the animals vaccinated even after 14 months still kept enough antibody titers to inhibit infection.

FMD situation during 2008 – 2014

No	Province	2008		2009		2010		2011		2012		2013		2014	
		OB	VL	OB	VL	OB	VL	OB	VL	OB	VL	OB	VL	OB	VL
1	Attapeu							2	10						
2	Bokeo	1	1					1	1	1	2	1	1		
3	Bolikhamxay	1	1												
4	Champasak	1	1	4	4	6	61	4	54	1	2	5	5	8	9
5	Huaphanh					2	16	11	55						
6	Khammouan			1	2	2	6	1	6			1	4		
7	Luangnamtha	2	2	1	1					2	11	2	6		
8	Luangprabang							1	2						
9	Oudomxay			1	5			1	1						
10	Phongsaly							7	17	3	3	1	1		
11	Saravan					3	6	6	29						
12	Savannakhet					3	4								
13	Sekong							4	5						
14	Vientiane Capital	24	44			6	10	20	44	2	2				
15	Vientiane Province					1	1	1	4						
16	Xayabury	2	4	1	4	5	20	8	25	7	11	2	4		
17	Xiengkhuang			2	2	2	10								
	Grand Total	**31**	**53**	**10**	**18**	**30**	**134**	**67**	**253**	**16**	**31**	**12**	**21**	**8**	**9**

Plan of FMD Vaccine Donation from Japan

Japanese Ministry of Agriculture and Fishery and Forestry (MAFF) can donate FMD Vaccines in 2015:

200,000 doses O Manisa (6PD50)

FMD Vaccine Production Plan in Myanmar

JICA Project (2015 or 2016)
JICA is now planning to establish FMD vaccine plant in Yangon.

500,000 – 1,000,000 doses O Type Vaccine by using roller tube bottles.

Members of Oishi team for these studies in Laos

(Japanese Samuries :)
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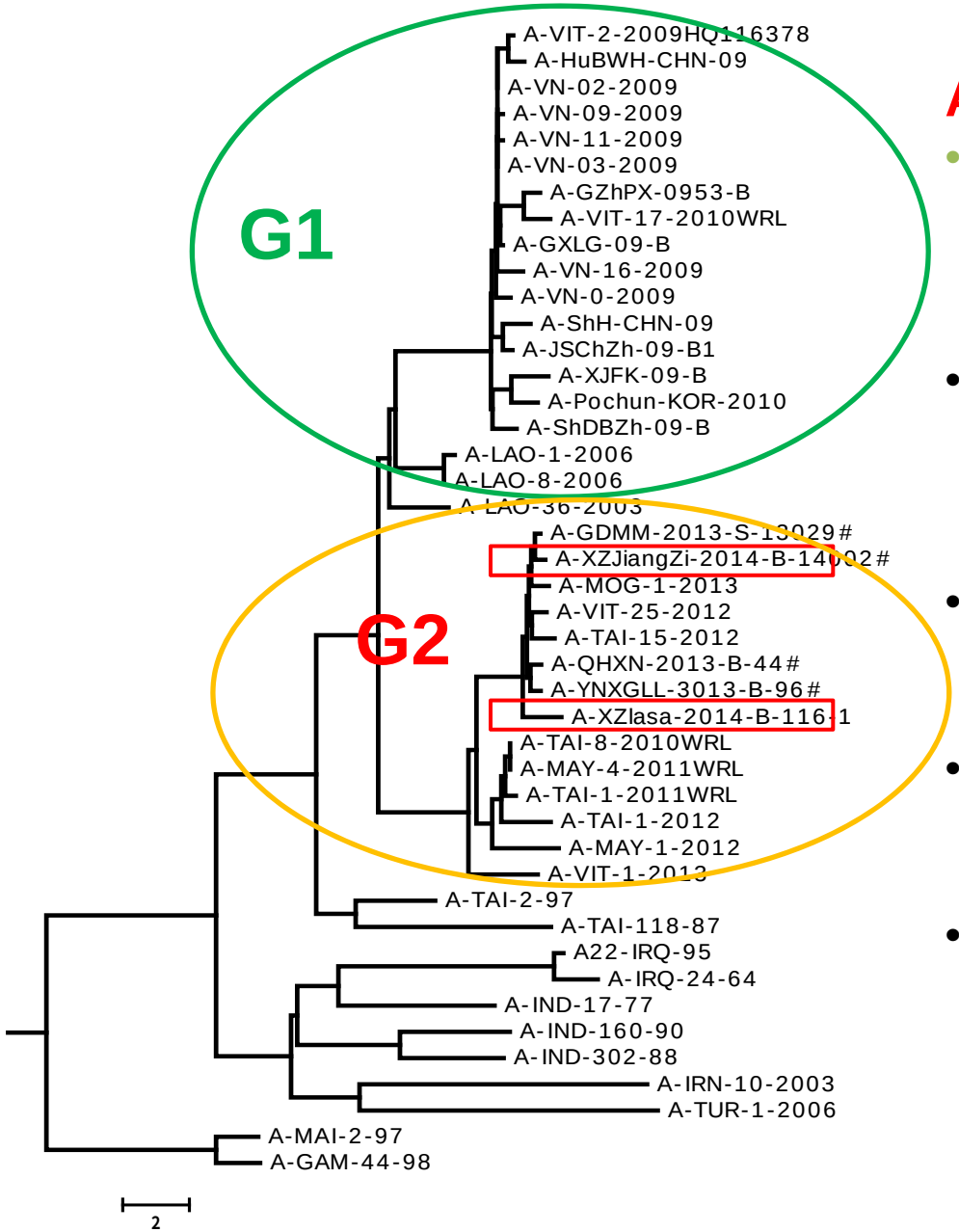
Manabu Yamada (Path, NIAH)
Kazuki Morioka (FMD, NIAH)
Katuhiko Fukai (FMD, NIAH)
Toshiyuki Tsutsui (Epi, NIAH)
Norihiko Muroga (Epi, NIAH)
Noriyoshi Ojima (DAH, MAFF)
Josuke Mago (AQS, Yokohama)
Yoshito Katagiri (AHC, Okinawa)

(OIE) Chantanee Buranathai
(Laos) Syseng KHOUNSY

MOLECULAR EPIDEMIOLOGY ANALYSIS



A/Sea-97 strain

- >99% identity with the virus collected in 2013; also located on the other genetic branch(named G2)
- low genetic relationship with the virus(named G1) found in China in 2009: about 91% identity
- Shared close relationship with viruses from SEA nations : over 98% homology
- FMDVs from SEA nations are appearing in East Asia continuously. What is driving?
- Regional joint prevention and control

Welcome Participants
OIE/FAO FMD Reference Laboratories Network Meeting
OIE/FAO口蹄疫参考实验室网络会议

OIE FMD Reference Laboratory in Lanzhou

Cooperation and Collaboration to Control FMD outbreaks Challenges for NO MORE FMD in East Asia