SEACFMD Laboratory Network Meeting
Lanzhou, China 26-28 September 2017

Current progress on FMD research in National Institute of Animal Health, Japan

Makoto Yamakawa, DVM, PhD
National Institute of Animal Health (NIAH)
National Agriculture and Food Research Organization (NARO)
JAPAN

NARO

History of FMD Outbreaks in Japan

- FMD has broken out only twice in 100 years
- Good animal quarantine system
- Geographical advantage as islands

} Effective to protect incursion of FMD

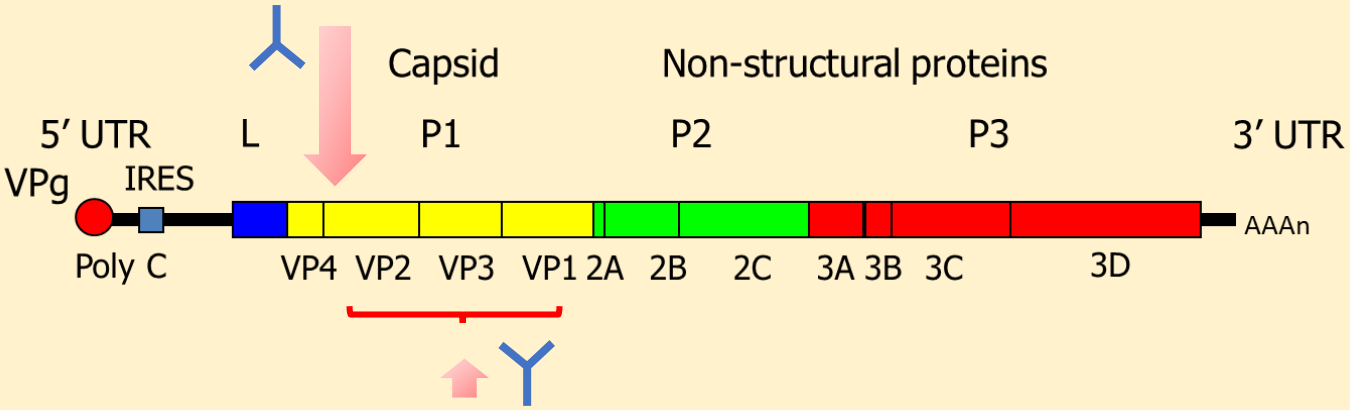
Year	Animal species	Place (prefectures)	No. of slaughtered animals	Serotype/topotype of the virus
1900-1908	cattle	18 prefectures	4,051	unknown
2000	cattle	Miyazaki Hokkaido	740	O/ME-SA topotype PanAsia lineage
2010	cattle pigs goats sheep	Miyazaki	297,808 (including vaccinated animals)	O/SEA topotype Mya-98 lineage

Our country is now completely free from FMD!

We have finally started FMD research after 2000 epidemic in Japan.

Development of antigen detection systems using monoclonal antibodies

MAb against highly conserved region that enable to react all serotypes

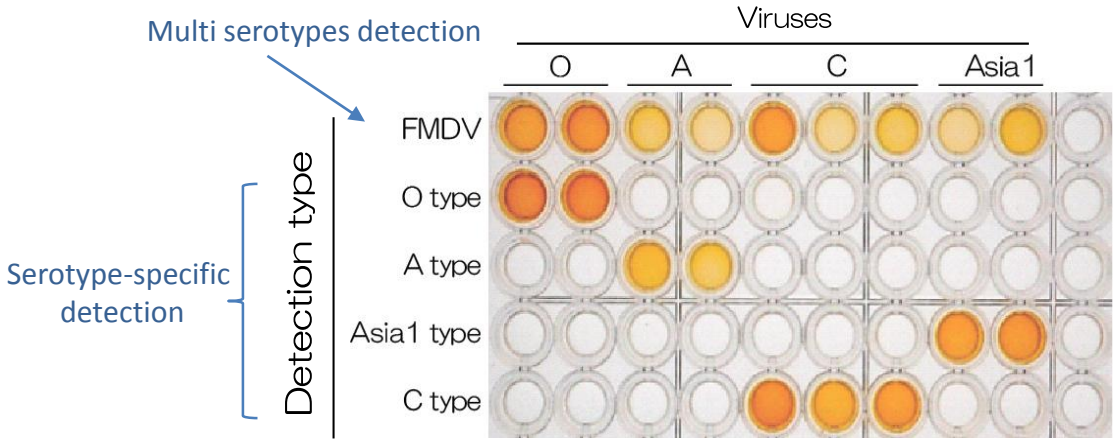


MAb against serotype- specific region that enable to distinguish each serotype

Production of monoclonal antibodies

Newly developed ELISA for FMDV-antigen detection

We have developed antigen-detection sandwich ELISA method using monoclonal antibodies against FMDV serotypes O, A, C and Asia1.



ELISA developed at Exotic Diseases Research Station is more sensitive than the international standard method (indirect sandwich ELISA), for detection of FMDV and identification of its serotype.

	Multi-serotypes	Serotype specific (O)	indirect sandwich
Positive rate (%): sample (No. of ELISA+ / No. of PCR+)	57 (102/178)	64 (114/178)	9 (15/176)
Positive rate (%): farm (No. of ELISA+ / No. of PCR+)	85 (66/78)	87 (68/78)	14 (11/78)

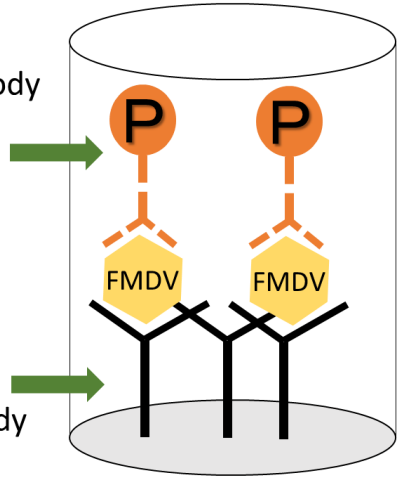
We have already obtained monoclonal antibodies against the other 3 serotypes, SAT1, 2 and 3.
We have inspected its utility at World Reference Laboratory of FMD (Pirbright Lab. in UK).

J. Clinical Microbiology 47(11) 3663-3668, (2009). PLOS ONE, 9(4) e94143, (2014).

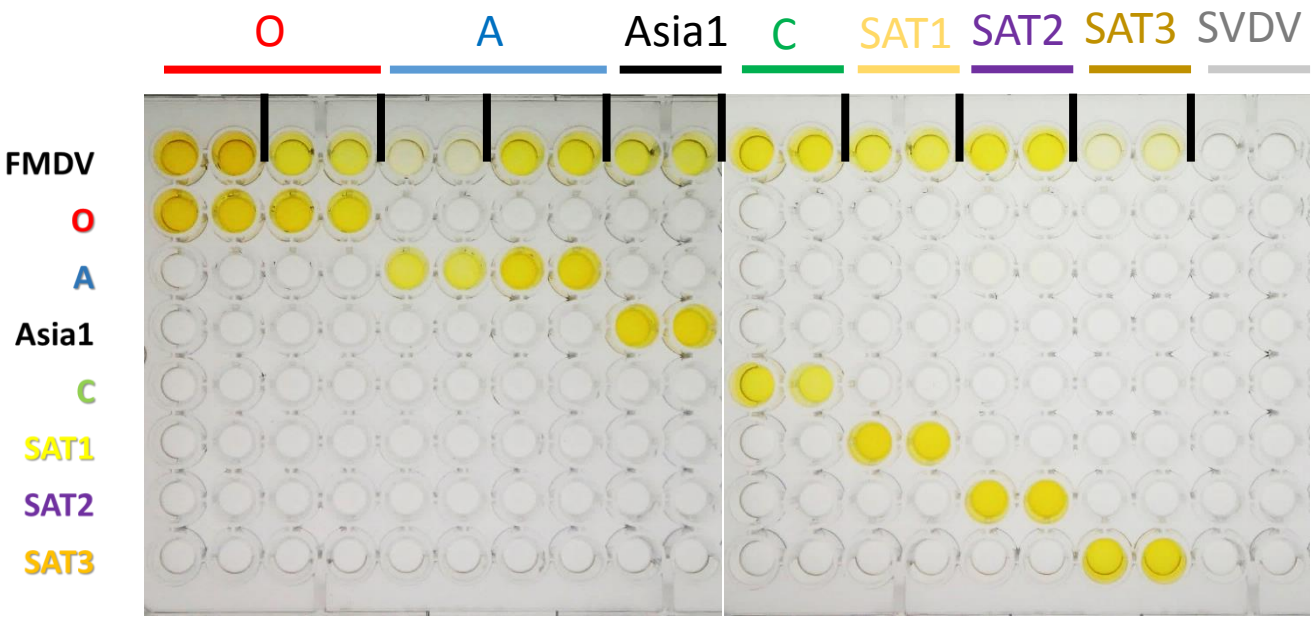
Now we can detect all serotypes of FMD virus and discriminate each serotype!

HRP-labeled multi-serotype reactive MAb
and each serotype specific MAbs for detecting antibody

FMDV (1H5+16D6)
O (70C4+CT5)
A (16C6+2A1)
Asia1 (12C7)
C (13F1)
SAT1 (22D1+31D6)
SAT2 (30F3)
SAT3 (7G3+11H3)

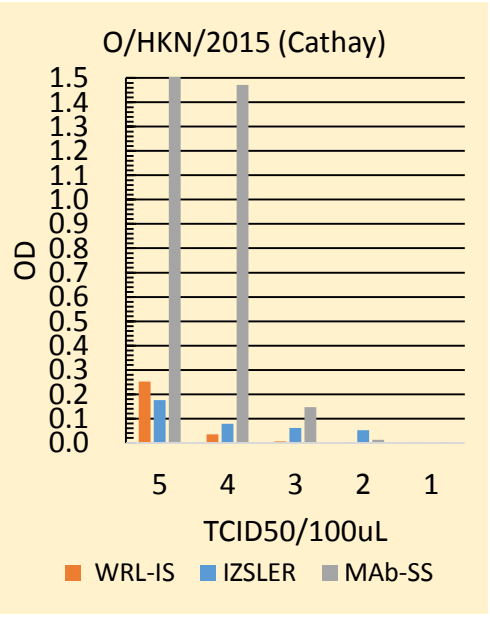


Multi-serotype reactive MAb (1H5) for capture antibody



The result of detection and differentiation of MAb-based sandwich ELISA

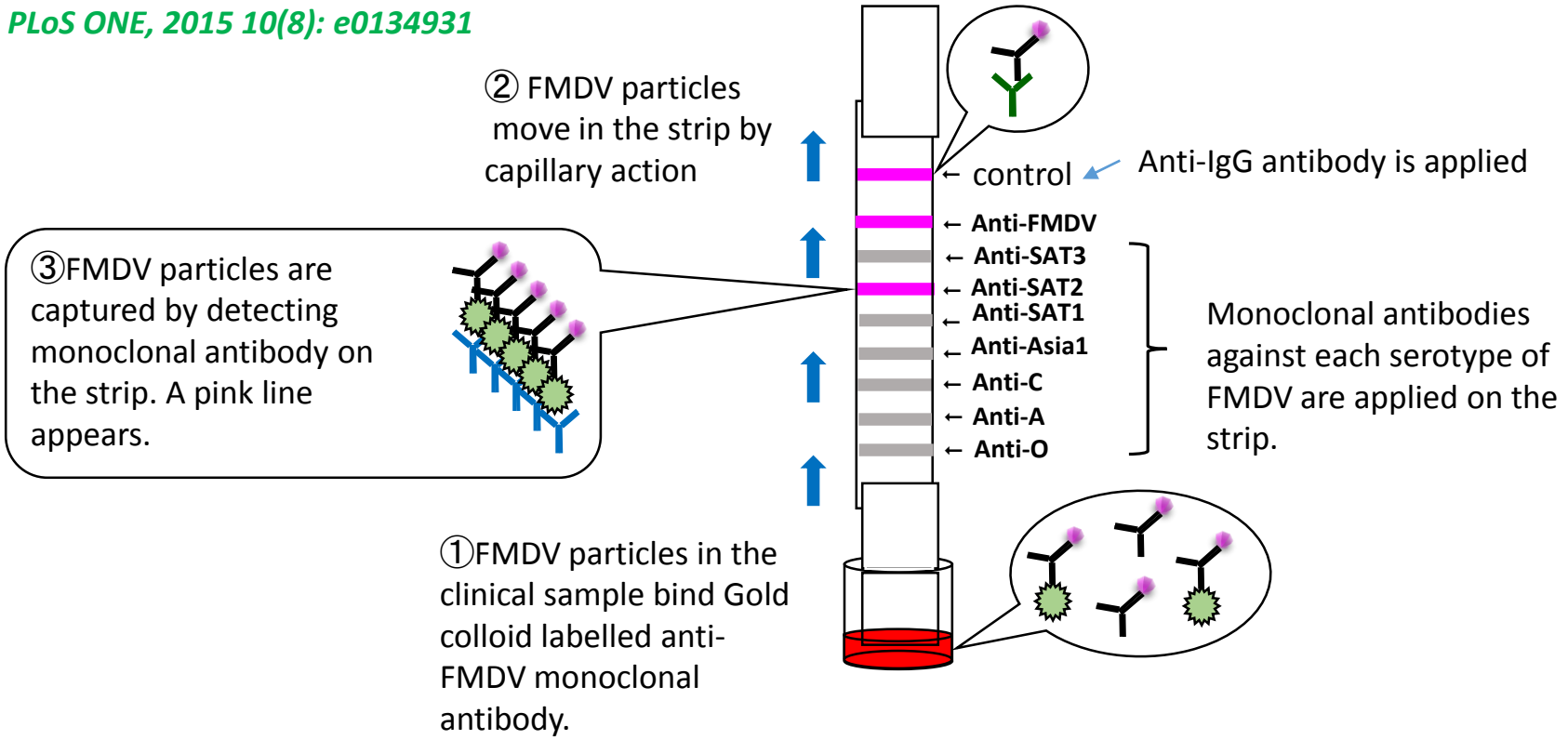
Comparison of sensitivities of each ELISA system



Development of a simple diagnostic method that can be used in the field to detect viral antigens

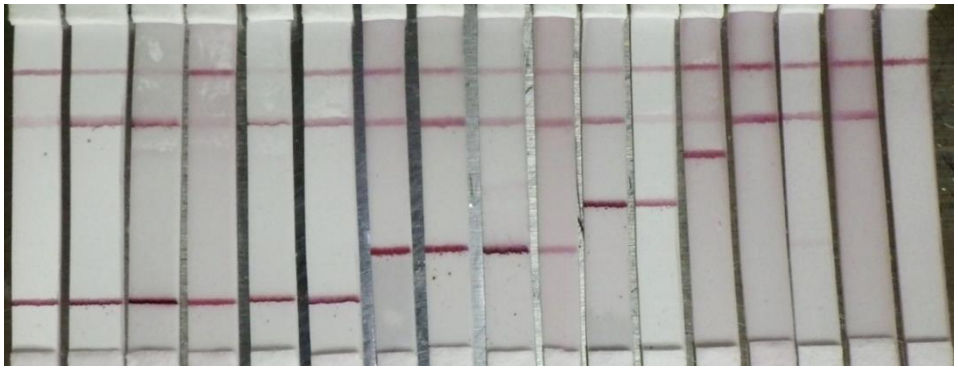
We have made an anti-FMDV monoclonal antibody reacting with all serotypes and seven serotype-specific monoclonal antibodies. We are now trying to develop lateral flow antigen detection system (immune-chromatography) for detecting viral antigens and for identifying serotypes simply and rapidly in collaboration with private companies.

PLoS ONE, 2015 10(8): e0134931



Immune-chromatography kit for detecting viral antigen (final version): a case of detection of SAT2

Antigen Detection and Serotyping by lateral flow antigen detection system (LFD) for Foot-and-Mouth Disease Virus



The result of FMDV serotyping &detection

PLoS ONE, 2015 10(8): e0134931

National Institute of Animal Health , Japan
Exotic Disease Research Station

collaboration

- The State Central Veterinary Laboratory (SCVL) in Mongolia
- Regional Reference Laboratory for Foot-and-Mouth Disease in the South East (RRLSEA), Thailand.
- The Pirbright institute (WRL)

Scientific technique research promotion program for agriculture, forestry, fisheries and food industry
Grant No. 28032B

consortium

Private companies in Japan

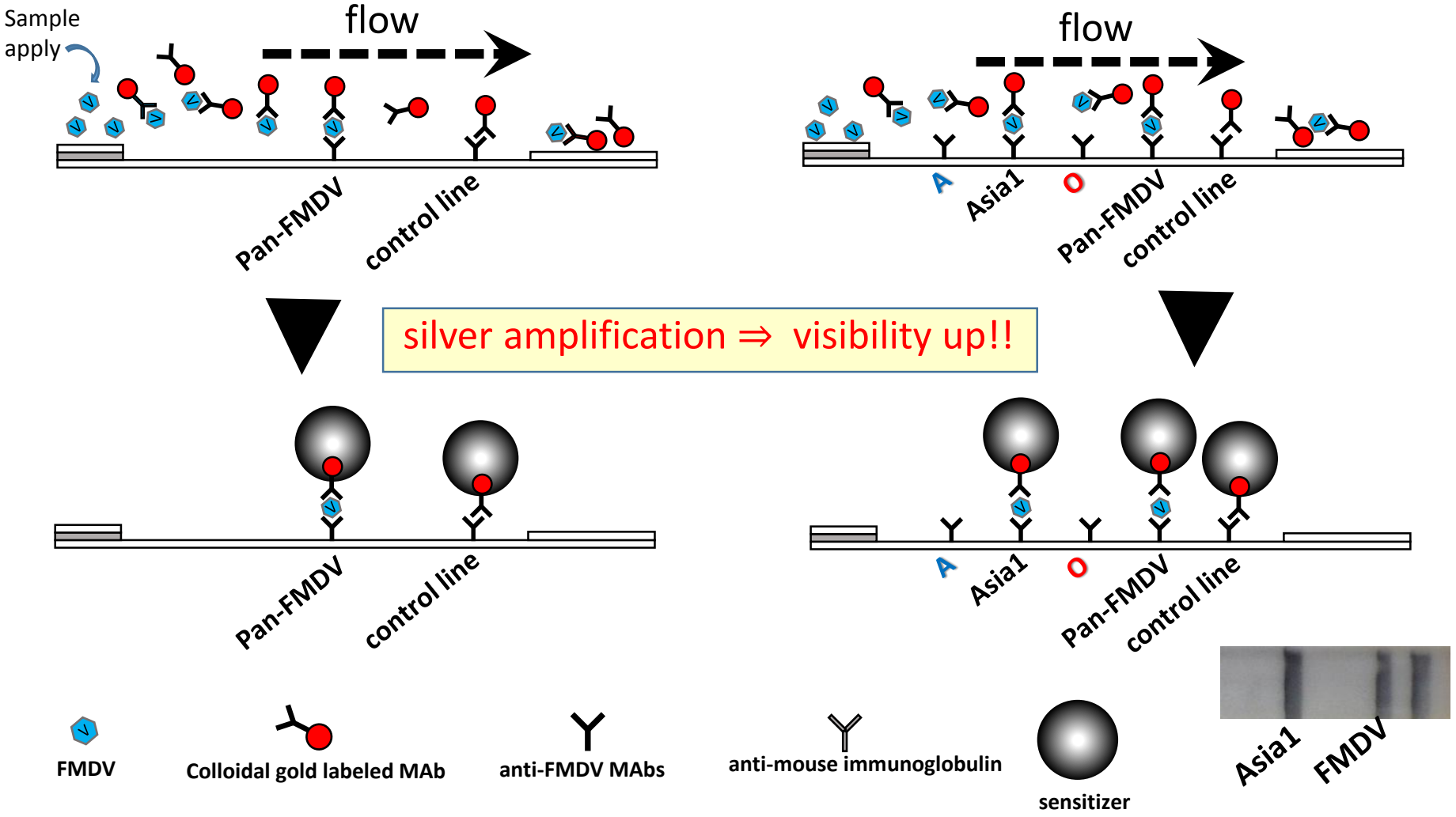
- ✓ **Sensitization**
- ✓ **Manufacturing**

- ✓ Validation using・・・
  - ➢ Field clinical samples
  - ➢ Epidemic FMDV isolates all over the world
- ✓ We can share developed LFD or ELISA for FMD control in Asia

Development of rapid antigen diagnosis kit

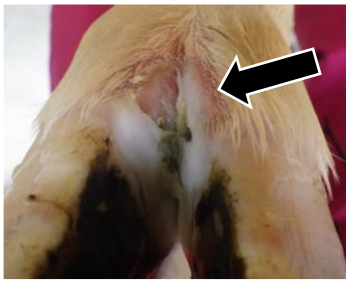
Ongoing: Pharmaceutical procedure for Japanese government

This is an advanced system for simple and rapid detection of FMDV.

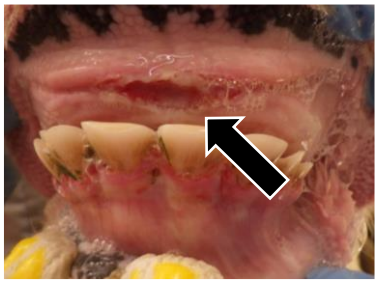


Mechanism of FMDV antigen detection and serotyping kit using silver amplification immunochromatography system

How to use lateral flow antigen detection system
(Immunochromatography kit)



Vesicular Epithelium



Vesicular Swab

Collect samples
from the lesions of
FMDV infected animals

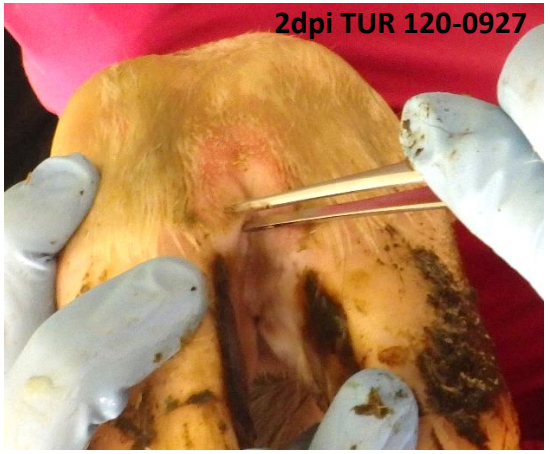
Homogenize

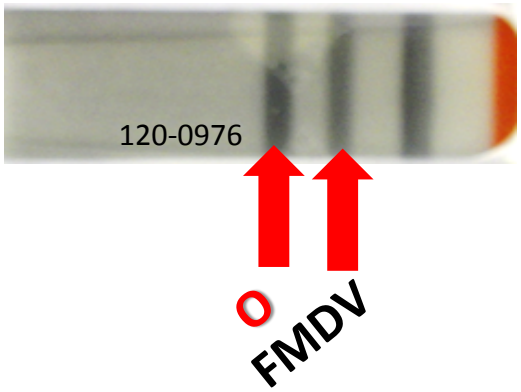
Swab

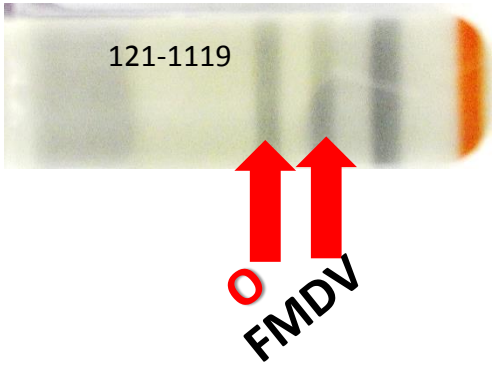
Make suspension
in the buffer

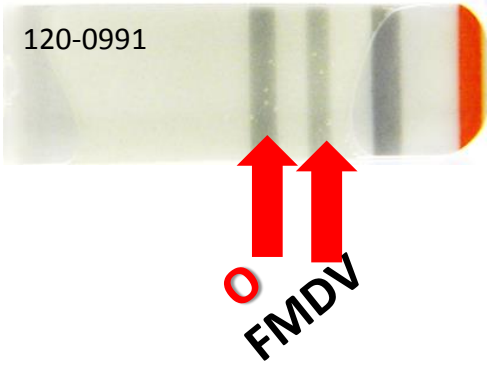
Apply to the device (Now improving!)

Silver amplification immunochromatography **(No Sensitization apparatus !!)**





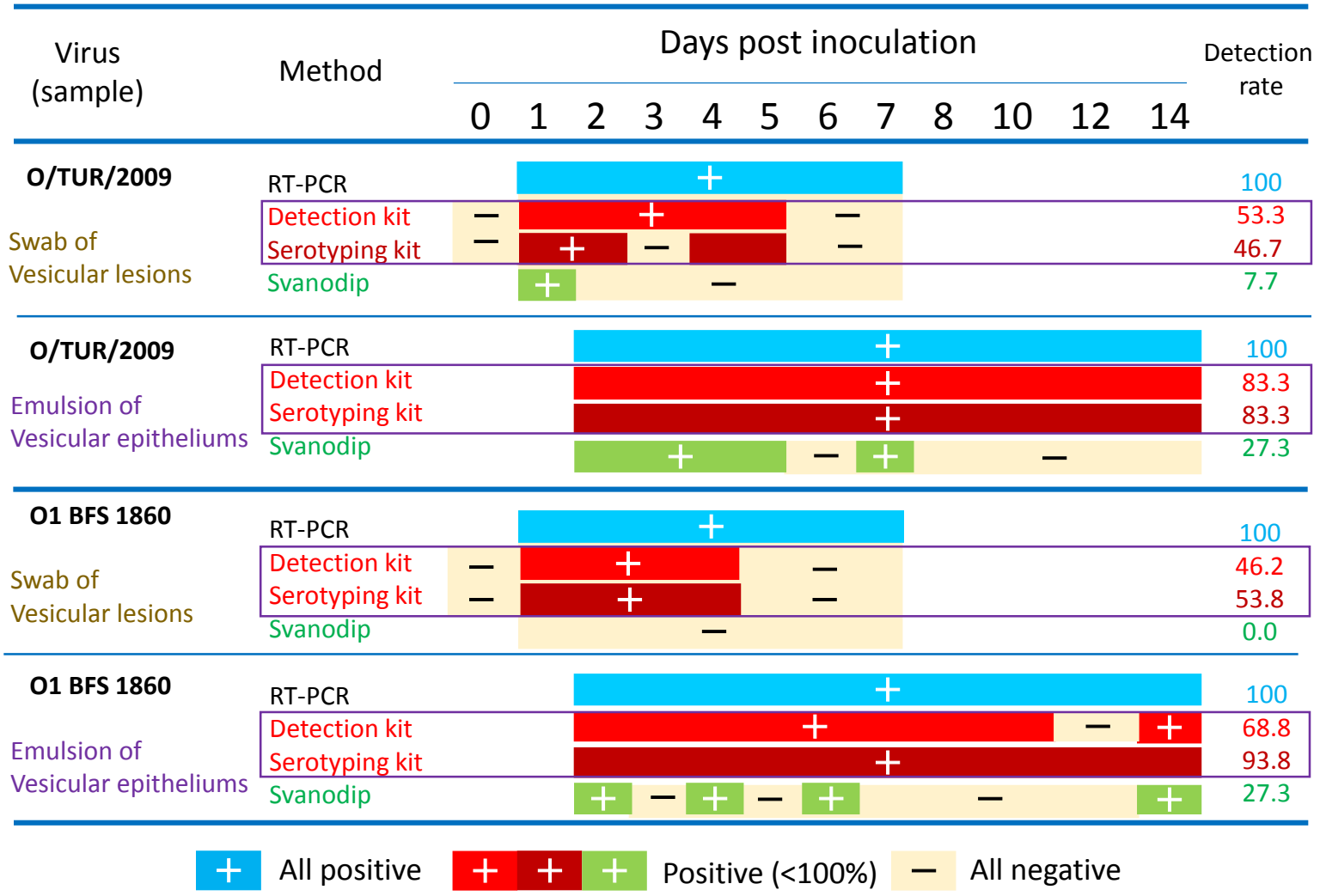




Detection and serotyping of FMDV using clinical samples obtained from cattle experimentally infected with Turkish strain of serotype O :

O/TUR/ 5/2009 (ME-SA topotype)

Time course of antigen detection from cattle experimentally infected with FMDV



Detection rates (total) from the PCR positive samples

- ✓ Virus isolation(LFBKαvβ6): 51.5%
- ✓ Our detection LFD: 66.2%
- ✓ Our serotyping LFD: 72.1%
- ✓ Svanodip: 19.4%

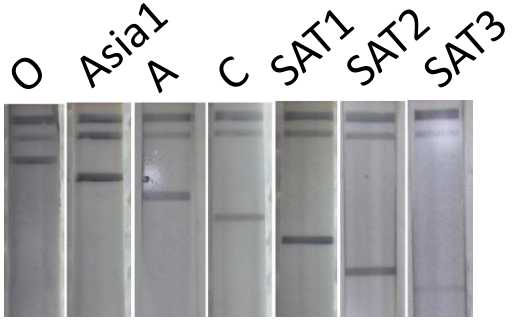
Our LFD system can detect positive cows throughout the experiment for 14days

Summary

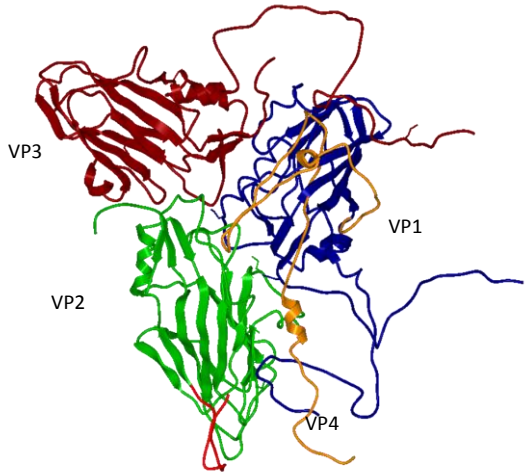
- Our developing kit does not require any apparatus.

- The first diagnosis is also possible in remote provincial laboratories that do not have sufficient diagnostic facilities or in areas where traffic infrastructure is not well developed.
  ⇒ Facilitate submitting FMD sample and reporting.

- In the near future, we aim to develop a kit that makes it possible to distinguish all 7 serotypes at the same time.



- On the other hand, in order to maintain the utility of this kit, it is necessary to continue to keep an eye on the reactivity of the MAbs and the epidemic strains.

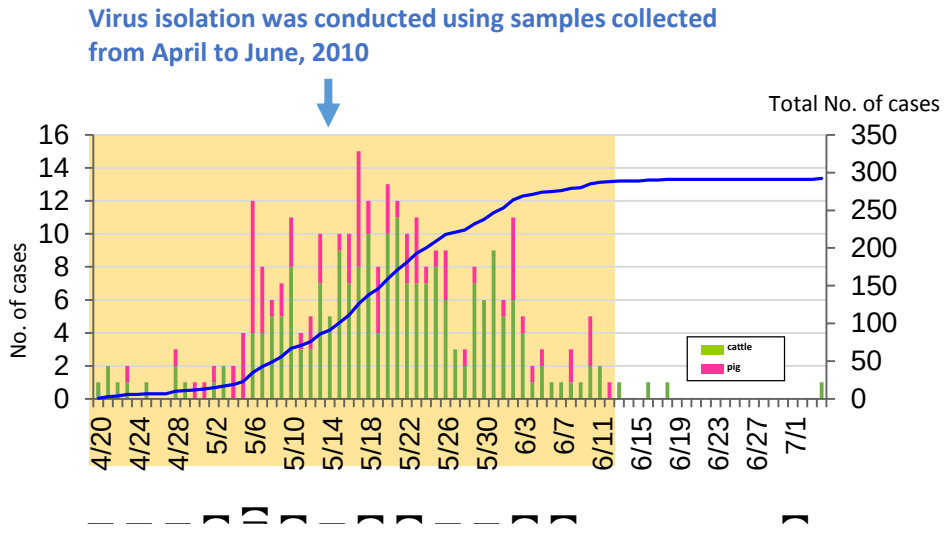


The other research activities:

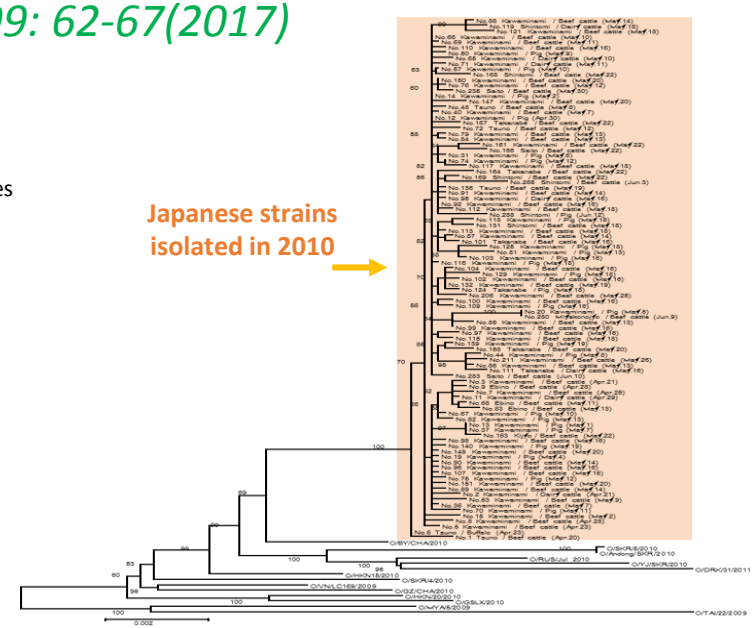
- Molecular analysis-

Genomic analysis of the Japanese strains of FMD virus (type O) isolated in 2010 outbreak

Veterinary Microbiology 199: 62-67(2017)



Date of outbreak
(2010 in Miyazaki Prefecture)



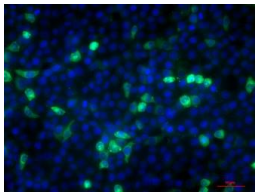
Phylogenetic analysis of L-fragment of FMDV (type O)

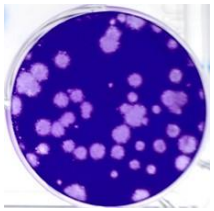
NGS provides high level information !

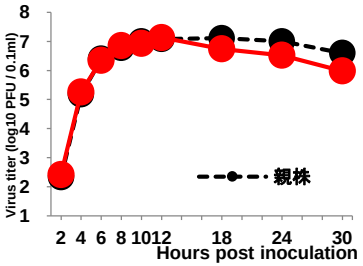
- We have isolated many strains of FMD virus from clinical samples of 292 cases in the 2010 epidemic in Japan.
- The L-fragment genes (approx. 7.8kb) of 104 strains were amplified by RT-PCR and sequenced by using a next generation sequencer.
- Nucleotide sequences of 2010 isolates showed more than 99.5% identity to the sequence of initial isolate obtained from the first case of the epidemic without any genetic deletion or insertion.
- These results indicated that a single strain of FMD virus was introduced from overseas and its nucleotide sequence has changed gradually during the epidemic.

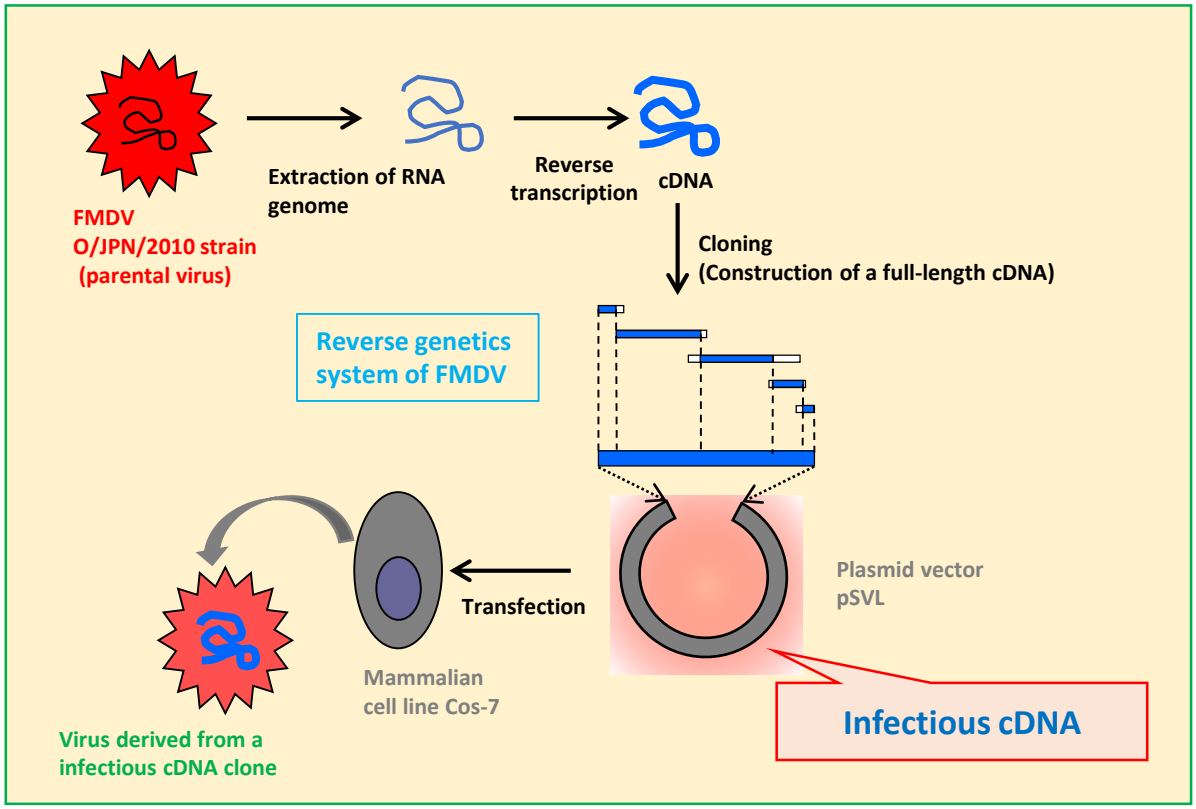
Construction and characterization of a infectious cDNA clone of FMDV

Research in Veterinary Science 106: 165-169(2016)









A full-length infectious cDNA of FMDV was constructed. The virus recovered from transfected cells retained the *in vitro* characteristics (antigenicity, plaque size, one step growth.....) and the *in vivo* pathogenicity of the parental strain. This clone should be useful to analyze determinants of pathogenicity and mechanism of virus replication and to develop genetically engineered vaccines against FMDV.

Thank you for your attention !

NARO