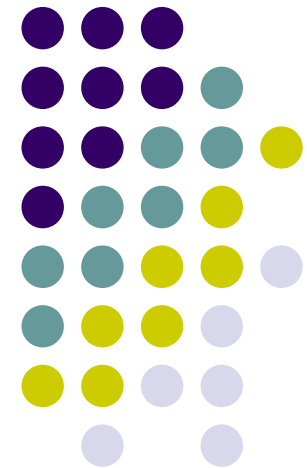


Foot and mouth disease situation and control strategies in the People's Republic of China – the current situation

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Contents



- Current situation of Asia 1 and A type FMD in P. R. China
- The work done by NRL of FMD facing Asia 1 and A type FMD
- The strategies for control and eradication of FMD



The situation of Asia 1 FMD

- 1. From 2005 to 2006 Asia 1 FMD have spread to 11 administrative provinces. Indicating the high pathogenicity of Asia 1/JS/WX/05 isolates.**
- 2. Since 2007, epidemic situations limits to northwest of China, Xinjiang, Qinghai, Gansu three provinces after compulsory inoculation with inactivated vaccine.**
- 3. However, in 2009 the situation become worse, another 6 provinces report new outbreak of Asia 1. these indicate that the Asia 1 FMD epidemic regions further expanded**

Asia 1

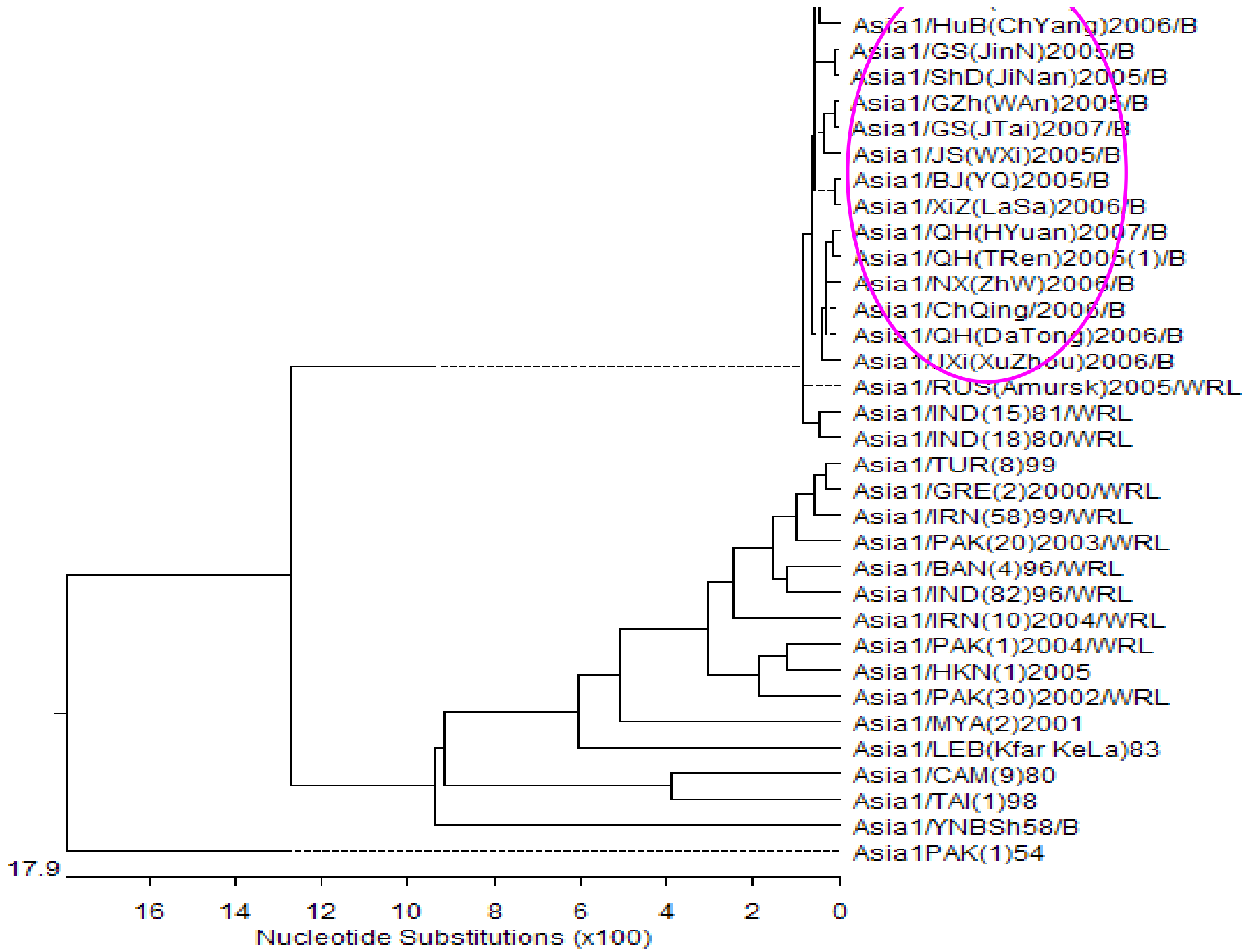
● 2005/06

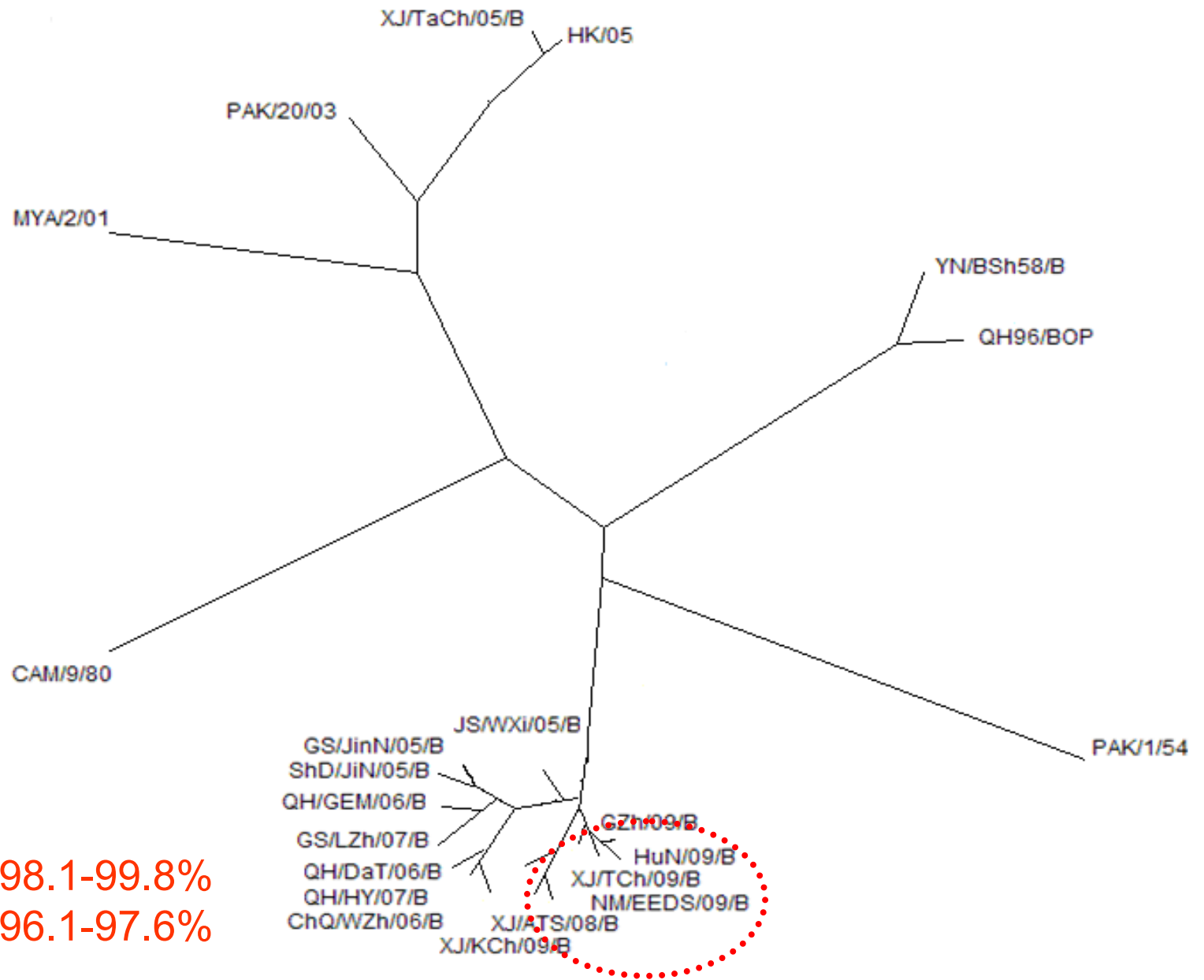
● 2007

○ 2008

● 2009







Homology

2005~2007 98.1-99.8%

2008~2009 96.1-97.6%

New evolution cause new outbreaks?

The changes in VP1 protein



| | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | | |
|---|--------------|----|----|----|----|----|----|----|--|--------------|
| 1 | TTTTGESADPVT | | | | | | | | | IND/18/80 |
| 1 | TTTTGESADPVT | | | | | | | | | JS/WXi/05/B |
| 1 | TTTTGESADPVT | | | | | | | | | ShD/JiN/05/B |
| 1 | TTTTGESADPVT | | | | | | | | | GS/JiN/05/B |
| 1 | TTTTGESADPVT | | | | | | | | | QH/DaT/06/B |
| 1 | TTTTGESADPVT | | | | | | | | | QH/HeN/06/B |
| 1 | TTTTGESADPVT | | | | | | | | | QH/GEM/06/B |
| 1 | TTTTGESADPVT | | | | | | | | | ChQ/WZh/06/B |
| 1 | TTTTGESADPVT | | | | | | | | | QH/HY/07/B |
| 1 | TTTTGESADPVT | | | | | | | | | GS/LZh/07/B |
| 1 | TTTTGESADPVT | | | | | | | | | NX/PL/08/B |
| 1 | TTTTGESADPVT | | | | | | | | | XJ/ATS/08/B |
| 1 | TTTTGESADPVT | | | | | | | | | GS/GGu/08/B |
| 1 | TTTTGESADPVT | | | | | | | | | XJ/KCh/09/B |
| 1 | TTTTGESADPVT | | | | | | | | | NM/EEDS/09/B |
| 1 | TTTTGESADPVT | | | | | | | | | XJ/TCh/09/B |
| 1 | TTTTGESADPVT | | | | | | | | | GZh/TZ/09/B |
| 1 | TTTTGESADPVT | | | | | | | | | HuN/HY/09/B |
| 1 | TTTTGESADPVT | | | | | | | | | Sch/GY/09/B |
| 1 | TTTTGESADPVT | | | | | | | | | ShX/HZh/09/B |
| 1 | TTTTGESADPVT | | | | | | | | | GX/LCh/09/B |
| 1 | TTTTGESADPVT | | | | | | | | | GX/LCh/09 |



V35→I, P45→L, S47→N, T50→I

| | 90 | 100 | 110 | 120 | 130 | 140 | 150 | 160 | | | |
|-----|----------|----------|-----------|-------|----------|--------|------------|----------|---------|-----------|--------------|
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALAH | RVNNRLPTS | IND/18/80 |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | JS/WXi/05/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | ShD/JIN/05/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | GS/JinN/05/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | QH/DaT/06/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | QH/HeN/06/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | QH/GEM/06/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | ChQ/WZh/06/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | QH/HY/07/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | GS/LZh/07/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | NX/PL/08/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | XJ/ATS/08/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | GS/GGu/08/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | XJ/KCh/09/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | NM/EEDS/09/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | XJ/TCh/09/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | GZh/TZ/09/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | HuN/HY/09/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | SCh/GY/09/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | ShX/HZh/09/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | GX/LCh/09/B |
| 241 | VHTGPVTW | PNGAPKTA | LNHTNPTAY | QKQPI | TRLALPYT | APHRVL | STVYNGKTTY | GEESRRGD | AALARRV | NNRLPTS | GX/LCh/09 |

N100→D

S140 →P

Enhance the virulence?

Immune evasion?

Persistent infection in ruminant?

The outbreak of A type FMD



Since the first report of A type FMD in Wuhan of Hubei province in 13 Jan, 2009, A type FMD were found in Shanghai, Jiangsu, Guangxi, Guizhou and Shandong subsequently.

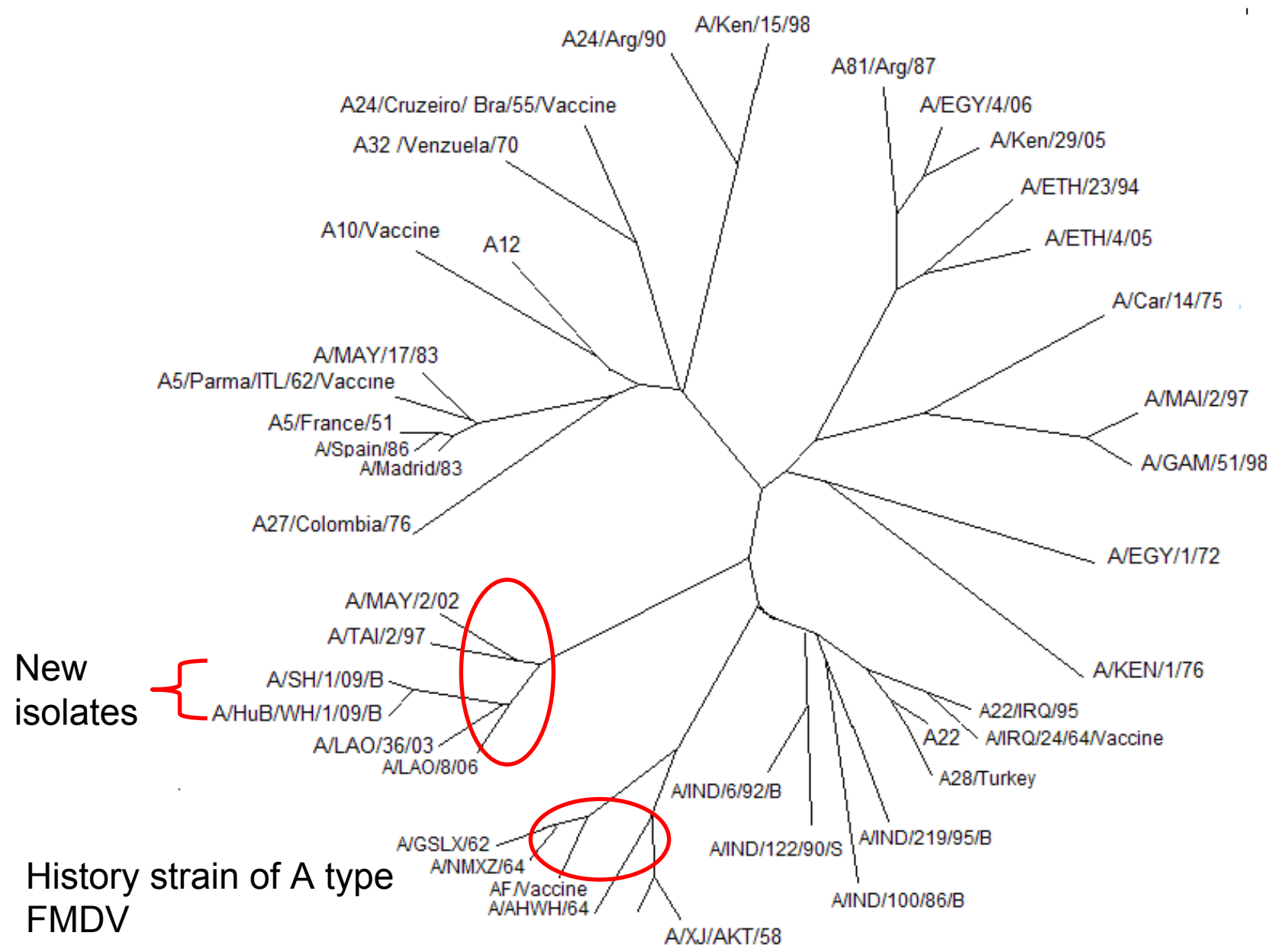




The phylogenetic analysis of the virus origin

The homology of VP1 gene from two isolates A/WH/09 and A/SH/09 were 98.4%. This indicated that FMD occurred in these two places were caused by the same virus agent. The VP1 gene homology were about 75.9%-79.9% when comparing A/WH/09 and A/SH/09 with some history stain of A type FMDV in China.

The VP1 gene from A/WH/09 and A/SH/09 were aligned with a lots of reference viruses. The result showed that the virus cause recent FMD outbreak in China was closely related to some published VP1 sequences of A/MAY/02(95.9%), A/Tai/97(95.7%) and A/Lao/8/06 (95.3%), and highly homological with A/Tai/08 virus compared by WRL, which indicated that the virus was introduced from Southeast Asia



Dendrogram of FMDV type A isolates based on nucleotide sequence VP1 coding region

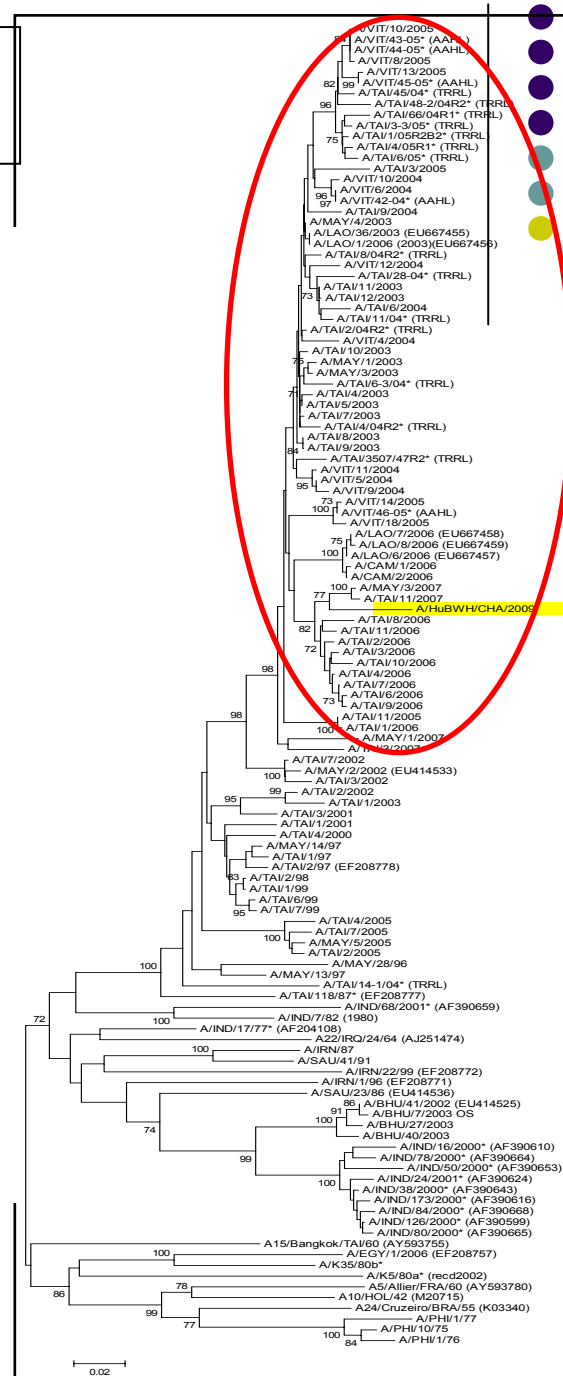
Report on FMDV A VP1 sequence received from LVRI, P.R. China in 2009

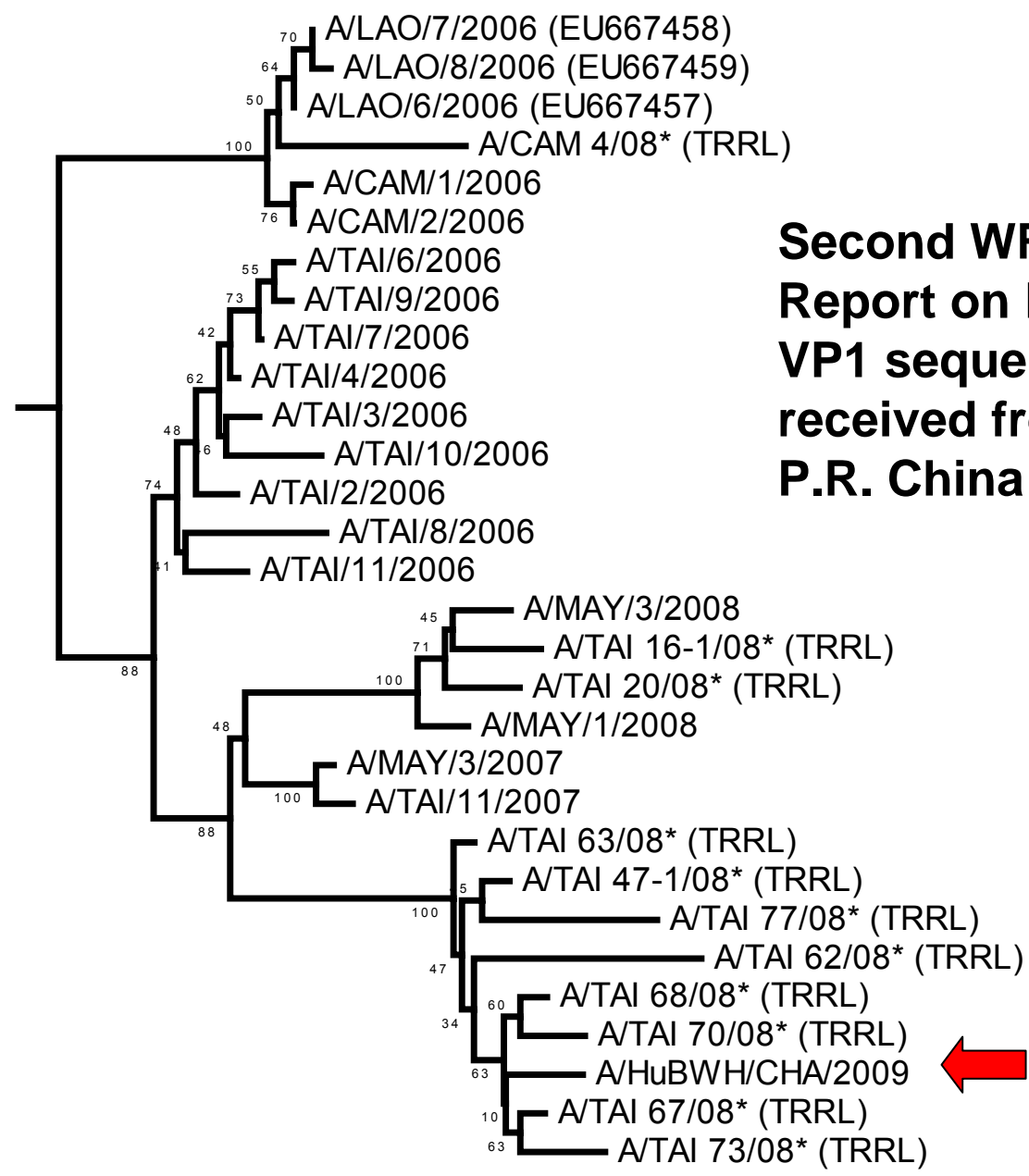
| Ten Most Closely Related Viruses | | | | | | |
|----------------------------------|-----------------------|----------|------------------|-----------------|--------------------|-----------------------|
| Pos. | Virus name | Filename | No. nt. compared | No. nt. matched | No. of ambiguities | % Identity Difference |
| 1 | A/MAY/3/2007 | MAY07-03 | 636 | 610 | 0 | 95.91 4.09 |
| 2 | A/TAI/11/2007 | TAI07-11 | 636 | 609 | 0 | 95.75 4.25 |
| 3 | A/TAI/2/2006 | TAI06-02 | 636 | 609 | 0 | 95.75 4.25 |
| 4 | A/TAI/29-2/06* (TRRL) | TAI06-AE | 636 | 609 | 0 | 95.75 4.25 |
| 5 | A/TAI/11/2006 | TAI06-11 | 636 | 608 | 0 | 95.6 4.4 |
| 6 | A/TAI/9/2006 | TAI06-09 | 636 | 608 | 0 | 95.6 4.4 |
| 7 | A/TAI/22-2/06* (TRRL) | TAI06-AB | 636 | 607 | 0 | 95.44 4.56 |
| 8 | A/TAI/24-2/06* (TRRL) | TAI06-AC | 636 | 607 | 0 | 95.44 4.56 |
| 9 | A/TAI/32-2/06* (TRRL) | TAI06-AF | 636 | 607 | 0 | 95.44 4.56 |
| 10 | A/TAI/38/06R1* (TRRL) | TAI06-AG | 636 | 607 | 0 | 95.44 4.56 |

| Relationships to Reference Virus Strains | | | | | | |
|--|---------------------------------|----------|------------------|-----------------|--------------------|-----------------------|
| Pos. | Virus name | Filename | No. nt. compared | No. nt. matched | No. of ambiguities | % Identity Difference |
| 1 | A/TAI/2/97 (EF208778) | TAI97-02 | 636 | 579 | 0 | 91.04 8.96 |
| 2 | A/TAI/118/87* (EF208777) | TAI87-AD | 636 | 562 | 0 | 88.36 11.64 |
| 3 | A/IRN/2/87 (EF208770) | IRN87-02 | 636 | 522 | 0 | 82.08 17.92 |
| 4 | A/SAU/23/86 (EU414536) | SAU86-23 | 636 | 520 | 0 | 81.76 18.24 |
| 5 | A/SAU/41/91 | SAU91-41 | 633 | 516 | 0 | 81.52 18.48 |
| 6 | A22/IRQ/24/64 (AJ251474)* | IRQ64-24 | 636 | 514 | 0 | 80.82 19.18 |
| 7 | A/IRN/1/96 (EF208771)* | IRN96-01 | 635 | 513 | 1 | 80.79 19.21 |
| 8 | A/IRN/22/99 (EF208772)* | IRN99-22 | 633 | 511 | 0 | 80.73 19.27 |
| 9 | A/IRN/1/2005 (EF208769) | IRN05-01 | 636 | 513 | 0 | 80.66 19.34 |
| 10 | A24/Cruzeiro/BRA/55 (AJ251476)* | BRA55-C | 636 | 499 | 0 | 78.46 21.54 |

nt, nucleotides*
*, not a WRLFMD reference number

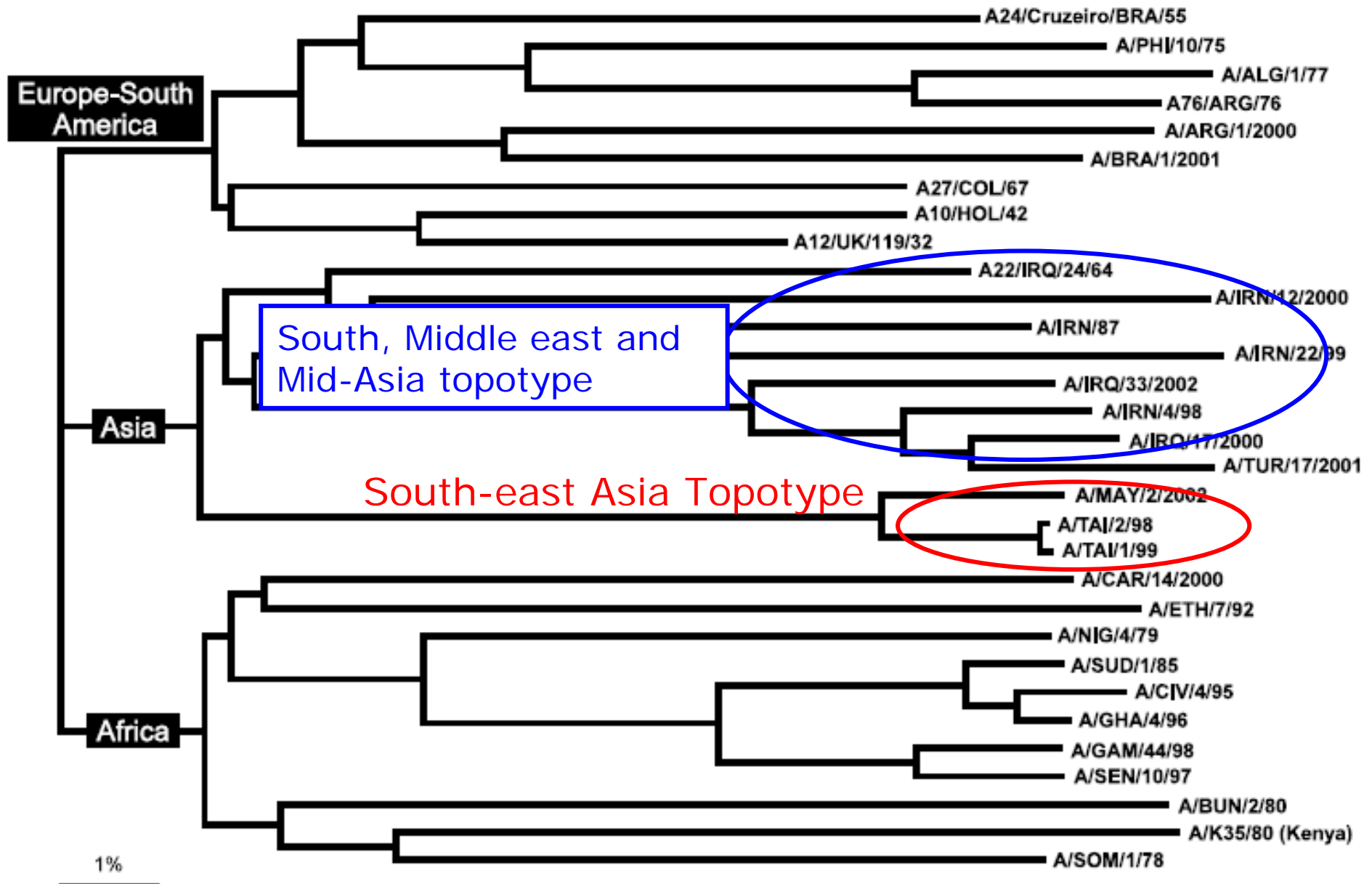
Analysis result by WRLFMD SE Asia Topotype





**Second WRLFMD
Report on FMDV A
VP1 sequence
received from LVRI,
P.R. China in 2009**

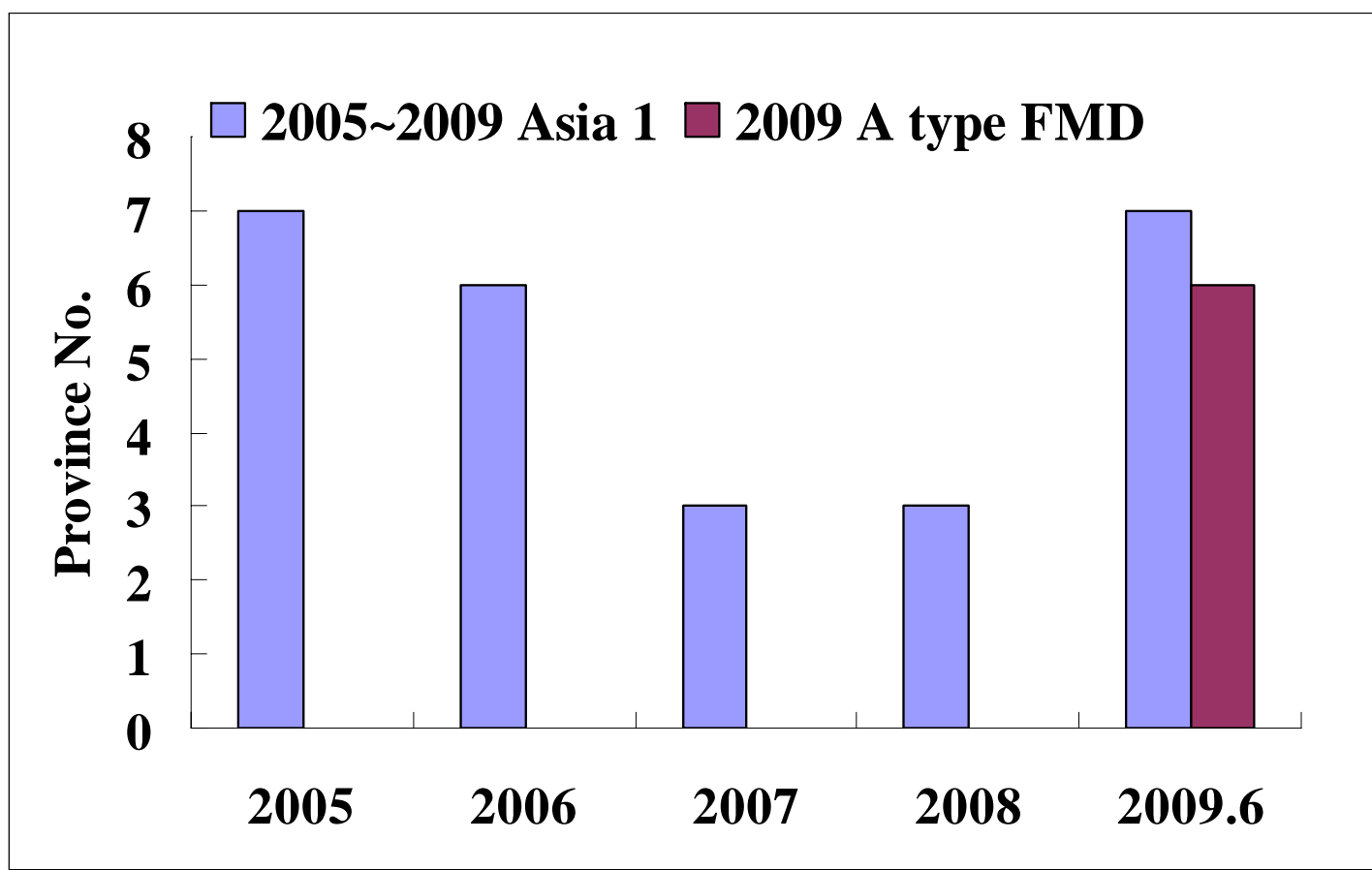
0.005



Unrooted Neighbour-joining tree showing the relationship between selected type A FMDVs based on complete VP1 gene sequences (Knowles and Samuel, 2003)



The possible routes for A type FMD introduction
Land, Water?



Brief summary the outbreaks of Asia 1 and A type FMD in recent years.

From 2005 to now, there are 17 provinces report outbreak of Asia 1 FMD, and 6 provincial administrative regions have found A type FMD incursion since the beginning of 2009. The situation of the FMD control become even more complicated.

The works done by NRL of FMD facing the Asia 1 and A type FMD



- Final diagnosis of the disease case
- Epidemiological surveillance and trace the origin of the outbreak
- Provide the diagnostic reagent for disease surveillance
- Study the virus pathogenicity to different host
- Culture and evaluation the virus seeds for vaccine production
- Training staffs for veterinary lab throughout the country.

Major lesson gotten from epidemiological investigation



- **Animal trade and transport is major reason for the disease transmission.**

Local investigation and molecular evidences indicated that all Asia 1 FMD outbreaks in 2009 were closely related to animal movement (mainly cattle). A type FMD introduction was related to animal trade between countries. These indicated that the animal administration and quarantine system were not effective in some extent.

Diagnostic tools for rapid diagnosis and disease survey provided by NRLFMD of China

- Liquid phase blocking ELISA(LPBE) kit for Asia 1 and A type FMD
- 3ABC-ELISA kit
- Multi-RT-PCR kit
- Typing RT-PCR kit
- Real time quantitative PCR
- Pen-side test strips
- Indirect sandwich ELISA for antigen typing



The pathogenicity of A/HuB/WH/09 to different natural hosts



- Cattle showed typical FMD symptoms in 1~2 days after inoculated within tongue with virus material (field virus materials or suckling mouse), such as blister in the tongue (severe) and foot (5/24 not severe), drooling, anorexia, fever and reluctant to movement.
- There is one field case of swine outbreak of A type FMD in Guizhou. However, Under experimental condition, pig and sheep show subclinical infection by contact with diseased cattle.

The antibody responses against A type FMD



- In 2 virus challenged cattle, A serotype antibody could be detected 6~8 days post challenge (dpc) and 3ABC antibody could be detected in 10~12dpc; virus nucleotide could be detected intermittently in blood and O/P fluids in 3~18 dpc.
- In 3 of 5 contact sheep, A serotype antibody could be detected after 12~18 days of exposure, and 3ABC antibody could be detected after 12~16days exposure. In all 5 sheep, FMD virus RNA nucleotide could be detected intermittently in blood and O/P fluids since 8th days post contact.
- In 5 contact swine, viremia can be detected in one pig by RT-PCR after contact 6 days with diseased cattle, and low level antibody response were detected after 20 day post exposure. But pig did not show clinical signs within 20 days experiment.



The capability of replication in BHK21 cell for A/HuB/WH/2009 and its potential for vaccine production

- BHK21 cell could show typical cytopathic effect (CPE) of FMDV after once passage, but BHK21 cell could not show complete CPE within 12 hours after 10~17 passage. The time of complete CPE was not stable in BHK21 cells.
- It is not easy for measuring the r value between A/HuB/WH/09 and A/FC/72 because the CPE is not stable when adapt the virus to BHK21 cell. So, we are not sure about the immune relationship between A/HuB/WH/09 and vaccine strain of A/FC/72.
- This work is still continuing.

The strategies for control and eradication of FMD



- Slaughter

Destroy all infected and suspicious animal

- Disinfection

- Animal movement control

- Vaccination

O and Asia 1 bivalent vaccine; no A type vaccine available

- Surveillance



Difficulties we are facing

- Policy
Pay more attention on FMD, make law to eliminate regional protectionism, frame out practical long term FMD eradication plan
- Economics
Improve the subsidy level to normal market price (now is only 1/3 of that) so that the farmer do not try to evade the sampling and inspect.
- Administration
More effective animal movement control and quarantine system. Establishment more perfect disease information system. Evaluation the risk for animal movement. Inhibit the animal move out from epidemic region.
- Surveillance
enhance disease surveillance, learn the detail of disease distribution and develop the disease forecast and forewarning system,

Acknowledge



- WRL of Pirbright
- Workers in National Reference Laboratory of China
- Deputy General Director Zhang Zhongqiu



- Thank you for your attention