

COUNTRY REPORT FROM JAPAN

Regional Workshop on Swine Disease Diagnosis
Beijing, P. R. China, 30 – 31 Oct 2019



WORLD ORGANISATION FOR ANIMAL HEALTH

Protecting animals, preserving our future

Basic information

- **Production size:**

Total no. of pig: 9,189,000

Sows: 823,700 (9.0%)

- **No. of pig farm: 4,470 (no backyard farming, no household)**

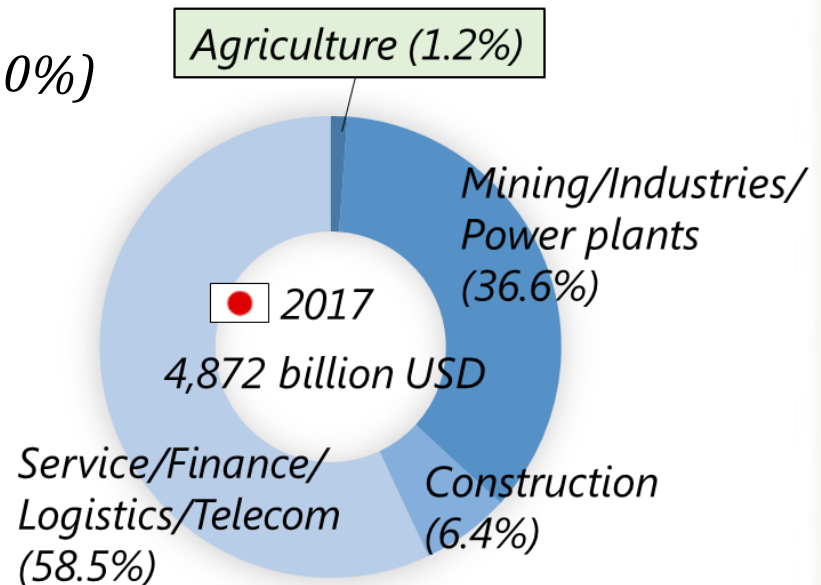
- **Domestic consumption of pork per year**

Total: 1,810,000 tons

Domestic production: 890,000 tons (49.0%)

Import: 925,631 (51.0%)

- **Share of GDP ratio: 0.4%**



Modified from the data of CAO, Japan

Updates on disease situation



- *Current status of major pig diseases of Japan (as of Aug/2019)*

(No. of farm)

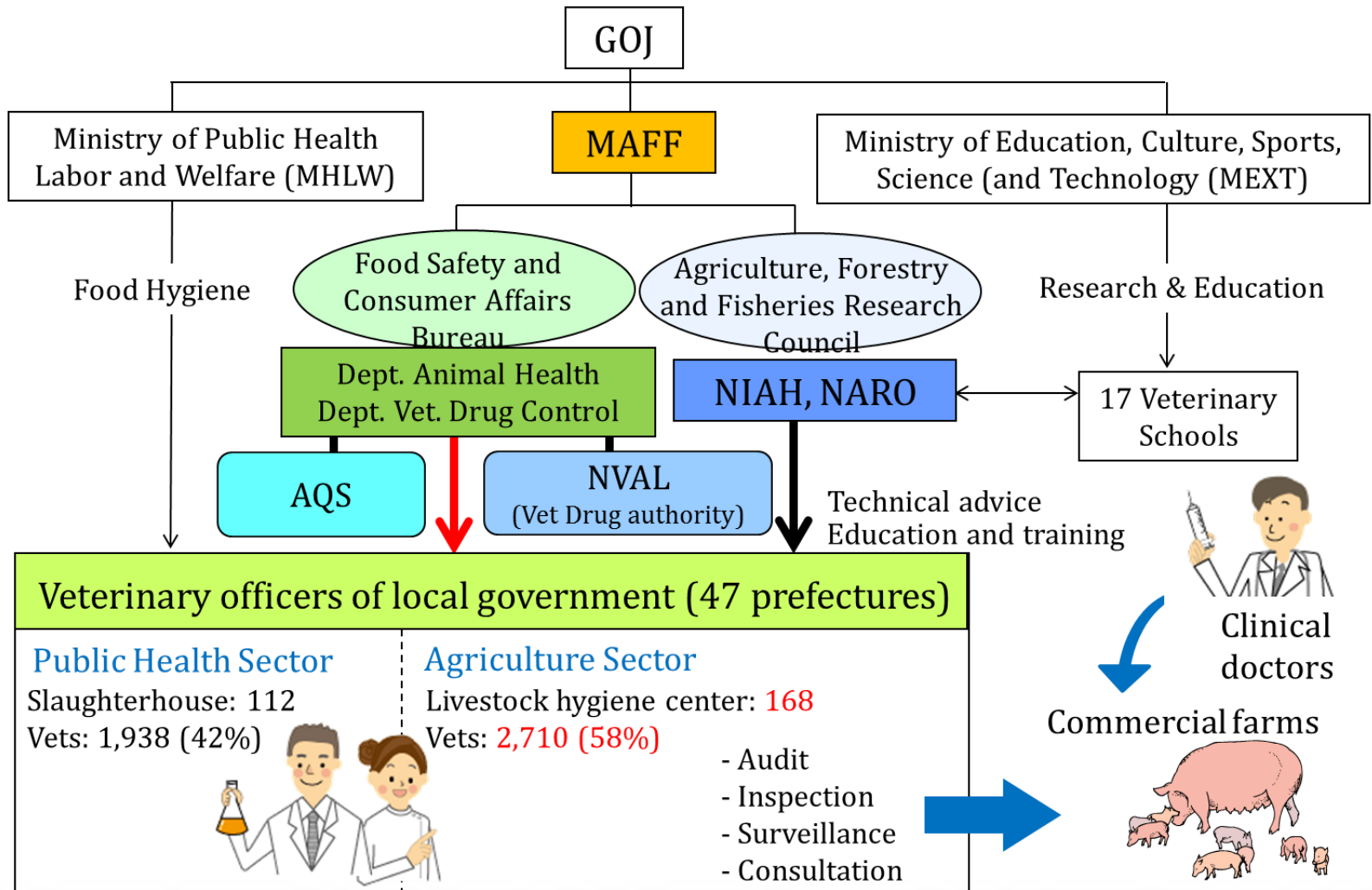
Year	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
FMD	9	0	0	0	0	0	0	0	0	0
CSF	0	0	0	0	0	0	0	0	10	36
PRRS (LP)	46	74	34	36	19	34	29	25	27	14
PED	NA	NA	15,589	44	836	217	87	66	33	104
AD	5	0	1	0	0	1	0	1	0	0
Erysipelas	878	717	726	750	786	856	588	561	466	214

(No. of animal)

Year	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
FMD	9	0	0	0	0	0	0	0	0	0
CSF	0	0	0	0	0	0	0	0	12	146,302
PRRS (LP)	29	28	87	157	39	131	82	545	153	33
PED	NA	NA	72,950	180	3,885	1,088	420	251	173	601
AD	2	0	3	0	0	5	0	4	0	0
Erysipelas	2,177	2,089	2,775	4,531	3,727	3,380	2,376	1,719	1,672	1,404

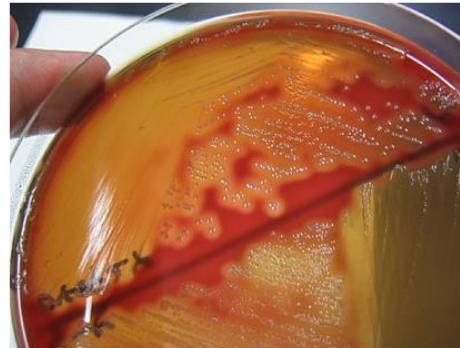
Basic information

- Veterinary System of Japan**



Basic information

- *Veterinary Officers of Local Government*
(Animal hygiene station: 166, officer: 2,084)



- Audit, Inspection of farms
- Diagnostic tests of animal diseases at pen-side and bench
- Preventive actions in case of the outbreak of contagious disease
- Consultation, education, exchange of information

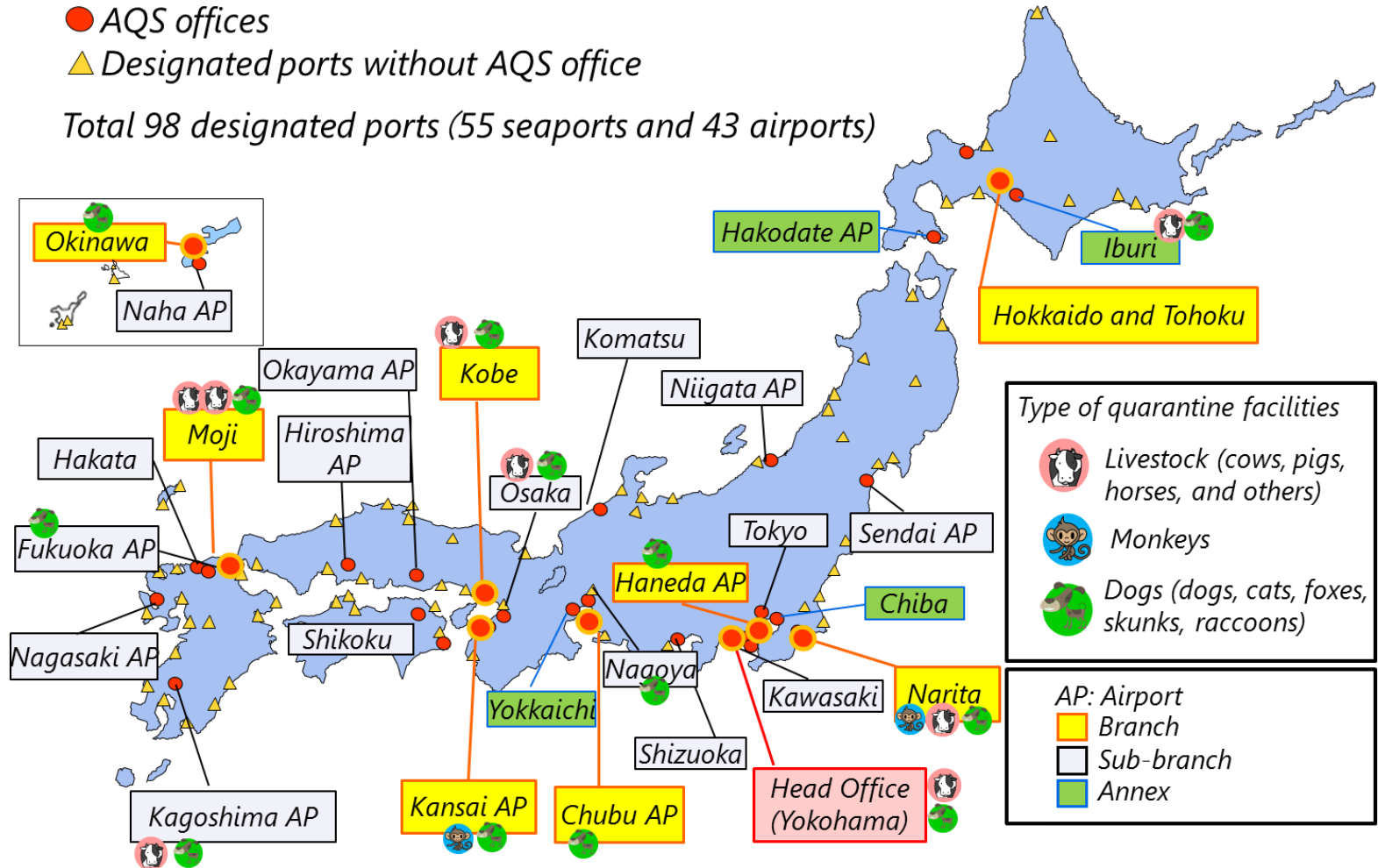
Basic information

- Animal Quarantine Service (Officer: 435)**

● AQS offices

▲ Designated ports without AQS office

Total 98 designated ports (55 seaports and 43 airports)



Basic information

- Daily Activity of Animal Quarantine Officers at Boarder Control**

Inspections of hand luggage



Shoe sole disinfection



Warnings for passengers on the restriction of meat products import



Interviews and awareness-raising



Quarantine detector dogs



Basic information

- ***National Institute of Animal Health:
National Reference Lab of Animal Diseases Diagnosis
(Researcher: 128)***
 - *R&D*
 - *Diagnostic service as a national reference laboratory*
 - *Education & Training*
 - *OIE Reference Laboratories
and Collaboration Center*
 - *FAO/OIE accredited Rinderpest
Holding facility*
 - *Resource bank*
 - *Outreach activities*



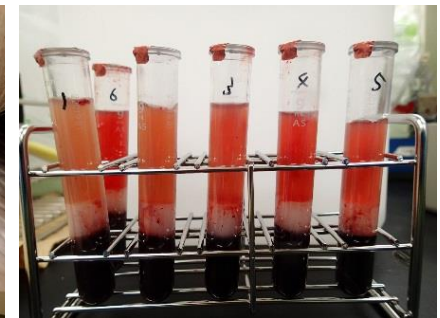
Swine diseases diagnosis (ASF/CSF/FMD)

- **Diagnostic capacity of our lab**
(Researcher: 10)
 - Cell culture (virus isolation/titration)
 - FAT
 - Immunoblotting
 - ELISA
 - Endpoint PCR/real-time PCR
 - DNA sequencing
 - Whole genome sequencing



Swine diseases diagnosis (ASF/CSF/FMD)

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 - Endpoint PCR/real-time PCR
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 - Whole genome sequencing
 - Animal experiments



What worked well

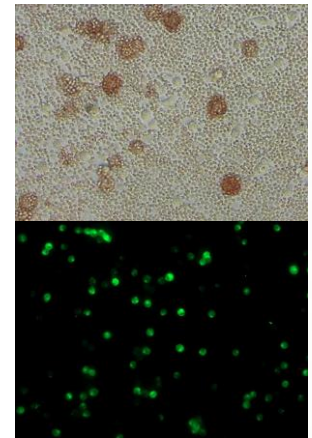
- **Successful isolation of multiple ASFV strains in pork meat products smuggled by oversea tourists by using pulmonary alveolar macrophage (PAM) cultures. (76 cases of PCR positive meat/meat products have been detected by AQS as of Oct/2019.)**
 - China 62; Vietnam 10; Laos 1; Cambodia 3

例数	アフリカ豚コレラ遺伝子検査陽性となった豚肉等の情報	写真
1例目	到着日：2018年10月1日 (北京発、新千歳空港着) 品目：ソーセージ (1.5kg) (税関検査) 遺伝子陽性確認日：2018年10月19日	
2例目	到着日：2018年10月14日 (上海発、羽田空港着) 品目：豚肉製品 (0.4kg) (検疫探知犬) 遺伝子陽性確認日：2018年11月9日	
3例目	到着日：2018年11月9日 (大連発、成田空港着) 品目：ソーセージ (2.5kg) (検疫探知犬) 遺伝子陽性確認日：2018年11月22日	
4例目	到着日：2019年1月12日 (上海発、中部空港着) 品目：ソーセージ (0.6 kg) (税関検査) 遺伝子陽性確認日：2019年1月25日 (ウイルス分離検査：陽性)	
5例目	到着日：2019年1月12日 (青島発、中部空港着) 品目：ソーセージ (1.3kg) (口頭質問) 遺伝子陽性確認日：2019年1月25日 (ウイルス分離検査：陽性)	
6例目	到着日：2019年1月16日 (瀋陽発、中部空港着) 品目：ソーセージ (0.5kg) (検疫探知犬) 遺伝子陽性確認日：2019年1月25日	
7例目	到着日：2019年1月12日 (上海発、羽田空港着) 品目：ソーセージ (0.1kg) (検疫探知犬) 遺伝子陽性確認日：2019年1月25日	
8例目	到着日：2019年1月26日 (延吉発、関西空港着) 品目：ソーセージ (0.3kg) (税関検査) 遺伝子陽性確認日：2019年2月5日	
9例目	到着日：2019年1月24日 (北京発、成田空港着) 品目：豚肉(燻製) (1.5kg) (口頭質問) 遺伝子陽性確認日：2019年2月7日	

← 1st case detected on 01/Oct/2018

← virus isolated

← virus isolated



What worked well

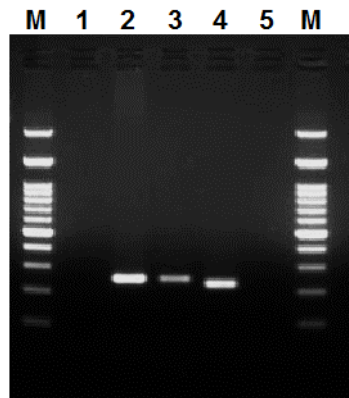
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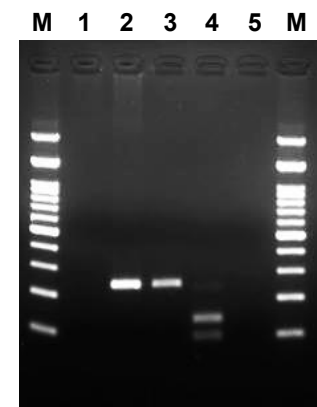
What worked well

- *Successful isolation of multiple ASFV strains in pork meat products smuggled by oversea tourists by using pulmonary alveolar macrophage (PAM) cultures. (So far 76 cases of PCR positive meat/meat products have been detected by AQS.)*
- ***Distribute endpoint PCR systems for ASF/CSF diagnosis to local animal hygiene stations to be used for screening dead/sick pigs and wild boars or confirmation of health status of pigs prior to movement.***



1: Extraction negative control
2&3: ASFV sample
4: Positive control
5: PCR negative control

Confirmation by
EcoRI digestion



1: Extraction negative control
2&3: ASFV sample
4: Positive control
5: PCR negative control

What worked well

Current systems for molecular diagnosis at local animal hygiene stations

	ASF	CSF
Method	<i>Endpoint PCR (modified King's)</i>	<i>Endpoint RT-PCR (Vilcek's) * IFA and ELISA combined</i>
Target	<i>Pig, wild boar</i>	<i>Pig, wild boar</i>
Test sample	<i>Whole blood (serum, spleen, tonsil, lymph node)</i>	<i>Serum (tonsil, spleen, kidney)</i>
Extraction	<i>Roche High Pure Viral Nucleic Acid Kit</i>	<i>Roche High Pure Viral RNA Kit</i>
Chemistry	<i>Takara Ex Taq Hot Start version</i>	<i>Invitrogen PrimescriptIII One-step RT-PCR with platinum Taq</i>
Detection	<i>Lonza SeaKem GTG agarose gel EP/ EtBr staining</i>	<i>Lonza SeaKem GTG agarose gel EP/ EtBr staining</i>
Confirmation of test results	<i>EcoRI digestion of positive control</i>	<i>BglII digestion of positive sample</i>

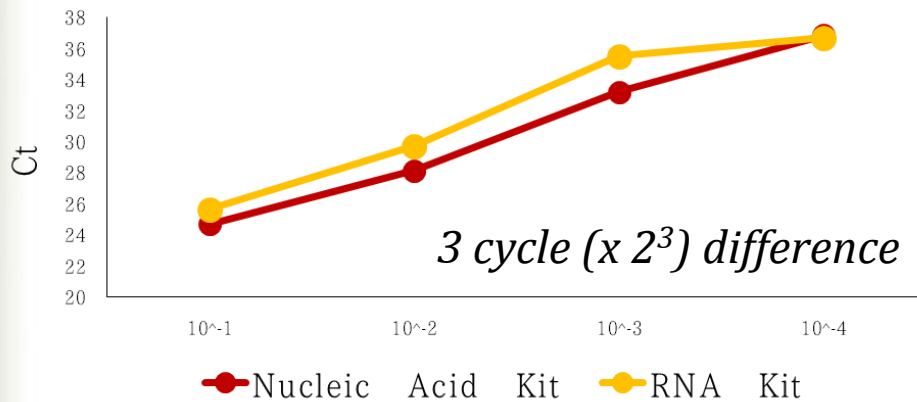
What worked well



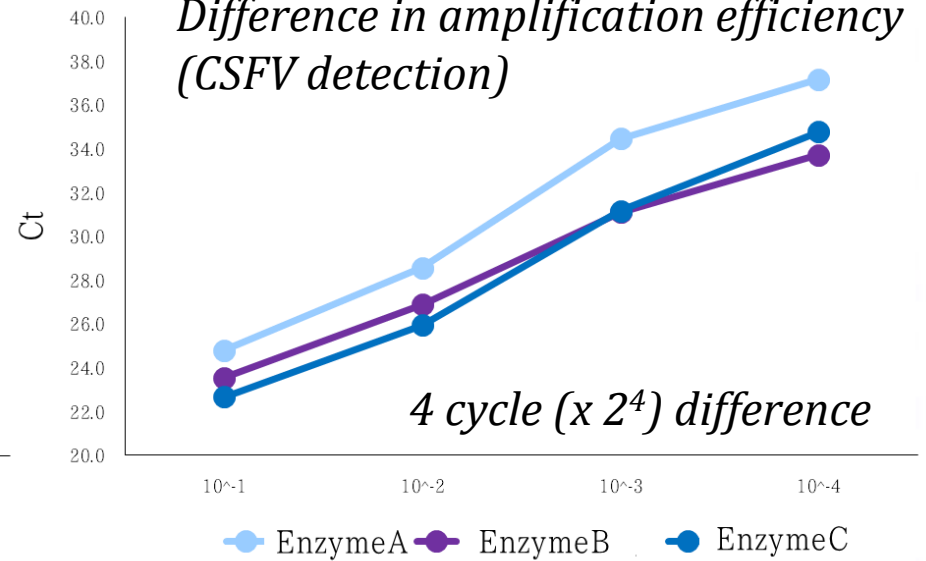
Points to be considered

- Choice of kit and chemistry

Difference in RNA extraction efficiency (CSFV detection)



Difference in amplification efficiency (CSFV detection)



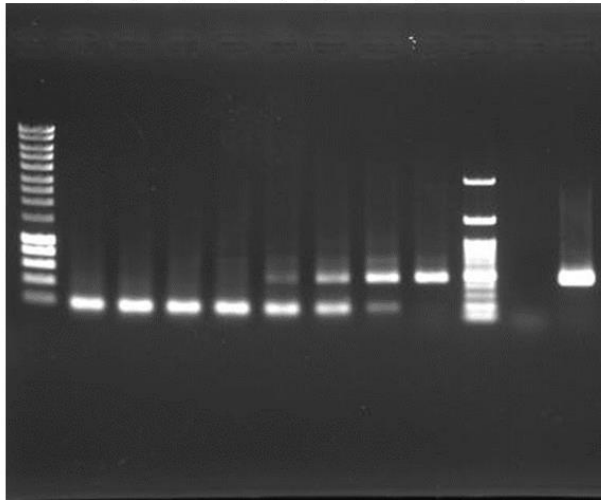
What worked well

Points to be considered

- *Optimization of procedures*

Adjustment annealing temperature in PCR with P72U/D primer set (ASFV detection in tissue homogenate)

	Sample A							NC	PC	
Annealing temp.	52.2	53.0	54.6	56.4	58.3	60.1	62.0	63.9	58.3	58.3



-50C/enzyme unknown (Bastos et al., 2003)
-52C/Taq polymerase (Lubisi et al., 2005)

What worked well



- *Successful isolation of multiple ASFV strains in pork meat products smuggled by oversea tourists by using pulmonary alveolar macrophage (PAM) cultures. (So far 76 cases of PCR positive meat/meat products have been detected by AQS.)*
- *Distribute endpoint PCR systems for ASF/CSF diagnosis to local animal hygiene stations to be used for screening dead/sick pigs and wild boars or confirmation of health status of pigs prior to movement.*
- ***Established stable procedures to generate various GM ASFVs.***

Challenges and possible solution



- ***Main limitations encountered in implementation apart from financial***
 - *Limited access to biological resources, especially low/moderate pathological viruses and antisera against different strains*
 - *Shortage of human resources*
 - *Limited space/equipment for animal experiments*
- ... and limitation of finance, of course!*

Thank you



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