Alternative way to test the efficacy of swine FMD vaccines: measurement of pigs median infected dose (PID_{50}) and regulation of live virus challenge dose

Dr. Dong Li

OIE/National Foot-and-Mouth Disease Reference Laboratory of China

State Key Laboratory of Veterinary Etiologic Biology, Lanzhou Veterinary Research Institute of Chinese Academy of Agricultural Sciences

Introduction

- 300 million pigs in China
- Vaccine doses:1200 million doses used
  - Sero type O
  - W/O/W double phases
  - 5 plants to produce
- Quality testing is needed

Abstract

- OIE: PD_{50} or PG test
- Protection against Generalization test in pigs
  - 10^4 $TCID_{50}$
  - Heel bulb or neck behind ear

- Experiments:
  - Strain adaption to suckling mice
  - Infective dose 1000 PID_{50}
  - 3 control pigs
  - Challenge on the neck behind ear

Materials and Methods:

- PID_{50}
  - Serial dilution(10^{-1} to 10^{-10}).
  - 4 pigs per dilution, 40 pigs
  - Challenge on the neck behind ear
  - Repeat from 10^{-5} to 10^{-8}
  - Karber calculation
- Vaccination
  - Conventional inactivated FMD type O vaccine
  - Neck behind ear
  - Serum sampling on 14 & 28 dpv
- Challenge
  - 28dpv
  - 1000 PID_{50} (2ml / pig)
  - Observed for 10 days
- PG test
  - 16 vaccinated pigs (2ml/pig)
  - 3 control pigs

Results :

- The PID_{50} was 6.5(table 1) .
- Serum antibody titrations: LPB-ELISA kit(WRL-IAH)
  - 14-28dpv
  - Cut-off 1.65

- Clinical observation:
  - At 2-4 days after challenge, blisters were observed on the 3 animals of the negative control group.
  - 16 vaccinated pigs were completely protected.

Table 1 Data for calculating PID_{50}

Virus dilution	Number of pigs	Number of Healthy pigs	Number of sick pigs	Number of accum-Ulated Healthy pigs↓	Number of accum-Ulated sick pigs	Sick ratio	Sick percentage (%)
10^{-1}	4	0	4	0	24	24/24	100
10^{-2}	4	0	4	0	20	20/20	100
10^{-3}	4	0	4	0	16	16/16	100
10^{-4}	4	0	4	0	12	12/12	100
10^{-5}	4	0	4	0	8	8/8	100
10^{-6}	4	1	3	1	4	4/5	80
10^{-7}	4	3	1	4	1	1/5	20
10^{-8}	4	4	0	8	0	0/8	0
10^{-9}	4	4	0	12	0	0/12	0
10^{-10}	4	4	0	16	0↑	0/16	0

Table 2   Serum titers at 14dpv and 28dpv of vaccinated pigs by LPB-ELISA and in vivo status of swine challenged with O/CHN/99

Pig's No	1	.2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17 CC	18 CC	19 CC
Titer 14 dpv (-log10)	1.3	1.3	1.0	1.3	1.8	1.6	1.0	1.0	1.0	1.0	1.6	1.3	1.3	1.6	1.6	1.6	<0.6	<0.6	<0.6
Titer 28 dpv (-log10)	1.8	1.9	1.6	1.8	2.2	2.1	1.6	1.3	1.6	2.1	1.9	1.8	1.9	1.8	2.1	1.8	<0.6	<0.6	<0.6
Challenge	P[a]	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	U[b]	U	U

a   P means the pig was protected after challenge 28dpv
b U means the pig was unprotected after challenge 28dpv

Discuss:

- There are two prevention and eradication strategies for FMD. "Slaughter policy" is definitely widely used in the developed countries. However, "vaccination policy" is carried out in developing countries due to high FMD prevalence and economic reasons. The FMD situation in China is currently not so favorable.

- Normally, a 10000 ID_{50} of virus challenge dose is used in cattle FMD vaccine efficacy testing and 10000 $TCID_{50}$ is used in pigs FMD vaccine for efficacy testing (OIE terrestrial manual 2009, Chapter 2.1.5 Foot-and-mouth disease). However, 10000 ID_{50} dose has been shown too strong in pigs as even high efficient vaccines cannot protect the animals, but 10000$TCID_{50}$ is too weak in pigs even 10^5 $TCID_{50}$ could not cause all control pigs showing clinical signs (interior data). Therefore, a challenge dose of 1000 PID_{50} was used in our experiment and the dose of $10^4 TCID_{50}$ was given up. The suckling mouse adapted strain was used here due to easier to pass and control than which passed in pigs.

- There are two ways to test FMD vaccine potency in cattle: PD_{50} test(within Europe☐or PG test (Protection against Generalization). Here, we accepted PG test which used widely in South America. Generally, 19 pigs are needed. 16 are vaccinated for 28 days, and other 3 are negative control. The vaccine final products will be qualified when the protected rate is equal or more than 12/16, and sickness rate for control is equal or more than 2/3.

Conclusion:

we measured pigs median infected dose(PID_{50}), used suckling mice passaged virus as intramuscular challenged strain, and determined 1000 PID_{50} as the challenge dose to test the efficacy of swine FMD vaccine.

VIROLOGY JOURNAL

Alternative way to test the efficacy of swine FMD vaccines: measurement of pigs median infected dose (PID$_{50}$) and regulation of live virus challenge dose

Dong Li*, Zeng-Jun Lu, Bao-Xia Xie, Pu Sun, Ying-Li Chen, Yuan-Fang Fu, Zai-Xin Liu*

Abstract

Foot-and-mouth disease in pigs is serious recently around the world. "Vaccination prevention" is still an important policy. OIE specifies 10,000 TCID$_{50}$(0.2 ml) of virulent virus for challenge test in pigs to test the potency of FMD vaccine by intradermal route inoculating the virus in the heel bulbs of one foot or by intramuscular route administering into one site of the neck behind the ear. Convenience and speediness are available in the process of potency test of commercial FMD vaccine. We selected the route of "administering into one site of the muscular part of the neck behind the ear" because of convenience and speediness. However, it was difficult to infect control pigs even up to 100,000TCID$_{50}$, so we changed the challenged virus from cell-passaged strain to suckling mice-passaged one, measured its PID$_{50}$ (pigs median infected dose) and defined the virus challenge dose as 1000 PID$_{50}$. Meanwhile, we arranged the number of control pigs from two to three for easy evaluation.

Findings

Foot-and-mouth disease (FMD) is an extremely contagious viral disease of cloven-hoofed domesticated as well as wild animals and has a great potential for causing severe economic loss. The causal agent, FMD virus (FMDV), is a member of the genus *Aphthovirus* in the family *Pdorna viridae* and occurs as seven distinct serotypes throughout the world: A, O, C, Asia1 and South African Territories (SAT) 1-3. Vaccination is the most important control and eradication strategy for FMD, especially the oil-adjuvant vaccine in developing countries [1-4]. The different processes for preparing vaccines against viral diseases are comprised by a sequence of steps which, although different in accordance with particular virus and processes selected, may be classified as follows: virus production, virus inactivation and vaccine formulation.

In recent years, the pigs infected with FMD have been reported around the Chinese mainland, such as Chinese Taipei, Chinese Hong Kong [5-9]. Furthermore, import of vaccines are not only expensive, they are not always suitable. Therefore research into vaccine development and improvement is vital. In order to test vaccine efficacy in swine, the protected rate as well as ID$_{50}$ and the challenge dose need to be determined. The OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE terrestrial manual 2009, Chapter 2.1.5 Foot-and-mouth disease) lists a number of criteria for testing FMD vaccines in pigs. These include challenge in the heel bulb of 1 foot with 10^4 TCID$_{50}$ of FMDV titered in a suitable pig cell culture system or administering into one site of the muscular part of the neck behind the ear. However, "challenge in the heel bulb" is too laborious and time-consuming during the commercial vaccine potency tests because more than hundred pigs need to be captured at the same time, so we choose "administering into one site of the muscular part of the neck behind the ear" as our challenge route. Most of the FMD vaccine companies in China select BHK-21 cell lines to propagate the swine vaccine virus strain, and we also use BHK-21 passaged virus as the challenge strain to keep the consistency. Unfortunately, we did not achieve a good result in our factual operation due to the control

* Correspondence: lidong.gsau@gmail.com; liuzaixin.hld@gmail.com
Key Laboratory of Animal Virology of Ministry of Agriculture, National Foot-and-Mouth Disease Reference Laboratory of China, State Key Laboratory of Veterinary Etiologic Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu, 730046 China

Aknowlegement

- Dr.Tom Wilianms & Dr. F.F. Maree's invitation for GFRA in South Africa

Thank you for your attention

19 April 2012 **Hazyview**