江苏省农业科学院
JIANGSU ACADEMY OF AGRICULTURAL SCIENCES

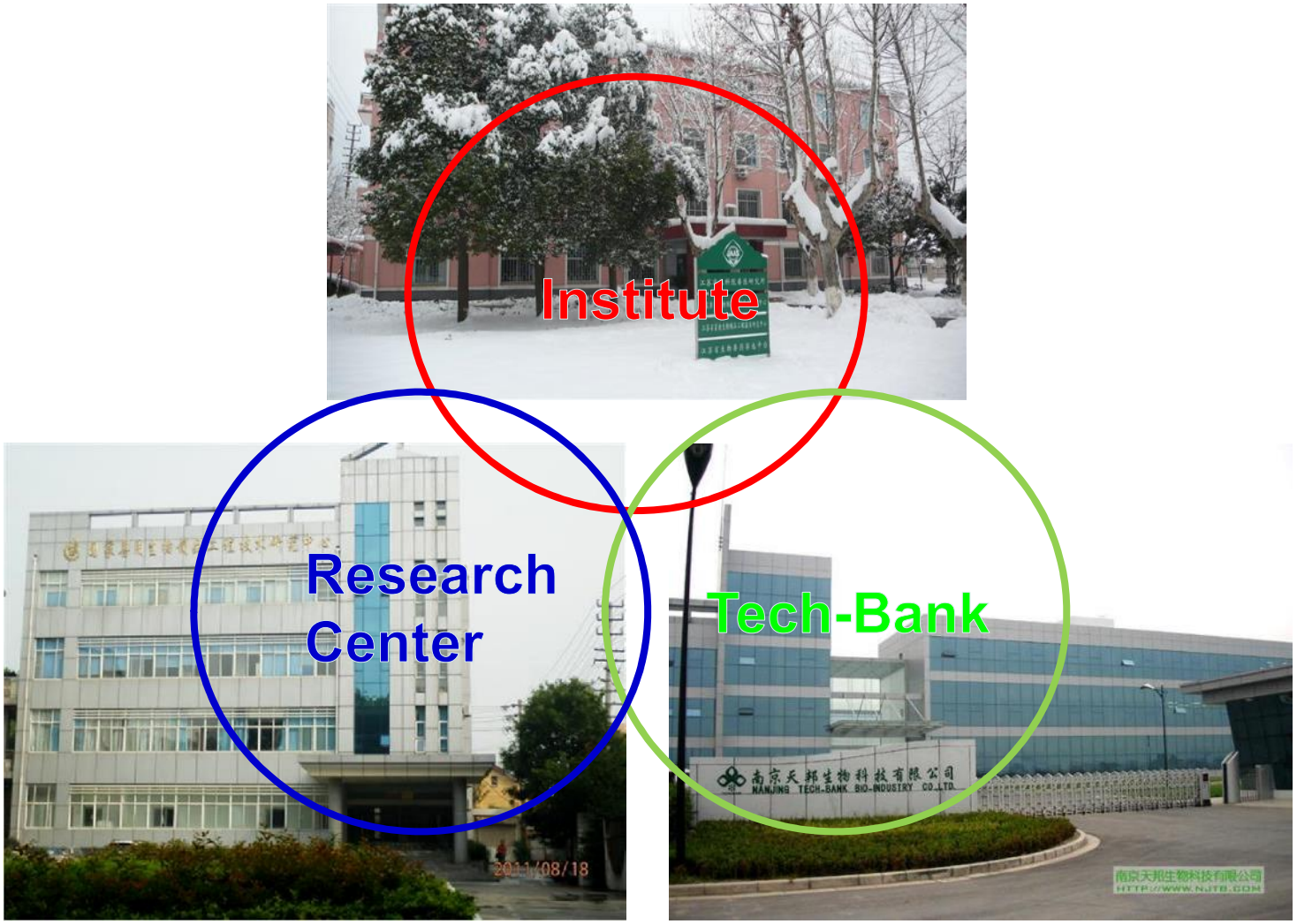
Molecular epidemiology of Mycoplasma hyopneumoniae

Guoqing Shao

Jiangsu Academy of Agricultural Sciences (JAAS)

27th, Oct.,2016，Brussels

About IVS JAAS



6 Vaccine licences

MHP vac research history---JAAS IVS

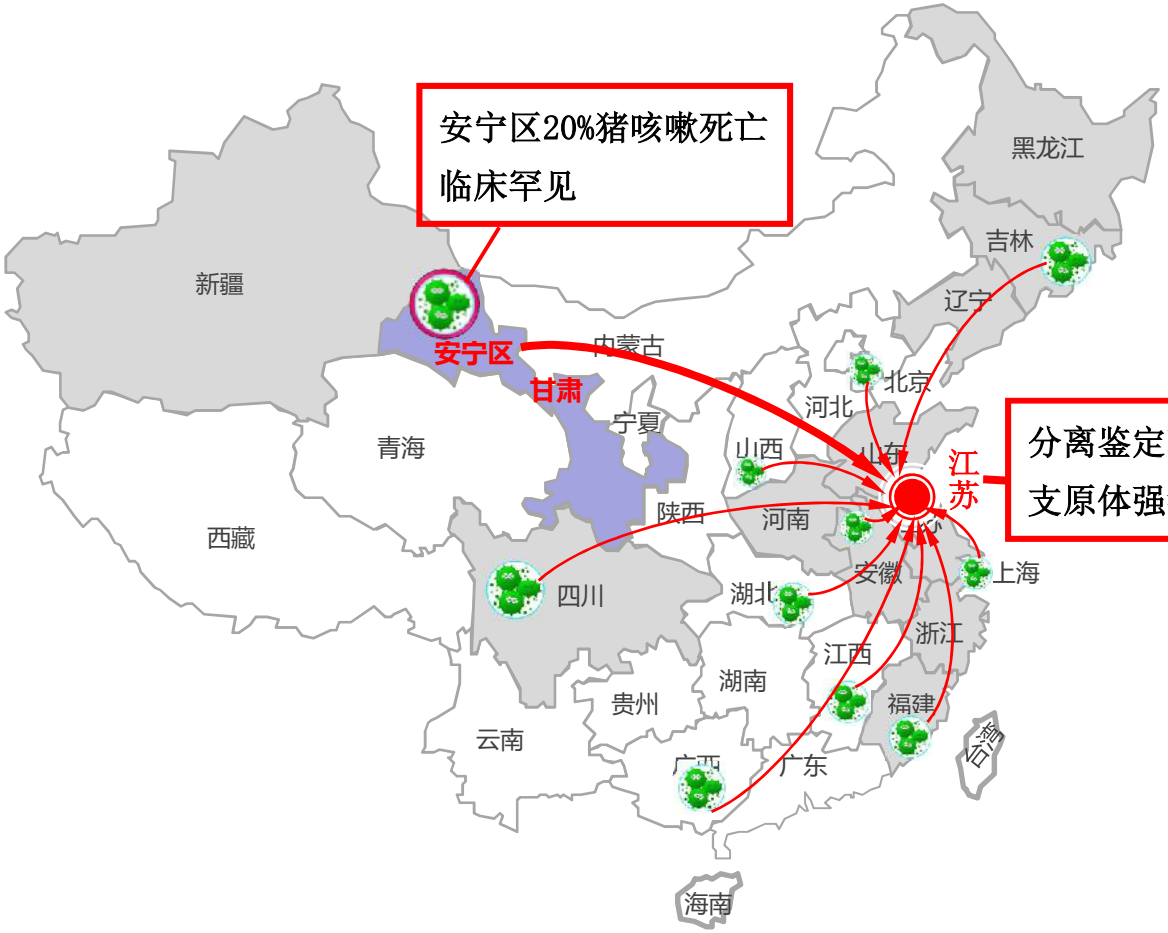
Prof. He, the 1st director

Prof. Jing, the 2nd director

Prof. Shao, the 3rd director

- **1957-1980** **isolation and cultivation of Mhp strain**
- **1978-1989** **cultivation of the attenuated vaccine (168 strain)**
- **1985-1994** **immunology research of attenuated vaccine**
- **1995-2001** **Applied Research of attenuated vaccine**
- **2000-2007** **Product Development and market**
- **2006-now** **upgrade of attenuated vaccine**
  new vaccine research (inactive vaccine, Genetic engineering vaccine)
  Basic research (Genomics & Proteomics)

➢Very virulent strain is key to a live vaccine



二花脸猪人工发病

其中耳号168猪
咳嗽气喘严重

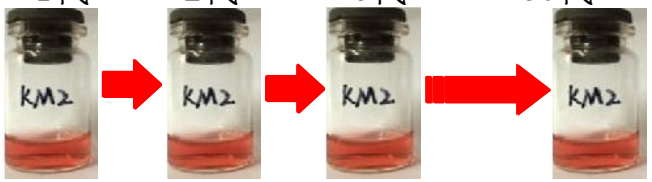
安宁系168强毒株

➢ Normal attenuation method do not work

安宁系168
强毒株

每隔20代进行猪毒力测试

+1代 +2代 +3代 +… +50代

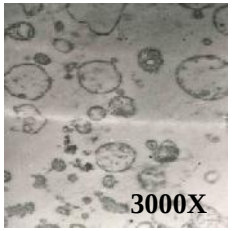


无细胞培养基连续传代致弱
毒力下降，但免疫原性也下降

活体猪感染回归，恢复免疫原性

完成322代间断致弱，5次猪体回归

F322代致弱株
毒力减弱
免疫原性稳定



3次固相
克隆纯化

发明克隆致弱株

The First Cell-free Culture attenuated

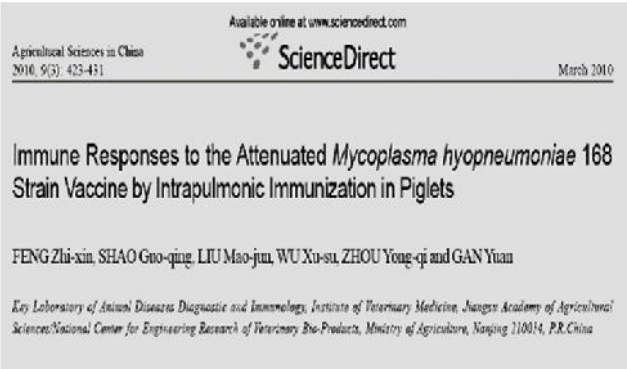
Vaccine inoculation -Intrapulmonary

Patent license

Production license

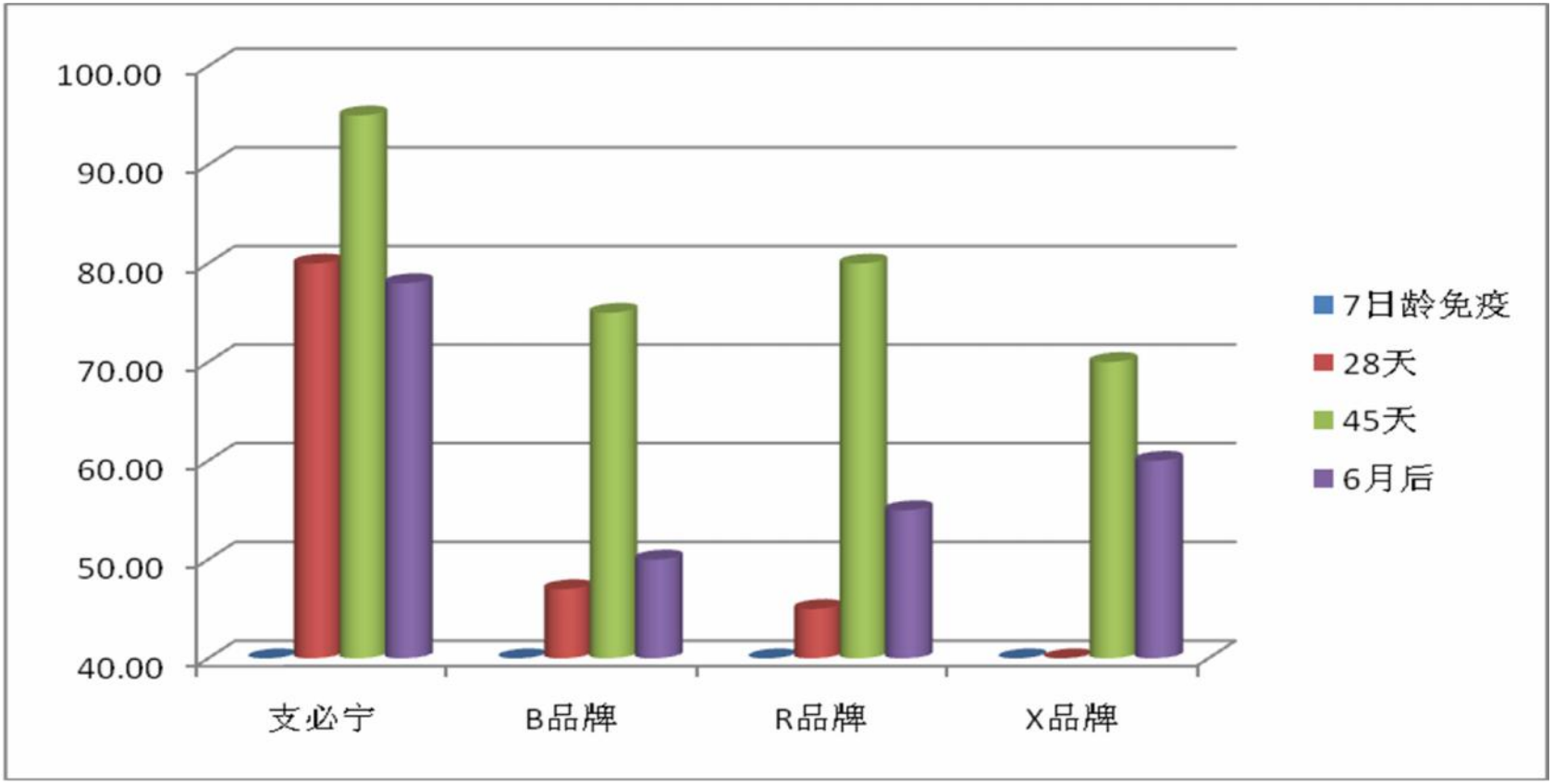
Vaccine product

Vaccine inoculation- Intrapulmonary



Good Immune protection

Efficacy study



Research on the attenuated Mhp aerosol vaccine

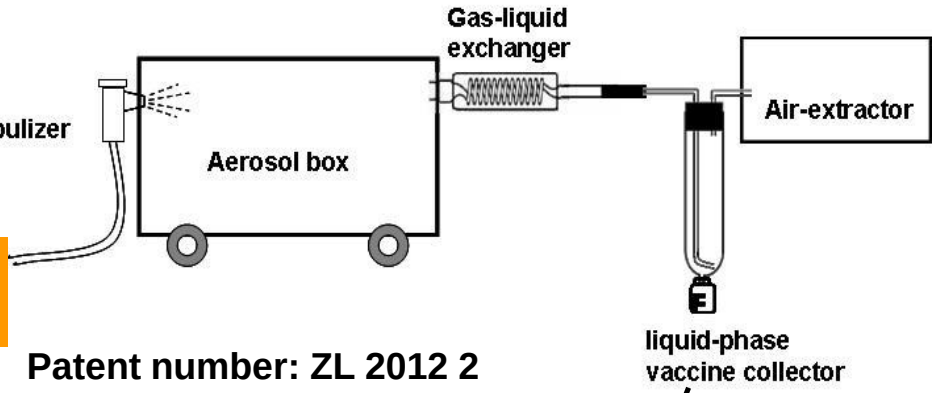
Is it aerosol vaccine?

No!

Key points of the spray vaccine

- **How to produce the aerosol vaccine?**
- **How to control the particle diameter of the aerosol vaccine?**
- **How to keep the viability of the aerosol vaccine?**
- **How to develop the spray model?**
- **How to calculate the immune dose of the aerosol vaccine?**

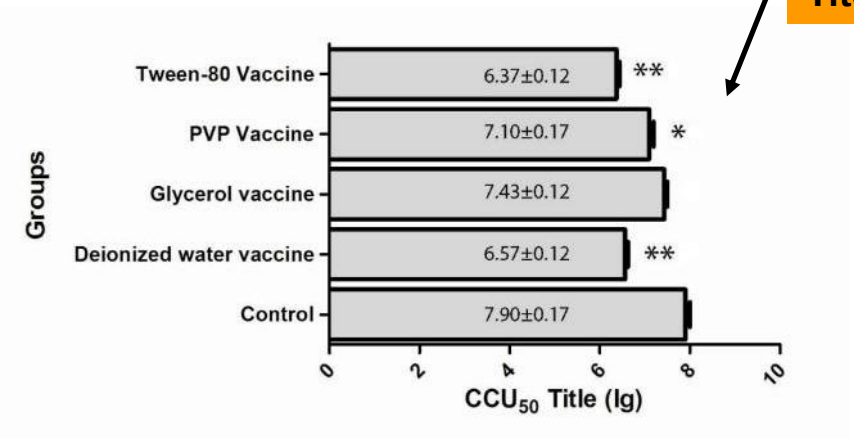
Viability of the aerosol vaccine



The attenuated vaccine solution

Patent number: ZL 2012 2 0357811.3

Titer detection



1 bar, 23.8°C(RT) and 70%(RH),

Factors:

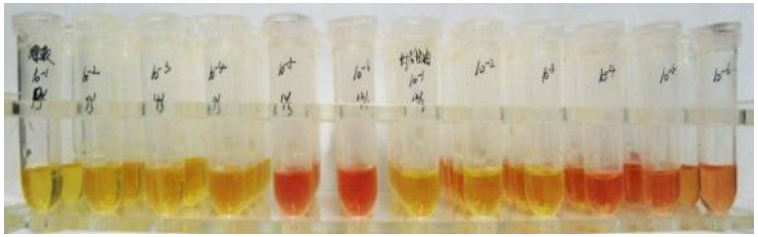
Shear force

Impact force

bubbling

Relative humidity

Excipient



---Feng et al., Veterinary Microbiology .2013. 167(3-4):417-24.

spray model ---a new Foolish Vaccination way

Veterinary Microbiology 167 (2013) 417–426

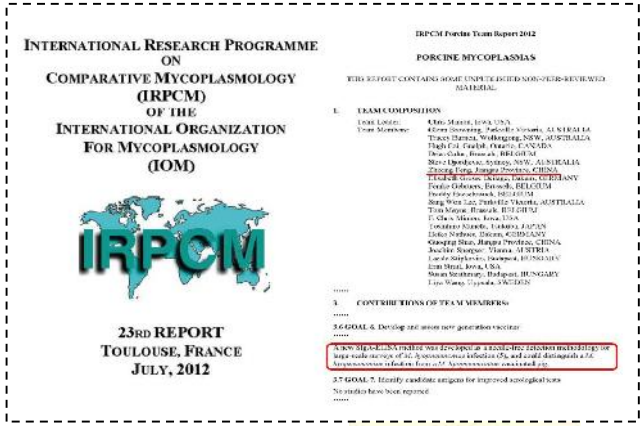
Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Development and validation of an attenuated *Mycoplasma hyopneumoniae* aerosol vaccine

Zhi-Xin Feng[a,1], Yan-Na Wei[a], Gui-Lan Li[a], Xiao-Ming Lu[a], Xiu-Feng Wan[b], G. Todd Pharr[b], Zhan-Wei Wang[a], Meng Kong[a], Yuan Gan[a], Fang-Fang Bai[a], Mao-Jun Liu[a], Qi-Yan Xiong[a], Xu-Su Wu[a], Guo-Qing Shao[a,*]


INTERNATIONAL RESEARCH PROGRAMME ON COMPARATIVE MYCOPLASMOLOGY (IRPCM) OF THE INTERNATIONAL ORGANIZATION FOR MYCOPLASMOLOGY (IOM)

IRPCM

23RD REPORT
TOULOUSE, FRANCE
JULY, 2012

IRPCM Porcine Team Report 2012

PORCINE MYCOPLASMAS

Veterinary Microbiology 142 (2010) 410–416

Contents lists available at ScienceDirect

Veterinary Microbiology

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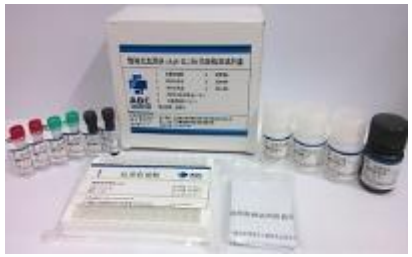
Short communication

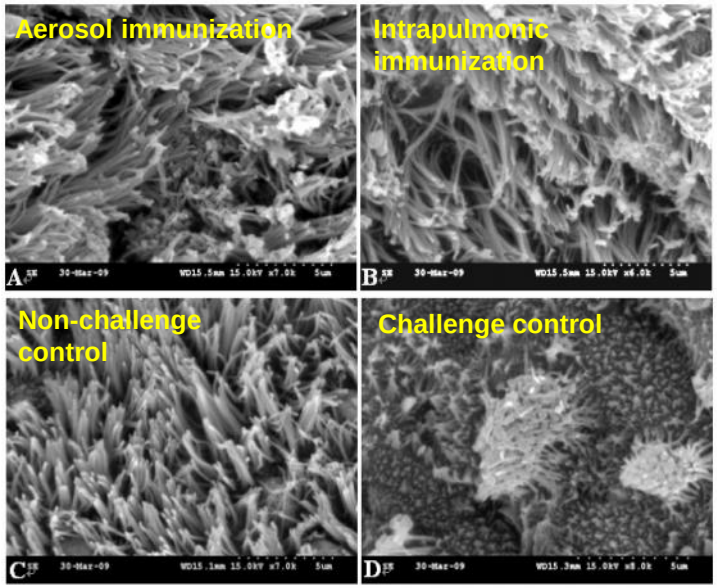
Development and validation of a SIgA-ELISA for the detection of *Mycoplasma hyopneumoniae* infection

Zhi-Xin Feng[a,b,c,1], Guo-Qing Shao[a,b,c,*], Mao-Jun Liu[a,b,c], Hai-Yan Wang[a,b,c], Yuan Gan[a,b,c], Xu-Su Wu[a,b,c]

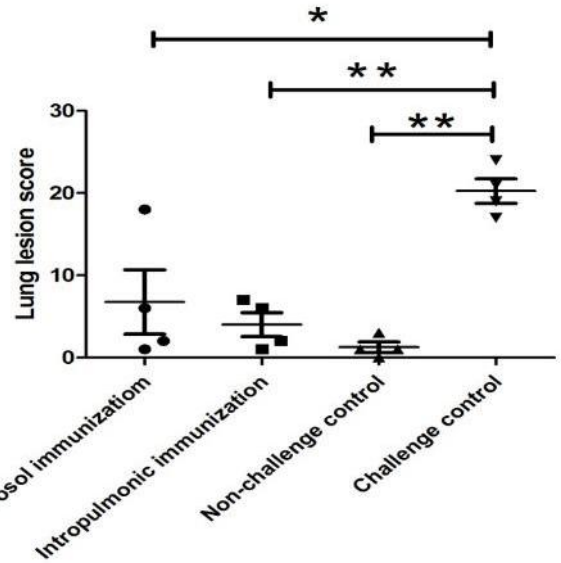


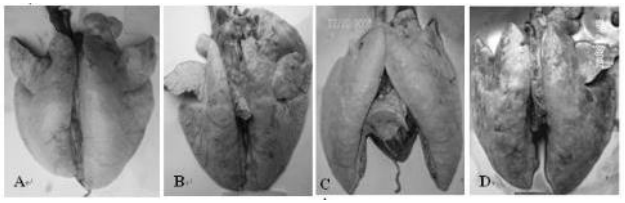
The attenuated *Mycoplasma hyopneumoniae* strain 168 vaccine was the first live attenuated vaccine for enzootic pneumonia, the most important bacterial disease of pigs throughout the world. The effective control of this disease in pigs ranks with the development of vaccines to control the viral diseases hog cholera and pseudorabies.

Protection against challenge



Scanning electron micrograph detection for the ciliated epithelial cells post-challenge





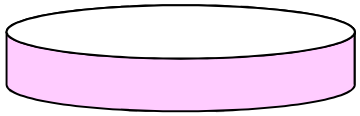
Lung lesion and score assess post-challenge

---Feng et al

PHD Research:Molecular epidemiology of MHP

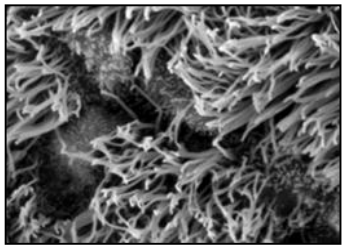
Etiological study-Expand the scope of epidemiology, get more field strains

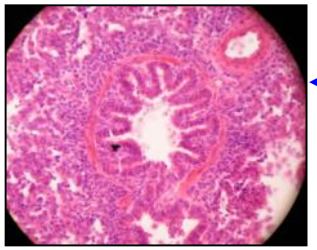
KM2 Liquid Medium



KM2 Solid Medium

Animal assay





Identification by Histology and Electron microscopy

Genetic diversity of Mycoplasma hyopneumoniae isolates

- **16S-23S intergenic spacer region**
- ***RFLP*、*RAPD*、*SSRP*、*AFLP*、*SNP*、*STMS*、*SCAR*、*CAPS*、*SPAR*、*ISTR***
- **MLVA（multiple loci VNTR analysis）Mhp have more than 22 VNTR loci**
- **P97(R1\R2)、 P146(P146R3)、H4（putative membrane protein）**
- **Genotype distribution of Mycoplasma hyopneumoniae in swine herds from different geographical regions. Vet Microbiol. 2015 Feb**

Thanks to

Thanks for all co-operatives !