Microbiological Identification and Analysis of Swine Lungs Collected from Carcasses in Swine Farms, China

Yunfeng Zha · Jiexiong Xie · Ye Chen · Chunya Wei · Wanjun Zhu ·
Jidang Chen · Haitao Qi · Liangquan Zhang · Long Sun · Xiaozhan Zhang ·
Pei Zhou · Zhenpeng Cao · Wenbao Qi · Minze Zhang · Zhen Huang ·
Guihong Zhang

Received: 4 March 2013 / Accepted: 19 March 2013 / Published online: 27 March 2013
© Association of Microbiologists of India 2013

Abstract The primary objective of this 3 years study was to determine the prevalence of porcine pathogens of the lungs of swine in swine farms in southern China. A total of 5,420 samples were collected from 200 swine farms. The bacterium that was most commonly isolated was *Streptococcus suis*, with 10.24 % of the samples being positive, 114 lungs (2.1 %) were positive for pseudorabies virus and 263 (4.85 %) were positive for classical swine fever virus; much lower than positive for PRRSV (15.1 %, $p = 0.023$) and PCV2 (13.8 %, $p = 0.038$). Lungs that were positive for PRRSV and/or PCV-2 have significantly

increased odds of being positive for any of the *S. suis* (9.79 vs. 0.44 %, $p = 0.003$).

Keywords Microbiological · Lungs · Abnormal pigs carcasses

Yunfeng Zha, Jiexiong Xie, Ye Chen these authors contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s12088-013-0396-x) contains supplementary material, which is available to authorized users.

Y. Zha · J. Xie · Y. Chen · C. Wei · W. Zhu · J. Chen ·
L. Sun · X. Zhang · P. Zhou · Z. Cao · W. Qi · M. Zhang ·
Z. Huang · G. Zhang (✉)
College of Veterinary Medicine, South China Agricultural
University, Guangzhou 510642, Guangdong, China
e-mail: guihongzh@scau.edu.cn

Z. Huang
e-mail: huangzhen325320@163.com

Y. Zha
Guangdong Animal Health Supervision Institute,
Guangzhou 510230, China

H. Qi
Beijing dabeinong swine technology Co., Ltd, 14F Zhongguancun
Building No. 27 Zhongguancun Street, Beijing 100080, China

L. Zhang
Guangzhou Lizhi Agriculture Co., Ltd, Guangzhou, China

While lungs have provided a historical sampling site for detecting normal flora and aided in elucidating clinical disease [1], lungs sampling should be also considered as a monitoring tool for identifying emerging and re-emerging swine pathogens including those with foodborne and zoonotic implications. Commensal bacteria, such as *Streptococcus porcinus*, *Streptococcus dysgalactiae*, as well as opportunistic pathogens, such as *Staphylococcus aureus*, *Staphylococcus hyicus*, and *Streptococcus suis*, can be isolated from swine lungs [2–4]. Additionally, some pathogens such as porcine reproductive and respiratory syndrome virus (PRRSV), Porcine circovirus type 2 (PCV2), *Salmonella* spp, and classical swine fever virus (CSFV) can persistently colonize lungs tissue, which may serve as a reservoir of infection [4]. The emergence of PRRSV in China in 1993 was documented when an unknown *arterivirus* was isolated from the lungs of affected pigs [5, 6]. Porcine circovirus type 2 is the primary causative agent of porcine circovirus-associated diseases (PCVADs) in pigs and causes significant economic losses in most pig-producing countries. More recently, an outbreak of *S. suis* infection causing mortality in humans was caused by an emergent, highly virulent strain of *S. suis*, a pathogen that persists in the lungs of clinically healthy pigs [7, 8]. Most infectious diseases are caused by bacteria which proliferate within quorum sensing (QS) mediated biofilms [9, 10]. Further documentation and surveillance of the normal flora of the swine lungs is pertinent to the

continued understanding of disease pathogenesis including zoonotic pathogens associated with swine and pork production. PCV2 and PRRSV, *S. suis* infection has been considered to be an important challenge to Chinese swine industry. The objectives of this study were to gather data to determine the prevalence of microbiological populations of the lungs of pig carcasses in swine farms in southern China, to determine if sampling the carcasses of normal (healthy slaughtered pigs) or abnormal (sicked and die pigs) hogs provides different microbiological profiles, monitor important pathogens that have health implications for both swine and humans.

Lungs were collected from swine carcasses in southern China from June 2010 to December 2012. A total of 5,420 samples were collected from 200 swine farms distributed throughout Guangdong, Zhejiang, Fujian, Guangxi Provinces, southern China. Bacteriology was quantified by culture and all bacteriological work, including setup, culture, identification, and typing, was done following standard operating procedures outlined in the manual of clinical microbiology. Testing for virology were carried out according to previously reported protocols [5, 7]. All animal research was conducted under the guidance of the Centers for Disease Control and Prevention (CDC)'s Institutional Animal Care and use committee in an association for assessment and accreditation of laboratory animal care International accredited facility. The comparison of categorical variables between microbiological populations was performed using a Chi square test where appropriate. Statistical significance was defined as $p < 0.05$. The data was analyzed with SAS software, Version 9.1 (SAS Institute, Cary, NC, USA).

The bacterium that was most commonly isolated was *S. suis*, with 10.24 % of the samples being positive, 114 lungs (2.1 %) were positive for pseudorabies virus (PRV) and 263 (4.85 %) were positive for CSFV; much lower

than positive for PRRSV (15.1 %, $p = 0.023$) and PCV2 (13.8 %, $p = 0.038$). lungs that were positive for PRRSV and/or PCV-2 have significantly increased odds of being positive for any of the *S. suis* (9.79 vs 0.44 %, $p = 0.003$). The frequency of individual pathogens was significantly ($p = 0.002$) associated with the carcasses of normal (healthy slaughtered pigs, 80.1 %) and abnormal (sicked and die pigs, 19.9 %) pigs (Table 1).

Little information is available in the literature about the usefulness of collecting tissue samples from abnormal pigs versus normal pigs for surveillance or research purposes. Most studies have focused on the risks of microbiological contamination of the carcass during the slaughter process. Here, we report for the first time the the prevalence of microbiological populations of the lungs of pigs in swine farms in southern China. We found a relatively high PRRSV and PCV2 prevalence, as well as bacteria and viruses co-infection. *S. suis*, the most frequently identified bacteria (10.24 %, 555/5420) in this study, has been reported as an important swine pathogen and as a pathogen with zoonotic potential [11] A previous China study using PCR testing methods reported that 98 % of swine farms were positive for *S. suis* [3]. The clinical and potential zoonotic significance of the findings in this study cannot be commented on further as typing was not performed. The high recovery rate of *S. suis* in this and previous studies coupled with the reported changes in virulence patterns seen in other parts of the world [8] warrants the continued surveillance of this pathogen with appropriate subtyping. Interestingly, lungs that were positive for PRRSV and/or PCV-2 have increased odds of being positive for any of the zoonotic pathogens (*S. suis*). It has been proposed that these 2 viruses may cause a reduction in the immune function of swine, making them more susceptible to other pathogens. It may be prudent to closely monitor such

Table 1 The frequency of individual pathogens isolated from 5,420 swine lungs and the univariable associations between lungs positivity for porcine circovirus type 2, porcine reproductive and respiratory

syndrome virus, pseudorabies virus, classical swine fever virus, and bacterial pathogens isolated from swine lungs collected from abnormal carcasses versus normal carcasses in swine farms

Pathogen isolated	Total number of positive lungs	Freq (%)	No. of lungs positive	
			Abnormal carcass	Normal carcass
PCV-2	820	15.1	684/820 (83.4 %)	136/820 (16.6 %)
PRRSV	747	13.8	632/747 (84.6 %)	115/747 (15.4 %)
PRRSV+PCV-2	368	6.79	332/368 (90.2 %)	36/368 (9.8 %)
PCV+SS	268	4.94	252/268 (94.0 %)	16/268 (6.0 %)
PRRSV+SS	179	3.30	175/179 (97.8 %)	4/179 (2.2 %)
PCV+PRRSV+SS	84	1.55	84/84 (100 %)	0/84 (0 %)
CSFV	263	4.85	219/263 (83.3 %)	44/263 (16.7 %)
PRV	114	2.10	93/114 (81.6 %)	21/114 (18.4 %)
SS	24	0.44	22/24 (91.7 %)	2/24 (8.3 %)
Total	2499	46.1	2001/2499 (80.1 %)	498/2499 (19.9 %)

abnormal pigs carcasses (sicked and die pigs) as potential sentinels for the microbiological populations in developing countries with extensive swine-raising industries. Further virological and serological studies in swine farm are urgently required in China to monitor swine infections.

Acknowledgments This study was supported by the Modern Agricultural Industry Technology System (CARS-36).

References

1. Wang XR, Ma HY, Zhu WG et al (2008) Investigation and isolations of isolates of PRRSV in “Swine high fever disease” pig herds. *Chin J Vet* 44(3):39240
2. Ke Yuehua, Wang Yufei, Yuan Xitong et al (2012) Altered transcriptome of the B-melitensis vaccine candidate 16 M delta vjbR, implications for development of genetically marked live vaccine. *Indian J Microbiol* 52(4):575–580
3. Das Jayshree, Jha DK, Policegoudra RS et al (2012) Isolation and characterization of antidermatophytic bioactive molecules from *Piper longum* L. Leaves *Indian J Microbiol* 52(4):624–629
4. Keffaber KK (1989) Reproductive failure of unknown etiology. American association of swine. *Practitioner Newslett* 1:1–10
5. Cai L, Ni JQ, Xia YG, Zi ZC, Ning K, Qiu P, Li XX, Wang BY, Liu Q, Hu DM, Yu XL, Zhou Z, Zhai XY, Han X, Tian KG (2012) Identification of an emerging recombinant cluster in porcine circovirus type 2. *Virus Res* 165:95–102
6. Lin K, Wang C, Murtaugh MP, Ramamoorthy S (2011) Multiplex method for simultaneous serological detection of porcine reproductive and respiratory syndrome virus and porcine circovirus type 2. *J Clin Microbiol* 49(9):3184–3190. doi:[10.1128/JCM.00557-11](https://doi.org/10.1128/JCM.00557-11)
7. Wenhui L, Zhongyan W, Guanqun Z et al (2012) Complete genome sequence of a novel variant porcine reproductive and respiratory syndrome virus (PRRSV) strain: evidence for recombination between vaccine and wild-type PRRSV strains. *J virol* 86(17):9543. doi:[10.1128/JVI.01341-12](https://doi.org/10.1128/JVI.01341-12)
8. Kovi RC, Dahiya S, Minakshi G et al (2005) Evaluation of new primers targeting serogroup specific genes for detection of bluetongue viruses by RT-PCR. *Indian J Microbiol* 45(2): 103–107
9. Kalia VC (2013) Quorum sensing inhibitors: an overview. *Biotechnol Adv* 31(2):224–245. doi:[10.1016/j.biotechadv.2012.10.004](https://doi.org/10.1016/j.biotechadv.2012.10.004)
10. Kalia VC, Purohit HJ (2011) Quenching the quorum sensing system: potential antibacterial drug targets. *Crit Rev Microbiol* 37(2):121–140. doi:[10.3109/1040841X.2010.532479](https://doi.org/10.3109/1040841X.2010.532479)
11. Ratra R, Lal SK (2008) Functional genomics as a tool in virus research. *Indian J Microbiol* 48(2):195–201. doi:[10.1007/s12088-008-0032-3](https://doi.org/10.1007/s12088-008-0032-3)