CONFERENCE SCENE

Latest developments on *Streptococcus suis*: an emerging zoonotic pathogen: part 1

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The first international workshop on *Streptococcus suis*, which is an important swine pathogen and emerging zoonotic agent, took place in Beijing, jointly organized by the Faculty of Veterinary Medicine, University of Montreal, Canada and the National Institute for Communicable Disease Control and Prevention, China CDC. The aim of the meeting was to gather together, for the first time, more than 80 researchers working on *S. suis*, from countries including China, Canada, Japan, The Netherlands, Germany, Thailand, the UK and Vietnam. This article, the first of a two-part report on this First International Workshop, reviews current aspects of the epidemiology and population genomics of *S. suis*, covers public health concerns and discusses questions about *S. suis* serotyping and molecular diagnostics.

KEYWORDS

- emerging zoonosis
- epidemiology
- genomics • molecular diagnostics • public health
- *Streptococcus suis*

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Relevance of *S. suis* research

Streptococcus suis is a swine pathogen responsible for important economic losses in the swine industry worldwide. It causes a wide variety of diseases in pigs, including meningitis, septicemia and endocarditis. Among the 35 serotypes originally described based on capsular polysaccharide (CPS) antigens, serotype 2 is not only prevalent in swine diseases, but is also considered to be an emerging zoonotic agent causing meningitis and streptococcal toxic shock-like syndrome (STSLS) in humans [1]. *S. suis* has gained more attention since recent recognition of its high prevalence in human meningitis cases in south-east and east Asia, and reports of outbreaks that resulted in high mortality rates [2,3]. The emergence of *S. suis* as a human pathogen has led to an explosion of research. As of November 2013, 371 *S. suis*-related articles were published during the previous 5 years and more than 140 in the last 20 months (PubMed database). Here, we discuss current aspects emerging from the First International *S. suis* Workshop held in China in 2013.

Advances in epidemiology & population genomics

The genome sequences of over 100 *S. suis* strains of different geographic origins have either been published or are available in public databases. Real-time genomic epidemiological evaluation of infectious agents, and in particular of those with potentially severe clinical outcomes, might limit detrimental effects of infections and prevent outbreaks. Dr Jianguo Xu (National Institute for Communicable Diseases Control and Prevention, China CDC) described a newly developed *S. suis* whole-genome sequence-based typing schema by which the minimum core genome of the species was defined. This method provides a clear division of groups containing human isolates and discriminates outbreak isolates, including those causing STSLS, from those causing sporadic, less severe disease [4]. This typing system will facilitate the application of genome data to *S. suis* epidemiology and surveillance. Using comparative genomics, Dr Baoli Zhu (Chinese Academy of Sciences, China) showed that *S. suis* type 2 has a polymorphic, highly mobile and mosaic genome. The 89K pathogenic island, so far unique to Chinese STSLS-causing strains, was found to be partially included in other virulent and avirulent strains [5]. Dr Duncan Maskell (University

of Cambridge, UK), again using whole-genome sequencing, described the population biology/structure of over 400 isolates from pigs in the UK and pigs and humans in Vietnam, which have detailed clinical histories or were isolated from tonsils of healthy pigs. Similar findings to those of Dr Xu were reported in terms of there being extensive recombination. Data analysis using discriminant analysis of principle components identified particular single nucleotide polymorphisms that are associated with disease. Studies on *S. suis* infection in Thailand have mainly focused on human isolates. Dr Potjanee Srimanote (Thammasat University, Thailand) reported the genetic linkage between *S. suis* serotype 2 isolates from pigs and humans during the 2007 infection outbreak in Thailand. A strong correlation between virulence gene profiles, random amplified polymorphic DNA patterns and multilocus sequence types (STs) was observed, confirming *S. suis* transmission from pigs to humans. Multilocus STs distinguished four STs prevalent in this country: ST1, ST104, ST233, and a new ST336 [6].

Advances in serotyping & molecular diagnostics: the dilemma of *S. suis* serotypes

S. suis serotyping is based on CPS composition and the use of antisera, which is expensive and time-consuming. There is also a need to complete knowledge on the diversity and evolution of *S. suis* CPS types. Dr Daisuke Takamatsu (National Institute of Animal Health, Japan) provided a comprehensive analysis of *S. suis* *cps* gene clusters. Albeit all CPS serotypes are synthesized by the Wzx/Wzy pathway, the number and combinations of *cps* genes differ among serotypes and, except for serotypes 1, 2, 1/2 and 14, all serotypes possessed serotype-specific gene(s). The evolution of the *S. suis* *cps* cluster diversity is the result of multiple gene transfer and recombination events, as well as small-scale mutations [7]. Interestingly, a large number of *S. suis* isolates, especially those from healthy pigs, remain untypable. Using whole-genome high-throughput sequencing, Dr Han Zheng (National Institute for Communicable Diseases Control and Prevention, China CDC) reported 12 new *cps* gene clusters among 'untypable' strains. The potential discovery of new serotypes remains to be validated.

Development of a practical and simple typing method for *S. suis* is desirable. Dr Chengping

Lu (Nanjing Agricultural University, China) reported the development of serotype-specific PCR tests for nine different *S. suis* serotypes [8]. Dr Masatoshi Okura (National Institute of Animal Health) developed a multiplex PCR-based *cps*-typing method for the 35 described serotypes, composed of grouping and typing steps: a first PCR detects *cps* genes conserved in multiple serotypes (grouping PCR), followed by a second PCR that detects serotype-specific *cps* genes within each group. Since *cps* gene clusters of serotypes 1/2 and 2 and those of serotypes 1 and 14 are almost identical to each other, the method does not discriminate between these serotypes [9].

Most PCR tests that are directly used with clinical specimens are directed against serotypes 2 (and 1/2) and 14 (and 1), responsible for the majority of human cases. Since PCR equipment is not always available in certain countries, Dr Prasit Tharavichitkul (Chiang Mai University, Thailand) described the rapid detection of these *S. suis* serotypes in streptococcal-positive hemocultures using the Quellung reaction with specific anti-CPS antiserum.

During the Workshop, a controversy arose regarding the taxonomy of *S. suis* strains. Serotypes 32 and 34 had previously been reported to belong to the species *S. orisratti*. PCR tests based on *gdh* or *16S rRNA* primers are largely used to identify a field strain as *S. suis*. Dr Tharavichitkul reported that *gdh* primers are less specific than those of *16S rRNA* [10]. Dr Ro Osawa (Kobe University, Japan) described a potentially more reliable PCR assay targeting the *recN* gene, which encodes a DNA recombination/repair protein. He proposed that the reference strains of serotypes 20, 22, 26 and 33, even if they are considered to be *S. suis* by the *gdh* or *16S rRNA* PCR tests, might not be *S. suis* based on DNA–DNA reassociation, as well as *sodA*- and *recN*-based phylogenies [11]. There is still a need for a consensus approach to define *S. suis* strains, especially for diagnostic laboratories. There is also a call for the complete genomic sequencing of the 35 serotypes to resolve this issue.

Highlights on zoonotic & public health concerns: the need of prevention campaigns

The danger of *S. suis* for humans and the educational and preventive measures campaigns in Asian populations at risk were discussed during

the workshop [12]. In Vietnam, consumption of raw or undercooked pork products has been related to large numbers of cases [2]. Dr Heiman Wertheim (Oxford University Clinical Research Unit, Hanoi, Vietnam) investigated the behavior of eating *tiết canh* (raw pig blood) and found a lower rate of consumption in urban regions compared with rural areas. While some rural people start eating *tiết canh* in their early childhood, most of them start to do so after getting married. Weddings are a common occasion where people are first exposed to this dish. In urban areas, people often eat *tiết canh* mainly for breakfast in food shops. Interestingly, manual workers and sales persons were more likely to consume *tiết canh* than office workers. To understand the potential risk of pig-derived products in the food chain, Dr Ngo Thi Hoa (Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam) investigated the presence of *S. suis* in pork byproducts collected from slaughterhouses and wet/retail markets in Vietnam, and found an 11% contamination rate with *S. suis* serotype 2 in internal organ samples. However, the potential of these isolates to cause disease remains to be evaluated [13]. Dr Kazunori Oishi (Osaka University, Japan) reported public health interventions in Thailand. Data suggest a high incidence rate (6.2/100,000) of *S. suis* infection in the general population in 2010, mostly related to consumption of raw pork products. A risk assessment of human infection was disseminated to the local residents and the implementation of the Food Safety Campaign to ‘stop consuming raw pork products’ started in 2011 [14,15].

In contrast to Asian countries, human *S. suis* infection is considered a swine-related occupational disease in western countries. Infection rates in the general population and in-risk groups are poorly known because *S. suis* infection is not a notifiable disease. Dr Constance Schultsz (Academic Medical Center, University of Amsterdam, The Netherlands) reported that the annual incidence rate of 3–5 per 100,000 individuals at risk in The Netherlands is an underestimate. The National Institute of Public Health of The Netherlands recently placed *S. suis* among its top ten priority zoonotic pathogens for which increased awareness and disease surveillance is warranted. Serotype 2 was identified as the main cause of human infections, whereas serotype 9 is much more common in invasive porcine infections in that country [16].

Future perspective: part 1

The precise functions of each *cps* gene and CPS structures are still unknown for most serotypes. It is also unclear why specific serotypes or STs tend to cause diseases in swine and/or humans. For a comprehensive understanding of this important virulence factor, further studies with experts in different fields will be necessary. Interdisciplinary studies will also be required to uncover the complex serological interrelationships between serotypes 1/2 and 2 and serotypes 1 and 14. Finally, a major conclusion of the workshop is the need for an international consortium to form a consensus on *S. suis* identification at the species level.

In terms of public health, epidemiological surveillance should continue and communication campaigns through mass media, social and health workers and educational programs must be intensified in populations at risk.

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References

Papers of special note have been highlighted as:
• of interest

- Gottschalk M, Xu J, Calzas C, Segura M. *Streptococcus suis*: a new emerging or an old neglected zoonotic pathogen? *Future Microbiol.* 5(3), 371–391 (2010).
- Wertheim HF, Nghia HD, Taylor W, Schultz C. *Streptococcus suis*: an emerging human pathogen. *Clin. Infect. Dis.* 48(5), 617–625 (2009).
- Comprehensive review on the clinical and epidemiological features of the *Streptococcus suis* infection in humans in Asia.
- Fulde M, Valentin-Weigand P. Epidemiology and pathogenicity of zoonotic streptococci. *Curr. Top. Microbiol. Immunol.* 368, 49–81 (2013).
- Chen C, Zhang W, Zheng H *et al.* Minimum core genome sequence typing of bacterial pathogens: a unified approach for clinical and public health microbiology. *J. Clin. Microbiol.* 51(8), 2582–2591 (2013).
- A typing system that facilitates the application of genome data to the fields of *S. suis* clinical medicine and epidemiology.
- Wu Z, Li M, Wang C *et al.* Probing genomic diversity and evolution of *Streptococcus suis* serotype 2 by NimbleGen tiling arrays. *BMC Genomics* 12, 219 (2011).
- Deep comparative genomic analysis of the 89K pathogenicity island.
- Maneerat K, Yongkiettrakul S, Kramomtong I *et al.* Virulence genes and genetic diversity of *Streptococcus suis* serotype 2 isolates from Thailand. *Transbound Emerg. Dis.* 60(Suppl. 2), 69–79, (2013).
- Okura M, Takamatsu D, Maruyama F *et al.* Genetic analysis of capsular polysaccharide synthesis gene clusters from all serotypes of *Streptococcus suis*: potential mechanisms for generation of capsular variation. *Appl. Environ. Microbiol.* 79(8), 2796–2806 (2013).
- First complete description of *cps* loci of all serotypes of *S. suis*.
- Wang K, Sun X, Lu C. Development of rapid serotype-specific PCR assays for eight serotypes of *Streptococcus suis*. *J. Clin. Microbiol.* 50(10), 3329–3334 (2012).
- Okura M, Lachance C, Osaki M *et al.* Development of two-step multiplex PCR assays for typing of capsular polysaccharide synthesis gene clusters of *Streptococcus suis*. *J. Clin. Microbiol.* doi:10.1128/JCM.03411-13 (2014) (Epub ahead of print).
- Le HTT, Sugiyama N, Duangsonk K, Tharavichitkul P, Osawa R. Phenotypic and PCR-based identification of bacterial strains isolated from patients with suspected *Streptococcus suis* infection in northern Thailand. *Jpn J. Infect. Dis.* 65(2), 171–174 (2012).
- Le HTT, Nishibori T, Nishitani Y, Nomoto R, Osawa R. Reappraisal of the taxonomy of *Streptococcus suis* serotypes 20, 22, 26, and 33 based on DNA–DNA homology and *sodA* and *recN* phylogenies. *Vet. Microbiol.* 162(2–4), 842–849 (2013).
- Horby P, Wertheim H, Ha NH *et al.* Stimulating the development of national *Streptococcus suis* guidelines in Vietnam through a strategic research partnership. *Bull. World Health Organ.* 88(6), 458–461 (2010).
- Nghia HD, Tu Le TP, Wolbers M *et al.* Risk factors of *Streptococcus suis* infection in Vietnam. A case–control study. *PLoS ONE* 6(3), e17604 (2011).
- Identification of most important risk factors associated with *S. suis* bacterial meningitis in parts of Asia.
- Takeuchi D, Kerdin A, Pienprongam A *et al.* Population-based study of *Streptococcus suis* infection in humans in Phayao Province in northern Thailand. *PLoS ONE* 7(2), e31265 (2012).
- Kerdin A, Dejsirilert S, Puangpatra P *et al.* Genotypic profile of *Streptococcus suis* serotype 2 and clinical features of infection in humans, Thailand. *Emerg. Infect. Dis.* 17(5), 835–842 (2011).
- Identification of the most important *S. suis* sequence types in human infections in Thailand.
- Schultz C, Jansen E, Keijzers W *et al.* Differences in the population structure of invasive *Streptococcus suis* strains isolated from pigs and from humans in The Netherlands. *PLoS ONE* 7(5), e33854 (2012).