Pathogenicity of One *Streptococcus suis Type 2* isolation to mice or to swine

ABSTRACT

Aims: The objective of this study was to identify SS2 and evaluate the virulence of ZHJ01 strain isolate, and whether the clinical and pathological outcome of a systemic infection caused by one serotype 2 when inoculated with ZHJ01 strain simultaneously. It will be better to clarify its epidemiologic, clinical, microbiologic characteristics and the pathogenesis mechanism of *S. suis type 2* in the area of Hubei province of China.
Study design: Pathogenicity of this *S.suis type 2* isolation to mice or to swine was monitored. *Swine S.suis type 2* isolation was inoculated to mice and pigs for the virulence test, and the observation of the clinical signs and pathological changes.
Methodology: In this study, the infected pigs suspected of *S.suis* disease in Jingzhou regions in Chinese province of Hubei has been studied. Proper serological typing can be performed using a co-agglutination test. The case of *Swine S.suis type 2* was determined by the virulence factor amplification based on PCR detection and bacterial isolation, identification.
Results: SS2 was determined by the extracellular protein factor (EF) based on PCR detection. Sequence analysis indicates that the isolate was very similar to nucleotide homology with others SS2 strains from different county or contries, and there was not much variation. LD_{50} of *S.suis type 2* for mice was 2.5 x 10^7cfu. LD_{50} of *S.suis type 2* for piglets was 3.92 x 10^9cfu.
Conclusion: The results of this experiment show that *Swine S.suis type 2* has a strong pathogenicity to pigs in Hubei province of China, and that provide more data to support the development of *S. suis* vaccine.

Keywords: Streptococcus suis type 2 ; Identification ; Biological trait; Experimental model; Median lethal dose (LD_{50})

1. INTRODUCTION

S.suis disease is a zoonotic infectious disease caused by *S.suis*, which can induce the death in humans. This microorganism has also been described as a pathogen for people in contact with swine or raw pork products [1]. *S.suis*, a Gram-positive encapsulated coccus, is a causative agent of serious zoonotic diseases with clinical manifestations of meningitis, septicemia, arthritis, pneumonia, endocarditis, and even acute death in pigs and humans [2]. In the epidemics that occurred in 2005 in the Chinese province of Sichuan, a marked part of patients infected with *S. suis* experienced a shock-like syndrome with a high mortality, that has been described in the preceding article devoted to epidemiology of the disease caused by this bacterium [3]. At present, genetic analysis of virulence and pathogenicity is challenging because *S.suis* produces multifactorial virulence factors [4] and because natural populations are characterized by high rates of recombination [5], creating many different genotypes. *S.suis* has been found in a total of 35 serotypes, the strongest virulence is type 2, type 1, type 9 and type 7. The genotype of *S.suis* isolation in different regions is not exactly same. Although the distribution of different serotypes varies depending on the geographical origins of the strains, *S. suis serotype 2* (SS2) is the most pathogenic of *S.suis* and the most prevalent capsular type among diseased pigs [6, 7].

Most studies have been done with the virulence of *S. suis 2*. Several virulence factors or candidates have been described, including capsule, muramidase-release protein and extracellular protein factor, suilysin, and adhesins [8]. *S.suis*

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outbreaks in pigs between 4 and 10 weeks of age usually, occurs throughout the year with no significant seasonality, and have a high morbidity and mortality rates. *S.suis type 2* infection may cause death in weaning piglets as well as growing pigs [9]. In pigs, *S. suis* is one of the economically most important pathogens in the pig industry causing primarily meningitis, arthritis and septicemia mainly in piglets and weaners [10].

S.suis type 2 has a certain correlation with the clinical diseases of pigs. Pigs in all ages could be infected by *S.suis type 2*, but most of them are endemic at 3-12-week-old piglets, especially at the peak of weaning and mixed groups [6]. The mouth and nasal cavity are the main invasive tissues of *S.suis type 2*, which are then settled and bred in the tonsils. In cases of arthritis, the earliest changes can be seen from the dilation and congestion of synovial vessels, and the surface of the joints may appear fibrin multiple serous inflammation, and the affected joint cystic wall may thicken, synovial membrane formation erythema, the amount of synovial fluid increased, and contains inflammatory cells. *S.suis type 2* causes bronchial pneumonia, meningitis, endocarditis, arthritis, arteritis and abscess in patients with diseased pigs. MRP and EF are considered to be two important virulence factors for *S.suis type 2*. According to the existence of MRP and its related proteins, *S.suis type 2* has the following phenotypes: MRP+EF+、MRP-EF-、MRP+EF* (EF analogues) 、MRP+EF-、MRP+EF-、MRP-EF+、MRP-EF+ etc. [11,12].

Diagnosis of *S.suis type 2* infection is based on clinical symptoms and pathological changes to confirm that the infection needs to be determined by pathogen separation and tissue examination. At present, the detection methods are mainly isolation culture and biochemical identification, serological identification, molecular level identification, stochastic amplification of polymorphic DNA analysis (RAPD) method [13]. According to the bacterial morphology, *S.suis* can be preliminarily identified, but it is difficult to determine its serotype, and the type of diagnostic serum can be used for PCR detection of latex or slide agglutination experiment [14].

The distribution of serotypes among clinical isolates differs between regions, and may also vary over time. Moreover, serotype 2 strains are heterogeneous, composed of a multitude of sequence types (STs) whose distribution greatly varies worldwide. That is endemically present and seems to be emerging in pigs [15]. The pathogenic mechanisms of *S. suis* is not completely defined. The objective of this study was to identify SS2, and evaluate the virulence of ZHJ01 strain isolate when inoculated simultaneously, and whether the clinical outcome of a systemic infection caused by one serotype 2. It will be better to clarify its epidemiologic, clinical, microbiologic characteristics and the pathogenesis mechanism of *S. suis type 2* in Chinese province of Hubei.

2. MATERIAL AND METHODS

2.1 Materials

2.1.1 Standard serum

S. suis type 2 standard serum prepared by the laboratory of the College of Animal Science of Yangtze University [16].

2.2 Experiment method

2.2.1 Isolation, Biochemical identification and Serotyping

A new *S. suis* isolate was collected from the tonsil of a sick pig housed in a pig farm in the affected areas of Zhijiang, Hubei Province, China. The isolate was designated as ZHJ01. Isolates were grown on Todd-Hewitt broth supplemented with 1% yeast extract (THY), and incubated at 37℃ without agitation [17]. If required, strains were cultured on THY agar plate (1.5% w/v) containing 6% (v/v) sheep blood and incubated at 37℃ for 48 h.

The biological properties of ZHJ01 were identified. The typical colonies after purification and culture were inoculated with glucose, lactose, raffinos, sorbitol, D (+)-sucrose, trehalose, 6.5%NaCl, D (-)-Salicin, hippurate, esculin hydrate, v-p, etc., 37 ℃ cultured 24h, and the test results were recorded. Gram staining was performed to identified the morphologies of ZHJ01, and the bacterial capsule was observed through transmission electron microscopy as described by Charland *et al* [18].

Proper serological typing, which is one of the most important features of the *S. suis* infection diagnosis, can be performed using a co-agglutination test. The operation details were described as previously published [17].

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2.2.2 DNA extraction and virulence factor amplification

Genomic DNA of ZHJ01 was extracted according to the manufacturer's instructions as the previously described method [19]. The quality and quantity of extracted DNA were measured by using a NanoDrop ND2000c spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Only the sample that had an OD260/OD280 ratio of approximately 2.0 and showed no degradation were used for PCR verification. DNA samples were stored in distilled H_2O at -20℃.

The primers of three main virulence factors were designed according to the sequence of GenBank (shown in Table 1). The presence of virulence genes was confirmed by polymerase chain reaction and sequencing [20]. Detection of virulence factors were performed using PCR amplification and DNA sequencing as previously described [21].

Table 1. PCR Primer set amplification conditions.

Gene	Primer	Amplication/bp	Sequence (5'—3')	GenBank number	Reference
MRP	mrp-1	1094	TTTATGCGTAGATCAAAT	AM946016	[22]
	mrp-2		GAGTTGTATTAATCTGATAAGCAGG		
EF	epf-1	1162	AACACAGTGACAGAAGCAGAGACAG	CP000407	[23]
	epf-2		CAAATTCCAAGTTACGTTTAGCAAG		
Sly	sly-1	1400	AAACTTATGAGAAAAAGTTCGCACT	NC009443	[24]
	sly-2		GCCAGATTACTCTATCACCTCATCC		

2.2.3 Bacterial strains cultivation and CFU counts

SS2 ZHJ01 is confirmed a virulent strain. Bacteria were grown overnight on sheep blood agar plates at 37℃, and isolates were inoculated into 5 mL cultures of Todd-Hewitt broth (THB) (Oxoid), which were incubated for 12 h at 37℃ with agitation. Working cultures were prepared by transferring 300 mL of the 12 h cultures into 30 mL of THB, which were further incubated for 3–4 h at 37℃ with agitation. Late log phase bacteria were washed twice in phosphate-buffered saline (PBS) (pH 7.4). The pelleted bacteria were then resuspended and adjusted to a concentration of 5.6 x 10^{10} CFU/mL. The inoculum for experimental infection was diluted in THB to obtain a final concentration according to the experimental Infection of mice and piglets. This final suspension was plated onto agar to accurately determine the CFU/mL.

The cultures of strain ZHJ01 were centrifuged at 10000g for 20 min at 4 ℃. The supernatant was removed and the precipitation was resuspended to the original volume with sterile phosphate buffered saline (PBS). From the bacterial suspension a 10-fold serial dilution from 10^7 to 10^3 CFU/ml was prepared in PBS, inoculated into THB (preheated 37 ℃) resulting in start inoculum of approximately 10^1, 10^3, and 10^6 CFU/ml. Broths were all incubated overnight at 37 ℃. The counts were performed after 24 h of incubation and 10-fold diluting it in PBS. Colonies were counted after incubation overnight at 37 ℃. The number of CFUs per mL of sample = average number of colonies (plate containing 30–300 colonies). The dilution factor of the plate counted [25].

2.2.4 Experimental infection of mice and piglet

For mice infection, mice of 6 weeks of age were acclimated to standard laboratory conditions of a 12-h light/12-h dark cycle with free access to rodent chow and water. A preliminary study was performed to verify the 50% lethal dose (LD_{50}) of the ZHJ01 strain and to determine the optimal bacterial dose and time points. Six-week old mice (six mice in each group) were randomly divided into 5 groups, and infected by intraperitoneal injection with SS2 in 1 ml volume. Three control mice were each injected with a 1 ml volume of the vehicle solution (sterile THB). The inoculum for experimental infection was diluted in THB to obtain a final concentration of 2.5 x 10^8 according to be adjusted to a concentration of 5.6 x 10^{10} CFU/ml described above. Experimental mice were inoculaed by intraperitoneal (ip.) injection with 1 ml of a suspension of strain at the following concentrations: 2.5 x 10^8 CFU/mL, 5 x 10^7 CFU/mL, 1 x 10^7 CFU/mL, 2 x 10^6 CFU/mL, 2 x 10^5 CFU/mL. The clinical symptoms, including activity, and the course of disease, were monitored daily after infection for 14 days post infection (d.p.i.). All mice were monitored once per two days for two weeks for mortality, clinical signs and assigned clinical scores and pathological scores as described [21, 27, 28]. LD_{50} of SS2 for mice was calculated by using the Reed-muench method [26].

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For swine infection, 30 piglets (four-week-old) were randomly divided into five groups. As mentioned above, the inoculum for experimental infection was diluted in THB to obtain a final concentration of $2.2{\times}10^{10}$ according to be adjusted to a concentration of $5.6{\times}10^{10}$ CFU/mL. Piglets were injected intramuscularly with a bacterial suspension of strain at the following concentrations: 2.2×10^{10} CFU/mL, 2.2×10^{9} CFU/mL, 2.2×10^{8} CFU/mL, 2.2×10^{7} CFU/mL, 2.2×10^{6} CFU/mL. Three control piglets were each injected with the vehicle solution (sterile THB). The infected piglets were carefully monitored for clinical symptoms, such as body temperature, appetite, daily activity, appetite, lethargy, movements, joints and lameness. Death piglets were immediately dissected. The score of pathological changes and clinical signs were observed [28], and the main tissues, organs and blood were isolated and collected, and used to evaluate bacterial load. All animal experiments were performed with the approval of the local ethics committee.

2.2.5 Histopathology and bacteriology identification in tissues and organs

The presence of bacteriological distribution in the tissues and organs from animal were identified by bacterial culturing and qualitative PCR after infection SS2. We evaluated bacterial colonization of blood samples from the liver, spleen, kidney, and brain. Small samples of these tissues and organs were trimmed, placed in 2 ml of PBS, at pH 7.4 and homogenized we prepared dilutions of 100 mL of each homogenate in PBS, from 101 to 104, and plated the suspensions onto THB agar. Blood samples (100 ml) were also plated. Colonies were counted and expressed as CFU/g, for organ samples, and CFU/mL, for blood samples.

3. RESULTS AND DISCUSSION

3.1 Morphology and culture characteristics of ZHJ01.

Gram staining showed that ZHJ01 is a Gram-positive coccus appearing in short chains under an optical microscope. Moreover, Serologic tests indicated that the isolate belongs to the *S. suis type 2*. When cultured on sheep blood agar, ZHJ01 formed slightly gray or semitransparent, wet, smooth, and glossy colonies. The strain showed a hemolysis, as reflected by the 2 mm diameter hemolytic rings on the plates.

3.2 Biochemical Results.

The strain isolate was inoculated with glucose, lactose, raffinos, sorbitol, d(+)-sucrose, trehalose dihydrate, 6.5%NaCl, d(-)-Salicin, hippurate, esculin hydrate, v-p, etc. in order to observe their biochemical characteristics. The biochemical test results of the bacterial isolate are shown in table 2.

Table 2. Biochemical test results of bacterial isolate

nt	Glucose	Lactose	Raffinose	Sorbitol	Sucrose	Trehalose	6.5%NaCl	Salicin	Hippurate
al s	+	+	−	−	+	(+)	−	(+)	−

+, means positive; -, means negative; (+), means positive and aerogenesis.

3.3 Virulence factor PCR results.

PCR products were electrophoresed on a 2% (wt/vol) agarose gel, stained with golden-view, and photographed under UV light, and derived from the sequences of a swine isolate of *S. suis type 2* producing the expected 1162-bp product of EF gene (shown in Figure 1).

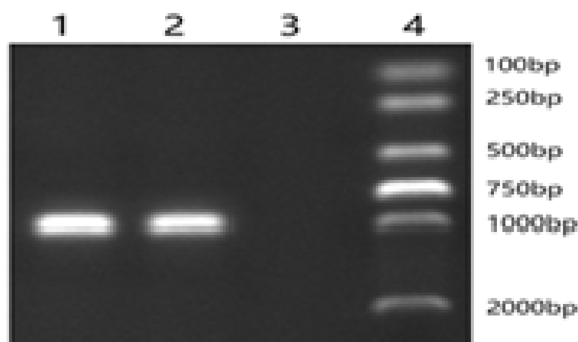

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Fig. 1. Results of PCR amplification of EF gene.

Lane 1: Positive control ; Lane 2: Isolated strains ; Lane 3: Negative control ; Lane 4: Marker 2000b

3.4 Animal experimental model.

3.4.1 Mice trail

To confirm host genetic differences in susceptibility to ZHJ01 infection, mice were used to determine mortality and clinical signs after infection. The inoculated mice showed expected clinical signs of disease such as depression-like behavior, rough appearance of hair coat and swollen eyes [23]. Mice injected with a dose of 10^7 CFU survived and were still active, while a high dose of 10^8 CFU was required for 100% mortality.

They caused moderate clinical signs associated with *S.suis type 2* infection, and relatively high mortality among inoculated mice. Mortality of mice was observed 3 days after the challenge. At a high dose (10^8 CFU per animal) all mice presented severe clinical symptoms associated with septicemia during the first 36 h post-inoculation (pi), including depression, swollen eyes, weakness, and prostration (shown in Table 3). All mice in the three groups died from septicemia during the first 48 h pi in the high dose trials. At an intermediate dose (10^7 CFU per animal), most mice also presented severe clinical symptoms and prostration during the first 72 h pi. Mice presented moderate clinical symptoms during the first 72 h pi, six mice were died. At a low dose (10^6 CFU per animal), the mice presented moderate clinical symptoms, and four mice were died. Finally, no mice in the lowest group (10^5 CFU per animal) showed clinical signs associated with *S.suis type 2* infection, with the exception of slight depression immediately after inoculation, which subsided after 24 hours post-inoculation.

The mortality rate of the first group was 100% with the largest inoculation concentration. There was no death record in the control group, and the rest of the group showed different mortality rates with different concentration of bacteria liquid (shown in Table 4). The bacteriological distribution in the tissues and organs from the nine dead animals in table 6. According to the proportion of death within one week, LD_{50} was calculated according to Reed-muench method [26], and the average value of five test measurements was calculated, LD_{50} was $2.5×10^7$cfu (shown in Table 5).

Table 3. Score of clinical symptoms in mice

Challenge dose/CFU	Number of infected mice/ Total	Score
2.5×10^8	6/6	3.33±0.41
5×10^7	6/6	3.00±0.55
1×10^7	5/6	2.67±0.75
2×10^6	4/6	1.67±1.17
4×10^5	0/6	0.50±0.00
Control	0/3	0.50±0.00

The score of clinical symptoms are expressed as mean ± SD obtained from three independent experiments, and following: 0.5, means normal; 1.5, means mild (dysregulation, difficulty walking, unstable standing) ; 2.5, means moderate clinical symptoms (trembling, twitching, drowsiness, blindness, diarrhea and poor appetite) ; 3.5, means dying or dead.

Table 4. Score of pathological changes in mice

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Challenge dose	Mice with pathological changes/ Total (Score of pathological changes)											
	Brain		Lung		Liver		Spleen		Kidney		Peritoneum	
2.5 x 10^8	6/6	3.0±0.0	6/6	2.0±0.0	6/6	2.8±0.4	6/6	3.8±0.4	6/6	3.7±0.5	6/6	3.8±0.4
5 x 10^7	6/6	2.8±0.4	6/6	1.7±0.5	6/6	2.7±0.5	5/6	3.2±1.2	4/6	2.5±1.2	6/6	3.3±0.5
1 x 10^7	1/6	1.2±0.4	1/6	1.2±0.4	2/6	1.3±0.5	1/6	1.2±0.4	0/6	1.0±0.0	5/6	2.0±0.6
2 x 10^6	0/6	1.0±0.0	0/6	1.0±0.0	0/6	1.0±0.0	0/6	1.0±0.0	0/6	1.0±0.0	0/6	1.0±0.0
4 x 10^5	0/6	1.0±0.0	0/6	1.0±0.0	0/6	1.0±0.0	0/6	1.0±0.0	0/6	1.0±0.0	0/6	1.0±0.0
Control	0/3	1.0±0.0	0/3	1.0±0.0	0/3	1.0±0.0	0/3	1.0±0.0	0/3	1.0±0.0	0/3	1.0±0.0

The score of pathological are expressed as mean ± SD obtained from three independent experiments in every group, and following: 4, means severe diffuse injury; 3, means moderate injury with many lesions; 2, means between normal and mild lesions; 1, means normal.

Table 5. LD50 determinations of mice challenged with SS2

Challenge dose/ CFU	Number of dead mice/ Total
2.5 x 10^8	6/6
5 x 10^7	3/6
1 x 10^7	0/6
2 x 10^6	0/6
4 x 10^5	0/6
Control	0/3

Mice were injected with S.suis type 2. According to Reed-Muench method, LD_{50} of S.suis type 2 for mice was 2. 5 x 10^7cfu.

Table 6. The numbers of bacterial count test in each organs (×10^7CFU)

Organ	Brain	Liver	Spleen	Lungs	Kidney	Blood
Intraperitoneal	0.27±0.14*	1.46±0.10	2.26±0.08	1.72±0.46	1.85±0.08	2.60±0.03*

*Data are represented as mean ± SD of three independent experiments based on bacterial count test, and the number of SS2 is detected in each organ. * indicates a significant difference (p <0.01).*

3.4.2 Piglets trail

To Animals were housed in isolated rooms in the experimental of College of Animal Science of Yangtze University, Hubei, China, and were fed non-medicated feed and water ad libitum. Any dead pigs were removed for routine bacteriological examination. LD_{50} values were estimated according to the method of Reed and Muench [26]. Results were calculated as mean values (± standard deviations) from three independent experiments.

The first group (2.2 x 10^{10} CFU) of *S. suis type 2* infected piglets presented clear clinical symptoms (100% morbidity). The typical course of disease could be divided into three phases: the early stage, acute progress phase, and convalescent period. Nine piglets displayed joint swelling, lameness, and crouching from 1–4 d.p.i. in the acute progress phase (5 d.p.i. to 11 d.p.i.), generally speaking 1–3 days after the emergence of early symptoms. Eight animals died with respiratory distress soon after developing the syndrome with joint swelling, lameness, and crouch, and two defecated flavo-green loose stool. Further, sixteen piglets exhibited crouching with joint swelling and stiff limbs, and unable to raise their heads (appeared at 5–7 d.p.i.) in table 8. The morbidity, mortality, and incidence rates of the severe and relatively ligter syndromes have changed via the clinical presentation for epidemiological investigation of *S. suis*.

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Piglets in the groups (1–2) developed slight fevers (38.5 ℃to 39.6 ℃), and exhibited significant levels of respiratory disease characterized by rapid and labored respiration at 3-6 DPI. At 7 DPI, the body temperatures of piglets challenged with SS2 risen to 40.0 ℃. All piglets in group 2 exhibited roughened hair coats at 9 DPI. These pigs had loss of appetite, elevated body temperatures (40.0 ℃ to 41.5 ℃), were reluctant to rise and were lame in one or more legs. Four pigs exhibited severe signs such as head tilt, nystagmus, tremors, ataxia, prostration and opisthotonus, and had fevers (>41 ℃) at 11 DPI. They were euthanized immediately. All of the piglets injected with SS2 strain developed most of the typical disease symptoms, including high fever, poor appetite, limping, shivering and dyspnea.

LD_{50} values of both strains had to be determined in series of experiments for which a large number of piglets is required. The determination of LD_{50} of *S.suis type 2* infected with piglet was 3.92 x 10^9cfu(shown in Table 9).

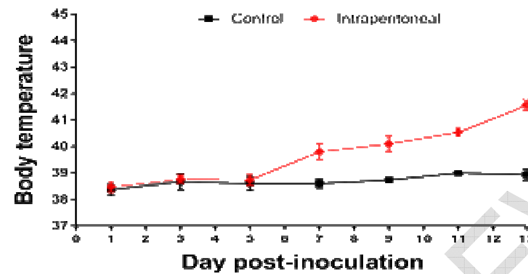

Fig. 2. The changing temperature of each pig in the infection periods

Clinical symptoms of the piglets after *S.suis type 2* infection. The body temperatures of each piglet were monitored continuously once per two days during the experimental period after intramuscular injection ((shown in Figure 1)). The piglet has been infected with *S.suis type 2* to death after 13d.

Table 7. Score of clinical symptoms in pig

Challenge dose/CFU	Number of infected pig/ Total	Score
2.2 x 10^{10}	6/6	5.7±0.8
2.2 x 10^9	6/6	5.0±1.1
2.2 x 10^8	5/6	3.8±2.0
2.2 x 10^7	2/6	1.7±1.2
2.2 x 10^6	0/6	1.0±0.0
Control	0/3	1.0±0.0

The score of clinical symptoms are expressed as means ± SD obtained from three independent experiments, and following: 0.5, means normal; 1.5, means mild (drowsiness, limping, swollen eyes; 2.5, means moderate clinical symptoms (poor appetite, shivering, dyspnea, blindness and prostration) ; 3.5, means dying or dead.

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Table 8. Score of pathological changes in piglets

Challenge dose	Piglets with pathological changes/ Total (Score of pathological changes)											
	Liver		Spleen		Lung		Brain		Kidney		Peritoneum	
2.2×10^{10}	6/6	6.0±0.0	6/6	5.7±0.8	6/6	6.0±0.0	6/6	5.7±0.8	6/6	5.3±1.0	6/6	5.7±0.8
2.2×10^{9}	6/6	5.3±1.0	6/6	5.7±0.8	6/6	5.3±1.0	5/6	4.5±2.0	4/6	3.3±2.0	5/6	4.5±2.0
2.2×10^{8}	2/6	1.7±1.2	2/6	2.0±1.5	3/6	2.2±1.5	2/6	1.7±1.2	1/6	1.2±0.4	4/6	3.7±2.3
2.2×10^{7}	0/6	1.0±0.0	0/6	1.0±0.0	1/6	1.2±0.4	0/6	1.0±0.0	0/6	1.0±0.0	1/6	1.2±0.4
2.2×10^{6}	0/6	1.0±0.0	0/6	1.0±0.0	0/6	1.0±0.0	0/6	1.0±0.0	0/6	1.0±0.0	0/6	1.0±0.0
Control	0/3	1.0±0.0	0/3	1.0±0.0	0/3	1.0±0.0	0/3	1.0±0.0	0/3	1.0±0.0	0/3	1.0±0.0

The score of pathological are expressed as mean ± SD obtained from three independent experiments in every group, and following: 4, means severe diffuse injury; 3, means moderate injury with many lesions; 2, means between normal and mild lesions; 1, means normal.

Table 9. LD50 determinations of piglets challenged with SS2

Challenge dose/ CFU	Number of dead mice/ Total
2.2×10^{10}	6/6
2.2×10^{9}	2/6
2.2×10^{8}	0/6
2.2×10^{7}	0/6
2.2×10^{6}	0/6
Control	0/3

Piglets were injected with S.suis type 2. According to Reed-Muench method, LD_{50} of S.suis type 2 for mice was 3. 92 x 10^{9}cfu.

Table 10. The numbers of bacterial count test in each organs (×10^{9}CFU)

Organ	Brain	Liver	Spleen	Lungs	Kidney	Blood
Intramuscularly	8.05±0.22*	0.23±0.09	0.25±0.10	9.95±0.12	0.23±0.09	13.0±0.13*

*Data are represented as mean ± SD of three independent experiments based on bacterial count test, and the number of SS2 is detected in each organ. Significant differences in levels between tissues and organ are marked. * indicates a significant difference ($p < 0.01$).*

Determination of bacteria in tissues and organs in table 10. The bacteriological distribution in the tissues and organs from the eight dead animals with 3–4 d.p.i. were identified by bacterial culturing and counting after the infection with ZHJ01.

3.5 Histopathological

Death piglets were histopathologically analyzed by necropsy. Gross lesions were noted in five pigs, in which a fibrinopurulent polyserositis was seen, and in four piglets, which had an exudative meningitis and arthritis. Brain, spleen, liver, kidney, heart, lung, joint, intestine and lymph node from the piglets infected with S.suis type 2 displayed conspicuous macroscopic lesions (Fig. 3), such as encephalemia and encephaledema (Fig. 3-A), kidney swelling and nephremia (Fig. 3-F).

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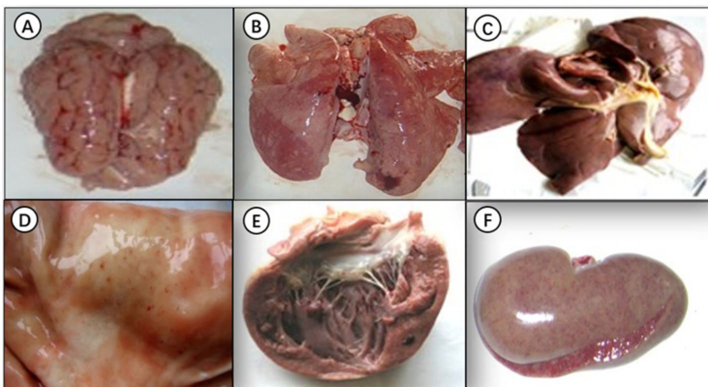

Fig. 3. Histopathology of *S.suis type 2* (SS2) disease caused by ZHJ01 strain.

Piglets were intramuscularly inoculated with S. suis type 2 strain. Four piglets that died from SS2 were dissected and histopathological analysis. (Figure 3) Gross pathology of the piglets at 4 d.p.i (day post-infection) . (A) Encephalemia and encephaledema; (B) Pulmonary congestion; (C) Light liver degeneration; (D) The petechia emerging on the surface of the stomach tract; (E) Cardipericarditis; (F) Kidney swelling and nephremia;

4. CONCLUSION

S.suis type 2 had caused a relatively high occurrence and abnormally elevated mortality. Through the results of bacterial isolation, culture, biochemical identification, serological test and PCR assay, the pathogen of the sick pig was identified as *S. suis type 2*.

The infected piglets showed joint swelling, lameness, and crouch at beginning, then developed into septic-like shock syndrome (SLSS) syndrome, at last the survivals showed physical activity impairment. Brain lesions, such as venous thrombosis, may directly contribute to the sequelae in human cases, were identified in the pigs [31]. In addition, the presence of *mrp*, *epf*, and *sly* genes, considered "classical" virulence markers mostly described for serotype 2 strains were studied [29].

The genes for the three virulence-associated factors, *epf*, *mrp* and *sly*, were frequently detected in clinical isolates, which supports their association with virulence. It is better collecting pathogens from parts such as lungs and tonsils, and the results are accurate. The results of this study suggest that EF genes seem to be a critical virulence factor for *S. suis type 2* infection, and EF genes seems to be strongly correlated to invasiveness of isolates in serotype-2, and might be a critical virulence factor for *S. suis serotype 2* infection, whereas the relevance of observation for pathogenicity remains to be elucidated.

In the long run, the prevention and control of swine infection should form the more strategic component of the public health program. Surveillance systems should be established to alert farmers and the general public if an infection outbreak in pigs is recognized [30]. More studies are needed to confirm their virulence potential. ZHJ01 strain (one isolated from a diseased pig from Zhijing, Hubei province, China) clearly presented a higher zoonotic and virulence potential.

In the current study, we assessed the properties of biological and the genome of sequence based on the *S. suis type 2* strain of isolation and sequence, and the results would offer more information, and might explain the distinction of the

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virulence of *S. suis* . As reflected by our results, *S. suis type 2* strain can display virulence in the piglet infection experiments. LD_{50} of *S.suis type 2* for mice was 2.5 x 10^7cfu. LD_{50} of *S.suis type 2* for piglets was 3.92 x 10^9cfu.

This has been shown in many studies carried out in several regions/countries. Results from some studies indicated that the predominant serotypes isolated from clinical cases have changed over the last decades [32]. This study can provide information for epidemiological investigation of *S. suis type 2* as well as aid in developing new vaccines for the control, and has great significance for further monitoring and effective prevention to *S. suis*.

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