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Establishment of a Low Serious Level of *Salmonella*-Challenged Pig Model

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Abstract

Salmonella choleraesuis is one of the most common *Salmonella* species affecting pigs. It can produce necrotizing enterocolitis, the septicemic disease, hepatitis, pneumonia, and cerebral vasculitis. In addition, some of *Salmonella* species in pigs have been associated with human foodborne illness. Commonly, *Salmonella* serotypes in pigs were associated with human diseases as *Salmonella typhimurium*, *Salmonella derby*, *Salmonella heidelberg*, *Salmonella worthington*, and *Salmonella infantis*. These *Salmonella* serotypes may cause mild to moderate diarrhea in pigs and may be resistant to multiple drugs. Salmonellosis in pigs was also seriously affects the economic loss of pig farmers. Therefore, the establishment of a suitable *Salmonella*-challenged pig model for the research and development (R&D) of vaccines or novel therapeutic and preventions drugs against *Salmonella* infection is very important. Based on the experiments, the specific pathogen-free (SPF) pigs were used in this experiment. Eight SPF pigs (8-week-old) were randomly divided into two groups (Normal control group and *Salmonella*-challenged group). After one month of *Salmonella* challenge, the observation of pigs' clinical symptoms, survival, the change of body weight, and the *Salmonella* secretion in sera and stool. Finally, all pigs (12-week-old) were sacrificed and performed the gross examination and the further histo-pathologic examination. It can be seen from our results of the development of *Salmonella choleraesuis*-challenged pig model which the disorder of the spirit, appetite, excretion, gait, and body appearance were found. However, the breathe disorder was not found in the *Salmonella choleraesuis*-challenged pigs. The disorder of the spirit, appetite, and excretion in pigs were found in the early phase of *Salmonella choleraesuis* challenge. The disorder of the gait and body appearance in pigs were found in the late phase of *Salmonella choleraesuis* challenge. All pigs were survival until the end of the experiment (100%; 0/8). The sera and stool were collected and detected *Salmonella choleraesuis*. Data were presented that *Salmonella choleraesuis* in sera was not found in all pigs until the end of the experiment. However, the stool was detected *Salmonella choleraesuis* before and post 3 days (D3), D6, D7, D10, D17, D24, and D31 challenge. The highest concentration of *Salmonella choleraesuis* in the stool of *Salmonella*-challenged pigs was detected on D6 challenge. After sacrifice of pigs, the lung, spleen, liver, and intestines organs were examined and collected. Under the gross examination, four organs were normal by a senior pathologic veterinarian. Under the histo-examination, alveolar space in the *Salmonella choleraesuis*-challenged group is less than that of the normal control group, and interstitial pneumonia is more serious in the *Salmonella choleraesuis*-challenged group than the normal control group. In addition, liver tissue necrosis can be seen in the *Salmonella*-challenged group. According to the results of this study, a low serious level of *Salmonella*-challenged pig model has been successfully established, which can be provided to the related researcher for R&D of vaccines or the novel therapeutic and prevention drugs in the future.

Keywords: Bacterial challenge pig model; Establishment; *Salmonella choleraesuis*; Salmonellosis

Introduction

Salmonella is an enteric pathogen that can infect animals and humans. In human, *Salmonella* is one of the major food safety hazards. Salmonellosis in pigs is caused by Gram-negative *Salmonella* genus. Pigs can be infected at each stage of pig production. Salmonellosis can causes fever, depression, septicemia, pneumonia, meningitis, arthritis and diarrhea in sows. Especially, the piglets are most sensitive to *Salmonella*. The weaning period is the highest risk period for *Salmonella* excretion. Ten- to 12-week-old pigs under cold stress and market-age pigs (18-22 weeks old) under heat stress are also at a higher risk of *Salmonella* infection. Additionally, *Salmonella*-infected pigs also served as the reservoirs to infect the more pig populations. Currently, *Salmonella* infection has also become a major concern in pig farms. In order to ensure a high level of pig performance, pig farmers should pay close attention to farm management focusing on *Salmonella* prevention and control [1-5].

Salmonella is capable of surviving at least 6 years in the environment. Therefore,the biosecurity practicesare very important for reducing *Salmonella* infection in the pig farms. According to the researches, the season and/or environmental temperature was associated with *Salmonella* prevalence in finishing pigs. Because piglet populationsare the diminished passive immunity and less developed active immunity. Therefore, many environmental stresses as grouping, a diet change, and moderate development of the gastrointestinal tract etccan facilitate the occurrence of Salmonellosis [6]. Taken together, *Salmonella* control in pig farms is an important issue which links to food safety. Effective *Salmonella* control on the farm is based on the prevention of *Salmonella* entering and spreading on a farm. In order to promote the development of vaccines or the clinical therapeutic and prevention drugs, the establishment of a bacterial challenge pigmodel with *Salmonellosis* suitable for R&D of vaccines or the clinical therapeutic and prevention drugs is very important and need.

Materials and Methods

Experimental Reagents

Experimental reagents included as phosphate buffered saline (PBS; Sigma-Aldrich®), Zoletil 50 (Vibac Laboratories), azaperone(Stresnil®), and xylose-lysine-deoxycholate agar (XLD agar; Creative®)

Bacterial Strains

Salmonella enterica subsp. Entericaserovar Choleraesuis (ATCC® 10708™) strain was ordered and challenged in pigs. This bacteria were grew on XLD agar under the 37°C and aerobic status

Animal Care

All animal experiments were approved by the Institutional Animal Care and Use Committee of Agricultural Technology Research Institute (ATRI), Taiwan. Eight 8-week-old specific pathogen free (SPF) pigs were ordered from ATRI, Miaoli, Taiwan (the approval No.: 107108) and experimented in the Laboratory Animal Center, National Pintung University of Science and Technology (NPUST)(the approval No.: NPUST-107-054). The 8 pigs were randomly grouped and each group was 4 pigs. These pigs were fed in the animal room under a 12-h light/dark cycle at 22-24°C and 70-75% humidity. Normal laboratory diet (FWUSOW industry, Taichung, Taiwan) and fresh water were supplied to pigs continuously ad libitum.

Experimental Animals and Grouping

Eight 8-week-old SPF pigs without salmonellosis were obtained from ATRI, Taiwan and performed the experiment in NPUST. All SPF pigs were randomly divided into two groups (4 pigs/group) as the normal control group and the *Salmonella*-challenged group.

Salmonella Challenge

Salmonella choleraesuis (8×10^8 CFU/pig) was challenged to 4 pigs by artificial oral administration. At the each designed experimental points, the detection of clinical symptoms, survival rate (%), the change of body weight (BW), bacterial excretion, and gross and histo-pathological examination were performed and compared between two groups.

Monitor of Clinical Symptoms and Survival Rate and Detection of Body Weight in Pigs

In this study, the monitoring of clinical symptoms and survival, and the detection of BW in each group were performed once per day. Six indexes of clinical symptoms as spirit, appetite, excretion, breathe, gait, and body appearance were applied and scored (Table 1).

Score	Spirit	Appetite	Excretion	Breathe	Gait	Body appearance
1	Normal	Normal	Normal	Normal	Normal	Normal
2	Inactive / weak	Suboptimal	Atherosclerosis	Slight	Slight limp	Petechial bleeding / Scabs
3	Lying down	Unable to eat	Watery diarrhea	Severe	Severe limp	Anemia / Jaundice

Table 1: Six indexes of clinical symptoms as spirit, appetite, excretion, breathe, gait, and body appearance.

Gross and Histo-Pathologic Examination

At the end of the experiment, all pigs were sacrificed and dissected. Collection of lung, liver, spleen, and intestine organs and the gross and histo-pathologic examination were performed by a senior pathologic veterinarian.

Collection of Peripheral Bloodand Stool

Collection of peripheral bloodand stool was performed before *Salmonella* challenge (Day 0) and post 3 days-, 6 days-, 7 days-, 10 days-, 17 days-, 24 days-, and 31 days-challenge with *Salmonella*. These sera and stool were collected and performed the detection of bacterial excretion.

Statistical Analysis

Statistical analysis was performed usingone-way analysis of variance(one-way ANOVA), Student's *t*-test, Fisher's exact test, and Kruskal-Wallis one-way ANOVA. Survival in the group comparisons was performed using Fisher's exact test. Clinical examination in the group comparisons was performed using Kruskal-Wallis test. Others in the group comparisons was performed using ANOVA. Differences between groups were considered statistically significant at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

Results

Average Daily Weight Gain in Pigs

From the beginning to the end of the experiment, the average daily weight gain (ADWG) of the normal control group was 0.53 ± 0.01 kg and ADWG of *Salmonella*-challenged group was 0.38 ± 0.07 kg. The average weight gain (AWG; %) of the normal control group was 368.71% and AWG (%) of *Salmonella*-challenged group was 297.90. AWG (%) and ADWG (kg) of the normal control group was significantly higher than that of *Salmonella*-challenged group (p <0.05-$p < 0.01$) (Table 2).

Group	No.	Average weight gain (%)	Average daily weight gain (kg)
Normal control group	4	368.71	0.53 ± 0.01
Salmonella-challenged group	4	297.90*	0.38 ± 0.07**

Table 2: The average weight gain and average daily weight gain of the *Salmonella*-challenged group and the normal control group. Data were presented as mean ± SEM. $^{*}p < 0.05$; $^{**}p < 0.01$.

The Survival Rate of Pigs Post *Salmonella* Challenge

After *Salmonella* challenge to pigs, all pigs were survival. The survival rate (%) was 100%.

Clinical Symptoms of Pigs Post *Salmonella* Challenge

The clinical symptoms of the pigs in each group be continuously monitored. *Salmonella choleraesuis*-challenged pigs were observed the disorder of the spirit, appetite, excretion, gait, and body appearance. However, the breathe disorder was not found in the *Salmonella choleraesuis*-challenged pigs. The disorder of the spirit, appetite, and excretion in pigs were found in the early stage of *Salmonella choleraesuis* challenge (D3-D5 post *Salmonella* challenge). The disorder of the gait and body appearance in pigs were found in the late stage of *Salmonella choleraesuis* challenge (D20-D31 post *Salmonella* challenge).

Macroscopic and Microscopic Lesions of Pig's Organs Post *Salmonella* Challenge

After sacrifice of pigs, the lung, spleen, liver, andintestine organs were examined and collected. Under the gross examination, three organs were normal by a senior pathologic veterinarian. Under the histo-examination, alveolar space in *Salmonella*-challenged group is less than that of the normal control group, and interstitial pneumonia in *Salmonella*-challenged group is more serious than the normal control group (100%; 4/4 vs. 0%; 0/4). In addition, liver tissue necrosis can be only seen in the *Salmonella*-challenged group (75%; 3/4 vs. 0%; 0/4) (Table 3).

	Normal control group	*Salmonella*-challenged group
Average percentageof interstitial pneumonia	0% (0/4)	100% (4/4)***
Average percentage of liver tissue necrosis	0% (0/4)	75% (3/4)***

Table 3: The average percentage (%) of interstitial pneumonia and liver tissue necrosis in two groups. Data were presented as mean values. ***$p < 0.001$.

Quantification of *Salmonella* in Sera in Pigs

Collection of sera on Day 0, Day 3, Day 6, Day 7, Day 10, Day 17, Day 24, and Day 31 post *Salmonella* challenge. *Salmonella* excretion was detected in sera by using XLD agar assay. Data presented that no bacteria colonies were found in the XLD agar. Therefore, the bacteremia was not found in sera in both groups.

Quantification of *Salmonella* in Stool

Collection of stool on Day 0, Day 3, Day 6, Day 7, Day 10, Day 17, Day 24, and Day 31 post *Salmonella* challenge.*Salmonella* excretion was detected in stool by using XLD agar assay. Data presented that *Salmonella* excretion was only occurred on Day 3-Day 10 post *Salmonella* challenge. After Day 10 post *Salmonella* challenge, *Salmonella* excretion was not detected on Day 17, Day 24, and Day 31 (Table 4).

	D0	D 3	D 6	D 7	D 10	D 17	D 24	D 31
Normal control group	0	0	0	0	0	0	0	0
Salmonella-challenged group	0	4,000***	20,000***	11,875***	3,000***	0	0	0

Table 4:*Salmonella* excretion in stool of *Salmonella*-challenged pigs and normal pigs was detected on Day 0, Day 3, Day 6, Day 7, Day 10, Day 17, Day 24, and Day 31 post *Salmonella* challenge in the experiment. Data were presented as mean. ***$p < 0.001$.

Discussion

Although all ages of pigs are susceptible for *Salmonella*, however salmonellosis is most common in the weaned and growing-finishing pigs [7-9]. Salmonellosis can induce the inflammation and necrosis of intestines and result in diarrhea that may be accompanied by generalized sepsis, seriously. *Salmonella choleraesuis* is the most common *Salmonella* species to infect pigs. This pathogen can cause necrotizing enterocolitis, hepatitis, pneumonia, and cerebral vasculitis, and septicemic disease. Additionally, *Salmonella typhisuis*is also frequent *Salmonella* species to infect pigs. *Salmonella typhisuis* infection can causethe necrotizing, non-suppurative inflammation of the intestines. The mucosa face of the intestines is frequently ulcerative. Occasionally, *Salmonella typhisuis* infection also generalized septicemia. Sources of infection

for *Salmonella choleraesuis* and *Salmonella typhisuis* are primarily asymptomatic carrier pigs, therefore, the prevention of *Salmonella* infections in the pig farms is a very important issue [10-12].

Numerous serotypes of *Salmonella*can infect pigs. Some of these *Salmonella* species have been verified to associate with human foodborne illness. Additionally, these *Salmonella* serotypes may cause mild to moderate diarrhea in pigs and may be resistant to multiple drugs [13-17]. Therefore, the establishment of a suitable *Salmonella*-challenged pig model for R&D of vaccines or novel therapeutic and preventions drugs against *Salmonella* infection is very important. In this study, a low serious level of *Salmonella*-challenged pig model has been successfully established, which can be provided to related researcher for R&D of vaccines or the novel therapeutic and prevention drugs.

Salmonellosis can cause diarrhea or generalized septicemia for the nursing pigs. This disease also cause the weanling or growing-finishing pigs are febrile and have liquid feces that may be yellow and contain shreds of necrotic debris. In this study, the development of *Salmonella choleraesuis*-challenged pig model was seen that the disorder of the spirit, appetite, excretion, gait, and body appearance. Additionally, all pigs were survival until the end of the experiment (100%; 0/8). Under the gross examination, four organs (lung, spleen, liver, and intestines) were normal. Under the histo-examination, alveolar space in *Salmonella*-challenged group is less than that of the normal control group, and interstitial pneumonia in *Salmonella*-challenged group is more serious than the normal control group. In addition, liver tissue necrosis can be seen in the *Salmonella*-challenged group. According to these results, a low serious level of *Salmonella*-challenged pig model has been successfully established in this study.

According to the reports, pigs infected with *Salmonella choleraesuis* can cause an inflamed, slightly thickened ileum and colon and mucosal ulceration on the mucosal surface of the intestines. Mesenteric lymph nodes (MLNs) are enlarged, edematous, and reddish. MLNs are hemorrhage may be seen in the acute *Salmonella* infection cases [18-20]. According to the results of this study, the gross examination was normal and low serious interstitial pneumonia and liver tissue necrosis were seen. Therefore, a low serious level of *Salmonella*-challenged pig model has been successfully established, which can be provided to related researcher for R&D of vaccines or the novel therapeutic and prevention drugs in the future.

Conclusion

Salmonella choleraesuis is one of the most common *Salmonella* species affecting pigs. Salmonellosis in pigs was also seriously affects the economic loss of pig farmers. Some of *Salmonella* species in pigs have been associated with human foodborne illness. Therefore, the establishment of a suitable *Salmonella*-challenged pig model for R&D of vaccines or novel therapeutic and preventions drugs against *Salmonella* infection is very important. Based on the results of this experiment, a low serious level of *Salmonella*-challenged pig model has been successfully established, which can be provided to related researcher for R&D of vaccines or the novel therapeutic and prevention drugs.

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Conflicts of Interest

The authors declare no conflict of interest.

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