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The Distributions of *S. enteritidis* in the Internal Organs of Chinese Guizhou Minipig after Orally Infected

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Abstract: Chinese Guizhou mini-pigs were orally infected with a high-virulence strain of *S. enteritidis*. The kinetics of the *S. enteritidis* genomic deoxyribonucleic acid loads was analysised by the real-time PCR assay and the histopathological examination in various tissues were investigated. The results showed that at 4 h postinoculation, high *S. enteritidis* deoxyribonucleic acid loads were observed in various organs of the infected pigs. Thereafter, the bacterial deoxyribonucleic acid loads increased by various amounts until 2nd day postinoculation. The time course of the appearance of bacterial antigens and tissue lesions in various tissues was coincident with the levels of the bacterial deoxyribonucleic acid loads at the infection sites.

Key words: *S. enteritidis*, quantitative study, mini-pig, PCR, infection sites, China

INTRODUCTION

Guizhou mini-pig is a traditional pig breed in China and much differed from commercial pigs either in the size or in the developmental characteristics. The slower growth rate and delayed maturation of the mini-pig lead to a higher fat deposition in adipose tissue and muscle. *Salmonella enteritidis* (*S. enteritidis*) is an enteric pathogen that colonizes the intestinal tract of a variety of animals and accounts for millions of cases of gastroenteritis and food-borne illness each year. It have became a significant public health problem (Braden, 2006). Generally, little is known about the distributions of *S. enteritidis* in pig after *S. enteritidis* infected. The purpose of this study was to apply real-time PCR assay to study the regular distrubition of *S. enteritidis* in the internal organs of pigs after orally inoculated.

MATERIALS AND METHODS

Bacterial strains: A high-virulence strain of *S. enteritidis* (Phage type 4; No.: 50338) was purchased from the National Center for Medical Culture Collection.

Experimental animals and samples: Three months old, 15-18 kg, Chinese Guizhou mini-pig free from *S. enteritidis* infection were used in the study. Prior to challenge with *S. enteritidis* all pigs were found to be negative for *S. enteritidis* specific antibodies and *S. enteritidis* specific antigens by an enzyme-linked immunosorbent assay and polymerase chain reaction, respectively (Gast and Beard, 1990; Deng *et al*., 2008). The pigs were maintained in isolation units in a biosecure animal building. In brief, a group of nine pigs were orally infected with a high-virulence *S. enteritidis* strain (Animal experiments were reviewed by an Institutional Animal Care and Use Committee (IACUC) for humane use of animal for experimental purposes).

Each pig was inoculated with 4.0×10^6 cells in 3 mL of water. The liver, spleen, lung, kidney, jejunum, ileum, rectum, cecum were analyzed by a fluorescent quencher polymerase chain reaction assay at postinoculation times of 30 min; 2, 4, 8, 12, 24 and 36 h and 2nd and 3rd day. At each time point, one pig were randomly selected from the infection group and their tissue samples were collected and processed for further analyses. Deoxyribonucleic acid extraction from the tissue samples was performed as described previously (Deng *et al*., 2008; He *et al*., 2010, 2011a, b).

Quantitative real-time polymerase chain reaction assay for detection of *S. enteritidis* deoxyribonucleic acid: In the previous study, we have established a serovar specific real-time polymerase chain reaction assay, the limit of detection was 7 copies μL^{-1} (Agron *et al*., 2001; Deng *et al*., 2008; He *et al*., 2010). Brifely, a real-time PCR assay was carried out using a real-time PCR core kit (R-PCR Version 2.1, TaKaRa, Japan) with an Icycler

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Table 1: Kinetics of *S. enteritidis* deoxyribonucleic acid loads in pig orally infected with a high-virulence strain determined by quantitative real-time polymerase chain reaction

	Time							Days	
Tissues	30 min	2 h	4 h	8 h	12 h	24 h	36 h	2	3
Liver	0.00±0.00	0.00±0.00	0.00±0.00	3.78±0.38	3.99±0.55	5.25±0.63	5.72±0.83	6.93±0.23	6.15±0.86
Spleen	0.00±0.00	0.00±0.00	0.00±0.00	3.32±0.22	3.66±0.66	5.63±0.57	7.31±0.55	9.94±0.35	9.45±0.36
Lung	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.37±0.18	4.57±0.16	8.56±0.36	5.26±0.34
Kidney	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	3.22±0.63	3.79±0.47	4.21±0.22	7.15±0.36	5.84±0.25
Jejunum	0.00±0.00	0.00±0.00	2.02±0.02	2.50±0.33	3.83±0.74	4.75±0.55	6.13±0.13	8.56±0.56	7.44±0.65
Ileum	0.00±0.00	0.00±0.00	2.40±0.50	2.99±0.20	4.22±0.24	5.41±0.21	6.45±0.56	8.69±0.27	7.75±0.15
Rectum	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	3.72±0.54	3.57±0.54	5.69±0.27	4.34±0.13
Cecum	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	4.33±0.24	5.66±0.96	7.67±0.65	5.35±0.38

Each time point represents the mean concentration of genomic deoxyribonucleic acid and is expressed as $\log_{10}$ of the bacterial genome copy number per gram of tissue tested±SD obtained from one pig; Min = Minute, h = hour, d = day

iQ™ real-time PCR detection system (Version 3.1, Bio-rad, USA) and was performed as described previously.

Histopathological examination: For histopathological examination, the paraffin-embedded sections were cut at 5 μm thickness and stained with haematoxylin and eosin as previously described (Mutinelli *et al*., 2003).

Statistical analysis: All samples were analyzed 3 times by the real-time polymerase chain reaction assay and concentrations of the target deoxyribonucleic acid detected were expressed as the mean $\log_{10}$ of the bacterial genome copy number per gram of tissue tested.

The real-time polymerase chain reaction data were analyzed using Version 11 of the SPSS software. The comparison of means was performed using Duncan's multiple-range test. A $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The distribution of *S. enteritidis* within the internal organs after oral challenge was determined by means of FQ-PCR over a 3 days period at intervals. The results showed that the jejunum and ileum tested positive for *S. enteritidis* at 4 h postinoculation.

Thereafter, *S. enteritidis* was consistently detected in all the samples at 24 h postinoculation; the last organ to show a positive result were the cecum, rectum and lung at 24 h postinoculation.

The copy numbers of *S. enteritidis* in each tissue reached a peak at 24 h and 2 days postinoculation. The spleen, jejunum and ileum contained high concentrations of *S. enteritidis* whereas, the liver and rectum exhibited low concentrations. The numbers of bacteria decreased at 2nd and 3rd day. The internal organs of the pigs in the control group did not show any positive results at any time point. The details are shown in Table 1. The results of quantitative real-time polymerase chain reaction and histopathological examination revealed that the time course of the appearance of bacterial antigens and tissue lesions in various tissues was coincident with the levels of the bacterial deoxyribonucleic acid loads at the infection sites. Furthermore, all the examined infected organs demonstrated vascular damage, severe hyperemia and hemorrhage. The following pathological features were observed in the organs examined: swollen tubular epithelial cells and nephrosis in the kidney, necrotic foci and varying degrees of hepatocyte fat degeneration of hepatocytes in the liver and slightly hyperemic and hemorrhagic cribriform changes in the brain. Severe hyperemia, hemorrhages and heterophil infiltration in the lungs were also observed. The control group did not generate any positive results at any time point of the study at any location. These results demonstrate that the wide spread dissemination of *S. enteritidis* to infection in various organs increases with progression of the infection.

CONCLUSION

In this study, the mechanism of colonization by *S. enteritidis* in the internal orgnas is not clear and requires further studies. This study will help in understanding the pathogenesis of *S. enteritidis* infection *in vivo* and may help in the development of a live salmonella vaccine in future.

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