Research Note: Hypervirulent arthritis-causing *Salmonella* Pullorum isolated from Chinese native chicken breeds significantly decreased growth performance of chicks

Peiyong Li, Mingyue Zhang, Guijuan Hao, and Shuhong Sun*

Department of Preventive Veterinary Medicine, College of Animal Science and Technology, Shandong Agricultural University, Tai'an, China

ABSTRACT Pullorum disease is characterized by white diarrhea resulting from infection by *S.* Pullorum, but arthritis associated with *S.* Pullorum infection has become increasingly frequent recently, especially in Chinese native chicken flocks. In this study, we isolated and identified 4 *S.* Pullorum strains from the Qingjiaoma chicken breeders with arthritis symptoms. The LD_{50} of the isolate 20JS04 was 1.33×10^6 CFU, which was considered as a highly virulent strain in chicks. Reproducible arthritis symptoms were observed in the experimentally chickens infected with the isolate 20JS04, and the disease occurrence was 27.78% (5/18). In addition to the characteristics of high virulence and induced-arthritis, our results confirmed that the arthritis-causing isolate 20JS04 had greater negative impact on BW, ADFI, and ADG, compared with the white diarrhea-causing *S.* Pullorum standard strain CVCC526 ($P < 0.05$). These results suggest that the pathogenic diversity of *S.* Pullorum in China deserves more attention and stringent measures should be taken to control salmonellosis.

Key words: *Salmonella* Pullorum, chicken, arthritis, virulence, growth performance

2022 Poultry Science 101:101575
https://doi.org/10.1016/j.psj.2021.101575

INTRODUCTION

Salmonella Pullorum (*S.* Pullorum), a septicemic pathogenic bacterium, is among the most important pathogens of poultry due to high mortality of chicks or poults, leading to severe economic losses. Although this pathogen has been eradicated from commercial poultry in many developed countries, it is still the main prevalent serovar isolated from chickens in many countries (Barrow and Freitas Neto, 2011; Song et al., 2020).

For growing and mature fowl, infected chickens may not exhibit any signs and cannot be detected by their physical appearance. For young chicks, after natural infection with *S.* Pullorum, chicks may manifest somnolence, depressed appetite, and adherence of chalky white material to the vent. Other signs such as purulent arthritis and joint lesions associated with *S.* Pullorum infection has occasionally been described (Shivaprasad, 2000). However, *S.* Pullorum has been frequently isolated from chickens with swollen joint and lameness in China (Guo et al., 2019).

In order to better understand the features of arthritis-causing *S.* Pullorum, in this study, we isolated and identified 4 *S.* Pullorum strains from the Qingjiaoma chicken breeders with swollen hock joint and assessed their virulence. We also compared the impact of arthritis-causing *S.* Pullorum strain on growth performance with white diarrhea-causing *S.* Pullorum strain.

MATERIALS AND METHODS

All procedures used in this study were approved by the Animal Care Committee of Shandong Agricultural University (P. R. China) and were carried out in accordance with the guidelines for experimental animals of the Ministry of Science and Technology (Beijing, P. R. China).

Bacterial Isolation and Identification

In a Qingjiaoma chicken breeder farm located in southeast China, a disease characterized by swollen hock joints and lameness broke out in a flock of 30-day-old chicken. The number of chickens was about 10,000 and the incidence of the arthritis symptoms was about 3%. Therefore, samples, including synovial fluid of swollen hock joint, joint lesion, liver, spleen and heart of chickens presenting lameness or arthritis symptoms

Received July 19, 2021.
Accepted August 26, 2021.

*Corresponding author: sunshuhong@sdau.edu.cn

were collected to isolate *Salmonella*. Buffered peptone water (4.5 mL) (**BPW**, Haibo Biotechnology, Qingdao, China) was added to each sample for pre-enrichment, according to a previously described method (Yang et al., 2019). After incubation at 37°C for 8 to 12 h, 0.5 mL of each pre-enriched culture was incubated in 4.5 mL of Tetrathionate Broth Buffer (**TTB**, Haibo Biotechnology) at 37°C for 24 h. After selective enrichment, one loopful of each broth culture was streaked onto xylose lysine tergitol 4 (**XLT4**, Haibo Biotechnology) agar and the plates were incubated at 37°C for 24 to 36 h. The presumptive *Salmonella* colonies were identified by polymerase chain reaction (**PCR**) assays using a specific target gene ipaJ (Xu et al., 2018). The PCR cycling conditions were as follows: 1 denaturation cycle at 95°C for 3 min, 30 cycles of denaturation at 95°C for 30 s, followed by annealing at 58°C for 45 s and elongation at 72°C for 50 s, and a final 10 min elongation cycle at 72°C. Positive colonies are purified and stored at −70°C in cryogenic cultures (LB broth with 20% glycerol) until subject to the subsequent tests described below.

Assessment of Virulence in Newly Hatched Chicks

To assess the pathogenicity of *S.* Pullorum isolate in newly hatched chicks, Qingjiaoma chicks were challenged by oral gavage with one of 4 isolates, named 20JS04. This strain was grown on Luria-Bertani (**LB**) agar plates and incubated at 37°C for 24 to 36 h. The culture was harvested, resuspended and adjusted to achieve a dose of $\sim 10^4$ to $\sim 10^9$ CFU in a volume of 1 mL for oral gavage (10 chicks per dose). The chicks were monitored for 1 wk and the number of dead chicks was recorded to calculate LD_{50} according to BLISS method.

Effect of Arthritis-causing S. Pullorum Isolates on Growth Performance

A total of 54 three-day-old Qingjiaoma chicks were randomly allocated to 3 groups with 3 replications, and each replication with 6 chicks. The meconium of the chicks was tested to ensure that they were not infected by *Salmonella*. Three groups were inoculated intramuscularly with the white diarrhea-causing *S.* Pullorum standard strain CVCC526 ($\sim 10^4$ CFU), the isolate 20JS04 ($\sim 10^4$ CFU) and PBS as negative control, respectively. The standard strain of *S.* Pullorum (CVCC526) was purchased from the China Veterinary Culture Collection Center (Beijing, China). The chicks were monitored for up to 3 wk, and the body weight, feed intake and mortality of each replication were recorded. The synovial fluid of swollen hock, liver, spleen, and heart of dead chickens were collected and carried out the reisolation and identification of *S.* Pullorum. The specific PCR and biochemical identification were performed to confirm that the reisolated *Salmonella* had the same characteristics as the challenge strain. Besides, the liver and spleen of the 20JS04 group were collected to evaluate pathological changes.

Statistical Analysis

Statistical analysis was performed using the Statistical Analysis Systems statistical software package (Version 8e; SAS Institute Inc., Cary, NC). One-way analysis of variance followed by Duncan's significant difference tests was used to determine the statistical significance of the differences between multiple experimental groups. The data are expressed as the means ± SE. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Locomotor disorders caused by the joint lesion of leg remain a challenge to the poultry industry, which represent an economic concern and a problem of animal welfare (Braga et al., 2016). Generally, *Escherichia coli, Enterococcus cecorum, Staphylococcus aureus, Mycoplasma synoviae* and avian reovirus are considered to be the main pathogen that can cause arthritis, synovitis and claudication. Our laboratory investigations showed that there are more and more diseased chickens presenting arthritis and lameness associated with *S.* Pullorum infection, especially in Chinese native chicken flocks. These arthritis-causing *S.* Pullorum isolates used to be reported in the 1990−1991 outbreak of Pullorum disease in commercial broilers in the United States (Salem et al., 1992). The synovial fluid of joints were also considered as a potential reservoir of carcass contamination caused by *Salmonella* (Sexton et al., 2018). In this study, we isolated and identified 4 strains of *S.* Pullorum from the pathological organs and the synovial fluid of swollen hock joint of chickens presenting lameness or arthritis symptoms (Figure 1A). The LD_{50} of one isolate 20JS04 on Qingjiaoma chicks infected by oral gavage was 1.3×10^6 CFU, and it was lower than that previously reported (6×10^6 CFU) for S06004 (Geng et al., 2014), which was considered as a highly virulent *S.* Pullorum strain in chicks. It was observed that most of chicks (22/25) died of acute infection within 24 h (Figure 1B). Actually, we assessed the pathogenicity of the isolate 20JS04 by intramuscular inoculation at first (range from 10^3 to 10^7 CFU), but all the chicks died within 48 h. The 20JS04 strain used in this study was also more virulent than SP1621, which was also isolated from chickens with arthritis in China (Guo et al., 2019). The results indicated that the virulence of arthritis-causing *S.* Pullorum may enhance in Chinese chickens.

The growth performance of the flock with an outbreak of arthritis was still not improved after eliminating of the diseased chickens and treating with antibiotics. Therefore, we compared the impact of arthritis-causing *S.* Pullorum strain on growth performance with white diarrhea-causing *S.* Pullorum strain. In addition to somnolence and depressed appetite, chicks developed arthritis and lameness with isolate 20JS04 at around 10-d post

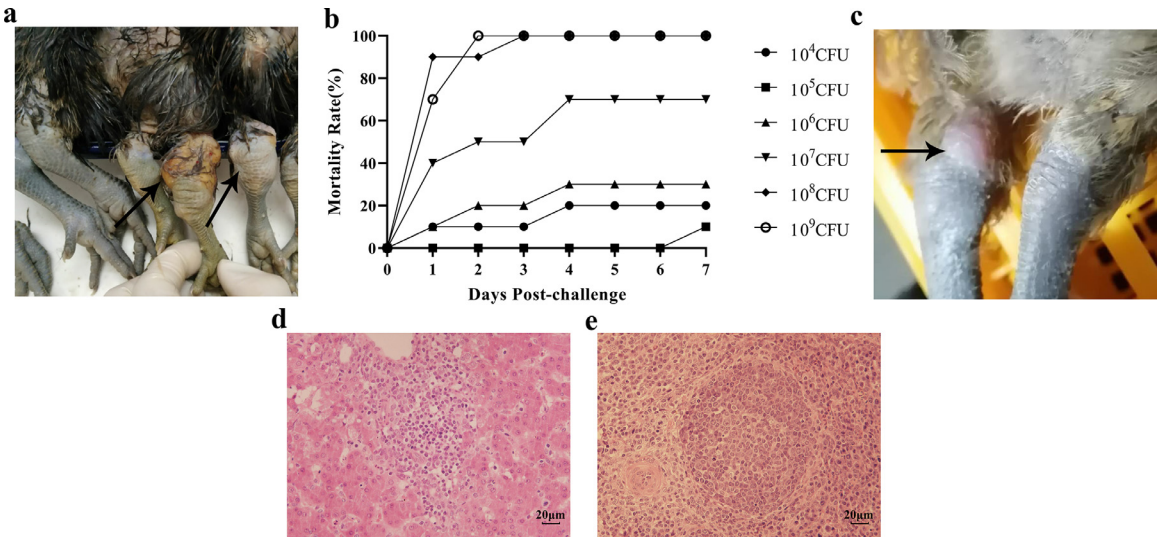

Figure 1. (A) Severe swollen hock joint of Qingjiaoma chicken breeders (arrow). (B) The mortality rate of chicks infected by oral gavage with a series of doses of arthritis-causing *S.* Pullorum isolate 20JS04. (C) Swelling of the hock joint of the experimental chick infection model (arrow). Pathological changes in the liver (D) and spleen (E) (400 ×).

Table 1. The body weight (BW), average daily feed intake (ADFI), average daily gain (ADG), feed conversion radio (FCR) and mortality of chickens in the different treatment groups[1].

Item	Control	CVCC526	20JS04	*P*-value
BW (g)	458.83 ± 0.93^{a}	407.67 ± 0.71^{b}	364.67 ± 0.11^{c}	<0.0001
ADFI (g)	34.93 ± 0.14^{a}	30.64 ± 0.29^{b}	25.16 ± 1.07^{c}	0.0001
ADG (g)	19.27 ± 0.07^{a}	15.60 ± 0.27^{b}	12.53 ± 0.65^{c}	<0.0001
FCR	1.81 ± 0.00^{b}	1.97 ± 0.03^{a}	2.01 ± 0.02^{a}	0.003
Mortality (%)	0^{b}	9.72 ± 5.01^{ab}	15.28 ± 1.39^{b}	0.0301

[a,b,c]Different superscripts within a row indicate significantly different means.
[1]Data represent the mean value (n = 3) for each treatment group.

infection (Figure 1C), and the incidence was 27.78% (5/18). Moreover, the pathological changes detected in the 20JS04 group included conspicuous inflammatory cell infiltrates in the liver (Figure 1D) and a significant reduction in the numbers of lymphocyte in the spleen (Figure 1E). Diarrhea and pasting of the vent feathers were the main clinical symptoms in the CVCC526 group, while no symptoms of arthritis were observed. Chickens in the control group were normal without any clinical symptom of salmonellosis. To confirm the *S.* Pullorum isolate 20JS04 was the causal agent for arthritis in chicken breeders, we reisolated *S.* Pullorum strain 20JS04 from experimentally challenged chickens with arthritic symptoms and confirmed these isolates by the specific PCR and biochemical identification. As shown in Table 1, the BW, ADFI and ADG of the 20JS04 group were significantly lower than the CVCC526 and control groups ($P < 0.01$). Compared with the control group, both the 20JS04 ($P = 0.0013$) and CVCC526 ($P = 0.0117$) groups had lower FCR, but there is no difference between 2 infected groups ($P = 0.3522$) (Table 1). Based on the above results, we could speculate that the *S.* Pullorum isolate may prefer to target to the joint, resulting in chickens developing gross lesions of joints or lameness accompanied with different degrees of limited mobility, and further impacting the feed intake of chickens. Although no study has yet conclusively shown how *S.* Pullorum enters the synovial fluid of joint, apart from the easily noticeable characteristics of high lethality and induced-arthritis, the effect of this kind of arthritis-causing *S.* Pullorum isolates on growth performance was also worth of attention.

In summary, we identified 4 arthritis-causing *S.* Pullorum isolates from the Qingjiaoma chicken breeders with swollen hock joints or lameness, and showed that one highly virulent isolate 20JS04 caused typical arthritis symptoms and had greater negative impact on growth performance than white diarrhea-causing strain. These results suggest that the pathogenic diversity of *S.* Pullorum in China should deserve more attention, which may increase the complexity and difficulty of salmonellosis control. Extensive testing and eradication programs should be strictly implemented to control salmonellosis in breeder farms.

ACKNOWLEDGMENTS

This work was supported by Shandong Provincial Agricultural Animal Breeding Project of China (2020LZGC013), the Fund of Shandong Agricultural Major Application Technology Innovation (SD2019XM009) and Talents Development Project of Zaozhuang.

DISCLOSURES

The authors have declared no conflicts of interest.

REFERENCES

Barrow, P. A., and O. C. Freitas Neto. 2011. Pullorum disease and fowl typhoid–new thoughts on old diseases: a review. Avian. Pathol. 40:1–13.

Braga, J. F. V., N. K. Chanteloup, A. Trotereau, S. Baucheron, R. Guabiraba, R. Ecco, and C. Schouler. 2016. Diversity of Escherichia coli strains involved in vertebral osteomyelitis and arthritis in broilers in Brazil. BMC Vet. Res. 12:140.

Geng, S., X. Jiao, P. Barrow, Z. Pan, and X. Chen. 2014. Virulence determinants of *Salmonella* Gallinarum biovar Pullorum identified by PCR signature-tagged mutagenesis and the spiC mutant as a candidate live attenuated vaccine. Vet. Microbiol. 168:388–394.

Guo, R., Z. Li, X. Zhou, C. Huang, Y. Hu, S. Geng, X. Chen, Q. Li, Z. Pan, and X. Jiao. 2019. Induction of arthritis in chickens by infection with novel virulent *Salmonella* Pullorum strains. Vet. Microbiol. 228:165–172.

Salem, M., E. M. Odor, and C. Pope. 1992. Pullorum disease in Delaware roasters. Avian. Dis. 36:1076–1080.

Sexton, T. Y., I. Geornaras, K. E. Belk, M. Bunning, and J. N. Martin. 2018. *Salmonella* contamination in broiler synovial fluid: are we missing a potential reservoir? J. Food. Prot. 81:1425–1431.

Song, Y., F. Wang, Y. Liu, Y. Song, L. Zhang, F. Zhang, X. Gu, and S. Sun. 2020. Occurrence and Characterization of *Salmonella* Isolated From Chicken Breeder Flocks in Nine Chinese Provinces. Front. Vet. Sci. 7:479.

Shivaprasad, H. L. 2000. Fowl typhoid and pullorum disease. Rev. Sci. Tech. 19:405–424.

Xu, L., Z. Liu, Y. Li, C. Yin, Y. Hu, X. Xie, Q. Li, and X. Jiao. 2018. A rapid method to identify *Salmonella* enterica serovar Gallinarum biovar Pullorum using a specific target gene ipaJ. Avian Pathol. 47:238–244.

Yang, J., Z. Ju, Y. Yang, X. Zhao, Z. Jiang, and S. Sun. 2019. Serotype, antimicrobial susceptibility and genotype profiles of *Salmonella* isolated from duck farms and a slaughterhouse in Shandong province, China. BMC Microbiol. 19:202.