Changes of Prevalent *Haemophilus parasuis* Serotypes in Pig Farms after Continuous Vaccination

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Abstract [**Objectives**] This study was conducted to evaluate the practical efficacy of *Haemophilus parasuis* (Hps) vaccines and determine whether the continuous use of vaccines leads to changes in Hps serotypes. [**Methods**] A swine production group that had used a bivalent vaccine of Hps serotypes 4 and 5 for two consecutive years were investigated and retrospectively analyzed. Data on Hps detection rate, nursery production and disease indexes, as well as serotype shifts after continuous vaccination, were collected to assess vaccine effectiveness. [**Results**] Continuous vaccination reduced the total nursery culling and mortality rate by 1.75%. Hps infection persisted in the farms, and the pathogen could still be isolated. However, serotyping revealed a significant shift in the dominant prevalent serotypes, indicating that the vaccine was indeed effective. [**Conclusions**] The matched Hps vaccine demonstrated effective results. However, whether replacing serotypes or adding more prevalent serotypes could further improve the control of Hps disease requires further investigation.

Key words *Haemophilus parasuis*; Pig farm; Vaccine

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Haemophilus parasuis (Hps) disease, also known as Glässer's disease, occurs throughout China and is particularly prevalent during the pig nursery stage[1-2]. It is one of the most severe bacterial diseases that co-infects with Porcine Reproductive and Respiratory Syndrome (PRRS). Clinically characterized by fever, joint swelling, dyspnea, polyserositis, arthritis, and neurological symptoms[3], this disease generally exhibits high mortality rates and causes significant economic losses to China's swine industry.

Hps is a Gram-negative short rod or filamentous bacterium with variable morphology. There are 15 major serotypes, with types 4 and 5 being most prevalent in pig farms and causing the greatest economic losses[4].

Currently, the prevention and control of Glässer's disease primarily rely on vaccines and sensitive antibiotics[5-6]. However, in practical farm management, many vaccine users perceive Hps vaccines as having low protective efficacy. Since drug-based prevention and control is proved to be effective against this disease, farmers tend to prefer medication over vaccination for disease management.

In this study, in order to explore the effects of Hps vaccines in practical use and the serotype changes of Hps under immune pressure in pig farms after using vaccines, a regional division of a pig farming group that had continuously used a bivalent vaccine of Hps serotypes 4 and 5 for two years was tracked and investigated.

Materials and Methods

Experimental materials

Test subjects The regional division of the pig farming group consisting of 4 breeding farms, 7 fattening farms, and some contract-growing farms, with a total of 18 000 sows. The farms had consistently used the Hps vaccine (containing primarily serotypes 4 and 5) for two years. Vaccination protocol: Sows were immunized with their fetuses 5 weeks before delivery, and piglets were injected with porcine circovirus vaccine at the same time separately on opposite sides at the age of 14 d, and strengthened once at the age of 28 d.

Main reagents The reagents used mainly included Hps antigen fluorescence quantitative detection kit, NADH, TSA, TSB, and *Staphylococcus aureus*, blood agar plate, type 1 - 15 standard strains, and standard serum.

Experimental methods

Changes in Hps detection rates in sows and nursery pigs were tracked in the two years before immunization (the data of only one year might be greatly influenced by other diseases) and from immunization to the present. Sow testing primarily used nasopharyngeal swabs, while piglet testing employed both nasopharyngeal swabs and pathological samples. The pre-vaccination tracking period was from March 2021 to February 2023, and the post-vaccination tracking lasted from March 2023 to December 2024 (the same applies below).

The comparison of overall morbidity, mortality, feed conversion ratio and other disease and production performance indexes in nursery pigs was conducted between the two-year pre-vaccination period and the post-vaccination period up to the present.

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For the isolated Hps strains, serotyping was performed to analyze changes in the main prevalent serotypes before and after vaccination.

Results and Analysis

Changes in Hps detection rate before and after vaccination

Pathological samples and nasopharyngeal swabs collected from the pig farms were tested to observe variations in Hps detection rate or positive rate.

Fig. 1 demonstrates that Hps could be detected in nasopharyngeal swabs from sows even without clinical symptoms, and the positive rate was not low. This might serve as an infection source for early-stage Hps disease in piglets. Compared with the pre-vaccination period, the sow carrying rate decreased by 4.1% during the two post-vaccination years. Since the nursery group primarily consisted of sampled pigs showing clinical symptoms or those died for diseases, the detection rate remained exceptionally high. It confirms the significant health impact of Hps on nursery pigs. Although the overall positive rate decreased by 8.9% after vaccination, it was still high.

Changes in disease and production indexes after vaccination

As this was a retrospective study, it was difficult to precisely attribute mortality or production changes specifically to Hps infection. Therefore, annual aggregated data were used for comparison, with overall index variations serving as the basis for analysis. Meanwhile, the farms maintained consistent feeding method, vaccination protocols of other vaccines, staff structure, sow sources, and genetic breeds throughout both pre- and post-vaccination periods. During data analysis, we excluded contemporaneous data from farms severely affected by PED or suspected ASF outbreaks.

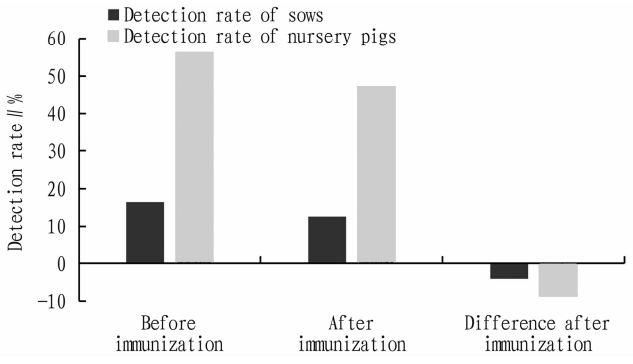

Fig. 1 Changes of Hps detection rate before and after immunization

The nursery stage is the primary period for Hps infection. Overall, the data in Table 1 reflected the impact of vaccination on various achievements in the nursery stage before and after immunization from a big-data perspective. In general, the total culling and mortality rate in the nursery stage decreased by 1.75% after vaccination.

Table 1 Changes in nursery pig morbidity and production indexes before and after immunization

Nursery index	Mortality[1]	Total culling and mortality rate[2]	Healthy pig rate	Feed conversion ratio	Deaths and culling within the first 7 d after transferring to fattening[3]	Total culling and mortality rate including deaths and culling within the first 7 d after transferring to fattening
Before immunization	4.14%	6.20%	90.4%	1.403	1.05%	7.25%
After immunization	3.06%	4.83%	90.2%	1.322	0.67%	5.50%

1. Mortality: The mortality index of nursery pigs was directly extracted without detailed statistics on whether deaths were caused by Hps.
2. Many nursery farms categorize culled pigs as sold, and we included this data in the total culling and mortality figures.
3. Most deaths and culling within the first 7 d after transferring to the fattening stage are a continuation of illnesses from the farrowing house, hence they were counted in the total culling and mortality of nursery pigs.

Serological changes in Hps isolates

A retrospective analysis was conducted on the serotyping of Hps strains isolated from pig farms in recent years to observe changes in prevalent serotypes before and after vaccination.

As shown in Table 2, even after two years of continuous vaccination, Hps could still be isolated from the farms, and Hps-related cases including those with typical symptoms still occurred. However, the proportion of original serotypes 4 and 5 significantly decreased after immunization.

Table 2 Changes in serotypes of Hps isolates

	Total number of isolates	Proportion of serotypes 4 and 5	Proportion of other serotypes	Difference for serotypes 4 and 5
Before immunization	132	68.18%	31.82%	
After immunization	96	29.17%	70.83%	−39.01%

Conclusions and Discussion

The nursery stage of live pigs is a critical period for Hps infection and should be the most important investigation stage[7]. However, in practice, it is difficult to determine whether a nursery pig's death is solely caused by Hps or whether changes in healthy pig rates and feed conversion ratios are due to a single factor. Therefore, we adopted a big-data retrospective approach. The feeding method, other vaccination programs, staff structure and sow sources and breeds remained largely unchanged before and after immunization in the regional division of the pig farming group, allowing the assessment of the changes before and after vaccination.

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In summary, maintaining the integrity of the low-temperature cold chain (≤4 ℃) and reducing processing time are the core strategies for controlling the microbial safety of raw milk. The adaptive growth and metabolic activity of psychrotrophic bacteria are the primary causes of dairy deterioration. Maintaining the temperature at ≤4 ℃ throughout storage and transportation and reducing the processing interval to within 18 h can effectively inhibit the proliferation of psychrotrophic bacteria. Special attention should be given to the metabolic activity of dominant psychrotrophic bacteria (such as *Pseudomonas*). Optimizing sterilization processes to deactivate their heat-stable hydrolytic enzymes is crucial for extending the shelf life of dairy products.

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Meanwhile, it should be noted that the detection rate of nasopharyngeal swabs does not necessarily reflect the incidence rate[8]. In sows, it might indicate a carrying state, while in piglets, it could signify either carrying or infection. Additionally, there were differences in sampling methods before and after vaccination. There was a tendency of increasing difficulty in collecting pathological samples, leading to a greater reliance on swab testing and isolation. Therefore, variations in sampling methods might introduce certain biases in positive rate. These represent potential operational and statistical limitations in this study.

The use of Hps vaccines has always been controversial, and many farms prefer to control the disease through medication. It is because users perceive Hps vaccines as having low protective efficacy, coupled with the fact that drug-based prevention and control are effective. Based on the data from the farms in this study, Hps infection still occurred in the nursery stage after vaccination, with typical clinical symptoms of Glässer's disease observed. PCR testing confirmed the presence of the pathogen, and some farms even reported relatively high incidence rates. It seems to confirm the perceived low efficacy of Hps vaccines. However, serotyping data reveals a different reality. Under the selective pressure of vaccination, the prevalence of serotypes 4 and 5 significantly decreased, and the dominant prevalent serotypes shifted to others. Infection caused by these alternative serotypes resulted in milder lesions compared with serotypes 4 and 5, which explained the overall reduction in nursery mortality observed in the big data analysis. The results unequivocally demonstrate that Hps vaccination is indeed effective.

If properly matched Hps vaccines are proved to be effective, can we anticipate that by enhancing surveillance to track prevalent serotype shifts and subsequently updating vaccine formulations to replace serotypes or include more prevalent serotypes, we could achieve better control of Glässer's disease? This hypothesis requires further investigation for validation.

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