XXII nd CONGRESS OF THE WVPA

World Veterinary Poultry Association

Verona (Italy)

September 4-8, 2023

Italian Branch of the World Veterinary Poultry Association

BOOK OF ABSTRACTS

S7-O5
SAFETY OF SG9R STRAIN: EVALUATION OF THE RISK OF REVERSION TO VIRULENCE

J.B. Paiva [1], P. Fernandes [1], L. Monteiro [1], E. Oliveira [1], N. Haga [1], A. Fonseca [2], N. Ikuta [2]

[1]Research and Development Department, BioCamp Laboratories Ltda., Campinas, São Paulo, Brazil
[2]Simbios Biotecnologia, Cachoeirinha, Brazil

Background
Salmonella enterica subsp. enterica serovar Gallinarum biovar Gallinarum 9R strain (SG9R) is a live attenuated vaccine to control fowl typhoid disease (FT). SG9R is a rough strain, which means that its LPS is incomplete by lacking the O-specific chain due to a nonsense mutation in the rfaJ gene which encodes an LPS 1,2-glucosyltransferase enzyme. This strain is the only S. Gallinarum live vaccine commercially available for FT control in the world, and it is extensively used in South America, including Brazil where FT remains an endemic problem. Concerns regarding FT include massive economic production losses and high mortality that can reach 80% in affected flocks of heavy and semi-heavy chicken lineages.In this study, efforts at reversion to virulence of the CampVac SG9R strain (BioCamp) were carried out through serial passages by susceptible hosts. It has included vaccination and re-isolation of the vaccine strain from internal organs, followed by phenotypic and genotypic stability characterization of all isolates.

Experimental Design
Therefore, ten groups of 20 Salmonella free-brown-laying hens were performed. Five successive passages starting with the master-seed culture and using one (10^8CFU/bird) and ten (10^9CFU/bird) doses via the intramuscular route were carried out. Clinical signs and mortality were daily recorded until 21 days post-vaccination (dpv). At 2, 5 and 7 dpv, three birds from each group were euthanized for the attempts to isolate the vaccine strain. Each isolate was individually characterized: biochemically by their growth on BGA and Rugai-Lysine modified medium; serologically with Salmonella-polyvalent somatic and flagellar antiserums (Poly-O and Poly-H, respectively), and group-D somatic antiserum (O9); and, 1% acriflavine agglutination test. One isolate from the spleen and one from the liver, those ones that remained longer in the birds were chosen to prepare a bacterial broth culture for the next passage. At 21 dpv, the remaining birds were euthanized for necropsy and gross lesions in internal organs were observed. At the end of the fifth passage, the genetic stability of 26 isolates was assessed by PCR, RFLP and sequencing analysis. A qPCR detecting invA gene confirmed Salmonella genera, a duplex qPCR for glpC and speC genes differentiated Salmonella Gallinarum biovar Gallinarum. The rfaJ gene from each isolate was amplified by PCR, and then RFLP and sequencing were employed to detect and characterize polymorphism patterns in this gene, respectively.

Results and Conclusions
No mortality was recorded in birds of any passage; no birds showed any clinical signs of fowl typhoid. Isolation of vaccine strain was possible in birds vaccinated with both doses, from all passages at all investigated time. Livers with slight green-yellowish colour and few necrotic foci were found in around 50% of birds at 21dpv, independently of the vaccine doses or vaccine passage. Some enlarged spleen with necrotic foci were also noticed. SG9R isolation was confirmed by means of biochemical tests, serology, acriflavine test and PCR-based techniques. The stability of attenuation on isolated strains was compared to the master-seed strain by using the combination of PCR, RFLP and sequencing of the gene rfaJ. All amplicons of the rfjA gene were identical to each other and the master-seed strain. The mutation on rfjA remained stable during the successive passages. All isolates showed the same pattern as the SG9R strain unlike the SG9S strain (the smooth parental strain) and SG wild-type strains. In view of the obtained results is possible to conclude that the rough 9R strain present in CampVac did not revert to virulence.

Keywords
safety guarantee assessment; SG9R vaccine strain; Salmonella Gallinarum; fowl typhoid.