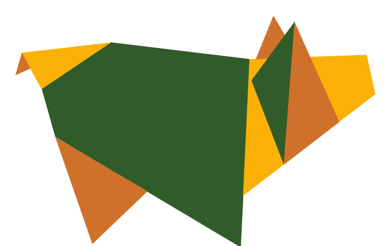

IPVS2022

26th international pig veterinary society congress - rio de janeiro - brazil

June 21st-24th

Proceedings IPVS2022

RIO DE JANEIRO/RJ, BRAZIL

Cellular and humoral immunity elicited by an Influenza A polyvalent virosomal vaccine in pigs

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Introduction

Influenza A virus (IAV) causes economic losses to the swine industry and public health concerns. The IAV is endemic in pigs and genetically and antigenically distinct virus lineages of subtypes H1N1, H1N2 and H3N2 circulate in different geographic regions with limited cross-protection (1, 2). The co-circulation of distinct IAV lineages associated with rapid viral evolution challenges the development of effective vaccines. The aim of this study was to evaluate the immunogenicity of an adjuvanted virosome-based influenza vaccine containing the hemagglutinin (HA) of H1N1pdm, H1N2 and H3N2.

Materials and Methods

Forty-three SPF pigs were randomized into three groups: G1: 10 non-vaccinated; G2: 30 vaccinated; and G3: 3 vaccinated – for long term immunity evaluation. Pigs from G1 received PBS injection, and pigs from G2 and G3 were vaccinated intramuscularly with a virosomal IAV vaccine on D0 and D14. Pigs were daily monitored for clinical signs, or any adverse effects related to vaccination. Blood and nasal swab samples were collected from all pigs on D0, D14 and D28. Pigs from G3 were also sampled on D60 and D90. Necropsy was performed on D28 for G1 and G2 and on D90 for G3. Nasal swab samples were evaluated by RT-qPCR for IAV (3). Serum samples were evaluated by hemagglutination inhibition (HI) (4) and serum virus neutralization (SVN) (5), using vaccine homologous viruses (H1N1pdm, H1N2 and H3N2) as antigens. For flow cytometry, splenocytes were labeled with CFSE for *in vitro* stimulation by the three vaccine viruses and stained with fluorochrome-labeled monoclonal antibodies RPE-CD8alpha and PerCP-Cy5-IFN-γ. Different cytokines (GM-CSF, IFNγ, IL-1α, IL-1ra, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18 and TNFα) were evaluated with MILLIPLEX MAP Porcine Cytokine/Chemokine Magnetic Bead kit (Merck Millipore), by Luminex platform. Differences between groups were evaluated using Kruskal-Wallis and Wilcoxon tests by SAS (6).

Results

No clinical signs were observed in pigs during the experiment. All nasal swabs collected on D0, D14, D28, D60 and D90 were negative for IAV. IAV antibodies were not detected in non-vaccinated pigs (G1). IAV antibodies for the three virus antigens were detected in the vaccinated group (G2) as follows: 20.7% of pigs developed antibodies for H1N1 (titers of 40 to 160), 48.3% for H1N2 (titers of 40 to 160) and 100% for H3N2 (titers of 160 to 640). Antibodies to H1N2 (titer 40) and H3N2 (titer 160) were detected in one out of three G3 pigs sampled on D90. Neutralizing-antibodies were detected in all vaccinated pigs (G2) with titers of 40 to 1280 for H1N1, 10 to 320 for H1N2 and 320 to 5120 for H3N2 viruses. On D90, one out of three pigs from G3 had antibodies to H1N1 (320) and H1N2 (20), and all three pigs had antibodies for H3N2 (titers of 40 to 320). Vaccinated pigs (G2 and G3) had increased $CD8^+$ $IFNy^+$ expression when compared to non-vaccinated pigs (G1) ($p<0.0001$). The $CD8^+$ $IFNy^+$ expression was higher for H3N2 virus, followed by H1N1 and H1N2. All cytokines evaluated by Luminex were expressed, but there was no difference between vaccinated and non-vaccinated pigs.

Discussion and Conclusion

A robust humoral and cellular immune response was induced in pigs through vaccination with a virosomal vaccine containing the HA genes of the most prevalent virus subtypes in pigs. Virosomal vaccines closely mimic the native virus, binding and fusing with host cells, contributing to a robust immunity (7). Specific HA antibodies and neutralizing activity for H1N1, H1N2 and H3N2 viruses were detected, which persisted for at least three months. Cellular immune response, with high expression of $CD8^+$ $IFNy^+$ T lymphocytes was elicited. HA-specific antibodies block virus attachment and entry into the cells (8) and cellular immune responses contribute to eliminate infected cells and reduce virus shedding, thus playing an important role during IAV infection (9). Vaccination of pigs with a polyvalent virosomal vaccine containing representative virus strains circulating in Brazil may reduce the IAV impact on the swine production and at human-animal interface.

Acknowledgments

Embrapa (12.13.10.004.04, 22.16.05.004.05) and Capes.

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