CFAES

FOOD ANIMAL HEALTH RESEARCH PROGRAM

Intranasal deliverable mannose surface conjugated chitosan-based influenza nanovaccine for nursery pigs

Sankar Renu, Ninoshkaly Feliciano-Ruiz, Anikethana Ramesh, Veerupaxagouda Patil, Yi Han, Jennifer Schrock, Gourapura J Renukaradhya
Food Animal Health Research Program, Department of Veterinary Preventive Medicine, OARDC, The Ohio State University, USA.

INTRODUCTION

Virulent swine influenza A virus (SwIAV) infection causes acute febrile respiratory disease in pigs of all ages. The triple reassortant 2009 pandemic SwIAV-H1N1 spillover to humans is evidence that pig can act as a mixing vessel for mammalian and avian influenza viruses. The commercial inactivated SwIAV vaccine is a multivalent formulation administered by intramuscular (IM) injection. It induces a systemic IgG with poor induction of secretory IgA (SIgA) antibody response in the airways. The SIgA antibody is important because SwIAV enters the body through airways and replicates primarily in the respiratory tract epithelial cells. Therefore, intranasal (IN) vaccination is the ideal approach to mimic the natural virus infection-induced mucosal immunity. However, IN delivered inactivated virus antigens are poorly immunogenic, and they need a suitable adjuvant and vaccine delivery system to trigger mucosal immune response (1).

Chitosan is a natural cationic copolymer derived from partial deacetylation of chitin, a component of crustacean and insect shells. Chitosan is composed of randomly distributed N-acetyl glucosamine and D-glucosamine residues with a net-positive charge. Chitosan is biocompatible and mucoadhesive and thus increases membrane permeability. Chitosan has amino and carboxyl groups which form hydrogen bonds with mucus glycoproteins resulting in adhesion of chitosan to epithelial lining. For these reason, chitosan nanoparticles (CS NPs) have been extensively investigated for mucosal based delivery of drugs, peptides and proteins (2).

Mannose receptor is a C-type membrane lectin, expressed on dendritic cells and macrophages. Thus, we surface conjugated a ligand mannose on CS NPs (mCS NPs) and loaded killed/inactivated SwIAV antigen (KAg) (mCS NPs-KAg), and IN delivered in nursery pigs. Further, the efficacy of mCS NPs-KAg nanovaccine was compared with a multivalent commercial influenza vaccine.

AIM

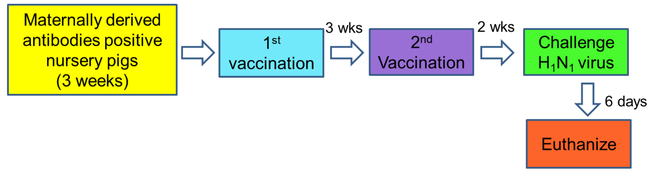
- ✓ To formulate mannose receptor targeted mCS NPs-KAg nanovaccine and characterize its localization in the airways, and immune tissue and cells.
- ✓ To evaluate the virus specific SIgA response and cross-protective efficacy induced by intranasal delivered mCS NPs-KAg nanovaccine in nursery pigs.

METHODS

Grouping of animals and vaccination

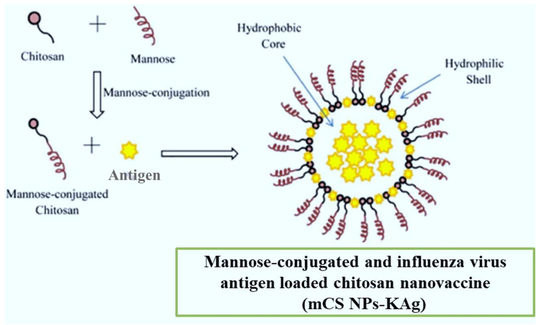
Group-1: Mock and no-challenge control (n=3)
Group-2: Mock and H1N1 virus challenge (n=4)
Group-3: IM Commercial vaccination and H1N1 virus challenge (n=4)
Group-4: IN mCS NPs-KAg nanovaccination and H1N1 virus challenge (n=4)

Experimental design

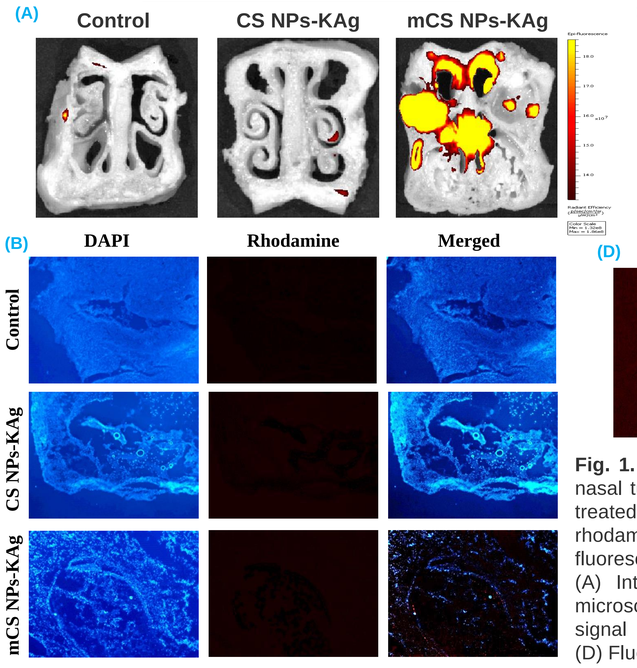


RESULTS

1. Formulation and internalization of Intranasal delivered mCS NPs- KAg nanovaccine in nursery pigs



Nasal turbinate



Mandibular lymph nodes

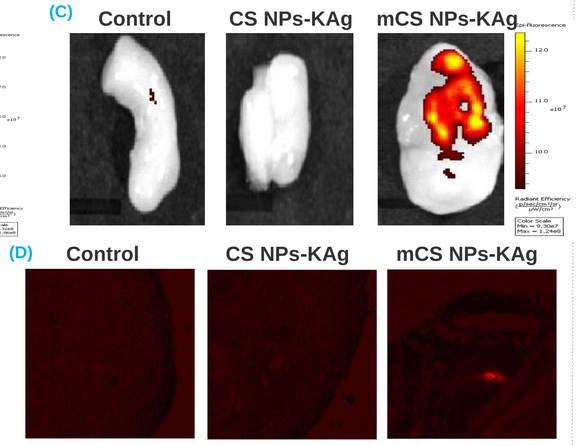


Fig. 1. Preparation and internalization of mCS NPs-KAg in pig nasal turbinate and lymph nodes germinal center. Pigs were IN treated with medium (control) or red color fluorescent dye rhodamine tagged CS NPs-KAg and mCS NPs-KAg for 4 h and fluorescence signal was detected in nasal turbinate using (A) Intravital imaging system (IVIS); and (B) Fluorescence microscopy. Six days after intranasal treatment fluorescence signal was detected in lymph nodes using (C) IVIS; and (D) Fluorescence microscopy.

2. *In vitro* uptake of mCS NPs-KAg in pig peripheral blood mononuclear cells (PBMCs)

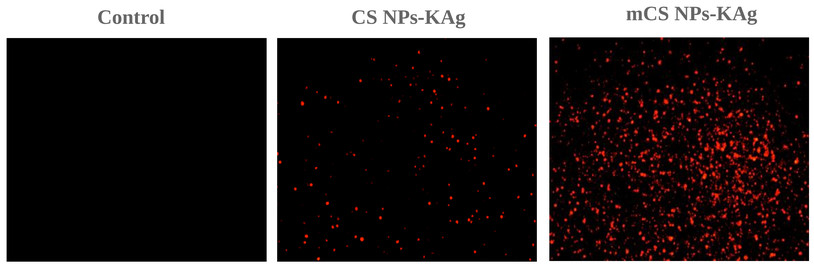


Fig. 2. *In vitro* uptake analysis of mCS NPs-KAg in immune cells. Pig PBMCs were treated with medium (control) or red color fluorescent dye rhodamine tagged CS NPs-KAg and mCS NPs-KAg for 4 h and observed in the red channel under a fluorescence microscopy (4× magnification).

3. mCS NPs-KAg nanovaccine IN delivery in pigs augmented vaccine virus specific SIgA antibody response

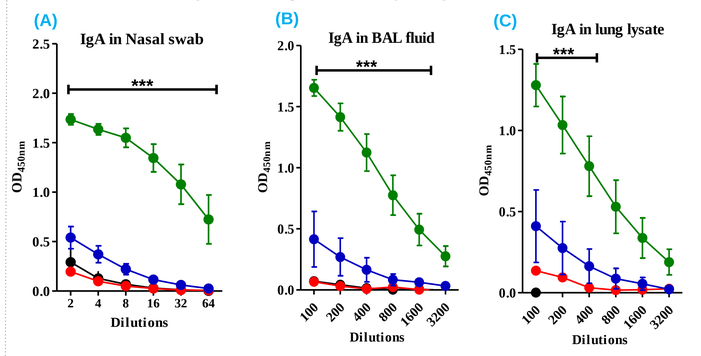


Fig. 3. mCS NPs-KAg nanovaccine induced increased vaccine virus specific SIgA antibody response. Pigs were vaccinated twice with mCS NPs-KAg or commercial vaccine and challenged at 35 days post prime vaccination. Samples collected at day post-challenge six were used for SIgA antibody analysis. Secretory IgA antibody response in (A) nasal swab; (B) BAL fluid; and (C) lung lysate samples were analyzed by ELISA. Data represent the mean value of three to four pigs ± SEM. Statistical analysis was carried out using two-way ANOVA followed by Bonferroni test. Asterisk refers statistical difference (***p<0.001) between the indicated groups. Ch: challenge

4. mCS NPs-KAg and commercial vaccine reduced the challenge virus load

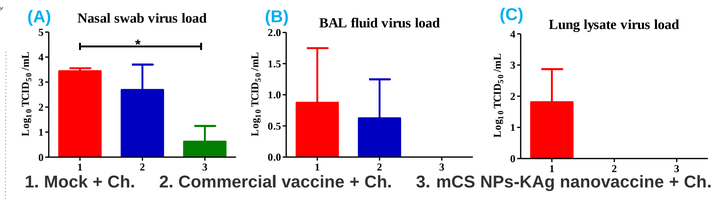


Fig. 4. mCS NPs-KAg nanovaccine reduced/cleared the heterologous challenge virus load. Challenge live SwIAV titer in (A) Nasal Swab; (B) BAL fluid; and (C) Lung lysate. Data represent the mean value of three to four pigs ± SEM. Statistical analysis was carried out using one-way analysis of variance followed by Tukey's post-hoc comparison. Asterisk refers statistical difference (*p<0.05) between the two indicated groups. Ch: challenge

CONCLUSIONS

- ✓ Designed and formulated pig immune cells targeting influenza nanovaccine.
- ✓ Intranasal delivered influenza nanovaccine was internalized by mucosal immune cells in nasal turbinate and lymph nodes germinal center.
- ✓ Maternally derived antibodies carrying nursery pigs vaccinated intranasal with mCS NPs-KAg induced significantly increased SIgA antibody response and reduced heterologous challenge virus load compared to commercial vaccine.

BIBLIOGRAPHY

1. S. Renu et al., Vet Microbiol. 2020;15:761-777.
2. S. Renu et al., Int J Nanomedicine. 2020; 242:108611.

ACKNOWLEDGEMENTS

This project was supported by the National Pork Board. Salaries and research support were provided by state and federal funds appropriated to OARDC, The Ohio State University. We acknowledge Dr. Juliette Hanson and animal care staffs for their help in animal studies. We thank Drs. Loic Deblais and Gireesh Rajashekara for their help in IVIS analysis.

THE OHIO STATE UNIVERSITY
COLLEGE OF FOOD, AGRICULTURAL, AND ENVIRONMENTAL SCIENCES