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Protection of White Leghorn chickens by U.S. emergency H5 vaccination against clade 2.3.4.4 H5N2 high pathogenicity avian influenza virus

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ABSTRACT

During December 2014–June 2015, the U.S. experienced a high pathogenicity avian influenza (HPAI) outbreak caused by clade 2.3.4.4 H5Nx Goose/Guangdong lineage viruses with devastating consequences for the poultry industry. Three vaccines, developed based on updating existing registered vaccines or currently licensed technologies, were evaluated for possible use: an inactivated reverse genetics H5N1 vaccine (rgH5N1) and an RNA particle vaccine (RP-H5), both containing the hemagglutinin gene of clade 2.3.4.4 strain, and a recombinant herpesvirus turkey vectored vaccine (rHVT-H5) containing the hemagglutinin gene of clade 2.2 strain. The efficacy of the three vaccines, alone or in combination, was assessed in White Leghorn chickens against clade 2.3.4.4 H5N2 HPAI virus challenge. In Study 1, single (rHVT-H5) and prime-boost (rHVT-H5 + rgH5N1 or rHVT-H5 + RP-H5) vaccination strategies protected chickens with high levels of protective immunity and significantly reduced virus shedding. In Study 2, single vaccination with either rgH5N1 or RP-H5 vaccines provided clinical protection in adult chickens and significantly reduced virus shedding. In Study 3, double rgH5N1 vaccination protected adult chickens from clinical signs and mortality when challenged 20 weeks post-boost, with high levels of long-lasting protective immunity and significantly reduced virus shedding. These studies support the use of genetically related vaccines, possibly in combination with a broad protective priming vaccine, for emergency vaccination programs against clade 2.3.4.4 H5Nx HPAI virus in young and adult layer chickens.

1. Introduction

In the past decades, high pathogenicity avian influenza (HPAI) has become one of the major zoonotic health issues that commercial poultry, wildlife, and humans have faced worldwide [1–3]. Outbreaks of HPAI in poultry and wild birds across continents have had a dramatic economic and social impact [4,5]. During December 2014–June 2015, the U.S. experienced the worst HPAI event for its poultry industry, with 21 states reporting Eurasian A/goose/Guangdong/1/1996 (Gs/GD) lineage HPAI H5N8, and Eurasian/North American reassortant H5N2 and H5N1 subtypes of clade 2.3.4.4 in commercial premises, backyard flocks, and wild birds [6–8]. Almost 50 million birds, primarily meat turkeys and layer chickens, died or were culled during the eradication program [9] and exports of U.S. poultry and poultry products to many different countries were banned [10], renewing interest in the development of vaccines for emergency use as preventative measure.

To reduce the economic impact on agriculture and a potential pandemic for humans, development of safe and effective vaccines that can protect from HPAI clinical disease and reduce or eliminate viral shedding in exposed birds has been recognized as a viable alternative approach to culling by decreasing risk of transmission in poultry and, ultimately, to humans [11,12]. The use of vaccines is a justifiable tool for control of HPAI when implemented properly

Abbreviations: APHIS, Animal and Plant Health Inspection Service; ABSL-2, animal biosafety level 2; ABSL-3E, animal biosafety level 3 enhanced; Gs/GD, Asian-origin A/goose/Guangdong/1/1996; Tk/MN/15, A/turkey/Minnesota/12582/2015 (H5N2); AI, avian influenza; CVB, Center for Veterinary Biologics; dpc, days post-challenge; DIVA, differentiation of infected from vaccinated animals; GMT, geometric mean titers; HI, hemagglutinin inhibition; HPAI, high pathogenicity avian influenza; LPAI, low pathogenicity avian influenza; MDT, mean death time; EID_{50}, mean embryo infectious doses; NVS, National Veterinary Stockpile; RRT-PCR, quantitative real-time RT-PCR; rFPV-H5, recombinant fowl-pox virus vectored vaccine; rHVT-H5, recombinant herpesvirus turkey vectored vaccine; rgH5N1, reverse genetics H5N1 vaccine; RP-H5, RNA particle vaccine; SEPRL, Southeast Poultry Research Laboratory; SPF, specific pathogen free; WS/05, A/Whooper Swan/Mongolia/3/2005 (H5N1).

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and in combination with strict epidemiological surveillance and biosecurity measures [5]. Indeed, immunization has been a more cost-effective and feasible tool than stamping-out programs alone in some developing countries [13–15]. Vaccination was not implemented in the 2014–2015 U.S. outbreak; however, the outline of an emergency vaccine bank and vaccination policy for use in the future may be strategic to shorten the time for development and approval for vaccines. Vaccines for inclusion in the U.S. National Veterinary Stockpile (NVS) and their potential field use should only be considered under certain conditions: (1) if the vaccine matching the circulating strains is available and targeted to high-risk poultry populations; and (2) if the vaccine enables or has the potential for differentiation of infected from vaccinated animals (DIVA) [12,16].

The objective of this study was to assess the immunogenicity and protective efficacy of three NVS-registered H5Nx vaccines, alone or in combination: an inactivated reverse genetics H5N1 vaccine (hereafter rgH5N1) and an RNA particle vaccine (hereafter RP-H5), both containing the hemagglutinin (HA) gene of A/Gyrfalcon/Washington/40188-6/2014 (H5N8) clade 2.3.4.4 strain, and a recombinant herpesvirus turkey vectored vaccine (hereafter rHVT-H5) containing the HA gene of A/Swan/Hungary/4999/2006 (H5N1) clade 2.2 strain. Studies were performed in day-old chicks and adult White Leghorn (egg laying-type) chickens against lethal clade 2.3.4.4 HPAI virus challenge.

2. Materials and methods

2.1. Animals

Eighty specific pathogen free (SPF) day-old chicks (for Study 1) and 110 SPF adult 61-week-old White Leghorn hens (for Studies 2 and 3) from the Southeast Poultry Research Laboratory (SEPRL) flock were utilized. Each group of birds was housed separately in negative pressured isolators with HEPA-filtered air within the animal biosafety level 2 (ABSL-2) facilities of SEPRL during the vaccination period. Subsequently, they were transferred to animal biosafety level 3 enhanced (ABSL-3E) facilities, housed in negative pressure HEPA-filtered isolators for the challenge period as indicated in Table 1. Birds had *ad libitum* access to feed and water throughout the experiment. All procedures were performed according to the requirements of the protocol approved by the Institutional Laboratory Animal Care and Use Committee.

2.2. Vaccines

Three types of vaccines were tested. First, an inactivated rgH5N1 vaccine contained the HA gene from A/Gyrfalcon/Washington/40188-6/2014 (H5N8) clade 2.3.4.4 strain with the polybasic cleavage site of the HA gene altered to a typical cleavage site sequence of low pathogenicity avian influenza (LPAI) virus, and the remaining 7 backbone segments obtained from the A/Puerto Rico/8/1934 (H1N1) common vaccine strain. The rgH5N1 virus was inactivated with 0.1% β-propiolactone (Sigma Aldrich, St. Louis, MO) and used to prepare an oil-in-water vaccine utilizing a mineral oil-based emulsion (Montanide ISA 70VG, SEPPIC, Paris, France) as previously described [17–20]. The vaccine was administered subcutaneously in a dose of 512 HA units/0.5 ml per bird. Second, a rHVT-H5 vaccine (Vectormune® AI, Ceva Animal Health, Lenexa, KS) was constructed by inserting the HA gene of the HPAI virus A/Swan/Hungary/4999/2006 (H5N1) clade 2.2 strain, with a modified cleavage site compatible with LPAI, into the genome of HVT FC-126 strain. The vaccine was prepared and administered subcutaneously in a dose of 2000 pfu/0.2 ml per bird, as per manufacturer's instructions. Finally, the RP-H5 vaccine (AlphaVax, Merck Animal Health, Ames, IA) contained the HA gene from A/Gyrfalcon/Washington/40188-6/2014 (H5N8) with a modified cleavage site compatible with LPAI. The vaccine was prepared and administered intramuscularly in a dose of $10^{7.0}$ RNA particles/0.5 ml per bird, as per manufacturer's instructions.

2.3. Virus

The influenza A isolate A/turkey/Minnesota/12582/2015 (H5N2) (Tk/MN/15) was used as challenge virus. The Tk/MN/15 virus was selected because it is poultry-adapted and is representative of the Midwest H5N2 cluster both phenotypically [21] and phylogenetically [22]. The virus was propagated and titrated by

Table 1
Summary of studies.

Study	Age	Vaccines[1] (age[2])	No. birds	Age[2] at challenge	Survivability[3]	Peak oral shedding (2 dpc)[4]	HI serology (pre-challenge)[5]	
							Vaccine strain as antigen	Challenge strain Tk/MN/15 as antigen
Study 1	Day-old	Sham	10	4w	0/10[a]	10/10 ($10^{6.0}$)[a]	0/10 (<2^3)	nd
		rHVT-H5 (1d)	10	4w	9/10[b] (90%)	4/10 ($10^{1.9}$)[b]	10/10 ($2^{4.5}$)	10/10 ($2^{3.3}$)
		Sham	20	7w	0/20[a]	20/20 ($10^{6.0}$)[a]	0/20 (<2^3)	nd
		rHVT-H5 (1d) + rgH5N1 (4w)	20	7w	20/20[b] (100%)	3/20 ($10^{1.9}$)[b]	20/20 ($2^{8.6}$ rgH5N1; $2^{9.6}$ WS/05)	20/20 ($2^{8.0}$)
		rHVT-H5 (1d) + RP-H5 (4w)	20	7w	20/20[b] (100%)	5/20 ($10^{1.9}$)[b]	20/20 ($2^{8.3}$ rgH5N1; $2^{8.0}$ WS/05)	18/20 ($2^{4.7}$)
Study 2	Adult	Sham	30	64w	0/30[a]	30/30 ($10^{6.4}$)[a]	0/30 (<2^3)	nd
		rgH5N1 (61w)	20	64w	20/20[b] (100%)	9/20 ($10^{1.9}$)[b]	20/20 ($2^{5.9}$)	20/20 ($2^{6.5}$)
		RP-H5 (61w)	20	64w	19/20[b] (95%)	16/20 ($10^{3.1}$)[c]	15/20 ($2^{5.6}$)	nd
Study 3	Adult	Sham	20	84w	0/14[a]	14/14 ($10^{7.7}$)[a]	0/14 (<2^3)	nd
		rgH5N1 (61w) + rgH5N1 (64w)	20	84w	17/17[b] (100%)	7/17 ($10^{2.7}$)[b]	17/17 ($2^{9.4}$)	nd

[1] rgH5N1 = inactivated oil emulsion vaccine with reverse genetic H5 gene insert from clade 2.3.4.4 (512 HAU/dose); rHVT-H5 = live recombinant herpesvirus turkey vaccine with H5 gene insert from clade 2.2 (512 HAU/dose); RP-H5 = RNA particle vaccine with H5 clade 2.3.4.4 hemagglutinin ($10^{7.0}$ RNA particles/dose); Tk/MN/15 = A/turkey/Minnesota/12582/2015 (H5N2); WS/05 = A/Whooper Swan/Mongolia/3/2005 (H5N1).

[2] d = day old; w = weeks old.

[3] Different superscript lowercase denotes statistically significant differences between vaccine and corresponding sham ($p < 0.05$); the numbers represent no. survivors/total.

[4] The numbers represent no. virus positive/total in group followed by mean virus shed titer. Different superscript lowercase denotes statistical significance of number of birds shedding between vaccine and corresponding sham by Fisher Exact or Chi square tests ($p < 0.05$). Different superscript uppercase denotes statistical significance of shedding titers between vaccine and corresponding sham by Mann-Whitney test ($p < 0.05$).

[5] The numbers represent no. serology positive/total in group followed by mean HI titers against vaccine virus or challenge virus. Positive defined as titers ≥2^3. nd = not determined.

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