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Avian influenza H5N1 vaccination efficacy in Egyptian backyard poultry

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ABSTRACT

Raising backyard poultry under low biosecurity conditions is a common practice in Egypt. While vaccination is routinely applied in Egypt in commercial settings to curb the spread of avian influenza viruses, it remains less commonly used in backyard settings. We assessed the immunogenicity and protective efficacy of a H5N1 vaccine based on a contemporary Egyptian clade 2.2.1.2 virus among turkeys, ducks, geese, and chickens raised together in a backyard setting. Results showed that this vaccine elicits an immune response in all tested species reaching up to a hemagglutination inhibition titer of 10 $\log_2$ after a booster dose. However, this response varied between species. When challenged, vaccinated birds survived and shed less virus in comparison with unvaccinated birds. However, unvaccinated ducks showed no symptoms of infection and survived the duration of the experiment. Moreover, vaccinated ducks shed more virus as compared to vaccinated birds of other species. Hence, we recommend avoiding mixing various species in the backyards of Egypt. Our data indicates that vaccination can be effective in the backyard setting in Egypt, although planning should consider the species covered.

1. Introduction

Poultry production in Egypt is classified into four sectors. Sector 1 includes industrial integrated farms raising more than 20,000 birds per cycle. Sectors 2 and 3 comprise commercial production systems raising 5000–20,000 and 500–5000 birds per cycle, respectively. Sector 4 refers to backyard poultry rearing [1]. In Egypt, backyard poultry production is in close geographical contact with Sector 2 and 3 farms that are commonly found in rural areas. Raising backyard poultry is considered as a main source of protein and sustenance for the clear majority of Egyptian farmers. Backyard poultry are generally raised without control conditions or biosecurity measures making them susceptible to acquiring and transmitting infectious diseases [2].

Avian influenza (AI) is a highly contagious viral disease that can affect several domestic bird species [3]. During the first wave of AI H5N1 outbreaks in Egypt in 2006, massive die-off of domestic poultry was recorded [4]. Therefore, the national veterinary authorities devised a comprehensive action plan to control the spread of the virus in Egypt. This included increasing public awareness through media campaigns, culling infected poultry, poultry movement restrictions and emergency vaccination [5,6]. Although the number of recorded H5N1 outbreaks decreased as a result of the implemented activities [6,7], the control strategy plan failed to restrain the spread of H5N1 virus in Egypt eventuating an enzootic situation [8]. Accordingly, two subclades of H5N1 viruses, 2.2.1 and 2.2.1.1, co-occurred in poultry from late 2009 through 2011 [9]. Subclade 2.2.1.1 viruses are thought to have emerged as vaccine escape mutants as a consequence of vaccine application [10]. Subclade 2.2.1 viruses evolved into a new phylogenetic cluster recently classified as clade 2.2.1.2 [11]. Those subclades (2.2.1, 2.2.1.1 and 2.2.1.2) were antigenically distinct [6]. Recently, Kayali et al. investigated the efficacy of 24 commercial inactivated avian influenza H5 vaccines that were authorized to use in Egyptian poultry [6]. Different influenza A/H5 viruses were thus used as vac-

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cine seed strains, including classical low pathogenic H5Nx viruses or H5N1 reassortants with surface glycoprotein genes (HA and NA) of H5N1 viruses in the genetic background of A/Puerto Rico/8/1934 (H1N1) strain. Abdelwhab et al., reviewed vaccine efficacy studies against different Egyptian H5N1 viruses under experimental conditions [12]. Interestingly, some of the tested vaccines were immunogenic and protected chickens when challenged by infection with Egyptian highly pathogenic H5N1 viruses under laboratory conditions [13–15]. However, the H5N1 and H5N2 experimentally prepared vaccines fared poorly when tested in the field in Egypt [16]. Previous studies on the cross-reactivity of commercial H5 poultry vaccines against H5N1 Egyptian isolates in the field setting in Egypt showed that only one vaccine based on an Egyptian H5N1 virus induced high cross-reactive antibody titers [17]. This discrepancy between the laboratory and field settings, regarding the protective response of H5-based vaccines, must be assessed knowing that backyard-bird rearing is wide spread in Egypt hence representing a major obstacle to the eradication of HPAI H5N1 virus from Egypt. However, the efficacy and the feasibility of AI vaccination in different poultry hosts in backyards is yet unevaluated. Here, we assessed the immunogenicity and efficacy of a reassortant/inactivated H5N1 vaccine based on a currently circulating Egyptian H5N1 clade 2.2.1.2 virus in different avian hosts which are commonly reared in Egyptian backyards.

2. Materials and methods

2.1. Virus

HPAI H5N1 isolate, A/chicken/Egypt/D10552B/2015 (clade 2.2.1.2, D10552B), was used for preparation of an experimental vaccine and laboratory challenge experiments. The pathogenicity of HA of D10552B virus (accession No. AMN14801) was attenuated by changing the multibasic amino acids at the cleavage site (KRRKKR) to be monobasic amino acid (R). For vaccine strains preparation, plasmid-based reverse genetics system was applied using six internal genes of A/Puerto Rico/8/34 (H1N1) and two surface LP-HA and NA genes of D10552B [18]. The generated vaccine strain, rgD10552B, was propagated in 10-day-old specific pathogen free-embryonated chicken eggs (SPF-ECE) (Koum Oshiem SPF Chicken Farm, Fayoum, Egypt). The mutation at the cleavage site of rescued rgD10552B virus was confirmed by sequencing. The rgD10552B virus was propagated in SPF-ECE for 3 passages and did not result in embryo death.

LPAI G1-like A/chicken/Egypt/S10489C/2015 (H9N2) isolate was used as antigen for HI assays against H9N2 viruses.

2.2. Vaccine

To make the experimental vaccines, the rgD10552B was propagated in SPF-ECE for three successive passages to a titer of $10^{7.5}$ EID_{50}/0.1 ml. Allantoic fluids were harvested, tested for sterility, and inactivated by addition of 0.1% formalin and mixed with Montanide ISA 70 VG (Seppic, France) in the ratio recommended by the manufacturer (30 antigen/70 adjuvant). The inactivated virus fluid was then examined for residual infectivity in SPF-ECE and tested for sterility and safety [19].

2.3. Vaccine efficacy in SPF chickens

SPF chicken eggs were hatched at the Centre of Scientific Excellence for Influenza Viruses, National Research Centre, Cairo, Egypt. Sera collected from 10 randomly selected chicks were tested for H5 and H9 antibodies resulting from maternal passively transferred immunity at weeks one and two of age. Using hemagglutination inhibition assay (HI) with Chicken red blood cells [20], the antibody titer against H5N1 and H9N2 viruses were monitored and corresponding Log_2 titer was calculated. Ten chickens received 0.5 ml of the vaccine by intramuscular injection through the thigh at 14 days old. One group was used as control and received 0.5 ml PBS. Weekly and up to the fourth week post vaccination (wpv), blood samples were withdrawn from chickens in each group to evaluate antibody titer against rgD10552B by HI.

At 4 wpv, five animals of each group were infected with 0.5 ml of challenge virus at a dose of $10^{6.5}$ EID_{50}/ml of HP D10552B virus via the natural route (i.e., intranasal, intraocular, and intratracheal) Birds were then monitored daily for morbidity and mortality. Cloacal and oral swabs were collected from each bird at days 2, 3, 5, and 7 post infection for virus titration in eggs. All animal experiments were approved by the Ethics Committee of the National Research Centre, Egypt.

2.4. Backyard trial

The trial was conducted during March and April 2016 concurrently at three different backyard sites in Kafr Elshekh, Sharkia and Gharbia governorates in the Nile Delta region of Egypt. Breading conditions typically applied in Egyptian backyard settings were implemented including allowing free range, supplementing the diet with crop waste or food leftovers, and using household water source for drinking. Forty-eight of each: chickens, Pekin ducks, domestic geese (White Embden geese), and turkeys raised at specific nursery farms were purchased at about 14–21 days of age as is the practice in Egypt [21]. To enable monitoring maternal immunity antibody (MIB) levels, 8 serum samples were collected per bird type and tested for H5 and H9 antibodies using HI. Sixteen of each bird type were distributed over the three backyard sites and housed at the same location. A week after placement for adaptation, 8 birds of each type per site were vaccinated with 0.5 ml of the experimental vaccine by intramuscular injection and 8 birds of each type served as unvaccinated controls. On vaccination day and on a weekly basis thereafter, blood was collected from 5 birds per type from each group to test for vaccine immunogenicity. In the 5th week post vaccination, a booster dose of 0.5 ml of experimental vaccine was administered. The birds remained on site until the 7th week post-first vaccination dose.

Before starting the experiment and weekly thereafter, we collected oropharyngeal swabs, cloacal swabs, surface swabs from each breeding site, water sample and air sample to monitor the presence of influenza A viruses (IAV) by PCR according to previously published methods [9,22] to ensure that the birds remained influenza-free during the study. Air sampling was done using a Coriolis cyclone sampler (Bertin Technologies, Montigny-le-Bretonneux, France). Ten milliliters of water with 0.01% Tween 20 was used in the sampling cones of the Coriolis sampler, which was run for a period of 10 min at 300 l/min.

2.5. Challenge of vaccinated backyard poultry with HPAI H5N1

Three animals from each group were randomly removed from all sites, placed in BSL-3 certified isolators and infected with 0.5 ml of challenge viruses at a dose $10^{6.2}$ EID_{50}/bird of HP D10552B virus via the natural route at 7 wpv. Birds were then monitored daily for morbidity and mortality. Cloacal and oral swabs were obtained from each bird at days 1, 3, and 5 post infections for virus titration in eggs.

2.6. Statistical analyses

Statistical analyses were done using GraphPad Prism V5 (GraphPad Inc., CA, USA). ANOVA with Tukey post hoc test was

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