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Pekin and Muscovy ducks respond differently to vaccination with a H5N1 highly pathogenic avian influenza (HPAI) commercial inactivated vaccine

Caran Cagle[a], Thanh Long To[b], Tung Nguyen[b,c], Jamie Wasilenko[a], Sean C. Adams[d], Carol J. Cardona[d,e,1], Erica Spackman[a], David L. Suarez[a], Mary J. Pantin-Jackwood[a,*]

[a] Southeast Poultry Research Laboratory, Exotic and Emerging Avian Viral Diseases Unit, Agricultural Research Service, U.S. Department of Agriculture, 934 College Station Road, Athens, GA 30605, USA
[b] National Centre for Veterinary Diagnosis, Department of Animal Health, Hanoi, Viet Nam
[c] Graduate School, Hanoi University of Agriculture, Hanoi, Viet Nam
[d] Department of Population Health and Reproduction, University of California, Davis, CA 95616, USA
[e] Veterinary Medicine Extension, University of California, Davis, CA 95616, USA

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ABSTRACT

Domestic ducks are key intermediates in the transmission of H5N1 highly pathogenic avian influenza (HPAI) viruses, and therefore are included in vaccination programs to control H5N1 HPAI. Although vaccination has proven effective in protecting ducks against disease, different species of domestic ducks appear to respond differently to vaccination, and shedding of the virus may still occur in clinically healthy vaccinated populations. In this study we compared the response to vaccination between two common domestic duck species, Pekin (*Anas platyrhynchos domesticus*) and Muscovy (*Cairina moschata*), which were vaccinated with a commercial inactivated vaccine using one of three different schedules in order to elicit protection to H5N1 HPAI before one month of age. Clear differences in responses to vaccination were observed; the Muscovy ducks developed lower viral antibody titers induced by the same vaccination as Pekin ducks and presented with higher morbidity and mortality after challenge with an H5N1 HPAI virus. When comparing the response to infection in non-vaccinated ducks, differences were also observed, with infected Muscovy ducks presenting a lower mean death time and more severe neurological signs than Pekin ducks. However Pekin ducks had significantly higher body temperatures and higher levels of nitric oxide in the blood at 2 days post challenge than Muscovy ducks, indicating possible differences in innate immune responses. Comparison of the expression of innate immune related genes in spleens of the non-vaccinated infected ducks showed differences including significantly higher levels of expression of RIG-I in Pekin ducks and of IL-6 in Muscovy ducks. Both duck species showed an up-regulation of IFNα and MHC-I expression, and a down-regulation of MHC-II. In conclusion, differences in response to infection and vaccination were observed between the two domestic duck species. This information should be taken into account when developing effective vaccination programs for controlling H5N1 HPAI in different species of ducks.

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1. Introduction

Highly pathogenic avian influenza (HPAI) subtype H5N1 virus infections are constantly monitored worldwide not only because of their negative effects on poultry, but also because of spread to humans and fear of a pandemic. The H5N1 HPAI viruses are widespread in poultry in Asia and have also spread to countries in the Middle East, Europe and Africa, causing great losses to commercial poultry production resulting from high mortality in affected flocks, loss of markets, as well as costs to prevent, manage, or eradicate the viruses and disease. Ducks have been implicated in the dissemination and evolution of H5N1 HPAI viruses [1–8]. In addition to their own economic importance, domestic ducks that are in contact with wild waterfowl and poultry function as key intermediates in the transmission of avian influenza and therefore are included in vaccination programs [9].

Since 2002, a number of H5N1 HPAI viruses have been found to cause disease and death in ducks [5,6,10–14], although the level of observed pathogenicity is not consistent among different H5N1 HPAI viruses [5,11], and the age and species of the ducks appear to influence the outcome of the infection [15–18]. Host immune responses most likely play a role in the differences observed in

* Corresponding author. Tel.: +1 706 5463419; fax: +1 706 5463161.
E-mail address: mary.pantin-jackwood@ars.usda.gov (M.J. Pantin-Jackwood).
[1] Present address: Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN 55108, USA.

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pathogenicity; however little is known about the immune response of ducks to AI virus infection. Vaccination has proven effective in protecting ducks against H5N1 HPAI and is being used in several countries to control the disease [19]; but virus infection may still occur in clinically healthy vaccinated populations, which may result in an endemic situation and in the emergence of antigenic variants [20]. Because of their proposed role in spreading H5N1 HPAI virus, it is of vital importance to control AI in domestic ducks. But improvement of AI control methods, including vaccination, depends on a better understanding of viral pathogenesis including host–pathogen interactions and host immune responses to infection.

Several studies have been conducted to evaluate vaccine efficacy in ducks against a HPAI virus lethal challenge [10,21–28]. Vaccine protection from infection and virus shedding varied depending on single or double-dose vaccination [26], and the challenge virus strain [21]. The majority of the published vaccine studies in ducks have been done in either Pekin (*Anas platyrhynchos domesticus*) [10,22–25] or mallard (*A. platyrhynchos*) ducks [29–31], and less research has been done using Muscovy ducks (*Cairina moschata*) [32,33], even though Muscovy ducks are economically significant as they are not only produced in Asia, but also represent 90% of the ducks hatched in France, the primary producer in Europe [33]. Vaccine efficacy studies in ducks conducted by Steensels et al. [25,32] using fowlpox-vectored AI vaccination (TROVAC AIV H5, rFP-AIV-H5) revealed oropharyngeal virus shedding in Muscovy ducks as late as 19 days post infection (dpi) while no shedding was detectable in Pekin ducks at any point after infection with the same HPAI H5N1virus. However, no research has been done comparing the responses of Pekin and Muscovy ducks to vaccination in the same study and under the same conditions.

In addition to response to vaccination, differences in virus pathogenicity among duck species have been reported. A comparison of three separate studies using either Pekin, Muscovy, or Mallard ducks all involving infection with viruses from the same H5N1 HPAI virus HA clade (2.2.1), dose, and mode of inoculation revealed differences in initial appearance of clinical signs and elapsed time to reach 100% mortality [33–35]. However, because the studies were done by different groups, multiple experiment variables could explain the differences. In a recent study, mallard and Muscovy ducks infected with different H5N1 HPAI viruses (HA clades 1, 2.3.2, and 2.3.4) showed clear differences in response to infection, with the Muscovy ducks presenting high mortality regardless of the virus given, contrary to the mortality in mallards which ranged from 0 to 100%, suggesting that Muscovy ducks are more susceptible to H5N1 HPAI virus infection [18]. Still, distinctions are not necessarily based on domestic versus wild ducks species differences. A single study comparing morbidity, mortality and viral shedding in five species of wild North American ducks with two different H5N1 HPAI viruses found only one of the five duck species became sick or died with either virus [15]. The two studies by Steensels et al. previously mentioned also showed differences in pathogenicity between Pekin and Muscovy ducks infected with the same clade 1 H5N1 HPAI virus, with only 20% of Pekin ducks presenting clinical signs compared to 100% of the Muscovy ducks [25,32].

Control of influenza virus infections in naive hosts is based on innate immunity and subsequent adaptive cytotoxic T and B cell immunity. The role of host immune elements in the control of AI virus infection in avian species is poorly understood. In addition, the potential contribution of host immune responses to the pathology observed in birds is largely undefined. Some studies have addressed individual innate immune genes expression in duck-origin cells infected with AI viruses by the use of RT-PCR assays [36,37], but studies exploring host gene expression in ducks infected with AI viruses have been very limited [38].

The purpose of this study was to compare the protection induced by vaccination against H5N1 HPAI in two types of domestic ducks, Pekin and Muscovy, using a commercially available inactivated vaccine. Three vaccine schedules were compared in order to determine which would confer the best protection before ducks were one month of age. The differences in pathogenesis and host's innate immune responses after infection with H5N1 HPAI virus were also examined. We expect to be able to explain the differences observed in the field in regards to vaccination and presentation of disease in these species of ducks.

2. Materials and methods

2.1. Virus and vaccine

The H5N1 HPAI virus A/Dk/Nam Dinh(VietNam)/NCVD-88/2007 (A/Dk/VN/88/07) (HA clade 2.3.4) was obtained from the National Center for Veterinary Diagnosis, Hanoi, Vietnam. The virus was inoculated into the allantoic cavity of 9 day-old embryonating chicken eggs and grown for 30 h at 37 °C. The allantoic fluid was harvested and frozen at −70 °C until further use. The commercially available inactivated reassortant avian influenza virus vaccine (H5N1 subtype, Re-1 Strain), produced by Harbin Veterinary Research Institute (Chinese Academy of Agricultural Sciences, Harbin, People's Republic of China) was used to vaccinate the ducks. This vaccine was produced by reverse genetics and derived it's HA and NA genes from A/Goose/Guangdong/96. This virus was attenuated by removing the multiple basic amino acids at the HA cleavage site. The six internal genes of this recombinant virus were derived from the high-growth A/Puerto Rico/8/34 (PR8) virus [39].

2.2. Duck vaccination experiment

One-day-old domestic white Pekin ducks and Muscovy ducks obtained from commercial farms were divided into groups as indicated in Table 1. Serum samples were collected from fifteen ducks of each species prior to vaccination to ensure that the birds were serologically negative for AI virus as determined by an ELISA test using IDEXX FlockChek AI MultiS-Screen (Idexx Laboratories, ME). Ducks were cared for and housed in accordance to an Institutional Animal Care and Use Committee approved animal use protocol at the Southeast Poultry Research Laboratory (SEPRL), Agricultural Research Service (ARS), United States Department of Agriculture (USDA), Athens, GA, USA. Experiments were performed in USDA-certified Biosafety Level 3-enhanced [40] facilities. Ducks had *ad libitum* access to feed and water.

Groups of ten ducks each were vaccinated subcutaneously in the nape of the neck with the recommended dose of Re-1 vaccine (0.2 ml of vaccine for the 1 day and 7 days-old ducks, and 0.5 ml for the 14 and 21 days-old ducks) following one of three different schedules (Table 1). Ten ducks were vaccinated at one day of age and at 14 days of age; ten only at 14 days of age, and ten at 7 and 21 days of age. Ten Pekin ducks and ten Muscovy ducks served as non-vaccinated infected controls and four Pekin and four Muscovy ducks served as non-vaccinated non-infected (sham inoculated) controls. At thirty days of age, blood samples were collected from all ducks for serology. At this same time, ducks from all vaccine schedules, including non-vaccinated controls were challenged intranasally via the choanal slit with $10^{5.0}$ EID_{50} of A/Dk/VN/88/07 H5N1 HPAI virus in 0.1 ml. Ducks were observed daily for clinical signs of disease. Oropharyngeal and cloacal swabs were collected at 3, 5, 7, 9, and 12 days post challenge (dpch) to quantify viral shedding. At 2 dpch body temperatures were taken from all ducks using a rectal thermometer. One-way ANOVA with Tukey's post-test was used to analyze body temperatures using Prism v.5.01