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Experimental co-infections of domestic ducks with a virulent Newcastle disease virus and low or highly pathogenic avian influenza viruses

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ABSTRACT

Infections with avian influenza viruses (AIV) of low and high pathogenicity (LP and HP) and Newcastle disease virus (NDV) are commonly reported in domestic ducks in many parts of the world. However, it is not clear if co-infections with these viruses affect the severity of the diseases they produce, the amount of virus shed, and transmission of the viruses. In this study we infected domestic ducks with a virulent NDV virus (vNDV) and either a LPAIV or a HPAIV by giving the viruses individually, simultaneously, or sequentially two days apart. No clinical signs were observed in ducks infected or co-infected with vNDV and LPAIV, but co-infection decreased the number of ducks shedding vNDV and the amount of virus shed ($P < 0.01$) at 4 days post inoculation (dpi). Co-infection did not affect the number of birds shedding LPAIV, but more LPAIV was shed at 2 dpi ($P < 0.0001$) from ducks inoculated with only LPAIV compared to ducks co-infected with vNDV. Ducks that received the HPAIV with the vNDV simultaneously survived fewer days ($P < 0.05$) compared to the ducks that received the vNDV two days before the HPAIV. Co-infection also reduced transmission of vNDV to naïve contact ducks housed with the inoculated ducks. In conclusion, domestic ducks can become co-infected with vNDV and LPAIV with no effect on clinical signs but with reduction of virus shedding and transmission. These findings indicate that infection with one virus can interfere with replication of another, modifying the pathogenesis and transmission of the viruses.

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1. Introduction

Avian influenza virus (AIV) and Newcastle disease virus (NDV) are two of the most economically important viruses affecting poultry worldwide (Alexander, 1995). These viruses transmit from their natural reservoirs, wild birds, to domestic birds initially producing subclinical infections and occasionally upper respiratory disease and drops in egg production (Swayne et al., 2013). More virulent forms of the viruses can arise and cause high mortality and great economic losses in poultry. Both, AIV and NDV are single-stranded, negative-sense RNA viruses. AIV's are type A Orthomyxoviruses and are classified as low pathogenicity (LP) and high pathogenicity (HP) viruses based on their virulence in chickens and the presence of multiple basic

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amino acids at the cleavage site of the hemagglutinin (HA) protein (Swayne et al., 2013). NDV's, also known as avian Paramyxovirus 1 (APMV1), are members of the genus *Avulavirus* in the Paramyxoviridae family (Miller and Koch, 2013). NDV's also vary in the type and severity of the disease they produce, and different pathotypes based on virulence in chicken and the sequences surrounding the protease cleavage site of the fusion (F) protein, have been described in poultry (Alexander and Senne, 2008; Miller and Koch, 2013). The original classification of NDV isolates into 1 of 3 virulence groups by chicken embryo and chicken inoculation as virulent (velogenic), moderately virulent (mesogenic), or of low virulence (lentogenic) has been abbreviated for regulatory purposes. Velogens and mesogens are now classified as virulent NDV (vNDV), the cause of Newcastle disease, whereas infections with lentogenic strains are the low virulence NDV widely used as live vaccines (Miller and Koch, 2013). The diseases produced by AIV and NDV remain one of the major problems affecting existing or developing poultry industries in many countries. Importantly, disease from vNDV and HPAIV are clinically indistinguishable.

Domestic ducks are economically important poultry, especially in Asian countries. Domestic ducks act as intermediate hosts of AIV between wild ducks and terrestrial poultry, with LPAIV's of many subtypes being isolated from domestic ducks (Huang et al., 2010; Kim et al., 2013). Historically, ducks naturally or experimentally infected with AIV's, including HPAIV's, only develop subclinical to mild disease. This dogma has been challenged since many Asian lineage H5N1 HPAIV's since 2002 have produced severe disease and mortality in ducks (Pantin-Jackwood and Swayne, 2009). Although waterfowl are a reservoir of NDV, the epidemiology of NDV in domestic ducks remains unclear. NDV has been isolated from domestic ducks in countries reporting endemic ND (Liu et al., 2009). Similar to AIV, genetically varied NDV found in domestic ducks suggests they may act as reservoir of different NDV genotypes and play a role in NDV epidemiology (Hu et al., 2010; Lee et al., 2009; Liu et al., 2009; Zhang et al., 2011b). In general, ducks show few if any clinical signs after NDV infection with strains lethal to chickens (Aldous et al., 2010; Anis et al., 2013; Dai et al., 2013; Otim Onapa et al., 2006; Tsai et al., 2004; Zhang et al., 2011a). However, some studies report NDV strains capable of causing clinical disease in ducks (Shi et al., 2011; Dai et al., 2014).

Natural co-infections of NDV and LPAIV have been documented numerous times in wild waterfowl and in domestic poultry (Couacy-Hymann et al., 2012; Dormitorio et al., 2009; Hanson et al., 2005; Molia et al., 2011; Rosenberger et al., 1974; Roussan et al., 2008; Shortridge, 1980). However, little is known on the interactions between these two viruses when simultaneously infecting poultry species including domestic ducks. We have previously demonstrated differences in virus shedding when chickens and turkeys were co-infected with a low virulence NDV and a LPAIV (Costa-Hurtado et al., 2014). Similarly, co-infection of mallard ducks with low virulence wild bird isolates of NDV and LPAIV did not affect the ability of the ducks to become infected with either virus but a minor effect on virus shedding was found (França et al., 2014).

Domestic ducks likely become co-infected with low and high virulence NDV, LPAIV and HPAIV in countries where these viruses circulate in poultry. It is not clear if co-infections exacerbate the diseases caused by these viruses, or if infection with one virus would interfere with infection by another. An effect of co-infection on virus replication could affect virus shedding and consequently transmission of the viruses to other hosts. This is information is important because it helps understand the epidemiology of these viruses in field situations aiding in the control of AI and NDV. The objective of this study was to examine co-infection of domestic ducks with a virulent NDV and a LPAIV or a HPAIV by infecting the ducks simultaneously or sequentially with the viruses. Pathogenesis (clinical signs, lesions), duration and titer of virus shed, seroconversion, and transmission were evaluated.

2. Materials and methods

2.1. Viruses

The following viruses were obtained from the Southeast Poultry Research Laboratory (SEPRL) virus repository: virulent NDV (vNDV): APMV-1/duck/Vietnam (Long Bien)/78/2002; LPAIV: A/Mallard/OH/421/1987 H7N8; and HPAIV: A/duck/VN/NCVD-672/2011 (H5N1). The APMV-1/duck/Vietnam, Long Bien/78/2002, was initially isolated from ducks in a Vietnamese poultry market and belongs to genotype VIId. This virus produces severe disease and death in chickens (Susta et al., 2011). The LPAIV is a wild duck isolate that has an infectious dose of 10^1 EID_{50} for ducks (E. Spackman, unpublished data). The HPAIV belongs to HA clade 2.3.2.1B and is highly virulent for ducks (Cha et al., 2013). The viruses were propagated in embryonating chicken eggs (ECE) as previously described (Senne, 2008). Allantoic fluid was diluted in brain heart infusion (BHI) medium (BD Bioscience, Sparks, MD) in order to obtain an inoculum with $10^{6-7.5}$ 50% egg infectious dose (EID_{50}) per bird in 0.1 mL. A sham inoculum was made using sterile allantoic fluid diluted 1:300 in brain heart infusion (BHI) medium (BD Bioscience, Sparks, MD). The experiment was performed in biosecurity level-3 enhanced (BSL-3E) and animal BSL-3E facilities at the SEPRL, United States Department of Agriculture, Agricultural Research Service, and procedure were reviewed by the SEPRL institutional biosecurity committee.

2.2. Birds

Pekin ducks (*A. platyrhynchos var. domestica*) were obtained at 1 day of age from a commercial hatchery. Serum samples were collected from 15 ducks to ascertain that the birds were serologically negative to NDV and AIV. At two weeks of age the ducks were housed in self-contained isolation units ventilated under negative pressure with inlet and exhaust HEPA-filtered air, and maintained under continuous lighting. Feed and water were provided with *ad libitum* access. Birds were cared for