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Quantitative transmission characteristics of different H5 low pathogenic avian influenza viruses in Muscovy ducks

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ABSTRACT

EU annual serosurveillance programs show that domestic duck flocks have the highest seroprevalence of H5 antibodies, demonstrating the circulation of notifiable avian influenza virus (AIV) according to OIE, likely low pathogenic (LP). Therefore, transmission characteristics of LPAIV within these flocks can help to understand virus circulation and possible risk of propagation. This study aimed at estimating transmission parameters of four H5 LPAIV (three field strains from French poultry and decoy ducks, and one clonal reverse-genetics strain derived from one of the former), using a SIR model to analyze data from experimental infections in SPF Muscovy ducks. The design was set up to accommodate rearing on wood shavings with a low density of 1.6 ducks/m^2: 10 inoculated ducks were housed together with 15 contact-exposed ducks. Infection was monitored by RNA detection on oropharyngeal and cloacal swabs using real-time RT-PCR with a cutoff corresponding to 2–7 EID50. Depending on the strain, the basic reproduction number (R_0) varied from 5.5 to 42.7, confirming LPAIV could easily be transmitted to susceptible Muscovy ducks. The lowest R_0 estimate was obtained for a H5N3 field strain, due to lower values of transmission rate and duration of infectious period, whereas reverse-genetics derived H5N1 strain had the highest R_0. Frequency and intensity of clinical signs were also variable between strains, but apparently not associated with longer infectious periods. Further comparisons of quantitative transmission parameters may help to identify relevant viral genetic markers for early detection of potentially more virulent strains during surveillance of LPAIV.

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1. Introduction

Avian influenza virus (AIV) infection is widespread in many different species of wild and domestic birds. Wild waterfowl (especially Anseriforms and Charadriiforms) are the natural reservoir of all currently described subtypes, combining 16 different hemagglutinin and 9 neuraminidase subtypes, and usually shed the virus without any symptoms (Alexander, 2000; Fouchier and Munster, 2009). Infection of terrestrial gallinaceous poultry, occurring by direct spillover from wild birds or indirect transmission of AIV, is usually asymptomatic or results in mild respiratory disease or drop in egg production (Pantin-Jackwood and Swayne, 2009). However, more severe symptoms with high mortality have also been associated with several different epizootics of H5 and H7 highly pathogenic (HP) AIV since 1959. Notably, these strains only rarely caused severe clinical signs or mortality following infection in domestic duck species, although Muscovy ducks were more susceptible to H5N1 HPAIV-induced disease than Pekin ducks (Cagle et al., 2011, 2012; Guionie et al., 2010; Pantin-Jackwood et al., 2013; Pantin-Jackwood and Swayne, 2009; Phuong et al., 2011). It could further be demonstrated that some of the outbreaks had actually taken place after evolution of the virus from low pathogenic (LP) to HP phenotype by nucleotide insertion in the hemagglutinin gene, following introduction and circulation of the virus in domestic species (Swayne and Halvorson, 2008). Therefore, H5 and H7 AIV are notifiable to OIE, the World Organization for animal health (OIE, 2013). To try and prevent such events, compulsory active surveillance of domestic bird flocks focused on H5 and H7 AIV has been implemented in the European Union, and results from these serological surveys point out domestic ducks and geese species as having the highest apparent seroprevalence for H5 and H7 subtypes (EURL AI, 2012).

Experimental and field data for both LP and HPAIV have already been obtained to estimate AIV transmission parameters between SPF chickens (Bouma et al., 2009; Claes et al., 2013; Gonzales et al., 2011; van der Goot et al., 2003, 2005), conventional native or broiler chickens in experimental settings (Poetri et al., 2009, 2011), conventional layer chickens in experimental settings (Gonzales et al., 2012a; Spekreijse et al., 2011), layer and multiplier chickens (Bos et al., 2009; De Jong et al., 2009; Gonzales et al., 2012b), mixed production type chickens (Soares Magalhães et al., 2010; Tiensin et al., 2007), turkeys (Bos et al., 2008, 2009, 2010; Comin et al., 2011; Saenz et al., 2012) and pheasants (van der Goot et al., 2007). Between-flock transmission parameters are also available from field infection data in undefined poultry species, aggregated at the farm/holding level (Boender et al., 2007; Garske et al., 2007; Mannelli et al., 2007; Mulatti et al., 2010; Stegeman et al., 2004) or at a higher village or sub-district level (Marquetoux et al., 2012; Ward et al., 2009; Zhang et al., 2013). HPAIV transmission parameters have also been estimated from transmission experiments in Pekin ducks (van der Goot et al., 2008) and teals (van der Goot et al., 2007), or obtained from field data on domestic duck flocks (Soares Magalhães et al., 2010) and wild birds (Iglesias et al., 2011; Penny et al., 2010). However, no such quantitative studies have been set up to study transmission of LPAIV in domestic duck species, despite their pivotal epidemiological role as an intermediate between virus pools in wild aquatic birds and terrestrial poultry. The two main species of domestic ducks and their hybrid are all highly receptive to AIV infection but usually express very few symptoms. Moreover Muscovy ducks are of great economic value, since it accounts for more than 80% of duck meat production in France and serves as part of the breeder stock for mule duck production. The risk of spillover from healthy virus shedding ducks within this specific population is therefore a major control point.

The present work was intended to fill this gap and compare experimentally estimated transmission parameters for three different H5 LPAIV field strains in SPF Muscovy ducks. One of these strains was used to regenerate a reverse-genetics-derived AIV that was also compared. This comparison would serve to check that the wild-type regenerated AIV could be transmitted the same way as the original field strain, and that molecular genetic markers of transmission could be identified this way.

2. Material and methods

2.1. Viruses

Three French H5 LPAIV field strains were selected based on previously described genotypes and full sequences: H5N1 A/duck/France/05066b/2005 (H5N1/05066b), H5N3 A/mallard/France/061054/2006 (H5N3/061054), and H5N2 A/chicken/France/03426/2003 (H5N2/03426) (Briand et al., 2010). The H5N1 LPAIV strain was recognized as a mixture of two subpopulations based on obtained full genomic sequences obtained by Sanger's method (Sanger et al., 1977). Two nucleotide variations were observed in the H5 coding sequence, yielding A200E and T341A mutations in the H5 protein: the latter lay within the precursor protein cleavage site (the given amino acid numbering refers to the H5 protein with its signal peptide).

The H5N1/05066b LPAIV was also regenerated using reverse genetics, after cloning all eight full segments from the original strain, as previously described (Hoffmann et al., 2000). The recombinant virus (H5N1/05066b rec) coding and non-coding sequences were checked against the parental field strain: the obtained clonal strain had the 200E and 341A markers on the H5 protein and corresponded to the most frequent virus subpopulation present in the parental strain multiplication used for transmission studies, as could be checked on the chromatograms for the parental sequence.

All virus stocks were grown and titrated on SPF chicken eggs before use: titration endpoints were calculated by the method of Reed and Muench.

2.2. Ducks

Specific pathogen free (SPF) Muscovy ducks (*Cairina moschata domestica*) were produced in Anses Ploufragan/Plouzané. They were reared in air-filtered holding facilities