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Circulation of influenza in backyard productive systems in central Chile and evidence of spillover from wild birds

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ABSTRACT

Backyard productive systems (BPS) are recognized as the most common form of animal production in the world. However, BPS frequently exhibit inherent biosecurity deficiencies, and could play a major role in the epidemiology of animal diseases and zoonoses. The aim of this study was to determine if influenza A viruses (IAV) were prevalent in backyard poultry and swine BPS in central Chile. Through active surveillance in Valparaiso and Metropolitan regions from 2012 – 2014, we found that influenza virus positivity by real-time RT-PCR (qRT-PCR) ranged from 0% during winter 2012–45.8% during fall 2014 at the farm level. We also obtained an H12 hemagglutinin (HA) sequence of wild bird origin from a domestic Muscovy duck (*Cairina moschata*), indicating spillover from wild birds into backyard poultry populations. Furthermore, a one-year sampling effort in 113 BPS in the Libertador Bernardo O'Higgins (LGB ÓHiggins) region showed that 12.6% of poultry and 2.4% of swine were positive for IAV by enzyme-linked immunosorbent assay (ELISA), indicative of previous exposure of farm animals to IAV. This study highlights the need for improved IAV surveillance in backyard populations given the close interaction between domestic animals, wild birds and people in these farms, particularly in an understudied region, like South America.

1. Introduction

Influenza A viruses (IAV) continue to cause diseases outbreaks in animals, including humans, and birds worldwide (Morens et al., 2013). Close interactions between human, swine, and wild and domestic bird viruses can lead to zoonotic spillover events, and subsequently the generation of novel viral strains through periodic exchanges of viral genes (Daszak et al., 2000; Neumann et al., 2009). Poultry production practices, trade of poultry, poultry products and spillover from wild birds have all been recognized as pathways by which avian influenza virus can spread locally and worldwide (Karesh et al., 2005; Gilbert et al., 2006; Kilpatrick et al., 2006; van den Berg, 2009).

Smallholder production, i.e. the many diverse forms of production including backyard productive systems (BPS), is practiced by most rural households throughout the world and presents a major IAV risk (FAO, 2010). BPS are typically defined as smallholder production farms, breeding less than 100 poultry and 10 pigs. These farms are generally maintained for sustenance farming and occasionally generate revenues from animals or product sales. Most concerningly, they generally have poor to absent biosecurity and are often located at the interface between wild and domestic animals making them prime locations for zoonotic and reverse zoonotic transmission of diseases (FAO, 2008; Smith and Dunipace, 2011; Hamilton-West et al., 2012). Indeed, the majority of BPS owners employ limited biosecurity measures and have limited knowledge of animal diseases; therefore, sick animals may be handled, sold, slaughtered and consumed without considering the risk to human health (Iqbal, 2009; FAO, 2010).

In Chile, there are two different realities in term of poultry and swine production. Commercial and industrial farming are very industrialized, operating both breeding and processing units with high biosecurity standards. Some of the bigger companies have even achieved a total integration of the production chain, by integrating food production, breeding and slaughter in one company (APA-ASOHUEVO, 2006; Hamilton-West et al., 2012). Moreover, live animal markets in Chile are uncommon and slaughtering of animals outside a certified facility is prohibited by law. However, in rural areas it is still possible to

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find slaughtering of animals in small size backyard breeding farms for household consumption of eggs and meat. Little is known about IAV in BPS in areas where IAV surveillance is lacking, such as South America (Butler, 2012). Backyard production systems in Chile represent more than 150,000 farmers, raising more than 3.7 million poultry and more than 400,000 pigs (Hamilton-West et al., 2012). Reports from other South American countries are limited to a few seroprevalence studies done in Argentina (Buscaglia et al., 2007), Ecuador (Hernandez-Divers et al., 2006), and Peru (Tinoco et al., 2015). Previous reports of IAV in BPS in Chile have been performed only during short seasons or at limited locations (Bravo-Vasquez et al., 2016; Bravo-Vasquez et al., 2017). Briefly, these studies demonstrated that IAV prevalence, as detected by qRT-PCR, was 27% of infected BPS in an area around a wetland during fall 2014 (Bravo-Vasquez et al., 2016). Seropositivity of backyard swine at a BPS level in this same study ranged between 42% and 60% in spring 2013 and fall 2014. Another study detected 32% seropositivity in backyard swine, but did not report IAV prevalence in poultry (Bravo-Vasquez et al., 2017). Therefore, the aim of this study was to determine the IAV prevalence in backyard poultry and swine in central Chile.

2. Materials and methods

2.1. Biosecurity and ethics statement

All sampling activities and protocols were approved by the ethics and biosecurity committee of Faculty of Veterinary Science (FAVET), University of Chile, by the Chilean National Commission for Technological Research (CONICYT), and by the St Jude Children's Research Hospital Institutional Animal Care and Use Committee (IACUC).

2.2. Study area and sample size

This study was carried out in central Chile, including the Valparaiso, Metropolitan and Libertador General Bernardo O'Higgins (LGB O'Higgins) regions. BPS were defined as rural households having up to 100 poultry (Hamilton-West et al., 2012) and up to 10 swine. Only BPS registered in a government subsidized development program were invited to participate in this study. Data presented in this study was gathered from two complementary active surveillance studies, carried out in adjacent locations and at overlapping time points.

Sampling included all poultry species and pigs present in the farm. A minimum of 5 poultry and 5 swine samples per farm were collected as described below. In cases where there were less than 5 animals on the farm, all animals were sampled. Due to lack of information regarding the influenza virus prevalence within BPS in South America and by estimating a high prevalence of IAV at the BPS if the animals would get infected, we assumed a prevalence ≥ 40% and 95% confidence to determine sample size, in order to identify at least one positive animal in each farm. The number of animals present in the farm and sensitivity and specificity of diagnostic tests were considered to adjust the sample size according to formula Eqs. (1) and (2) (Salman, 2003).

$$n = \frac{\log\ (1-c)}{[\log[Sp(1-p) + (1-Se)p]]} \quad (1)$$

Where,

n = Sample size
c = Desired confidence level
p = Disease prevalence
Se = Diagnostic test sensitivity Sp = diagnostic test specificity

$$n_c = \frac{n}{1 + \frac{n}{N}} \quad (2)$$

Where,

n_c = Corrected sample size
n = Sample size obtained with formula 1
N = Population (animals in the backyard farm)

2.3. Sampling and samples analysis

Cloacal and tracheal swabs were collected from poultry in BPS in the Valparaiso and Metropolitan regions throughout four seasons: winter (June–July) 2012; summer (March) 2013; spring (October-November) 2013; and fall (April) 2014) using disposable sterile swabs and stored in cryovials containing 1 mL Universal Transport Media, UTM™ (Copan Italia S.P.A). Samples were kept at 4 °C during sampling and stored at −80 °C until analysis. RNA extraction and real-time RT-PCR (qRT-PCR) analysis were performed at St. Jude Children's Research Hospital, Memphis, TN, USA as described (Karlsson et al., 2013). Briefly, viral RNA extraction was performed on 50 µL of swab sample on a Kingfisher Flex Magnetic Particle Processor (Thermo Fisher Scientific, Waltham, MA, USA) using the Ambion MagMax-96 AI/ND viral isolation kit (Life Technologies Corporation, Grand Island, NY, USA). Sample screening was done by qRT-PCR (Bio-Rad CFX96 Real-Time PCR detection System) with the TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems, Foster City, CA, USA). Specific primer/probes for the influenza matrix gene were used for the qRT-PCR reaction as described (WHO, 2009). Samples with a cycle threshold value (Ct) ≤38 were considered positive (Shu et al., 2011) and viral isolation on all samples with Ct ≤ 35 embryonated chicken eggs was attempted in as described (Lira et al., 2010).

2.4. Sequencing

Single stranded DNA was obtained using SuperScript Vilo™ (Life Technologies Corporation, Grand Island, NY, USA). Amplicons were obtained using Q5 High-Fidelity DNA polymerase (New England BioLabs, Ipswich, MA, USA) using universal oligonucleotide primers, as described elsewhere (Hoffmann et al., 2001). Further sequencing was performed by Sanger sequencing at the University of Wisconsin-Madison Biotechnology Center. Sequence is available under GenBank accession number KX101133.

2.5. Phylogenetic analysis

All publicly available avian H12 sequences at the Influenza Virus Resource (IVR) at NCBI greater than 1500 base pairs were used (n = 174). Duplicated sequences were removed prior to the phylogenic analysis. BEAST version 1.8.2 was used for the analysis (Drummond et al., 2005; Drummond et al., 2006; Drummond et al., 2012). An HKY85 substitution model was applied and we used time-stamped sequence data with a lognormal relaxed-clock Bayesian Markov chain Monte Carlo (MCMC) method. For each analysis, the Bayesian skyline coalescent tree prior model was used (10 groups). The starting tree was selected randomly. We performed four independent analysis of 50 million generations, sampled every 15000 generations. We then combined the output after removing burn-in (typically 10–20% of sampled chain). Twelve thousand estimated trees and parameters were summarized. FigTree version 1.4.2 was used for visualization of the annotated phylogenetic tree. The timing of the introduction of the H12 subtype into Chile was estimated by analyzing the times-scaled maximum clade credibility (MCC) tree.

2.6. Seroprevalence

During summer (December-March) 2012 and autumn (Abril-May) 2013, blood was collected from BPS located in LGB O'Higgins Region. Briefly, 1–3 and 3–5 mL of blood were collected from the brachial vein of each bird and from the marginal ear vein of each pig, respectively into a 6 mL vacutainer tube. Samples were kept at 4 °C during transport, centrifuged at 1300 g for 10 min and then stored at −20 °C until