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Presence of influenza viruses in backyard poultry and swine in El Yali wetland, Chile

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ABSTRACT

In South America little is known regarding influenza virus circulating in backyard poultry and swine populations. Backyard productive systems (BPS) that breed swine and poultry are widely distributed throughout Chile with high density in the central zone, and several BPS are located within the "El Yali" (EY) ecosystem, which is one of the most important wetlands in South America. Here, 130 different wild bird species have been described, of them, at least 22 species migrate yearly from North America for nesting. For this reason, EY is considered as a high-risk zone for avian influenza virus. This study aims to identify if backyard poultry and swine bred in the EY ecosystem have been exposed to influenza A virus and if so, to identify influenza virus subtypes. A biosecurity and handling survey was applied and samples were collected from BPS in two seasons (spring 2013 and fall 2014) for influenza seroprevalence, and in one season (fall 2014) for virus presence. Seroprevalence at BPS level was 42% (95% CI:22–49) during spring 2013 and 60% (95% CI 43–72) in fall 2014. rRT-PCR for the influenza A matrix gene indicated a viral prevalence of 27% (95% CI:14–39) at BPS level in fall 2014. Eight farms (73% of rRT-PCR positive farms) were also positive to the Elisa test at the same time. One BPS was simultaneously positive (rRT-PCR) in multiple species (poultry, swine and geese) and a H1N2 virus was identified from swine, exemplifying the risk that these BPS may pose for generation of novel influenza viruses.

1. Introduction

Wild birds, especially those related to aquatic environments, are considered as main reservoirs of influenza A virus (Webster and Hulse, 2004). Considering that all influenza A virus subtypes have the potential to contribute to the emergence of a pandemic strain through genetic reassortment, and the introduction of an avian or swine influenza virus in the human population may set the stage for influenza pandemic (Webster and Hulse, 2004; Olsen et al., 2006; Van Reeth, 2007). The World Health Organization, Food and Agriculture Organization of the United Nations and the World Organization for Animal Health, advise increasing the global surveillance of influenza virus to improve the preparedness and response to human and animal threats (WHO, 2005).

Chilean poultry and swine production are highly integrated at the industrial level where few companies represent more than 80% of the production, operating with high biosecurity standards (Hamilton-West et al., 2012). Nevertheless, other kinds of animal production are present in Chile, specifically backyard productive systems (BPS) for swine and poultry breeding. BPS are recognized as an important component of small farmers' livelihoods. Poultry species, like chicken, ducks and geese are the more commonly bred animals in BPS, followed by pigs. Usually both, poultry and pigs, do not represent the main economic activity of the farm, but are considered an important contribution to the household's food security and economies' (Randolph et al., 2007). In general, BPS have severe biosecurity deficiencies, which is concerning given that they represent an interface for interactions between domestic species, wild animals and humans (Iqbal, 2009; Conraths et al., 2011).

Given its more than 4000 km of coast, it is not surprising that Chile has several wetlands where thousands of local and wild birds migrating from the northern hemisphere nest and feed each winter. One of the most important wetlands in Chile is the National reserve "El Yali" (EY), located in Valparaíso Region. EY is a natural

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protected area of 520 ha, belonging to the Convention on Wetlands of International Importance especially as waterfowl habitat (Ramsar Convention) (Vilina et al., 2002). The EY ecosystem has a surface bigger than 11.500 ha and 130 different bird species have been described in EY, represented by the orders *Tinamiformes*, *Ciconiiformes*, *Accipitiformes*, *Passeriformes*, *Phoenicopteriformes*, *Falconiformes*, *Strigiformes*, *Galliformes*, *Columbiformes Anseriformes*, *Caprimulgiformes*, *Apodiformes*, *Piciformes*, *Charadriiformes*, *Gruiformes*, *Pelecaniformes*, *Podicipediformes* and *Sulidiformes*. EY is considered a priority site for influenza A virus introduction (SAG, 2006), since at least 22 are considered inter-hemispheric migratory species. Several BPS are located close to the reserve and within the ecosystem (Hamilton-West et al., 2012) raising concerns about the potential for these systems to be sites for emergence of new influenza strains.

To date, scarce data on influenza A virus in Chile are available. In domestic poultry, H7N3 (A/chicken/Chile/4977/02, GB|AY303634), pH1N1 (A/turkey/Chile/28317-6504-3/2009, GB| GQ866225) and H4N8 have been identified (SAG, 2013). In backyard poultry, there is just one study (Jimenez-Bluhm, unpublished results) which identified an H12NX in a domestic Muscovy duck (*Cairina moschata*) (A/Muscovy duck/Chile/3/2013, GB|KX101133) in central Chile. Therefore, this study aims to identify if backyard poultry and swine bred in BPS in the ecosystem of EY wetland have been exposed to influenza A virus, and if so, to identify viral strains.

2. Materials and methods

2.1. Sampling and samples analysis

The study units were backyard farms located in a radius of 3 km from the EY reserve boundaries. These farms were identified and invited to participate in the study. As BPS, we considered the productive units having up to 100 poultry (Hamilton-West et al., 2012) and up to 50 pigs.

BPS were sampled in two seasons (spring 2013 and fall 2014) for influenza A virus seroprevalence, and in one season (fall 2014) for prevalence. Since there was not information available regarding to influenza viruses within farm prevalence, 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 (Salman, 2003). Nevertheless, a minimum of five samples were collected, and in cases when there were less than five animals in the farm, all of them were sampled. Sampling included all poultry species and pigs present in the farm.

Blood was collected from the brachial vein of birds (1–3 mL) and from the marginal ear vein of pigs (3–5 mL) and placed into a 6 mL vaccutainer®. Samples were kept at 4 °C during transportation and serum was obtained by centrifugation at 1,300g for 15 min at Faculty of Veterinary Science of University of Chile (FAVET), and then stored at −20 °C until analysis.

To screen for the presence of influenza A virus, pools of cloacal swabs (poultry) and nasal swabs (swine) were collected in tubes containing 1 mL universal viral transport medium (Healthlink®). Samples were collected using disposable sterile swabs, and at least one pool per animal species present in the farm was collected. Each pool consisted in at most 9 swabs from animals of the same species (Ladman et al., 2012), and if more than 9 samples had to be collected, another pool of the same specie was added. Samples were maintained at 4 °C until arrival to FAVET, where the samples were stored at −80 °C until analysis.

Influenza A virus seroprevalence was determined using the ELISA assay (IDEXX Influenza A Ab Test) following manufacturer's instructions (Sensitivity: 95.4% and Specificity 99.7% for poultry, and Se: 95.3% and Sp: 99.6% for pigs) (Idexx, 2016). Plates were read using an INMUNSKAN Plus (BDSL) microplate reader. We considered a BPS positive if at least one sample gave positive results.

RNA extraction was performed using Trizol LS following manufacturer's instructions (Invitrogen). Purified RNAs were protected by adding RiboLock (Life Technologies) and then stored at −80 °C until amplification. RNA was amplified using real time reverse transcriptase PCR (rRT-PCR) in Mx3000P™ Stratagene (Agilent Technologies). Specific primers and probe were used in order to detect influenza A virus matrix gene as described by CDC (2009). The reaction mixture consisted of 3 μL of RNA, 5 μL of TaqMan Fast Virus 1-Step Master Mix (4x) (Life Technologies), 0.6 μL of each Inf A forward and reverse primers, 0.4 μL Inf A probe and 10.4 μL of RNAase free molecular grade water for a 20 μL reaction. The thermal profile included a 50 °C reverse transcription cycle during 5 min, a 95 °C cycle for AmpliTaq® Fast DNA Polymerase UP activation during 20 s and 40 amplification cycles (95 °C during 3 s and 60 °C during 30 s). Samples under 38 threshold value (Ct) were considered positive. Swine influenza virus subtyping was carried out using H1/H3 and N1 specifics primers and probes assays (Richt et al., 2004; CDC, 2009; Gunson et al., 2010). Subtype was confirmed by Sanger sequencing at St. Jude Children Research Hospital (SJCRH) using segment specific primers as described by Hoffmann et al. (2001). We considered BPS positive if at least one sample gave positive results.

In each BPS, a questionnaire was applied by a semi-structured interview to backyard owners to collect information regarding to animal handling and biosecurity measures.

2.2. Data analysis

A database was built into a Microsoft Excel® spreadsheet which included the information collected in field activities and laboratory results. Firstly, descriptive statistics were provided to characterize the BPS, taking into consideration structure, biosecurity and trade elements. Then influenza A virus prevalence and seroprevalence were estimated. Finally, the independent variables obtained in the survey were tested as risk factors for the presence of antibodies against influenza A virus (ELISA) and influenza A virus (rRT-PCR), by a logistic regression model (Dohoo et al., 2009) using R statistical software (http://www.r-project.org).

3. Results

3.1. BPS characterization

Forty six BPS were identified in the study site, from which 40 agreed to participate in the study. The total number of animals present in these farms was 1798 chickens, 45 turkeys, 34 pigs, 25 geese and 5 ducks. The median animals by farm were 30 domestic chickens (min:6, max:80), 0 turkeys (min:0, max:23), 0 geese (min:0, max:14), 0 ducks (min:0, max:5), and 0 pigs (min: 0, max: 20). Fifty five percent of the owners breed animals for sales to neighbors, family and tourists. The other 45% of the owners primarily keep animals for household consumption. In terms of confinement, 70% of the households keep their animals in a mixed confinement system, i.e., free ranging during the day and confined during night, while 25% are free-range and 5% are permanently confined. When asked how they handled mortalities, the most common answers were that they burnt (43%) or buried (23%) their dead animals, while 13% throw their dead animals in the garbage, or throwing them far away (10%). Three percent of the owners did nothing, leaving the dead animals just as they found them. It is important to mention that 10% of the participants did not want to answer this question.

Table 1
Management and biosecurity conditions in BPS that keep poultry and swine, close to the El Yali reserve.

Variable	Definition and Classification	N°	%
Farm management and biosecurity conditions			
Aim animal husbandry	Sale	22	55
	Household consumption	18	45
Confinement	Mixed stabling	28	70
	Free ranging	10	25
	Permanent stabling	2	5
Mortality handling	Burn dead animals	17	42.5
	Bury dead animals	9	22.5
	Throw dead animals to the garbage	5	12.5
	Throw dead animals far away	4	10
	Do not want to answer	4	10
	Do nothing with dead animals	1	2.5
Contact with wild birds	Animals do contact wild birds	29	72.5
	Animals do not contact wild birds	11	27.5
Contact with neighbor's animals	Animals do contact neighbor's animals	39	97.5
	Animals do no contact neighbor's animals	1	2.5
Main buyer	Household consumption	17	42.5
	Neighbors and family	14	35
	Neighbors, family and tourists	9	22.5

When asked if they had support of veterinarians, only 2 owners said they did (5%) (Table 1).

3.2. *Influenza a virus seroprevalence and prevalence at BPS level*

For the first period 31 BPS were sampled, and 42% (95% CI:22–49) of these BPS were influenza A virus seropositive, which increased to 60% (95% CI 43–72) during the 2014 sampling (40 BPS sampled). Nine BPS were seropositive in both sampling periods (Fig. 1). Twenty seven percent (95% CI: 14–39) of BPS were positive to the rRT-PCR assay for influenza A virus M gene (Ct value < 38) (Fig. 1). Ct values ranged from 33.69 to 37.82. Regrettably, even though these values are well below the rRT-PCR Ct cutoff value of 38, they are considered high for attempting direct subtyping of the samples or viral isolation in embryonated chicken eggs (Karlsson et al., 2013), thus no subtype information could be obtained. Eight farms (73% of rRT-PCR positive farms) were also positive to the Elisa test at the same time. Interestingly, one BPS was influenza A virus positive simultaneously for multiple species (poultry, swine and geese). From that farm, a swine virus was subtyped as an H1N2.

4. Discussion

Avian influenza viruses are circulating in wild birds in South American, mainly in the form of low pathogenic virus (Pereda et al., 2008; Bruno et al., 2009; Karlsson et al., 2013; Mathieu et al., 2015; Nelson et al., 2016). However, a persistent gap in knowledge about avian influenza virus in South America exists primarily due to a lack of surveillance (Olson et al., 2014). This lack is even more evident when looking for specific information in backyard populations. This study identifies serological and virological evidence of influenza A virus circulating BPS situated close to the EY wetland. Other studies in South American countries show a variety of results. Argentina conducted a surveillance program for influenza A virus in backyard poultry during 1998–2005 where all samples were negative. While Ecuador has found 11% influenza A virus seropositivity, Peru has reported backyard swine antibody prevalence to pH1N1 of 0% at pre-pandemic, 8% at the peak of the human pandemic (October 2009), and 24% in April 2010 and 1% in October 2011 (post-pandemic) (Hernandez-Divers et al., 2006; Buscaglia et al., 2007; Tinoco et al., 2015). Diverse studies have showed seasonality in the prevalence of influenza virus (Halvorson et al., 1985; Li et al., 2004; Park and Glass, 2007). This phenomenon has been attributed to higher population densities due to pre migratory congregation of waterfowl and due to a higher proportion of immunologically naïve fledglings to avian influenza virus (Webster et al., 1992). This is consistent with our results, since BPS had lack of biosecurity conditions and contact among domestic and wild birds with wild birds is highly probable. During the different seasons there are different populations of wild birds in El Yali wetland. To date, a population

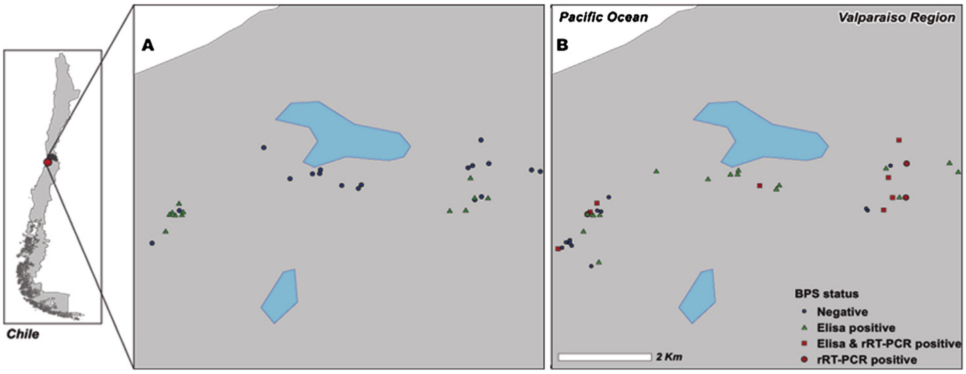

Fig. 1. Spatial distribution and influenza status of BPS close to the El Yali reserve, during 2013 and 2014.
Results of sampling performed during spring 2013 (A), and fall 2014 (B)

of 130 wild bird species have been described in the study site, however, of these, at least 22 are considered inter-hemispheric migratory species, which begin to arrive to South America during spring, staying in Chile up to fall. During fall, migratory birds begin to migrate to the northern hemisphere, leaving only the residents species in Chile, while during spring and summer we can see that resident species mingle with migratory species. Influenza virus is also known to be more prevalent in younger birds, which might influence the discussion of the results (Olsen et al., 2006), for wild bird populations, resident birds have their nesting period in southern hemisphere spring-summer season, so during fall, more younger individuals can be expected, increasing the prevalence of influenza virus in these populations. In BPS we can expect the same dynamics, since farms usually do not have light management, so younger birds are present during spring-summer seasons. However, due to poor flock management and recordkeeping at most BPS, it was not possible to have the precise information regarding to the age of the sampled birds, which added to the difficulty to distinguish between, for instance, a chicken of more than 1 year with one of only 6 months of age.

On the other hand, given the dynamics of backyard production, there is a possibility that the animals were consumed or traded during the study period, therefore, what we see in the different sampling seasons do not necessarily correspond to the same animals. Thus, further studies are needed to address properly seasonality trends.

The BPS' characterization shows similar results to what has been described before in a previous study in Chile (Hamilton-West et al., 2012), where people raising animals for household consumption and sales generate a flow of animals/products that are produced in an environment where a lack of biosecurity conditions exists. Even though, when analyzing through logistic regression the different variables as possible risk factors for the presence of influenza antibodies and/or virus, no association was observed. This could be explained by the little variability of management and biosecurity conditions observed between the different BPS. Still, the ways in which animals are handled reflect a high likelihood of contact between domestic animals, wild birds and humans. In this sense, the backgrounds that are taking place in this area of high-risk where there are migratory birds altogether with BPS, give rise to the conditions for the emergence of new variants with zoonotic components (Iqbal, 2009; Conraths et al., 2011).

In the present study an H1N2 virus was identified. This is the first report of swine influenza virus from pigs in backyard in Chile. Previous studies have demonstrated the presence of H1N2 subtypes from a human origin in industrial swine farms with decades of independent evolution (late 1980′s and early 1990′s) and evidence of reassortment with swine viruses. It would be interesting to investigate if the BPS H1N2 virus is related with H1N2 obtained from industrial farms or whether it has a parallel evolution in BPS (Nelson et al., 2014). Additionally, in the same backyard from which this isolate was identified, positive poultry and geese samples were also found, representing a risk for the reassortment of genomic segments from different viruses (Alexander, 2007). Thus, the BPS H1N2 virus may represent a risk for both human and animal population.

5. Conclusion

Influenza A viruses are circulating in BPS located close to El Yali wetland, where yearly several inter hemispheric migratory bird species arrives and cohabitate with other important number of resident wild birds. Surveillance activities must be increased in order to identify if influenza viruses in BPS are related to viruses circulating in wild birds populations and also preventive measures, such as enhanced biosecurity, must be engaged in BPS to reduce the risk for both, public and animal health in high risk areas for influenza virus.

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References

Alexander, D.J., 2007. An overview of the epidemiology of avian influenza. Vaccine 25, 5637–5644.

Bruno, M.G., David, L.B., Eliana, I., Rosa, I.G., Tadeusz, J.K., Yeny, T., Merly, M.S., Stephen, L., Bo, S., Alexander, K., Armando, E.G., Joel, M.M., 2009. Avian influenza in wild birds, Central Coast of Peru. Emerg. Infect. Dis. J. 15, 935.

Buscaglia, C., Espinosa, C., Terrera, M., De Benedetti, R., 2007. Avian influenza surveillance in backyard poultry of Argentina. Avian Dis. 51, 467–469.

CDC, 2009. CDC Protocol of Realtime RT-PCR for Influenza A(H1N1).

Conraths, F.J., Schwabenbauer, K., Vallat, B., Meslin, F.-X., Füssel, A.-E., Slingenbergh, J., Mettenleiter, T.C., 2011. Animal health in the 21 st century—a global challenge. Prev. Vet. Med. 102, 93–97.

Dohoo, I., Martin, W., Stryhn, H., 2009. Veterinary Epidemiologic Research, 2nd ed. Charlottetown, VER inc, pp. 395–422, Chapter 16.

Gunson, R., Maclean, A., Davies, E., Bennett, S., Miller, R., Carman, W.F., 2010. Development of a multiplex real-time RT-PCR that allows universal detection of influenza A viruses and simultaneous typing of influenza A/H1N1/2009 virus. J. Virol. Methods 163, 258–261.

Halvorson, D.A., Kelleher, C.J., Senne, D.A., 1985. Epizootiology of avian influenza – effect of season on incidence in sentinel ducks and domestic turkeys in minnesota. Appl. Environ. Microb. 49, 914–919.

Hamilton-West, C., Rojas, H., Pinto, J., Orozco, J., Herve-Claude, L.P., Urcelay, S., 2012. Characterization of backyard poultry production systems and disease risk in the central zone of Chile. Res. Vet. Sci. 93, 121–124.

Hernandez-Divers, S.M., Villegas, P., Prieto, F., Unda, J.C., Stedman, N., Ritchie, B., Carroll, R., Hernandez-Divers, S.J., 2006. A survey of selected avian pathogens of backyard poultry in Northwestern Ecuador. J. Avian Med. Surg. 20, 147–158.

Hoffmann, E., Stech, J., Guan, Y., Webster, R.G., Perez, D.R., 2001. Universal primer set for the full-length amplification of all influenza A viruses. Arch. Virol. 146, 2275–2289.

Idexx, 2016. IDEXX Influenza A Ab Test, from https://www.idexx.com/livestock-poultry/poultry/avian-influenza.html.

Iqbal, M., 2009. Controlling avian influenza infections: the challenge of the backyard poultry. J. Mol. Genet. Med. 3, 119–120.

Karlsson, E.A., Ciuoderis, K., Freiden, P.J., Seufzer, B., Jones, J.C., Johnson, J., Parra, R., Gongora, A., Cardenas, D., Barajas, D., Osorio, J.E., Schultz-Cherry, S., 2013. Prevalence and characterization of influenza viruses in diverse species in Los Llanos, Colombia. Emerg. Microbes Infect. 2.

Ladman, B.S., Spackman, E., Gelb Jr., J., 2012. Comparison of pooling 11 or 5 oropharyngeal swabbings for detecting avian influenza virus by real-time reverse transcription-PCR in broiler chickens. Avian Dis. 56, 227–229.

Li, K.S., Guan, Y., Wang, J., Smith, G.J.D., Xu, K.M., Duan, L., Rahardjo, A.P., Puthavathana, P., Buranathai, C., Nguyen, T.D., Estoepangestie, A.T.S., Chaisingh, A., Auewarakul, P., Long, H.T., Hanh, N.T.H., Webby, R.J., Poon, L.L.M., Chen, H., Shortridge, K.F., Yuen, K.Y., Webster, R.G., Peiris, J.S.M., 2004. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. Nature 430, 209–213.

Mathieu, C., Moreno, V., Pedersen, J., Jeria, J., Agredo, M., Gutiérrez, C., García, A., Vásquez, M., Avalos, P., Retamal, P., 2015. Avian influenza in wild birds from Chile, 2007–2009. Virus Res. 199, 42–45.

Nelson, M., Culhane, M.R., Rovira, A., Torremorell, M., Guerrero, P., Norambuena, J., 2014. Novel human-like influenza a viruses circulate in swine in Mexico and Chile. PLoS Curr. 7.

Nelson, M.I., Pollett, S., Ghersi, B., Silva, M., Simons, M.P., Icochea, E., Gonzalez, A.E., Segovia, K., Kasper, M.R., Montgomery, J.M., Bausch, D.G., 2016. The genetic diversity of influenza a viruses in wild birds in Peru. PLoS One 11, e0146059.

Olsen, B., Munster, V.J., Wallensten, A., Waldenström, J., Osterhaus, A.D.M.E., Fouchier, R.A.M., 2006. Global patterns of influenza a virus in wild birds. Science 312, 384–388.

Olson, S.H., Parmley, J., Soos, C., Gilbert, M., Latorre-Margalef, N., Hall, J.S., Hansbro, P.M., Leighton, F., Munster, V., Joly, D., 2014. Sampling strategies and biodiversity of influenza a subtypes in wild birds. PLoS ONE 9, e90826.

Park, A.W., Glass, K., 2007. Dynamic patterns of avian and human influenza in east and southeast Asia. Lancet Infect. Dis. 7, 543–548.

Pereda, A.J., Uhart, M., Perez, A.A., Zaccagnini, M.E., La Sala, L., Decarre, J., Goijman, A., Solari, L., Suarez, R., Craig, M.I., Vagnozzi, A., Rimondi, A., König, G., Terrera, M.V., Kaloghlian, A., Song, H., Sorrell, E.M., Perez, D.R., 2008. Avian influenza virus isolated in wild waterfowl in Argentina: evidence of a potentially unique phylogenetic lineage in South America. Virology 378, 363–370.

Randolph, T.F., Schelling, E., Grace, D., Nicholson, C.F., Leroy, J.L., Cole, D.C., Dentment, M.W., Omore, A., Zinsstag, J., Ruel, M., 2007. Invited Review: role of livestock in human nutrition and health for poverty reduction in developing countries. J. Anim. Sci. 85, 2788–2800.

Richt, J.A., Lager, K.M., Clouser, D.F., Spackman, E., Suarez, D.L., Yoon, K.-J., 2004. Real–time reverse transcription–polymerase chain reaction assays for the detection and differentiation of north american swine influenza viruses. J. Vet. Diagn. Invest. 16, 367–373.

SAG, 2006. Proyecto nacional de vigilancia epidemiológica en enfermedades aviares exóticas de la lista a de la OIE y en bronquitis infecciosa renal 2004–2007.

SAG, 2013. Carta Nº 2208. Respuesta a solicitud Nº AR006W-0000900, sobre acceso a la información Pública Ley Nº20.285.

Salman, M.D., 2003. Animal Disease Surveillance and Survey Systems, Methods and Applications, 1st ed. Blackwell Publishing, pp. 47–66, Chapter 4.

Tinoco, Y.O., Montgomery, J.M., Kasper, M.R., Nelson, M.I., Azziz-Baumgartner, E., Gilman, R.H., Bausch, D.G., Gonzalez, A.E., 2015. Transmission dynamics of pandemic influenza a (H1N1) pdm09 virus in humans and swine in backyard farms in tumbes, Peru. Influenza Respir. Viruses.

Van Reeth, K., 2007. Avian and swine influenza viruses: our current understanding of the zoonotic risk. Vet. Res. 38, 243–260.

Vilina, Y.A., Cofré, H.L., Silva-García, C., García, M.D., Pérez-Friedenthal, C., 2002. Effects of El Niño on abundance and breeding of black-necked swans on el yali wetland in Chile. Waterbirds, 123–127.

WHO, 2005. WHO Global Influenza Preparedness Plan.

Webster, R., Hulse, D., 2004. Microbial adaptation and change: avian influenza. Rev. Sci. Tech. Off. Int. Epiz. 23, 453–465.

Webster, R.G., Bean, W.J., Gorman, O.T., Chambers, T.M., Kawaoka, Y., 1992. Evolution and ecology of influenza-a viruses. Microbiol. Rev. 56, 152–179.