NOTE

Avian Pathology

Ecuadorian mainland industrial poultry production is free of H5/H7 Avian influenza virus: National surveillance program in 2016

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ABSTRACT. Avian influenza (AI) is a disease caused by influenza viruses type A that belong to the *Orthomyxoviridae* family. AI induces high economic losses in poultry production worldwide. Due to a possible outbreak, a national surveillance program was needed. From April to July 2016, 152 industrial poultry farms were randomly sampled. All samples were analyzed by competitive ELISA for Influenza type A viruses. Suspicious and positive sera were further analyzed by Hemagglutination Inhibition (HI) in order to serotype H5 or H7 low pathogenic avian influenza virus (LPAIV). The farms sampled showed 94.08%, 3.95% and 1.97% of negative, positive and suspicious results, respectively. However, serotyping revealed all positive and suspicious samples were negative to H5/H7 LPAIV. Our results show the absence of AI in the mainland Ecuadorian industrial poultry production.

KEY WORDS: broiler, Ecuador, high pathogenicity avian influenza, laying hens, low pathogenicity avian influenza

Poultry production is one of Ecuador's most important industries. In 2016, the annual consumption of poultry was estimated at 33.1 kg per capita [26] and egg consumption accounted for 165 units per capita [23]. Thus, diseases that reduce the efficiency of bird growth may lead to economic losses and present risk to the poultry industry [9]. Avian influenza (AI) is one such disease that imposes substantial economic loss due to low productive performance, high mortality rates (up to 100%), depopulation of infected farms, transport restrictions, and culling of animals in farms within the affected region [1, 9, 11, 21].

AI is caused by RNA viruses belonging to the *Orthomyxoviridae* family [15]. This family includes influenza viruses A, B, C and D [13]. Influenza A viruses are the etiological agents of avian influenza [5]. Avian Influenza A viruses are classified based on: 1) Viral surface antigens, hemagglutinin (H1-H16) and neuraminidase (N1-N9) [1] and 2) Pathogenicity in chickens, including low pathogenicity avian influenza (LPAIV) and high pathogenicity avian influenza viruses (HPAIV) [15].

According to the Sanitary Code for Terrestrial Animals developed by the World Organization for Animal Health (OIE), avian influenza is an infection of poultry caused by any HPAI type A viruses, as well as, H5 and H7 subtypes of LPAIV. When detected in poultry, notification is obligatory [15]. HPAIV strains cause a highly fatal systemic disease that include severe respiratory signs among birds, can be easily transmitted to other species, including humans [16, 22].

To the best of our knowledge, there has been no report of HPAIV outbreaks in Ecuador [14]. In 2015, Ecuador's Agriculture Quality Assurance Agency (AGROCALIDAD) notified the OIE non-outbreak status of HPAI in the Ecuadorian poultry industry. Hence, Ecuador has been considered an AI-free country like the majority of South American countries [17]. However, in the last few years, AI outbreaks have been reported in other countries in the Americas, alerting stakeholders of the poultry industry to the risk of AI dissemination within the continent.

Previous studies suggested that the risk of AI introduction to a susceptible population can be associated to the type of poultry production system (multi-age farm, poor biosecurity measures), presence of wild migratory birds (natural reservoirs of AI), legal and illegal trade of live birds, and direct contact with infected fomites [7]. All of these risk factors are present in Ecuador. Consequently, Ecuador's Regulation and Control Phyto and Zoosanitary Agency (AGROCALIDAD) implemented a national surveillance program in 2016 [4]. Building on these emerging risks within the poultry industry, this study aimed to determine the presence of AI viruses, specifically H5 and H7 subtypes in Ecuadorian poultry farms located in the mainland area of Ecuador.

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This cross-sectional study was carried out from April to July 2016, as part of the national avian influenza surveillance program. Our sample was calculated based on the 1,802 registered poultry farms in Ecuador [3]. Sample size was calculated for each type of production (broiler chicken, broiler breeding, and laying hens) using ProMESA v1.62 software, considering the formula to detect the presence of the disease in a population with 95% of confidence and 50% of prevalence [25]. A total of 152 farms were randomly selected and sampled independently of farm location. In this way, it was guaranteed that each element had the same opportunity to be included in the sample (Table 1).

From each farm, 25 animals were blood sampled for further laboratory diagnosis at the Animal Health laboratory of AGROCALIDAD. A farm was categorized as positive to Influenza A virus if at least one sample of the 25 showed antibodies against the virus. The screening of AI was firstly carried out with a commercial kit Screen® Influenza A Antibody Competition Multi-species20 (ID.vet genetics, France). All positive and suspicious samples diagnosed by ELISA were further analyzed by Hemagglutination-inhibition assay (HI) to identify H5 and H7 serotypes in accordance with OIE recommendations [15]. To this, the blood samples were heat treated (56°C) and H5N2 (Aphis; A/turkey/Minnesota/3689-1551/1981) and H7N3 (Aphis; A/chicken/Chile/176822/02) virus strains with their corresponding hyperimmune antisera were used.

From the 152 sampled farms tested by ELISA, six farms were positive (3.95%), three were suspicious (1.97%) and 143 were negative (94.08%) to Influenza A. The number of positive farms to Influenza A, were equally distributed according to type of production: two (7.14%) in broiler farms, 2 (2.94%) in laying hen farms, and two (3.57%) in broiler breeding farms (Table 2).

Table 1. Distribution of sampled poultry farms according to its location and type of production

Location (Province)	Broiler chickens		Broiler-breeders		Laying hens		Total
	Registered Farms [a]	Sampled farms	Registered Farms [a]	Sampled farms	Registered Farms [a]	Sampled farms	
Azuay	210	12 [b]	3	1	4	1	14
Bolívar	26	1	-	-	1	1 [b]	2
Cañar	32	2	-	-	1	0	2
Carchi	11	0	1	1	-	-	1
Chimborazo	39	2 [b]	-	-	19	5	7
Cotopaxi	20	1	2	0	41	10 [b]	11
El Oro	253	10	2	1	3	1	12
Esmeraldas	6	0	1	1	-	-	1
Guayas	59	3	7	3	1	1	7
Imbabura	48	5	5	2 [c]	-	-	7
Loja	47	2	1	0	1	1	3
Los Ríos	27	0	5	4	-	-	4
Manabí	98	3	1	1	50	5	9
Morona Santiago	19	1	-	-	-	-	1
Napo	8	0	6	3	-	-	3
Orellana	17	1	-	-	-	-	1
Pastaza	45	3	8	6 [b]	-	-	9
Pichincha	220	12	8	3 [b]	32	5	20
Santa Elena	15	0	4	1	1	1	2
Santo Domingo	155	8	4	1	5	2	11
Sucumbíos	11	1	-	-	1	0	1
Tungurahua	44	1	-	-	149	22 [c]	23
Zamora Chinchipe	24	0	-	-	1	1	1
Total	1,434	68	58	28	310	56	152

a) Data based in 2015 poultry census. b) Positive sera in at least a farm (Chimborazo presented 3 positive sera in the same farm). c) Suspicious sera in at least a farm (Tungurahua presented 2 suspicious sera in different farms).

Table 2. Frequency of Influenza viruses in Ecuadorian poultry farms according to its type of production

Farms	AI diagnosis by ELISA			
	N	Negative (%)	Suspicious (%) [a]	Positive (%) [a]
Laying hens	56	52 (92.86)	2 (3.57)	2 (3.57)
Broilers	68	65 (95.59)	1 (1.47)	2 (2.94)
Broiler breeding	28	26 (92.86)	0 (0.00)	2 (7.14)
Total	152	143 (94.08)	3 (1.97)	6 (3.95)

a) Samples were further analyzed by hemagglutination-inhibition assay and were negative for H5N2 and H7N3 IALP subtypes.

Suspicious cases were identified in both broiler and laying hen farms whereas no suspicious cases were identified in broiler breeding farms. However, after confirmation with HI test, suspicious and positives farms to Influenza "A" were all negative to H5N2 and H7N3 subtypes. That is, 6 farms were found to be positive and 3 were found to be suspicious to Influenza type A; However, all were negative to H5 and H7 serotypes.

No relationships were found between Influenza "A" positive or suspicious farms, since they were from different companies, type of poultry and location. However, the presence of those viruses in broiler chickens and laying hens farms could be attributed to their proximity to urban centers. Broiler breeder farms, on the other hand, were located near to wildlife areas (foothills of the Andes and Amazon region).

Reports and studies in South America have been limited. However, in 2016, an outbreak of H7 LPAIV subtype in Chile was reported, leading to the closure of poultry products to international trade [19]. While a suspicious outbreak caused by H7 LPAIV was reported in Colombian broilers, after an in-depth analysis no pathogenic variant was found [10]. Furthermore, in 2017, four HPAIV outbreaks were reported in Mexico, two outbreaks caused by H5N2 and H7N9 serotypes affected broilers farms and two caused by H7N3 affected laying hen farms, broiler, and combat birds [16].

Although, there is no report of AI in the Ecuadorian poultry production, outbreaks may occur as a consequence of the type of poultry production system (intensive and multi-age farms), legal and illegal trade of live birds, and migratory wild birds (natural reservoirs of AI virus) [7]. In fact, studies carried on by Otte *et. al.* and Leibler *et. al.* have shown that the occurrence and spread of AI was associated with overcrowding in intensive poultry production [12, 18]. Despite that 90% of poultry production in Ecuador utilizes an intensive production system [26], our results showed an absence of OIE notifiable AI viruses among industrial poultry farms.

Increasingly, H5 and H7 subtypes have been found in wild birds in recent years [2]. Phylogenetic studies have shown that transboundary movement of migratory birds (especially aquatic birds) is associated with the spread of AI viruses [8, 20]. These findings pose a great concern due to the high diversity of birds in Ecuador including migratory birds such as *Anas discor* (Blue-winged teal), *Anas acuta* (Northern pintail), and *Anas cyanoptera* (Cinnamon teal) [6]. In addition, more than 300 species of endemic aquatic birds are found in Ecuador [6] and could act as natural reservoirs of AI viruses [27]. As a consequence, wild birds may play an important role in the spread of AI viruses among susceptible species, including humans. Therefore, further studies are needed to elucidate the possible participation of these birds in the transmission pathway of AI in Ecuador.

It is important to identify the limitations of our study. The start sample size was estimated in 163 poultry farms based in 2015 poultry census for each production type. Since some selected farms were not available at the time of sampling (some of them had been closed or were not in production), they could not be included in the study. However, all provinces were sampled in at least one type of poultry (broiler, laying hen, and broiler breeding). This work did not consider backyard poultry farms, which are also susceptible to AI viruses [15]. Ecuador does not have updated information related to the number of backyard farms, but it is estimated that 10% of broiler chickens are produced using this method [26]. Consequently, our results do not represent the health status of poultry production as a whole. Therefore, complementary studies would be necessary to understand their participation in the spread of AI viruses. However, our study certainly represents the reality of the health status in industrial poultry production, since the sampling covered all the mainland provinces in Ecuador. We also used the recommended diagnosis test by the OIE that has 93 and 100% of sensibility and specificity [24], respectively; Therefore, we do not expect spurious results.

Our results showed the absence of H5 and H7 subtypes in the mainland Ecuadorian industrial poultry production. However, based on available information, several risk factors for AI introduction into Ecuador are present. It is therefore necessary to continue the surveillance program.

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