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

José Luis MEDINA1), Katherine LUGO1), Javier VARGAS1,2), Nataly MORALES2), Ana BURGOS1), Evelyn Pamela MARTÍNEZ1), David ORTEGA-PAREDES1) and María REVELO1)*

1)Faculty of Veterinary Medicine and Animal Husbandry, Central University of Ecuador, Quito, 170521, Ecuador
2)Ecuador’s Agriculture Quality Assurance Agency, AGROCALIDAD, Quito, 170516, Ecuador

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.

*Correspondence to: Revelo, M.: mcrevelo@uce.edu.ec
©2019 The Japanese Society of Veterinary Science
This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: https://creativecommons.org/licenses/by-nc-nd/4.0/)
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 (A/turkey/Minnesota/3689-1551/1981) and H7N3 (A/chicken/Chile/17682/02) 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 | Sampled farms    | Registered Farms | Sampled farms | Registered Farms | Sampled farms |
| Azuay               | 210              | a) 12 b)         | 3            | 1       | 4              | 1             | 14          |
| Bolivar             | 26               | -                | 1            | 1       | 1              | 1             | 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 discors (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.

REFERENCES

1. AAP (American Association of Avian Pathologists). 2013. Diseases of Poultry, 13th ed., John Wiley & Sons, Chichester.
2. Afanador-Villamizar, A., Gomez-Romero, C., Diaz, A. and Ruiz-Saenz, J. 2017. Avian influenza in Latin America: A systematic review of serological and molecular studies from 2000–2015. PLoS One 12: e0179573. [Medline] [CrossRef]
3. AGROCALIDAD (Ecuadorian Agro quality assurance agency). 2015. II Ecuadorian poultry census, MAGAP (Ministry of Agriculture Livestock Aquaculture and Fisheries) http://sinagap.agricultura.gov.ec/index.php/resultados-censo-nacional/file/591-reporte-de-resultados-censo-nacional-completo (in Spanish) [accessed on December 21, 2017].
4. AGROCALIDAD (Ecuadorian Agro quality assurance agency). 2016. Agreement No. 40–Contingency Plan for Avian Influenza. Official Registry of the Republic of Ecuador 743: 17–18 (in Spanish).
5. CDC (Centers for Disease Control and Prevention). 2017. Influenza type A viruses. https://www.cdc.gov/flu/avianflu/influenza-a-virus-subtypes.htm [accessed on December 21, 2017].
6. CERO (Ecuadorian Ornithological Records Committee). 2018. Official Checklist. https://ceroecuador.wordpress.com/official-checklist/ [accessed on May 29, 2018].
7. Gonzales, J. L., Elbers, A. R. W. and Beens, N. 2017. Risk factors of primary introduction of highly pathogenic and low pathogenic avian influenza virus into European poultry holdings, considering at least material contaminated by wild birds and contact with wild birds. EFS4 Support Publ. 14: EN-1282 [CrossRef].
8. Houston, D. D., Azem, S., Lundy, C. W., Sato, Y., Guo, B., Blanchong, J. A., Gauger, P. C., Marks, D. R., Yoon, K. J. and Adelman, J. S. 2017. Evaluating the role of wild songbirds or rodents in spreading avian influenza virus across an agricultural landscape. PeerJ 5: e4060. [Medline] [CrossRef]
9. Jones, P., Niemi, J. and Tranter, R. 2017. Production diseases: the costs to poultry producers. http://www.fp7-prohealth.eu/knowledge-platform/newsletter-articles/production-diseases-the-costs/ [accessed on March 9, 2018].

doi: 10.1292/jvms.19-0253
10. Karlsson, E. A., Ciudoderis, K., Freiden, P. J., Seufzer, B., Jones, J. C., Johnsson, J., Parra, R., Gongora, A., Cardenas, D., Barajas, D., Osorio, J. E. and Schultz-Cherry, S. 2013. Prevalence and characterization of influenza viruses in diverse species in Los Llanos, Colombia. *Emerg. Microbes Infect.* 2: e20. [Medline] [CrossRef]

11. Laanen, M., Maes, D., Hendriksen, C., Gelaude, P., De Vliegher, S., Rosseel, Y. and Dewulf, J. 2014. Pig, cattle and poultry farmers with a known interest in research have comparable perspectives on disease prevention and on-farm biosecurity. *Prev. Vet. Med.* 115: 1–9. [Medline] [CrossRef]

12. Leibler, J. H., Otte, J., Roland-Holst, D., Pfeiffer, D. U., Soares-Magalhaes, R., Rushton, J., Graham, J. P. and Silbergeld, E. K. 2009. Industrial food animal production and global health risks: exploring the ecosystems and economics of avian influenza. *EcoHealth* 6: 58–70. [Medline] [CrossRef]

13. Mostafa, A., Abdelwhab, E. M., Mettenleiter, T. C. and Pleschka, S. 2018. Zoonotic Potential of Influenza A Viruses: A Comprehensive Overview. *Viruses* 10: 497. [Medline] [CrossRef]

14. OIE (World Organisation for Animal Health). 2017. World Animal Health Information Database, (WAHIS) Interface. http://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home [accessed on May 8, 2018].

15. OIE (World Organisation for Animal Health). 2018. Avian Influenza (Infection with Avian Influenza Viruses). In: OIE Terrestrial Manual, 8th ed. OIE, Paris, pp 821–843.

16. OIE (World Organisation for Animal Health). 2018 Update on avian influenza in animals (types H5 and H7), Anim. Heal. World. http://www.oie.int/en/animal-health-in-the-world/update-on-avian-influenza/2018/ [accessed on January 21, 2018].

17. OIE (World Organisation for Animal Health). 2018. Self-declared disease status. Anim. Heal. World. http://www.oie.int/en/animal-health-in-the-world/self-declared-disease-status/ [accessed on May 7, 2018].

18. Otte, J., Pfeiffer, D., Soares-Magalhaes, R., Burgos, S., Roland-Holst, D. 2008. Flock size and HPAI risk in Cambodia, Thailand, and Viet Nam. HPAI Res Br 5–4.

19. SAG (Agricultural and Livestock Service). 2017. Status of cases of detection of avian influenza virus of Low Pathogenicity in production birds in Chile–2017, Chile. (Public report) (in Spanish).

20. Senne, D. A., Suarez, D. L., Stallnecht, D. E., Pedersen, J. C. and Panigrahy, B. 2006. Ecology and epidemiology of avian influenza in North and South America. *Dev. Biol. (Basel)* 124: 37–44. [Medline]

21. Siekkinen, K. M., Heikilä, J., Tammiranta, N. and Rosengren, H. 2012. Measuring the costs of biosecurity on poultry farms: a case study in broiler production in Finland. *Acta Vet. Scand.* 54: 12. [Medline] [CrossRef]

22. Spickler, A. R. 2016. Influenza Aviar. CFSPH, (The Cent. Food Secur. Public Heal. http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.php?lang=es [accessed on December 29, 2017].

23. Telégrafo, E. 2017. The Ecuadorian consumes 165 eggs a year, El Telégrafo (in Spanish).

24. Terregino, C. 2009. Evaluation of sensitivity and specificity of a commercial competitive avian influenza type A antibody ELISA kit (IDVET® Screen Influenza A). OIE-FAO and National Reference Laboratory for Newcastle Disease and Avian Influenza, Padua.

25. Thrushfield, M. 2007. Surveys. pp 228–246. In: Veterinary Epidemiology, 3rd ed., Blackwell Science, Oxford.

26. Vinuela, C. 2017. Salmonella and Campylobacter in broilers at slaughter age: a possible source for carcasses contamination in Ecuador. Ghent University, Gent.

27. Webster, R. G., Bean, W. J., Gorman, O. T., Chambers, T. M. and Kawaoka, Y. 1992. Evolution and ecology of influenza A viruses. *Microbiol. Rev.* 56: 152–179. [Medline]