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Influenza A, and *Salmonella* spp. in Backyard Poultry Eggs in Guatemala City

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ABSTRACT

Influenza A and salmonellosis are two of the most relevant zoonotic infectious diseases. Influenza A is one of the main threats to public health worldwide and is considered one of the causative agents of pandemics. Salmonellosis, meanwhile, has been identified by the World Health Organization as one of the four main causes of diarrheal diseases in the world. Poultry is an important source of both influenza A and *Salmonella* spp. but little is known about these potential threats in poultry products in Guatemala. The presence of influenza A virus antibodies and *Salmonella* spp. was studied in backyard poultry eggs sold in the El Guarda market in Guatemala City. 377 backyard poultry eggs were collected throughout seven months and sampled for hemagglutination inhibition test to determine the presence of antibodies to influenza virus A (H5N2 and H7N3) and cultured for *Salmonella* isolation. The eggs of chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*), quail (*Coturnix coturnix*), mallard duck (*Anas platyrhynchos*) and muscovy duck (*Cairina moschata*) were sampled. Twenty-six percent of the eggs carried H5N2 antibodies, 27% carried H7N3 antibodies and 1.3% carried *Salmonella* spp. The presence of *Escherichia coli* inside the sampled eggs was an incidental common finding. These results suggest that backyard poultry eggs sold at markets could be a potential source of influenza A virus and *Salmonella* for the human population. The evidence found in the sampled eggs also shows that these potential pathogens are circulating in backyard poultry populations in Guatemala.

Key words: Food security, One Health, Public health, Zoonosis

INTRODUCTION

Influenza A and salmonellosis are the most relevant zoonotic infectious diseases (Russell et al., 2014; Lee et al., 2015). Influenza A is one of the main threats to public health worldwide and is considered as one of the highly contagious infectious entities that can cause pandemics at any time (WHO, 2005). Salmonellosis, meanwhile, has been considered by the WHO (2018) as one of the four leading causes of diarrheal diseases in the world. Enteric *Salmonella* causes 1.3 billion cases of gastroenteritis and 3 million deaths worldwide (Bhunia, 2018) and it is, without a doubt, the most widespread foodborne disease in Latin America (Gil and Samartino, 2001). These pathogens are two of the infectious and contagious entities that represent a permanent risk to public health whose study should be a permanent priority to ensure their prevention, control, and eradication.

Sick poultry and its products can be taken to the markets to sell for human consumption. For this reason, markets are considered as reservoirs of diseases such as avian influenza and salmonellosis (Wray et al., 1991; Cardona et al., 2009; Singh et al., 2010). Therefore, it is important to investigate the risk to humans posed by the commercialization of poultry and its products in urban markets where many people come together, who could not only become infected but spread infectious agents.

The eggs of various poultry species that are sold in markets, can be a source of zoonotic pathogens. Influenza A virus of subtype H5N2 has been detected in eggs during disease outbreaks (Cappucci et al., 1985). Highly pathogenic H5N1 influenza virus has been isolated in table eggs after a mutation of a vaccine virus in chickens (Kilany et al., 2010). The eggs have also been considered as the main vehicle for enteric *Salmonella* infection in humans (Telzak et al., 1990; Braden, 2006; Bhunia, 2018).

Published data about the detection of influenzavirus A and *Salmonella* spp. in backyard poultry eggs sold in Guatemalan markets are practically non-existent even

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though animal products not only represent a possible source of infection for humans, but also provide information about the circulation of pathogens in the environment. In response to this gap of knowledge, the presence of antibodies against two variants of Influenza A virus (H5N2, H7N3) and *Salmonella* spp was investigated in chicken, duck, turkey and quail eggs that are being sold for human consumption in the El Guarda market –which is popularly considered as the most important place for the trade of animals and their products in Guatemala City. Present findings provide useful public health information and epidemiological data about pathogen circulation in the backyard poultry population that represents almost half of the national poultry farming in Guatemala.

MATERIALS AND METHODS

Study site

El Guarda is a traditional market located in Guatemala City (N 14 ° 36'48.77"; W 90 ° 32'20.08") and is the main center for the sale of domestic and wild animals and their products.

Study design and sample collection

A longitudinal study of exploratory scope was carried out for the present investigation. Seven backyard poultry eggs selling points were located in the El Guarda market and randomly sampled. Eggs were randomly collected from various poultry species from each of these selling points (Table 1). The eggs were collected every week from February to October 2019. Samples were taken from shell and yolk of all the eggs.

Table 1. Number of eggs collected and sampled from each species of backyard poultry in the El Guarda market, Guatemala

Scientific name	Common name	N
Gallus gallus	Chicken	234
Anas platyrhynchos	Mallard duck	57
Cairina moschata	Muscovy duck	29
Meleagris gallopavo	Turkey	30
Coturnix coturnix	Quail	27
Total		377

N: Number of sampled eggs

Sample transportation and laboratory procedures

The samples were transported in padded boxes, made of expanded polystyrene, to the Regional Reference Laboratory of Animal Health (Larrsa), at the Veterinary and Animal Husbandry Faculty, University of San Carlos of Guatemala, in Guatemala City.

Influenzavirus A antibodies were investigated by hemagglutination inhibition tests performed according to standard procedures (OIE, 2018a), using Merial (Italy) H7 antigens, Larrsa (Guatemala) H5 antigens, Merk (Germany) isotonic PBS, Charles Rivers (USA) positive control, Transferpette (Germany) micropipettes, Nunc (Denmark) V-bottomed microtiter plates and a Barnsted (Germany) orbital shaker.

Salmonella spp. isolation was performed according to standard procedures (OIE, 2018b), using Puritan (USA) sterile cotton swabs, Merk (Germany) and Difco (USA) culture media, Difco (USA) peptonated water, a Thermo Scientific (USA) incubator, a Labconco (USA) laminar flow hood and Biometieux (France) API identification kits.

RESULTS

The findings of the present study indicated antibodies against influenza A virus in several sampled eggs. Some eggs carried antibodies against H5N2, some against H7N3 and some against both. Chicken eggs were the most commonly positive eggs to both H5N2 and H7N3 influenza A antibody subtypes. However, the H7N3 variant was also detected in eggs from ducks (both species), turkeys and quails. *Salmonella* spp. was found in chicken and mallard duck eggs. Although this was not an initial objective of this study, *Escherichia coli* organisms were frequently isolated both from the shell and the interior of the sampled eggs. Table 2 shows the frequencies of positive reactors to influenza A antibodies, and the carriers of *Salmonella* and *E. coli* between poultry species and table 3 shows the distribution of positive samples in the sampled egg selling points at the El Guarda market. Figures 1 and 2 show the frequencies of H5N2 and H7N3 antibody titers in the sampled eggs (all species).

Table 2. Frequency of positive samples to influenza A (H5N2 and H7N3) antibodies, *Salmonella* spp. and *Escherichia coli* in backyard poultry eggs in the El Guarda market, Guatemala

Scientific name	N	H5N2 antibodies	H7N3 antibodies	H5N2 + H7N3 antibodies	*Salmonella* spp.	*Escherichia coli*
Gallus gallus	234	99	85	69	3	168
Anas platyrhynchos	57	0	1	0	2	48
Cairina moschata	29	1	2	0	0	25
Meleagris gallopavo	30	0	3	0	0	25
Coturnix coturnix	27	0	10	0	0	21
Total	377	100	101	69	5	287

N: Number of sampled eggs

Table 3. Frequency of positive samples to influenza A (H5N2 and H7N3), *Salmonella* spp. and *E. coli* in backyard poultry eggs in the El Guarda market, Guatemala, according to the selling point.

Selling point	N	H5N2	H7N3	*Salmonella* spp.	*E. coli*
A	89	43	49	2	61
B	50	9	8	0	36
C	85	21	18	0	71
D	28	4	4	0	23
E	60	8	12	3	43
F	57	14	8	0	47
G	8	1	2	0	6

N: Number of sampled eggs

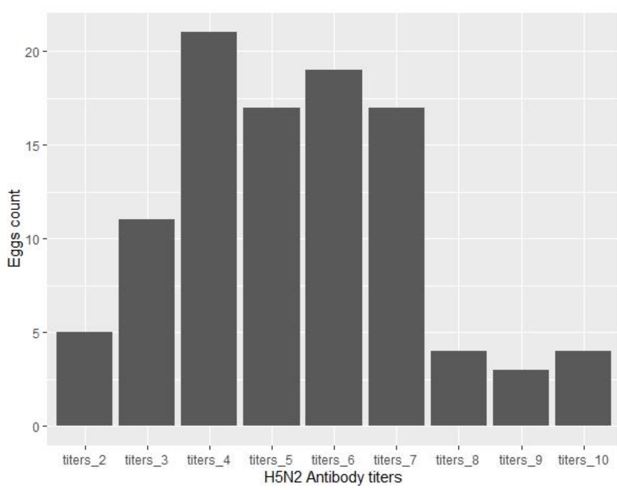

Figure 1. Frequency of logarithmic antibody titers of avian influenza A H5N2 variant in the sampled eggs.

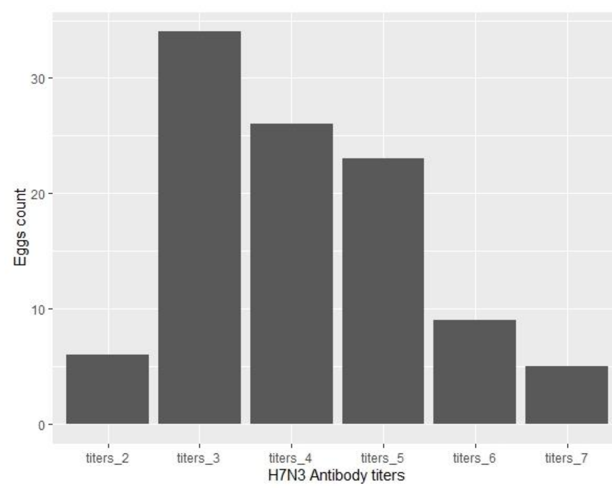

Figure 2. Frequency of logarithmic antibody titers of influenza A virus subtype H7N3 in the sampled eggs.

DISCUSSION

The observed frequencies of influenza A virus subtypes H5N2 and H7N3 antibodies in the sampled chicken eggs (42.3% and 36.3% respectively) are epidemiologically interesting especially considering that they probably come from unvaccinated backyard populations. There is some evidence indicating that Guatemalan peasants do not vaccinate their backyard poultry (Aguilar-Miller et al., 2016; Aquino-Sagastume et al., 2016; Mérida-Ruiz et al., 2016). In recent years the government passed a law for massive vaccination against the H7N3 type, however, this vaccination was intended to cover only those backyard poultry populations inside a 3 km radius around commercial poultry farms (Ministry of Agriculture, Livestock and Food, Guatemala, 2019). Therefore, the detected blood antibodies would indicate field exposure to influenza viruses and, by extension, the possibility of viral particles passing to the eggs from viremic chickens.

Another important issue to consider was the fact that, in backyard conditions, a hen lays around 30 eggs per year, in batches of about 10 eggs (Sonaiya et al., 1999). This means that if a market vendor wishes to maintain a constant supply of eggs throughout the year, he is forced to purchase eggs coming from several hens from several backyard flocks, from many parts of the country, suggesting that influenza A virus subtypes H5N2 and H7N3 could be ubiquitous in Guatemalan rural landscapes.

On the other hand, the presence of circulating antibodies against influenza virus A in mallard ducks, muscovy ducks, turkeys, and quails is an uncommon finding for Guatemala and perhaps the first published report. These antibodies could be either the result of vaccination response or a challenge with field viruses. Although a H5N1 vaccine designed for chicken induced immunity in ducks and geese (Tian et al., 2005) it is unlikely that the government vaccination program has covered the entire territory of Guatemala.

The findings of the present study also support the previous observations of influenza A subtypes H5N2 and H7N3 variants in both wild and farm avian populations of Guatemala (Gonzalez-Reiche et al., 2012; Gonzalez-Reiche and Perez, 2012; Jarquin et al., 2015; Lee et al., 2015). Although the antibodies found in the eggs may come from vaccinated individuals, there is some evidence of post-vaccination outbreaks in poultry populations (Kilany et al., 2010).

In this study, *Salmonella* was detected in chickens and mallard ducks, but the frequencies were rather low. *Salmonella* had previously been reported in chicken meat in Guatemala (Jarquín et al., 2015). Salmonellosis cases have also been reported in humans in some provinces of the country (Díaz et al., 2015).

An incidental but significant finding of the present study was the overall frequency (76.43%) of *E. coli* isolations from the interior of the sampled eggs. Not only does this indicate a pathogen-permeable egg but also a public health concern because *E. coli* from domestic animal populations use to become multiresistant (Krumperman, 1983; Kojima et al., 2005; Sayah et al., 2005). It is known that antibiotic-resistant *E. coli* present in chicken meat can colonize the human bowel after consumption (Linton et al., 1977). The contamination of foods with bacteria from farm animals has been identified as a relevant problem, especially from the One Health approach (Van den Bogaard and Stobberingh, 2000).

From a public health perspective, the presence of *Salmonella* and *E. coli* and the possible presence of influenza A viruses is relevant in a country like Guatemala, where the consumption of raw eggs with orange juice and the feeding of young children with boiled under-cooked eggs is traditional.

Finally, although 18 influenza A virus subtypes have been found in migratory ducks in Guatemala (Gonzalez-Reiche et al., 2017), the governmental surveillance system is only looking for two subtypes (H5N2 and H7N3). This situation generates a knowledge gap about the subtypes that could be circulating in poultry farm and backyard populations, as well as in poultry products for human consumption.

CONCLUSION

Antibodies against influenza A, subtypes H5N2 and H7N3 were common findings in the eggs of backyard poultry. This means that, under certain conditions, the eggs could also be a source of viral particles for consumers. *Salmonella* spp. was not a frequent finding in this study and *Escherichia coli* (both outside and inside most of the studied eggs) was an incidental finding.

DECLARATIONS

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Competing interests

The authors have declared that no competing interest exists.

Consent to publish

Author's contribution

DG-C conception, design and drafting the manuscript, MD-R conception, design, interpretation of data and reviewing the manuscript, CV-S fund management, data analysis and reviewing the manuscript, ML-L data analysis and interpretation and reviewing the manuscript, EA, CA, CE and JB sampling, management of samples and reviewing the manuscript.

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