

**UNIVERSIDAD DE SAN CARLOS DE GUATEMALA
FACULTAD DE MEDICINA VETERINARIA Y ZOOTECNIA
ESCUELA DE MEDICINA VETERINARIA**



**CIRCULATING ANTIBODIES AGAINST AVIAN
INFLUENZA AND NEWCASTLE DISEASE IN
SEMI-CAPTIVE PEACOCKS IN SOUTHWESTERN
GUATEMALA - ANTICUERPOS CIRCULANTES
CONTRA LA ENFERMEDAD DE INFLUENZA AVIAR Y
NEWCASTLE EN PAVORREALES SEMI CAUTIVOS
EN EL SUROESTE DE GUATEMALA**

Claudia Melissa Castillo Grijalva

Médica Veterinaria

GUATEMALA, MAYO DE 2021

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PRESENTADO A LA HONORABLE JUNTA DIRECTIVA DE LA FACULTAD

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FACULTAD DE MEDICINA VETERINARIA Y ZOOTECNIA
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ACTO QUE DEDICO A:

A mis padres:

Augusto y Claudia por darme la vida, por amarme y apoyarme incondicionalmente.

A mis abuelos:

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Dated: June 11, 2020

PAPER ACCEPTANCE CERTIFICATE

To:

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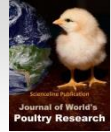
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Circulating Antibodies against Avian Influenza and Newcastle Disease in Semi-Captive Peacocks in Southwestern Guatemala

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ABSTRACT

Avian Influenza and Newcastle disease are the two most important diseases of poultry and are globally considered as threats to public health and economy. There is little information published about these diseases in peacocks and other common backyard poultry in Guatemala. Therefore, an exploratory serosurvey was conducted to determine the presence of circulating antibodies to Avian Influenza (AI) and Newcastle Disease (ND) viruses in a semi-captive population of peacocks in southwestern Guatemala. Additionally, the circulation of antibodies to these pathogens in backyard chickens, ducks, and turkeys from a neighboring community was explored. Blood samples were obtained from 48 peacocks, 30 chickens, 6 ducks, and 4 turkeys. The samples were processed in the Regional Reference Laboratory for Animal Health, at the Veterinary Medicine Faculty, University of San Carlos of Guatemala, located in Guatemala City. Antibodies against AI virus were investigated by Agar Gel Immunodiffusion, and antibodies against ND virus were examined using Hemagglutination Inhibition. No antibodies against AI virus were detected. Most of the samples (97.7%) were negative for antibodies against ND virus, except for two turkeys that carried low antibody titers. The findings of the present study indicate that no virulent strains of AI or ND viruses were circulating in the investigated site.

Keywords: Avian influenza, Epidemiology, Newcastle disease, Serology, Zoonoses

INTRODUCTION

Avian Influenza (AI) and Newcastle Disease (ND) are the most important diseases of poultry (Alexander, 2000; Capua and Marangon, 2006), and are considered global threats to public health and economy (Wong and Yuen, 2006). AI viruses have been a common cause of epidemics and pandemics (De Jong and Hien, 2006; Alexander, 2007; Peiris et al., 2007; Monto and Fukuda, 2020), and their ability to mutate and become more pathogenic or more capable to invade other host species makes them relevant to animal and public health.

Due to this mutation capacity, numerous subtypes of influenza A virus have evolved. On the other hand, Newcastle disease is one of the threats to farm economy and poultry production, not only because it causes economic losses due to mortality, but also because many countries have sanitary barriers that prohibit importations

from countries where the disease is endemic (Miller and Koch, 2013). Both AI and ND are known to affect a wide variety of hosts (Stallknecht and Shane, 1998; Ito and Kawaoka, 2000; Baigent and McCauley, 2003; Swayne and King, 2003). These pathogens have already been found in Guatemala in some avian species in certain areas of the country (Gonzalez-Reiche et al., 2016; Mérida et al., 2016; Gonzalez-Reiche et al., 2017). However, published information about AI or ND in peacocks or backyard fowl species in southwestern Guatemala is practically inexistent.

In response to this gap of knowledge, the presence of circulating antibodies against AI and ND viruses was investigated in a semi-captive population of peacocks in southwestern Guatemala. Additionally, the circulation of antibodies against these pathogens was explored in backyard chickens, ducks, and turkeys from a neighboring community.



MATERIALS AND METHODS

Study site

A population of 94 peacocks (*Pavo cristatus*) kept in semi-captive conditions in an amusement park located at Retalhuleu department, in southwestern Guatemala (coordinates 14°35'41" N 91°36'42" W) was studied. The study site was a fenced area of 128 thousand square meters surrounded by small villages and hamlets where peasants strongly depend on family agriculture and animal raising to survive (Figure 1). In the park facilities, peacocks roamed freely among visitors (one million visitors a year), and frequently came into the contact with wild birds. To explore the antibody circulation in the surroundings, a sample of backyard fowl was also studied in households of the neighboring community, San Martín Zapotitlán, Retalhuleu, Guatemala.

Species and sampling

A sample of 48 peacock individuals was calculated and randomly sampled based on 50% prevalence, 95% confidence interval, and 0.1 margin of error. Additionally, thirty chickens (*Gallus gallus*), four turkeys (*Meleagris gallopavo*), and six ducks (*Anas platyrhynchos*) were sampled by convenience. All sampled birds were adults. Three millimeters of blood was taken from the ulnar vein using 3cc syringes with 23g X 1.5" needles, while gently holding the birds by hand. The blood samples were put into test tubes without anticoagulant, centrifuged for four minutes at 3,000 rpm, transferred to plastic straws, and transported to be processed in the Regional Reference Laboratory for Animal Health (Larrsa), at the Veterinary Medicine and Animal Husbandry Faculty, University of San Carlos of Guatemala, in Guatemala City.

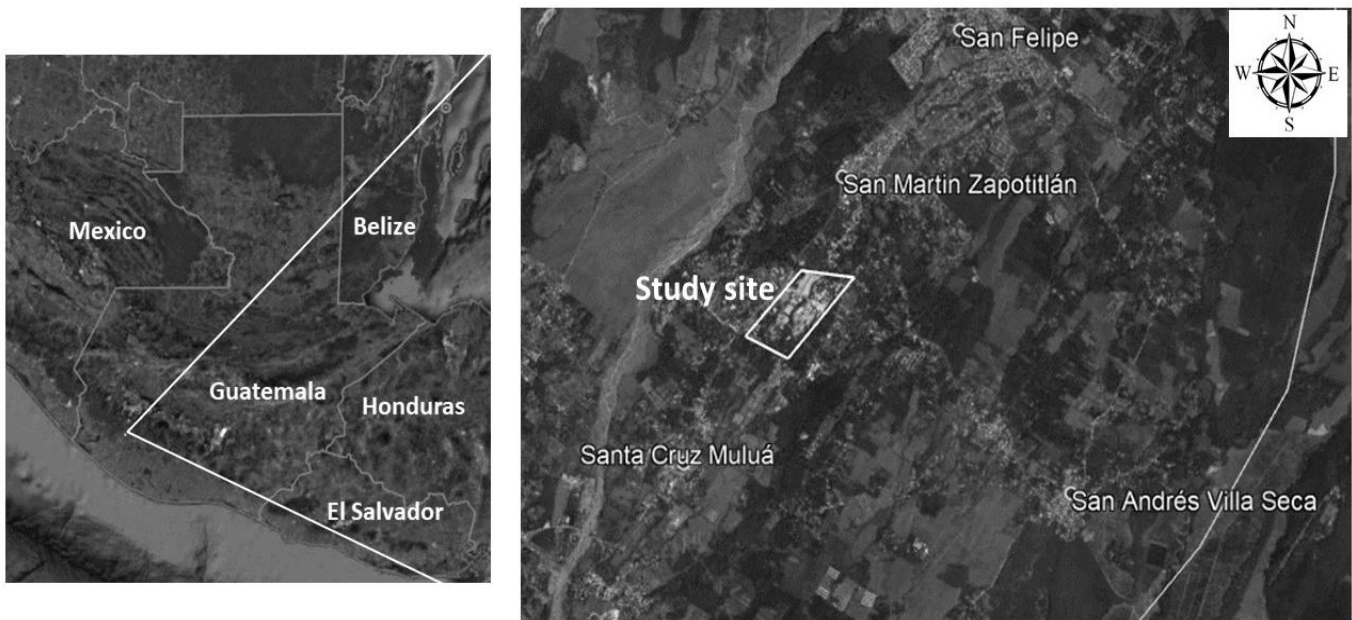


Figure 1. Study site location in Retalhuleu, southwestern Guatemala.

Laboratory procedures

Antibodies against AI virus were studied by agar gel immunodiffusion (GID) tests, performed according to standard procedures (OIE, 2018a), using Type I molecular biology grade agarose (Calbiochem, USA), NaCl and pH 7.2 phosphate buffer (Merk, Germany) and AI antigen (Larrsa, Guatemala).

Antibodies against ND virus were studied by hemagglutination inhibition tests performed according to standard procedures (OIE, 2018b), using a 1% chicken red blood cell solution (Larrsa, Guatemala), local ND antigens

(Larrsa, Guatemala) isotonic PBS (Merk, Germany), positive control (Charles Rivers, USA), 10-100 μ l unichannel micropipettes (Transferpette, Germany), 10-100 μ l multichannel micropipettes (Transferpette, Germany), micropipette tips (Transferpette, Germany) V-bottomed microtiter plates (Nunc, Denmark) and an orbital shaker (Barnstead, Germany). HI antibody titers were considered positive if there was inhibition of hemagglutination at serum dilutions of 1/16 (2^4 or $\log_2 4$, when expressed as the reciprocal, as is customary in Larrsa).



Ethical approval

This research was approved by the Bioethics Committee of the Graduate School, Veterinary Medicine and Animal Husbandry Faculty, University of San Carlos of Guatemala.

RESULTS AND DISCUSSION

No circulating antibodies against AI and ND viruses were found in the sampled peacocks. Antibodies were also not found in the backyard birds of the neighboring community, except for two turkeys that had low antibody titers (Log_2 5 and 6) against ND virus (Table 1).

Table 1. Frequency of positive blood samples to antibodies against Avian Influenza and Newcastle disease in peacocks.

Bird species	Number of samples	Avian Influenza antibodies	Newcastle Disease antibodies
<i>Pavo cristatus</i>	48	0	0
<i>Gallus gallus</i>	30	0	0
<i>Meleagris gallopavo</i>	4	0	2
<i>Anas platyrhynchos</i>	6	0	0
Total	88	0	2

Note: the titers of the positive *Meleagris gallopavo* samples were 5 and 6 (log_2 reciprocal).

Although peacocks are susceptible to some AI and ND virus strains (Munir et al., 2012; Desingu et al., 2016; Umar, 2017; Wajid et al., 2017), and even though the studied population was kept in semi-captive conditions and in frequent contact with people, and wild birds that were possible sources of infection (Rehan et al., 2019), no antibodies were found in the sampled individuals. It was also highly likely that the rest of the population did not have antibodies either, especially considering that the entire flock slept every night at the same roosting site, and therefore, if the population were susceptible, any outbreak of AI or ND would have readily spread due to disease ecology determinants (Beldomenico and Begon, 2010).

The absence of antibodies against AI virus observed in the sampled peacocks in the present study was consistent with previous studies in the USA by Hollamby et al. (2003) and in Hong Kong by Ellis et al. (2004), but inconsistent with studies in the Kingdom of Saudi Arabia by Ismail et al. (2010) and in Iraq by Rashid et al. (2017). On the other hand, the absence of antibodies against ND virus was consistent with the findings of Ibitoye et al. (2013) in Nigeria, and inconsistent with the findings of Vijayarani et al. (2010), Khulape et al. (2014) and Desingu

et al. (2016) in India; Sadiq et al. (2011) in Nigeria; Chumbe et al. (2015) in Peru and Munir et al. (2012) and Mustafa et al. (2015) in Pakistan.

The first interesting consideration when interpreting our findings was that GID test is a screening assessment that detects antibodies against all subtypes of AI virus (Jenson, 2014). In that sense, there was not only the absence of antibodies against the previously isolated subtypes in Guatemala, but also against all subtypes. The second consideration would be the cause underlying the absence of antibodies in almost all the sampled birds (including the backyard poultry from the neighboring community). Failure to find antibodies could mean that past epizootic outbreaks have swept birds –as has been previously reported in Guatemala (Lepe-López et al., 2020)– and have left no immune history. It could also mean that the circulating virus strains were not pathogenic enough to stimulate the production of antibodies in the studied birds. It has also been suggested that in backyard poultry, a lack of immune response could be the result of infectious bursal disease, chronic aflatoxicosis, or vitamin A deficiency (Awan et al., 1994). However, this was unlikely to be the underlying cause, at least in the case of the peacocks, because the population had veterinary care and adequate nutritional management.

The lack of evidence of the circulation of virulent strains of AI and ND viruses in the studied landscape is epidemiologically noticeable considering that in Guatemala, more than 26 million backyard chickens are being raised –and probably being trade– in practically all the country (Ministry of Agriculture, Livestock and Food, Guatemala, 2015). In fact, antibodies against AI and ND viruses have been detected in some backyard chicken populations (Aguilar-Miller et al., 2016; Aquino-Sagastume et al., 2016; Mérida-Ruiz et al., 2016) as well as in commercial poultry (Lee et al., 2004) and in wild birds (Gonzalez-Reiche et al., 2012; Gonzalez-Reiche et al., 2016). On the other hand, 758 species of birds are distributed in Guatemala, and at least 240 are migratory (Eisermann and Avendaño, 2018). This host diversity seems to be accompanied by a virus diversity because recently, 19 Influenzavirus A subtypes were isolated from migratory ducks in Guatemala, including the H7N3 subtype (Gonzalez-Reiche et al., 2017).

It is important to notice that, depending on the virus strain and the avian host species, some low pathogenicity AI viruses are unable to infect hosts stimulating only low or imperceptible immune responses (Alexander et al., 1978; Alexander et al., 1986). This differential species-dependent immune response was also observed for the ND



virus (Eze *et al.*, 2014). During an H5N1 AI outbreak in a natural park in Hong Kong, several species of birds resulted infected, but peacocks were among the non-affected species (Ellis *et al.*, 2004).

The absence of antibodies in the backyard poultry of the neighboring community found in the present study could mean that no virulent strains of AI or ND viruses were circulating in the landscape. In a backyard poultry national Serosurvey for AI and ND in Oman, the bird seroprevalence was 37.5% and 42.1% respectively, and the flock seroprevalence was 84% and 90% respectively (Al Shekaili *et al.*, 2015) but this massive seroprevalences seemed unlikely for Guatemala where previous data suggest a patchy distribution of these pathogens in the backyard poultry population.

On the other hand, the finding of two seropositive turkey individuals to antibodies against ND virus in the present study is rather difficult to explain, considering that all the other sampled specimens did not show circulating antibodies against ND virus. These two antibody-carrier turkeys could have been recently added to the population by the peasants.

Considering the evidence found in the present study, more research needs to be done to establish the distribution pattern of Influenza and Newcastle viruses in the avian host populations throughout the country, mainly, at the human-animal interface (Chaudhry *et al.*, 2020). This would enable the design of sound intervention policies to assure commercial and backyard poultry productivity and public health.

CONCLUSION

Considering that the population was not recently-established and based on the absence of antibodies and on the fact that no significant mortality was observed in recent years, the results indicate that no AI or ND viruses are circulating in the studied population of peacocks.

DECLARATIONS

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Competing interests

The authors have declared that no competing interest exists.

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MC-G conception of the idea, drafting the manuscript and field sampling DG-C conception of the idea, drafting and editing the manuscript YT field sampling and reviewing the manuscript CV-S and ML-L conception of the idea and reviewing the manuscript BS laboratory procedures, and reviewing the manuscript. All authors checked and confirmed the final version of the article.

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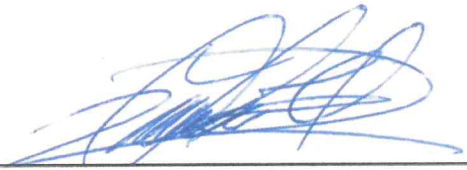


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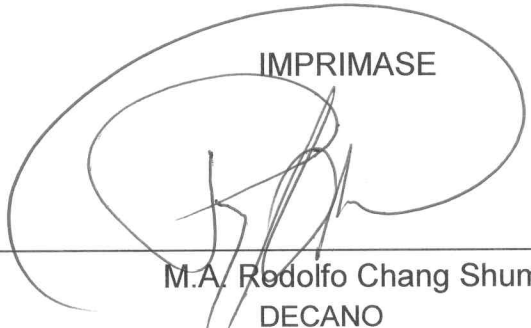
CIRCULATING ANTIBODIES AGAINST AVIAN INFLUENZA AND
NEWCASTLE DISEASE IN SEMI-CAPTIVE PEACOCKS IN
SOUTHWESTERN GUATEMALA

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