

Viruses of Economic Importance in Backyard Poultry in Guyana, South America

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Abstract: Poultry continues to be an economically important industry in Guyana. Despite efforts to curb outbreaks of viral infections on poultry farms in Guyana, viral diseases are thought to cause significant production losses and mortality. Currently, there is little information about the viruses circulating on poultry farms in Guyana. This study sets out to identify which viruses of backyard poultry, with worldwide and regional significance, are circulating in Guyana. Active surveillance was carried out to detect the presence/absence of antibodies for selected poultry viruses of potential economic significance in Guyana. Serum samples were collected from unvaccinated backyard poultry and tested using selected Enzyme-Linked Immunosorbent Assay (ELISA) detection kits. Results from the 261 samples taken from 55 backyard poultry farms showed that 98% of the birds tested positive for antibodies to chicken anemia virus (CAV), 54% for Infectious Bursal Disease Virus (IBDV), 73.5% for Newcastle Disease Virus (NDV), 60% for Infectious Bronchitis Virus (IBV) and, 3.3% for Avian Influenza Virus (AIV). However, no samples tested positive for antibodies to Avian Leukosis Virus (ALV). This study aided in understanding viruses circulating naturally and potentially causing disease in backyard poultry in Guyana.

Keywords: Guyana, Backyard Poultry, Viruses, Antibodies, Seroprevalence

ABBREVIATIONS

Aiv: Avian Influenza

Alv: Avian Leukosis

Cav: Chicken Anemia Virus

Elissa: Enzyme Linked Immunosorbent Assay

Gdp: Gross Domestic Product

Glda: Guyana Livestock Development Authority

Ibdv: Infectious Bursal Disease Virus

Ibh/Hps: Inclusion Body Hepatitis/Hydro-Pericarditis Syndrome

Ibv: Infectious Bronchitis Disease Virus

Ndv: Newcastle Disease

1. INTRODUCTION

The poultry industry is of high economic importance and substantially contributes to the Gross Domestic Product (GDP) of many Latin American and Caribbean countries, including Guyana (Jordan et al, 2018b). Due to the human population accelerating growth, there has been a significant increase in poultry meat and egg production worldwide (Mottet and Tempio, 2017). Backyard poultry contributes to the sustainability of many rural populations livelihoods in developing countries like Guyana (Conan et al 2012). It serves as a critical source of income and nutrition; however, disease outbreaks play a significant role in the profitability of this sector (Maikasuwa, 2011). Backyard poultry production contributes 8% to global egg production and 2% to global poultry meat production (Mottet and Tempio, 2017).

However, backyard poultry farms are generally characterized by poor hygiene and biosecurity, making poultry disease control challenging in backyard systems (Sonaiya, 2007). But then again, it has been suggested that backyard poultry play a marginal role in some disease outbreaks such as avian influenza (Bavinck et al, 2009) & (Smith and Dunipace, 2011) in other parts of the world.

Guyana, located in South America, is an agriculturally diversified country divided into four natural and ten administrative regions (numbering 1-10). Commercial quantities of broilers and layer birds are reared mainly in four of the ten administrative regions (Regions 3, 4, 5, and 6). This thriving poultry industry contributes significantly to the country's GDP. According to the Guyana Agriculture Statistics Yearbook 2018 (Guyana Agriculture Statistics Yearbook, 2018), table eggs local production was approximately 20, 28, and 32 million eggs. Poultry meat was recorded in kilograms as 32, 30 and, 42 million for the period 2016 to 2018, respectively.

Backyard poultry farming is generally carried out in Guyana's rural parts, where households rear a few chickens in their yards for supplementing their diets with eggs and meat. Any surplus of eggs and meat are sold mainly within the local community. Similarly, in Guyana these birds are "slaughtered and prepared on-site with no abattoir processing and the meat and other products are consumed on site or locally, and never enter the industrial circuit" (Capua et al, 2002). Backyard birds worldwide, including in Guyana, are mainly reared under extensive or partially confined systems, are not vaccinated, survive on kitchen scraps, and forage around for grass, worms, and insects (Sonaiya, 2007). The custom of rearing backyard poultry is so widespread in Guyana that it is carried out on small scale commercial broiler and layer establishments and very near to larger commercial poultry establishments, with inadequate and insufficient biosecurity measures in place.

There are currently no nationally recommended poultry vaccine protocols in place in Guyana, except for the Animal Movement and Disease Prevention Sale of Chicken Regulation No. 2 of 2008. The government of Guyana instituted this regulation following an outbreak of Inclusion Body Hepatitis (IBH) / Hydropericardium Syndrome (H.S.) in broiler birds in 2006 to enforce the use of IBH vaccines. As such, each producer/hatchery vaccinates their birds based on vaccine availability and on whether a particular

disease is considered a threat. Some producers do not vaccinate their birds as some customers dislike the wet appearance of chicks after vaccination. In contrast, others find it laborious and time-consuming to vaccinate and sell on the same day of the hatch, thus recommending field vaccination as a disease control measure. A few extensive commercial producers research with external private companies to evaluate antibody titers for certain viruses so they can ensure that their birds are sufficiently protected through vaccination.

This research, the first of its kind to be conducted in Guyana, aimed to collect baseline data on the circulation of economically significant viruses in Guyana. Through the detection of antibodies to Chicken Anemia Virus (CAV), Infectious Bronchitis disease virus (IBV), Infectious Bursal Disease virus (IBDV), Avian influenza Virus (A.I), Avian Leukosis Virus (ALV) and, Newcastle Disease Virus (NDV) in unvaccinated backyard poultry in Guyana.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out between 2018-2020 in four administrative regions (3, 4, 5 and, 6) of Guyana, South America. The study area extended along the coastline of Guyana along the Atlantic Ocean, where poultry production is concentrated (**Fig. 1**). Areas were selected due to backyard poultry prevalence and a convenient sampling method was used for surveyed locations

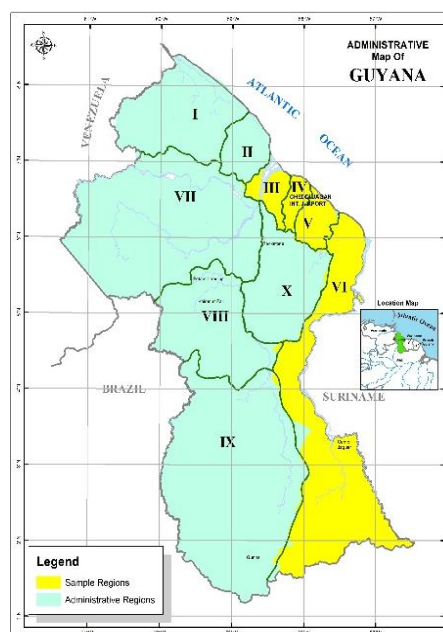


Fig1. Administrative regions of the Cooperative Republic of Guyana, highlighting sampled regions.

2.2 Study Population

Unvaccinated backyard creole/indigenous chickens older than 12 months, which appeared healthy at the time of sampling, were selected from a total of fifty-five (55) farms/households (Table 1). The selection of the farms/households

was based on the owners' willingness to allow samples to be taken from their birds (opportunistic sampling), since owners of backyard poultry are very selective as to which birds in a flock can be sampled (particular birds may be considered good layers, and broody hens or fighters).

Table1. Number of backyard poultry premises/farms surveyed and sampled per region.

Region	Number of farms sampled (number of sampled birds)
#3	8 (43)
#4	4 (25)
#5	29 (128)
#6	14 (65)
Total	55 (261)

2.3 Sampling protocol

Approximately 2.5ml of blood was taken from the brachial (wing) vein of unvaccinated adult birds. Samples were transported to the laboratory in coolers with ice packs. Extracted serum was stored in cryovials at -20 until time for testing.

Serum samples were analyzed using commercial enzyme-linked immunosorbent assay (ELISA) test kits from IDEXX Laboratories following the manufacturer's instructions (Table 2) to test for the presence of antibodies against, CAV, IBD, IBDV, AIV, ALV & NDV. Absorbance values were obtained using a microplate reader, set at an optical density of 650nm for each disease test kit.

2.4 Serological testing

Table2. Enzyme-linked immunosorbent assay (ELISA) kits used for antibody detection.

No.	Target pathogen	Kit particulars	Manufacturer	Lot#
1	Chicken Anemia Virus (CAV)	Ab screening test for CAV in chicken serum	IDEXX	DN649
2	Infectious Bursal Disease Virus (IBDV)	Ab screening test for IBD in chicken serum	IDEXX	MM083
3	Infectious Bronchitis Virus (IBV)	Ab screening test for IBDV in chicken serum	IDEXX	CN449
4	Newcastle Disease (NDV)	Ab screening test for NDV in chicken serum	IDEXX	BR857
5	Avian Influenza Virus (AIV)	Ab screening test for A.I. in chicken serum	IDEXX	BN312
6	Avian Leukosis Virus (ALV)	Ab screening test for ALV-subgroups A&B in chicken serum	IDEXX	AP770

2.5 Statistical Analysis

XChekPlus Software by IDEXX was used for reading and interpreting ELISA results. The presence or absence of antibodies is determined by relating the A (650) value of the unknown positive control mean. The positive Control is standardized and represents significant antibody levels to the pathogen in chicken serum. The relative level of antibody in the sample is

determined by calculating the sample to positive ratio as stipulated by the kit insert.

3. RESULTS

From the 261 backyard chicken sera samples collected on 55 farms. A total of 98% of unvaccinated creole birds tested positive for antibodies against CAV, while 60% tested positive for IBDV antibodies. 54% tested positive for IBV antibodies, 3.3% for AIV, and

73.5% of birds had antibodies for NDV. However, no antibodies were detected against ALV in the backyard chickens tested from the four regions, as indicated in **Table 3**.

Table3. Percentage of birds that tested positive by region

Re g.	Numb er of farms (sampl es per region)	% CA V	%IB V	%IB DV	%AI V	%AL V	%ND V
#3	8 (43)	100	46.5	42	9.3	0	58.1
#4	4 (25)	100	72	96	0	0	Not tested
#5	29 (128)	98.4	54.7	43.8	4	0	73.4
#6	14 (65)	93.8	43	58.5	0	0	89.2
% Av e		98%	54%	60%	3.3%	0	73.5%

Key:

CAV: Chicken Anaemia Virus

IBV: Infectious Bronchitis Disease Virus

IBDV: Infectious Bursal Disease Virus

AIV: Avian Influenza

ALV: Avian Leukosis

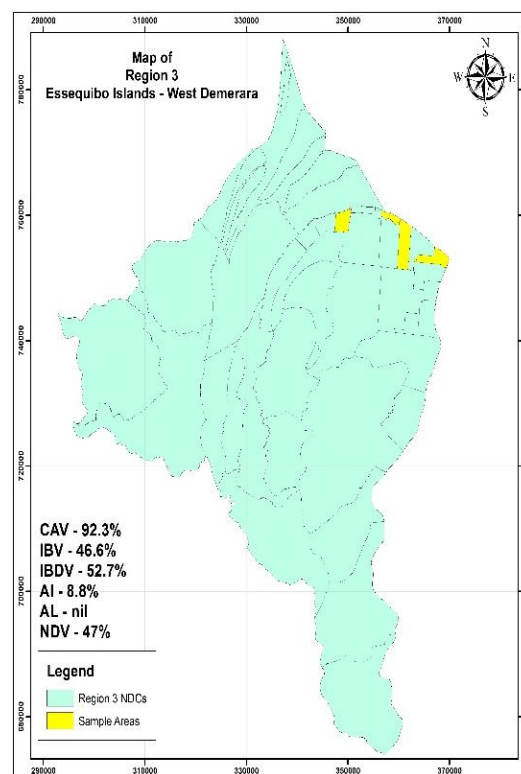
NDV: Newcastle Disease

The majority of sampled birds (ranging from 93.8-100%) in all four regions had antibodies to CAV. Between 43 and 54% of the sampled birds from regions 3, 5, and 6 had antibodies to IBV, whereas 72% of birds in region 4 were IBV antibody positive. Region 4 also had the highest percentage of birds (96%) that were positive for antibodies to IBDV, with 42, 43.8, and 58.5% of sampled birds being IBDV antibody positive in regions 3, 5, and 6.

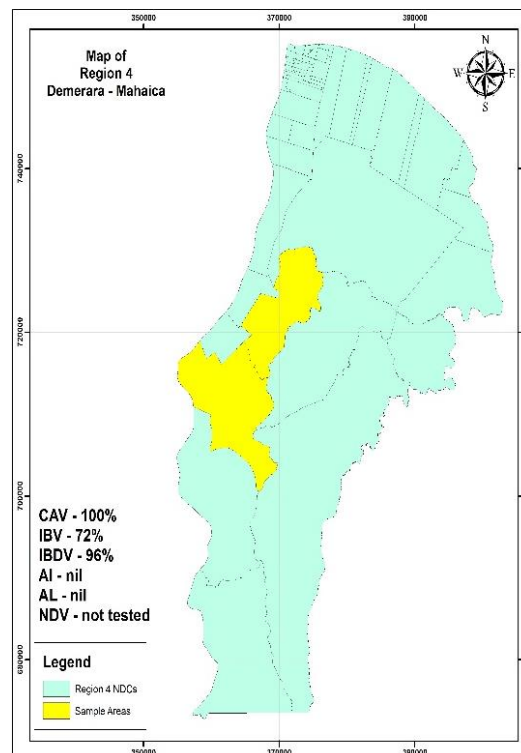
As for AIV, no birds tested antibody positive in regions 4 and 6; however, 9.3% (4 of 43) tested positive in region 3 and 3.3% (5 of 123) in region 5.

The highest percentage (89.2%) of NDV antibody-positive birds was observed in region 6, with 73.4 and 58.1% of birds testing positive for antibodies to NDV in Regions 5 and 3, respectively. No birds in any of the four Regions tested positive for antibodies to ALV. More localized information showing the Neighborhood Democratic Councils (NDC) within the four

Regions where sampling took place can be observed in **Fig. 2 and 3**

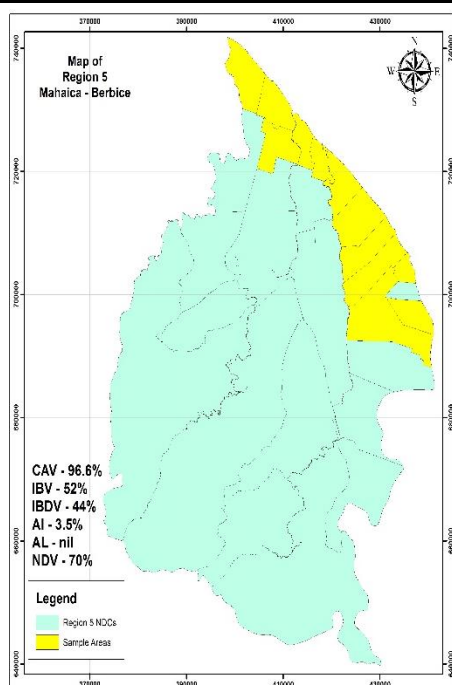


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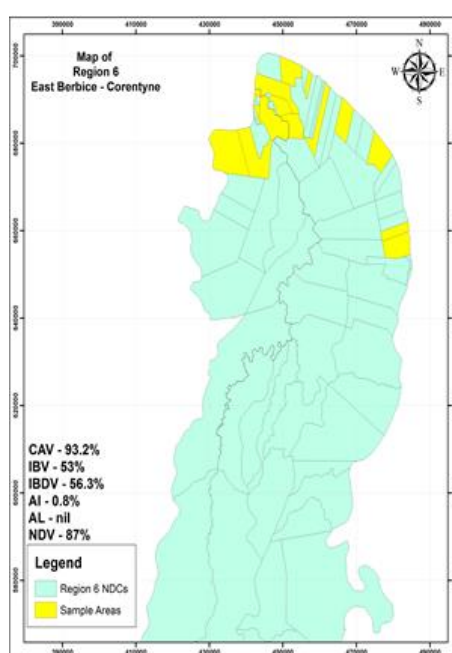


B

Figure2. Maps of Administrative regions showing the Neighborhood Democratic Councils (NDC) sampled (in yellow) and results obtained. (A) Region#3 Essequibo Island – West Demerara (B) Region#4 Demerara Mahaica



C



D

Figure3. Maps of Administrative regions showing the Neighborhood Democratic Councils (NDC) sampled (in yellow) and results obtained. (C) Region#5 Mahaica Berbice and (D) Region#6 East Berbice -Corentyne

4. DISCUSSION

Backyard poultry farming can be described as small-scale family-based poultry farming, which involves semi-scavenging flocks of mostly local breeds in rural communities (Sonaiya, 2007). The practice of rearing backyard/creole poultry within the confines of one's surroundings is a widespread occurrence in rural and semi-rural

areas of Guyana. The biosafety practices within these operations are practically non-existence and compounded by mixing different species, including ducks, turkeys, guinea fowl, pigs, and small ruminants. Backyard poultry farming in Guyana is carried out within very close proximity to commercial farms and even on the same premise of some smaller commercial holdings, thus increasing the risk of transmitting pathogens from the backyard to commercial birds. Additionally, a significant number of farmworkers working on commercial poultry farms are engaged in the rearing of backyard chickens, ducks, and other forms of livestock at their homes.

Insufficient disease control measures and poor management practices can result in high levels of morbidity and mortality in poultry due to infectious diseases (Bell, 2009). A *postmortem* survey of disease conditions in backyard poultry obtained from two laboratories over 4 and 12 years, respectively in the USA, revealed that the highest cause of mortality and morbidity were viral pathogens (Crespo and Senties, 2015). Viral pathogens that are capable of causing diseases of significant importance in poultry include; CAV, IBV, IBDV, AIV, ALV, and NDV. These diseases are known to cause substantial problems in the backyard and commercial poultry operations globally. In this study, we observed a seroprevalence of 98% in birds from all regions in Guyana tested for antibodies to CAV. This result is consistent with the high percentage of birds that were antibody positive for CAV in a study conducted in North-Eastern Ecuador, where approximately 90% of backyard chickens tested antibody positive for CAV (Hernandez-Divers et al, 2006). However, in a similar seroprevalence study carried out in Grenada, a lower prevalence of 10.3% was recorded (Sharma et al, 2015). It has been documented that CAV is likely to increase the mortality and morbidity of chickens infected with inclusion body hepatitis/hydro-pericarditis syndrome (IBH/HPS) (Toro et al, 2000). During 2006-2007 Guyana suffered a significant outbreak of IBH/HPS in its major poultry producing regions, so much so that vaccination was introduced (Jordan et al, 2018b). Observing the very high levels of antibodies detected against CAV in this study raises questions as to the role that CAV might have played in escalating this disease outbreak.

Knowledge of the CAV infection status of backyard poultry is vital concerning the adoption of effective prevention strategies in commercial

flocks due to the close association with the backyard and commercial flocks in Guyana. These results emphasize the need to increase levels of biosecurity in the backyard bird population in Guyana and also to consider the introduction of CAV vaccination in both the commercial and backyard sectors (Barrios et al, 2009).

Previous studies conducted by Dos Santos et al, (2008) in Rio Grande, Brazil, and (Jordan et al, 2018a) in Trinidad and Tobago exhibited slightly higher prevalence levels, where 65% of birds were tested positive for antibodies to IBV respectively compared to 54% obtained in Guyana which was very similar to those obtained in Grenada, 54.28% (Sabrinath et al, 2011). In another study carried out in Uberlandia, Minas Gerais, Brazil, 87.5% of backyard birds were found to be antibody positive to IBV which could have been as a result of the natural infection or live vaccine spread (Batista et al, 2020). Molecular characterization of the IBV strains circulating naturally in Guyana should be conducted to determine if field strains, vaccine strains, or novel strains are circulating. A recent study in neighboring Trinidad (Jordan et al, 2020), identified a new lineage of IBV in poultry, while in mainland South America two well-defined lineages of IBV were determined to be circulating in poultry (Marandino et al, 2015).

IBDV antibodies were detected in 60% of the birds tested throughout the different regions, compared to 67.5% in Trinidad (Jordan et al, 2018a), 80.2% in Rio Grande do Sul State Brazil (Dos Santos et al, 2008), and 100% in North-Eastern Ecuador (Hernandez-Divers et al, 2006). Most regional territories inclusive of Guyana, vaccinate birds in their commercial operations against IBDV. Notwithstanding this, very virulent strains of IBDV (vvIBDV) have been reported to be circulating in South American countries, causing disease in vaccinated birds (Banda and Villegas, 2004) and (Mosle and Choudhury, 1972). Again, molecular analysis and characterization of the circulating IBDV strains in Guyana are necessary to determine whether circulating strains are derived from vaccine or field strains, since Guyana is geographically linked to South America, politically linked to the Caribbean, and commercially linked to the United States of America, from where much of the commercial poultry originates.

AIV antibodies were detected in 3.5% of birds, with the highest prevalence observed in region 3.

In Ecuador, a seroprevalence study found that 11% of birds tested positive for antibodies to AIV (Hernandez-Divers et al, 2006). However, a survey carried out in neighboring Trinidad and Tobago found no circulating antibodies to AIV in their backyard poultry (Jordan et al, 2018a). Researchers in Grenada, however, reported a seroprevalence of 59.5% for antibodies against AIV in backyard poultry flocks (Sharma et al, 2015). Subsequently, there were no reported signs of AIV in the sampled birds in Guyana, and no increases in mortality levels had been observed on the farms. It is more likely that a low pathogenic avian influenza (LPAI), rather than a high pathogenic avian influenza virus (HPAI), was circulating in the birds.

Despite seeing evidence for tumor-related histopathology consistent with ALV infection in commercial flocks following increased mortality, no antibodies were detected for ALV in the backyard birds sampled in this study. Notwithstanding, research conducted by Mosele and Choudhury-Uddin et al (1972) as cited by Begum et al (2016) demonstrated that backyard birds appeared to be resistant to this disease; however, the inability to detect antibodies in this study may also have been a result of the specificity of the commercial kit used in this study to distinguish two of the six known subgroups of ALV.

In Trinidad and Tobago, 10% of unvaccinated backyard birds tested positive for antibodies to NDV (Jordan et al, 2018a), whereas in this study in Guyana, 73.5% of birds tested were positive for NDV. A similar seroprevalence study in Southern Brazil found that 33.8% of birds were positive for anti-NDV antibodies (Marks et al 2014). While further afield, research carried out in the Hue Province of Vietnam found that 28.4% of unvaccinated backyard birds were positive for antibodies to NDV (Quang et al 2002). Since there were no clinical signs nor increased mortality observed in the backyard birds that were tested in Guyana, the antibodies to NDV that were detected may have been a result of infection with a lentogenic strain or possibly due to the circulation of a vaccine strain (Alexander et al, 2012). Vaccination, using live vaccines against NDV, is routinely carried out in Guyana's commercial poultry sector; as such, these vaccine strains may be circulating in backyard birds. Further work, including the molecular characterization of circulating NDV strains, is necessary to answer these questions since NDV remains a constant threat to poultry producers' worldwide, despite the availability and global

employment of ND vaccinations since the 1950s (Kapczynski et al, 2013), as was highlighted, ND remains endemic among commercial birds in many countries of Asia and Africa despite intensive vaccination programs (Alexander et al, 2012). Birds in Region four were not tested for antibodies to NDV; however, considering the high seroprevalence for NDV observed in the other three regions, it can be assumed that birds in this Region would also be seropositive for NDV.

The results obtained in this study demonstrates evidence of the circulation of viruses that have an immuno-suppressive effect on the host, such as CAV and IBDV, and are suggestive that local birds in Guyana are likely to be at a heightened risk of succumbing to disease when infected with circulating pathogens. This situation is made worse by the low levels of biosecurity, uncontrolled mixing of species, and poor hygienic surroundings that these birds are kept under. However, since these local breeds of backyard birds are well adapted to the environments that they live in and, through natural selection, are likely to be more resistant to locally circulating prevalent pathogens, adverse climate conditions, poor nutrition, and other stressors “this immense biodiversity has ensured their survival in diverse ecological zones by naturally being selected for survival fitness” (Msoffe et al, 2014 p.4). As such, even though these backyard birds are challenged by many pathogens, they may not be adversely affected by them.

It is a common practice for male chickens (cocks) to be bought from commercial layer houses after the growing stage and introduced into backyard farms to improve egg production quality. Besides, lay-out hens are also routinely purchased and introduced into backyard farms with the same objective. Since both of these categories of birds may have been vaccinated while in the commercial system, the presence of some antibodies may be as a result of vaccines, either directly through vaccination or indirectly through the circulation of live vaccine strains, as was demonstrated when chicken embryo origin (CEO) Infectious Laryngotracheitis (ILT) vaccine viruses were detected circulating in healthy unvaccinated chickens in a vaccinated region of Brazil (Chacón et al, 2015).

In Guyana's commercial poultry sector, vaccination is mainly practiced against NDV, IBV, IBDV, Marek's Disease Virus (MDV), and IBH. However, vaccination is not routinely practiced by all commercial poultry producers of

day-old chicks locally. Vaccination varies between hatcheries, with one hatchery stating that it was too time-consuming to administer the vaccines at the hatchery in time for customers to pick up due to the high volume of chicks produced. They were therefore advocating field vaccination, which in practice is rarely carried out. The constraints affecting the practice of field vaccination are the inability to maintain the required cold chain for vaccines between purchase from local suppliers and the time of administration and difficulty sourcing adequate dose size (Wakenell, 2016). Some farmers also feel that it is not cost-effective to purchase a 3000-dose vial of the vaccine when they only have a small number of birds to vaccinate, as also expressed by Crespo and Senties (2015). Therefore, many smaller commercial broiler and layer producers in Guyana opt not to vaccinate their birds, and vaccination is not carried out in backyard birds.

5. CONCLUSIONS

This study demonstrated antibodies circulating against CAV, IBV, IBDV, AIV, and NDV in unvaccinated backyard poultry across the significant poultry producing regions of Guyana. No antibodies were observed against ALV in the sampled population. These pathogens are of potentially significant importance to the poultry industry from both an economic and a public health perspective. The general lack of biosecurity measures, which is associated with the practice of rearing backyard poultry in Guyana, and the circulation of immunosuppressive viruses in the birds, means that the backyard birds in Guyana are likely to be at heightened risk of being adversely affected by many pathogens that may have a significant impact on their health. The creation of national vaccination programs should, therefore, be considered. Additionally, backyard poultry populations should be included in routine disease surveillance programs since these backyard systems may play a significant role in spreading viruses and influencing the diversity of circulating viruses in Guyana, thus putting the commercial poultry sector at higher risk from disease outbreaks.

6. ACKNOWLEDGMENTS

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