Research Note

An exploratory investigation of Newcastle disease virus serum antibodies in backyard chickens of Trinidad with geographical considerations

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Backyard chickens in rural communities of developing countries provide a potential means for the production of resilient, integrated small poultry farming systems. Despite this, they are often affiliated with the transmission of the economically important Newcastle disease virus (NDV). Presently, in Trinidad, information gaps exist regarding the backyard chicken population. To promote a better understanding of the serological status to NDV in this farming system, 303 unvaccinated backyard chickens from 11 farms across five counties in Trinidad were assessed for the presence of serum NDV antibodies using two commercial enzyme–linked immunosorbent assays (ELISAs). The study also explored the potential application of egg yolk samples for serological testing using a chloroform–free technique. In addition, data on farm locations, demographics, vaccination status, general management and biosecurity practices were obtained using a cross–sectional survey. An association was identified between the ID Screen® Newcastle Disease Conventional Vaccines (NDVS–CV) ELISA and the ID Screen® Newcastle Disease Nucleoprotein Indirect (NDVNP) ELISA (χ^2_{1df}, P = 0.001). The results indicate the presence of two different NDV antibody types in the serum of unvaccinated backyard chickens across farms in Trinidad. Differences in test seropositivity are suggestive of the influence of geospatial, general management, biosecurity and husbandry factors. These results can provide a useful background in the design of prospective NDV studies that aim at bridging the knowledge gap to promote situational awareness.

Keywords: Backyard chickens, Newcastle disease, serum antibodies, enzyme–linked immunosorbent assay, Trinidad, West Indies

Free–range backyard chickens are frequently associated with rural communities in developing countries and have been implicated in Newcastle disease virus (NDV) transmission (Awan et al. 1994). The presence of multiple backyard chicken farms has been recognized across Trinidad, in addition to a well–established commercial poultry industry (Evans 2015; Brown Jordan et al. 2018c). Worldwide, for over a decade, commercial poultry systems have come under scrutiny on the grounds of animal welfare outcomes and quality of products. Due to an enhanced level of animal welfare awareness by consumers, the demand for free–range eggs and meat has increased (Miao et al. 2005; Stadig et al. 2016). Free–range systems in Trinidad should therefore not be viewed as a threat or vilified, but rather seen as a means to improve animal welfare while lowering production costs and creating opportunities for more resilient, integrated small farming systems. Such free–range systems may include benefits like improved quality of eggs and meat and a reduction in the use of antimicrobials (Pettersson et al. 2016; Hedman et al. 2020; Miao et al. 2005).

Consequently, from a governance

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perspective, the legitimacy[1] aspect dictates that there is a need to examine animal health factors that can impact these systems (Huggins 2020a; Huggins 2020b).

From an epidemiological perspective, backyard chickens can act as sentinels thereby providing useful information on the occurrence of Newcastle disease (ND) in Caribbean poultry populations (Bolfa et al. 2019). The free–range conditions under which chickens are kept provide opportunities for interactions with wild avian reservoir species for ND, particularly those that are migratory. Furthermore, other factors may render these chickens susceptible to ND, including being reared on a mixed species farm, low levels of farm biosecurity and an unvaccinated status (Conan et al. 2012, Brown Jordan et al. 2018a). Newcastle disease virus replication in backyard chickens can pose a potential threat to commercial poultry operations located in close proximity as they can facilitate virus transmission (Alsahami et al. 2018). From a socio–economic context, backyard chickens reared in developing countries are known to provide a valuable source of income and high–quality protein to many residents of rural communities. Moreover, they are often associated with a minimum degree of investment (Guèye 2005). In Trinidad, such chickens can additionally be kept as a hobby, typically being located on family residences in flock sizes of 100 birds or less (Brown Jordan et al. 2018a). The vast majority of backyard chickens are referred to as "common fowls" which are usually of mixed ancestry and therefore do not possess distinguishing characteristics of any particular breed (Williams 1943).

Although NDV is of global significance to backyard and commercial chickens, existing studies conducted in the Caribbean region are few in number. Virulent NDV genotype V isolates have been reported in the Caribbean Community (CARICOM) member state, Belize, that is closely associated with the Caribbean region (Susta et al. 2014). The occurrence of virulent NDV genotype XVI isolates has been reported in the Dominican Republic (Courtney et al. 2013). The last reported occurrence of NDV in Jamaica was in 1969 (Handistatus 2003; Brown Jordan et al. 2018b). In Trinidad, reference was first made to a "mystery disease" that affected backyard poultry in a 1949–1959 special course report (Jones 2016).

In the Caribbean serological studies conducted on NDV in backyard chickens have been described for St. Kitts, Grenada and Trinidad. On the island of St. Kitts, an NDV antibody seroprevalence of 31% has been reported in backyard chickens (Bolfa et al. 2019). Previously conducted seroprevalence studies in unvaccinated, indigenous backyard chickens of Grenada have indicated NDV antibody seroprevalences of 99% in 2006 and 66.3% in 2015 (95% CI; 61.5% – 71.1%) (Sharma et al. 2015). A 47.7% (95% CI, 41.2 – 54.2%) NDV antibody seroprevalence has been reported in layers and a 48.2% (95% CI, 41.8 – 54.6%) level in broilers of Grenada (Sharma et al. 2014). A previous study in Trinidad indicated a 10% (95% CI: 4 – 23%) NDV antibody level in a small selection of unvaccinated backyard chicken farms (Brown Jordan et al. 2018a). However, it remains unknown whether NDV antibodies are produced against circulating viral vaccine strains in backyard chickens of Trinidad. Additionally, although egg yolks have successfully been used as an alternative to serum samples in NDV serological studies in several countries, their potential applications have not been explored under local settings.

Further studies are therefore warranted to gain a better understanding of the NDV status in unvaccinated, backyard chickens of Trinidad and to guide future research. The current study aimed to assess the sera of backyard chickens in Trinidad for the presence of NDV antibodies using two types of commercial enzyme–linked immunosorbent assays (ELISAs). The study further explored cross–matched egg yolk samples from backyard chickens in Trinidad for the presence of NDV antibodies.

[1] This is a new aspect of governance identified by Huggins 2020.

Materials and methods

Ethical approval

This study was approved by the Campus Ethics Committee of The University of the West Indies, St. Augustine, Trinidad and Tobago.

Informed consent

Verbal informed consent was obtained from participants prior to administering a questionnaire.

Study area and design

The study was conducted in Trinidad from April 2018 to December 2019. Trinidad is the larger of the twin island Republic of Trinidad and Tobago (T&T) (10° 41' 30.5" N and 61° 13' 21" W), located in the southernmost region of the Caribbean archipelago. Trinidad was historically divided into eight counties that included Caroni, Mayaro, Nariva, Saint Andrew, Saint David, Saint George, Saint Patrick and Victoria (Figure 1). The layout of these counties has been retained for the present study. A sampling frame (a complete list) of all backyard chicken farms in Trinidad and Tobago was unavailable at the time of the study. Farms were therefore selected based on convenience sampling under the guidance of the Poultry Surveillance Unit of Trinidad and Tobago, using a preliminary list of backyard farmers. Selection was focused on farms in proximity to wet land areas where migratory birds that are known reservoirs of NDV were most likely to have contact with backyard chickens. Backyard farms were placed into cohorts depending on the population of chickens: less than 10, 10 – 49 and 50 – 99 (Figure 1 and Table 1). Five farms were placed into the population cohort of 50 – 99 and another five into the 10 – 49 cohort. One farm was placed into the population cohort of less than 10. Backyard chickens of an unvaccinated status without clinical signs of NDV infection were included in the study. Sampled backyard chickens were placed into one of three age categories, younger than 6 months (14 chickens), 6–12 months (87 chickens) or older than 12 months (202 chickens). All farms included in the study had a flock size of less than 100 birds. Cross–matched, recently laid eggs were collected to determine NDV antibody levels in yolks. A total of 28 egg yolks were matched to serum samples.

A questionnaire was administered to farmers for data collection on sampling dates, farm location, demographics, NDV vaccination status, presence of multiple species on farm premises, sources of original and replacement flocks, proximity to commercial farms and general management and biosecurity practices.

Sample collection and handling

Approximately 2.0 mL of blood was aseptically collected in red top tubes from each chicken using the wing vein. Samples were kept in a plastic cooler with ice packs for transportation to the Immunology Laboratory at the School of Veterinary Medicine, The University of the West Indies for processing. Serum separation was done by centrifugation at 5000 rpms for 10 minutes in a refrigerated centrifuge (Beckman, USA). All serum samples were immediately transferred to cryogenic storage tubes (ThermoFisher Scientific, USA) and kept at –20 °C until required for laboratory tests.

Egg yolk extraction

Egg yolks were manually separated from egg whites prior to homogenization. Each egg yolk sample (20 µl) was pre–diluted in commercial ELISA wash solution (80 µl of 1X) (ID.vet, Grabels–France) in triplicate, then stored at –20 °C prior to use in an ELISA test as per manufacturer's recommendations.

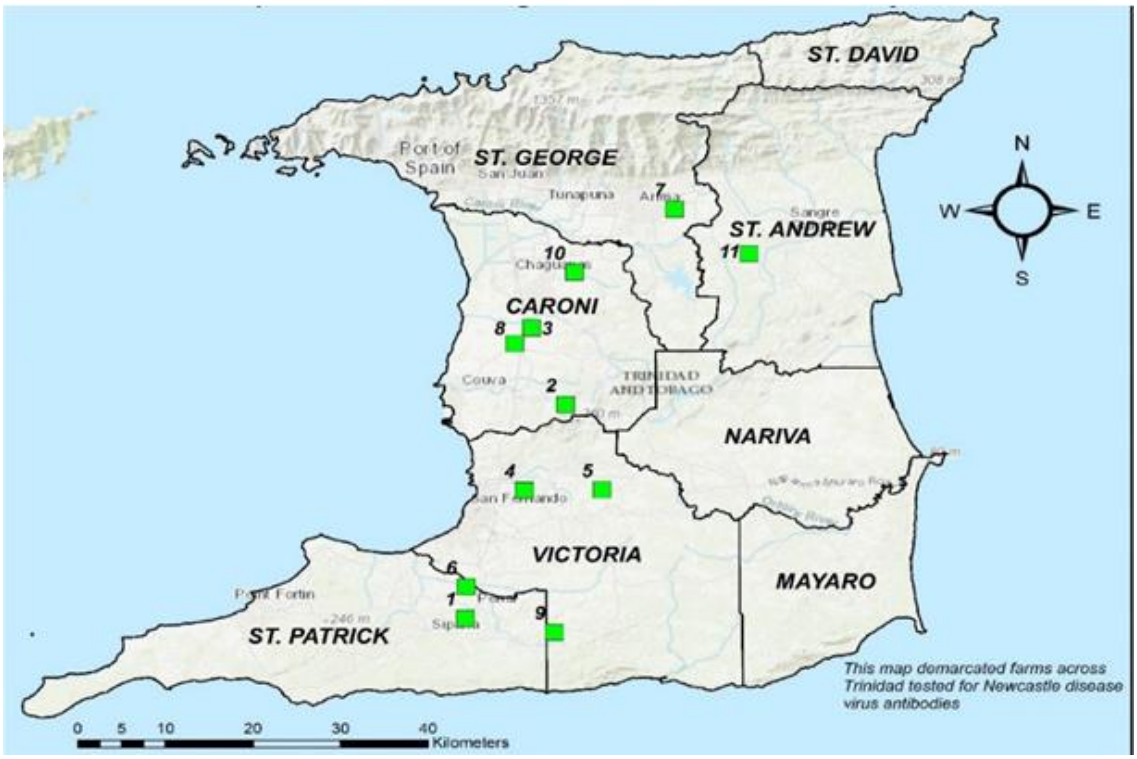

Figure 1: Map of Trinidad showing farm locations for the study
Source: Esri, HERE, Garmin, Intermap, increment P Corp, GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordinance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo©, OpenStreetMap contributors, and the GIS User Community.

Table 1: Sample site locations, population cohorts, sample sizes and positive serum test results for ID Screen® Newcastle Disease Conventional Vaccines (NDVS–CV) enzyme–linked immunosorbent assays (ELISAs)

Farm	Location	Population cohort	Sample size	Number of positive test results for NDVS–CV ELISA (serum)	Percentage positive test results for NDVS–CV ELISA (serum)	Number of positive test results for NDVNP ELISA (serum)	Percentage positive test results for NDVNP ELISA (serum)
1	St. Patrick	50 – 99	20	0	0	0	0
2	Caroni	50 – 99	30	15	50	13	43
3	Caroni	10 – 49	37	17	46	24	65
4	Victoria	50 – 99	30	25	83	30	100
5	Victoria	10 – 49	31	1	3	9	29
6	St. Patrick	50 – 99	17	0	0	0	0
7	St. George	10 – 49	33	1	3	3	9
8	Caroni	10 – 49	27	6	22	2	7
9	Victoria	<10	5	1	20	0	0
10	Caroni	10 – 49	32	1	3	8	25
11	St. Andrew	50 – 99	41	5	12	3	7

Laboratory tests

Serum samples were tested using the ID Screen® Newcastle Disease Nucleoprotein Indirect (NDVNP) (ID.vet, Grabels–France) and ID Screen® Newcastle Disease Conventional Vaccines (NDVS–CV) (ID.vet, Grabels–France) commercial ELISA kits. Egg yolk samples were tested using the ID Screen® NDVNP ELISA for egg yolk application with protocol version 1016. All procedures were conducted according to the manufacturer's instructions.

Statistical analyses

The data were expressed as frequencies and percentages. Pearson chi–square tests were applied to the data. Statistical significance was set at P value ≤0.05. A logit model was developed with the conventional ELISA test as the dependent variable and the nucleoprotein ELISA test as the independent variable. Data analysis was conducted with the statistical programme STATA 16 (College Station, Texas, USA).

Results

Questionnaire

Eleven farm owners across 5 counties in Trinidad participated in the cross–sectional study. All backyard chicken farms in this study were owned by males; however, they were family–run operations. Of the 11 farms visited, four of the farmers were between 25 – 34 years; one was 45 – 54 years, three were between 55 – 64 years and one was over 65 years of age. The farmers reported an unvaccinated NDV status for backyard chickens on all farms. The most frequently occurring age category of backyard chickens was older than 12 months (202/303). Other poultry species were reportedly kept on seven of the 11 sampled farms. Ducks were kept on all seven farms. Guinea fowl were kept on two farms while turkeys and geese were found on one farm. Nine farm owners indicated that their farm premises had a barrier or fence. One farm was reported to have no barrier or fence and one owner declined to participate for this question. Eight farm owners reported that there was contact between backyard chickens and wild birds. Two farm owners indicated that there was no contact, while one owner declined to participate for this question. Three owners reported that their backyard farms were in proximity to commercial farms. Sixty–eight (22.4%) of the backyard chickens in the study were purchased from agricultural shops; 95 (31.4%) from other farmers; 60 (19.8%) from importers and 33 (10.9%) from local breeders. Twenty–seven backyard chickens (8.9%) were acquired on a welfare basis while 20 (6.6%) were obtained through on–farm breeding (Figure 2).

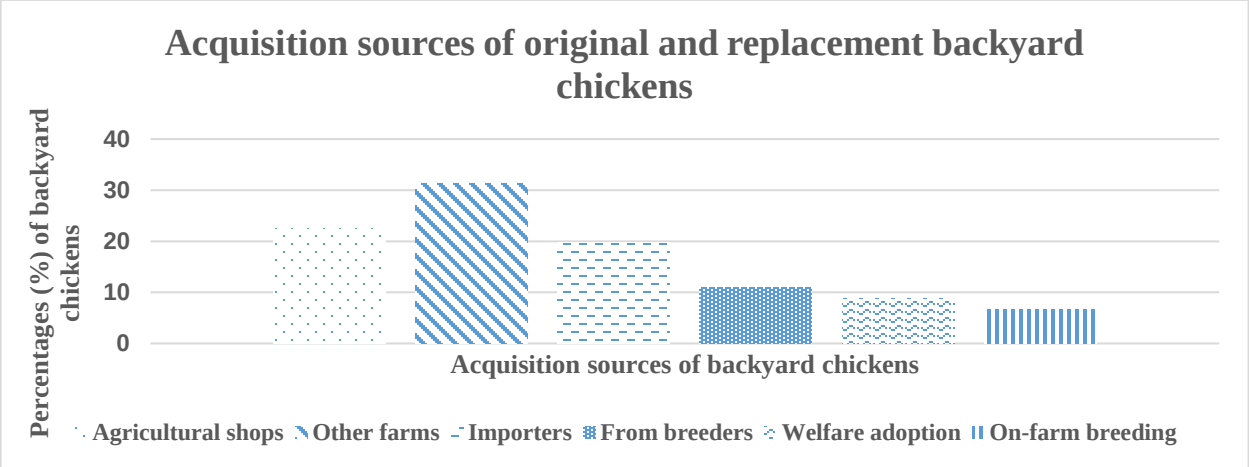

Figure 2: Acquisition sources of backyard chickens for 11 farms across 5 countries in Trinidad.

Enzyme–linked immunosorbent assays

A total of 303 serum samples obtained from unvaccinated backyard chickens on the 11 farms were assayed using commercial ELISAs, ID Screen® NDVNP and NDVS–CV. The number of birds sampled per farm ranged from five to 41. Overall, 30% (92/303) (Confidence Interval, CI, 95%: 25.4 – 36%) of all serum samples that were assayed generated positive results with the ID Screen® NDVNP ELISA. Twenty–four percent (72/303) (CI 95%: 19 – 29%) of all serum samples produced positive results with the ID Screen® NDVS–CV ELISA. Negative results were generated by both ELISAs for two farms using serum samples. Serum samples from one farm generated negative results with the ID Screen® NDVNP ELISA but positive results in 20% of the samples with the ID Screen® NDVS–CV ELISA (Figure 1 and Table 1). Proportions of positive ID Screen® NDVNP ELISA results on farms with at least one positive sample ranged from 7% (3/41) to 100% (30/30). Proportions of positive ID Screen® NDVS–CV ELISA results with at least one positive sample ranged from 3% (1/33) to 83% (25/30).

Twenty–three cross–matched egg yolk samples were tested with the ID Screen® NDVNP ELISA; NDV antibodies were detected in 52% (12/23) of the samples tested.

Model

An association was identified between the ID Screen® NDVS–CV ELISA and the ID Screen® NDVNP ELISA (χ^2_{1df}, P = .001). In going from a negative to a positive nucleoprotein indirect test, the odds of a positive conventional vaccines test increased by a factor of 11.9 (CI 95%: 6.4 – 22.0).

Discussion

In this study, we have shown the presence of NDV antibodies in the serum of unvaccinated backyard chickens in Trinidad using two commercially available ELISA kits, with an emphasis on geographical seropositivity. Antibodies were detected against the NDV nucleoprotein and purified NDV antigen specific to conventional vaccines in several farms, excluding those of St. Patrick and one originating from the county of Victoria (Table 1). Since convenience sampling was used in this exploratory investigation, inferences cannot be drawn to the wider target population and study findings are specific to the sample population. Nonetheless, data generated from this study can be used in the design of prospective studies and can offer insight from an "on-farm" basis.

To our knowledge, this is the first study to report the simultaneous detection of antibodies against conventional NDV vaccines and NDV nucleoprotein in sera of selected backyard chickens in Trinidad. A previous study, also based on convenience sampling, reported the detection of NDV–specific nucleoprotein antibodies at a level of 10% in 41 backyard chickens in five farms of Trinidad (Brown Jordan et al. 2018a). In the present study, a larger sample size was used for antibody detection with two types of commercial ELISA kits. Additionally, considerations were given to geographical location, demographic data, general management and biosecurity practices. Antibodies generated against the NDV nucleoprotein were also successfully detected in cross–matched egg yolks using a chloroform–free technique with the potential for future local applications. Such applications are useful from the perspective of minimising stress levels experienced by backyard chickens and in facilitating a convenient method of sample collection for NDV studies. Altogether, a 30% level was detected in all serum samples collected for evaluation of NDV–specific nucleoprotein antibodies. Overall, a 24% detection level was determined for all serum samples tested for anti–NDV antibodies produced against conventional vaccines. However, if the study results are interpreted from an "on-farm" perspective, then variation in test detection levels are observed (Figure 1 and Table 1). A farm in the county of Victoria

showed a 100% positive detection level for NDV-specific nucleoprotein antibodies and an 83% test positive level for antibodies produced against conventional vaccines. By contrast, another farm in the same county showed a 0% test positive level for the NDV-specific nucleoprotein but a 20% level for conventional vaccines. Interestingly, the backyard chickens from the latter were reported to have no contact with wild birds, unlike those from the previous farm. This farm was not close to the other two farms in the Victoria county, but it was close to two farms in St. Patrick which also had 0% test positive levels. While a 7% positive detection level was observed for NDV-specific nucleoprotein antibodies in a farm in Caroni, a 22% test positive level was detected for antibodies produced against conventional vaccines. This farm was reported as having a barrier or fence surrounding the premises and the absence of multiple poultry species on site. Backyard chickens, however, were still reported to have contact with wild birds. Another farm in Caroni was also reported to have enclosed premises with the absence of multiple poultry species on site but contact between backyard chickens and wild birds. By contrast, on this farm detection levels were 43% and 50% for NDV-specific nucleoprotein and anti-NDV antibodies produced against conventional vaccines, respectively. This supports the necessity to conduct further studies that take into account the wild bird migratory patterns based on geographical locations, especially for species that are highly susceptible to NDV. Notably, the Caroni Bird Sanctuary, an internationally declared wetland site that is protected, is in proximity to these backyard farms. This wetland site serves as the residence of over 100 avian species and is also the visiting site for migratory wild birds (Kumar 2018). Antibody levels were undetectable (0%) with both ELISAs on farms located in the county of St. Patrick that were reported as housing multiple poultry species along with contact between backyard chickens and wild birds.

Since backyard chickens are regarded as having an "unvaccinated" NDV status, these study findings may be suggestive of a possible circulating field strain of NDV linked to the use of "live attenuated" conventional vaccines, a scenario that was also mentioned in a previous study (Brown Jordan 2018a). In the present study, 27% of farm owners reported that their premises were in close proximity to commercial chicken farms. In the commercial layer industry of Trinidad and Tobago, live B_1 and LaSota NDV vaccines are typically administered on days 8 and 18 post-hatch. Another important consideration is that 19.8% of the backyard chickens in the study were reported to be imported, although the farmers had no vaccination records. This raises the possibility of *in ovo* vaccination with a turkey herpesvirus recombinant expressing the fusion (F) gene of NDV (rHVT-ND) prior to the arrival on farms. Therefore, future studies should be geared in the direction of confirmatory molecular diagnostic techniques for NDV genotyping, some of which offer the advantage of detecting mixed infections and low frequency variants (Bello et al. 2018). Due to practical limitations, it was not possible to conduct haemagglutination inhibition and neutralizing antibody tests at the time of the study since these required samples being sent overseas to reference laboratories. However, these are recommended for future studies on NDV in backyard chickens in Trinidad and Tobago and can be used to complement the monitoring of rHVT-F ND vaccine seropositivity. The study focused on farms that were close to wet land areas where migratory birds (reservoir hosts of NDV) can be found. The low accessibility to samples in some counties meant that there was little or no sampling in many parts of east, northwest and southeast Trinidad.

Conclusion

In this study, two types of commercial ELISAs were used to demonstrate the presence of NDV serum antibodies across unvaccinated, backyard chicken farms in Trinidad. The detected antibodies were produced against the NDV nucleoprotein or the purified NDV antigen, with the latter being used for monitoring vaccination

responses with conventional vaccines. Variations in test seropositivity were detected across farms and point to the presence of geospatial and other factors such as general biosecurity, management and husbandry practices. Further studies should include NDV genotyping, haemagglutination inhibition and neutralizing antibody testing across all counties. The outlined information gaps should be addressed to guide and support Government Governance, and Community Based Governance in these backyard poultry production systems, thereby supporting informed decisions now and in the future.

Declaration of competing interest

The authors declare that they have no financial or personal interests that could have appeared to influence the work presented in this paper.

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