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Research Article

Sero-epidemiological Survey of Avian Influenza and Newcastle Disease Antibodies in Japanese Quails (*Coturnix Coturnix Japonica*)

Osanyintuyi Shola Gabriel*, Adebayo Isaac Abayomi, Arogbodo Joseph Olowo

Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria

Abstract

The search for alternative and cheaper sources of animal protein has led to the introduction of quails to Nigeria. However, disease outbreaks constitute a limiting factor to the achievement of this goal. The objective of this study is to investigate the role of Japanese quails (*Coturnix coturnix japonica*) in the sustenance of the circulating strains of avian influenza virus (AIV) and Newcastle disease virus (NDV) in the Nigerian domestic chicken population. One hundred and twenty-two weeks-old unvaccinated Japanese quails and 210 twelve (12) day-old embryonated eggs of these birds were purchased from a reputable research center in Nigeria. Antibody was extracted from the egg yolk for maternal antibody evaluation and haemagglutination inhibition (HI) determination following standard procedures. The serum samples obtained were screened for avian influenza virus (AIV) and Newcastle disease virus (NDV) antibodies. Out of the 203 quail egg yolk extracts tested for the presence of HI antibodies, 3 (1.5%) and 136 (66.9%) were positive for AI and ND respectively. None of the serum samples was positive for antibodies against the two viruses. The positive egg yolk extracts yielded a minimum HI antibody titre of $\log_2 3$ ($\geq$1:8). The results obtained from this investigation showed that AI (H5N2) and ND maternal antibodies were present in Japanese quails, thus establishing the potential of these birds in the epidemiology of these two viral diseases. There is the need to routinely vaccinate local chickens and Japanese quails against ND. The national herd health programme should incorporate regular flock profiling as a way of routine sero-surveillance to forestall outbreak of these two viral diseases.

Keywords

Avian Influenza, Newcastle Disease, Japanese Quails, Antibodies, Sero-prevalence

1. Background

With a population of over 200 million people, Nigeria has, over the years, not been able to meet the Food and Agriculture Organization (FAO) recommended minimum protein intake requirement of 65gm per person/day. This has led to a search for alternative, cheaper sources of poultry products and the subsequent introduction of quail birds into Nigeria [8].

Apart from the escalating/galloping cost of feed materials in the poultry industry, disease outbreaks stand out as a significant limiting factor hindering the quest to achieve the national millennial goal as disease outbreaks could either lead to outright death of chickens or a more silent but devastating economic hardship as a result of poor production per-

*Corresponding author: sholagabriel78@gmail.com (Shola Gabriel)

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formance resulting to reduction in the quality and quantity of egg produced or slow growth rate arising from poor feed conversion rate.

Although quail is a hardy bird compared to other types of poultry, they can still be affected by the common poultry bacterial and viral diseases like salmonellosis, fowl cholera, colibacillosis, Reoviral infection, Marek's disease, Infectious Bursal Disease and Newcastle Disease.

Avian influenza is a disease of all domestic and wild birds characterised by respiratory, digestive and in some cases nervous signs with attendant high morbidity and mortality rates. Incubation period ranges from a few hours to 2 weeks, depending on the virus dose, route of infection and species of birds. An important feature of the avian influenza (AI) epidemic and the virus is the wide range of species of birds infected [11] and its potential for endemicity [6]. AI is worldwide in distribution [12, 7, 3] with a negative socio-economic impact, in addition to the public health significance of the disease.

Newcastle disease (ND) is a major viral disease of economic importance in poultry [5] and is rated as one of the greatest constraints to the development of rural poultry production in Nigeria and in most developing countries, causing serious threats [10]. ND is caused by virulent strains of Newcastle disease virus (NDV) and is notifiable to the World Organisation for Animal Health [14].

2. Objective

The objective of this study is to investigate the role of Japanese quails in the sustenance of the circulating wild viruses of Avian Influenza and Newcastle disease in Nigerian domestic chickens.

Materials and Methods

One hundred and twenty (120) 2-week-old unvaccinated Japanese quails (*Coturnix coturnix japonica*) were procured from a reputable Animal Research Centre in Nigeria and raised under hygienic conditions. They fed *ad-libitum* with commercial feed throughout the period of the experiment. In addition, two hundred and ten (210) embryonated eggs from Japanese quail were obtained from the same Research Centre.

Antibody was extracted from the embryonated quail eggs by dispensing 1 mL of Phosphate Buffered Saline (PBS) into test tubes into which 1 mL of the egg yolk had been dispensed, and 2 mL of chloroform was added. The mixture was shaken thoroughly and kept for one hour with intermittent shaking every 20 minutes. The tubes were centrifuged at 2,000 rpm for 10 minutes, thus separating the suspension into three phases. The supernatant was harvested with the aid of a Pasteur pipette and dispensed into labelled cryotubes and kept in the deep freezer (-20 ℃) until used.

Blood samples were aseptically collected from Japanese quails through the wing vein using filter papers, which were subsequently air-dried, labelled appropriately and kept in air-tight bags in the fridge until used.

The serum samples on the filter papers were eluted by puncturing the blood-saturated end of each filter paper with a file punch. Two of the punched discs of the filter paper eluates were placed into each well of micro-titre plates, 100 µl of PBS was dispensed in each well containing the disc of filter paper eluates and kept for 24 hours in a fridge (4 ℃). Exactly 25 µl of the eluate was harvested from each well after 24 hours and used as a source of serum in the HI test. The filter paper eluates and egg yolk extracts from the experimental birds and eggs, respectively, were tested for the presence of maternal antibodies against AI and NDV using a modified haemagglutination inhibition (HI) technique [2] with the adoption of heterologous red blood cell as indicator systems [1].

3. Results

Out of the 203 quail egg yolk extracts tested to detect the presence of AI and ND HI antibodies, 3 (1.5%) and 136 (66.9%) were positive for AI and ND antibodies, respectively. None of the filter paper eluates were positive for either of the two disease conditions. The distribution of the AI HI antibody titres showed that all the positive samples gave titres with ≥ 1:8 ($\log_2 3$).

Table 1. *Prevalence of Avian Influenza and Newcastle disease virus antibodies in embryonated eggs and filter paper eluates from experimental quails.*

Sample types	Avian Influenza		Newcastle disease	
	Total Number tested	Total Number positive	Total Number tested	Total Number positive
Egg yolk extract	203	3	203	136
Filter paper eluate	79	0	79	0

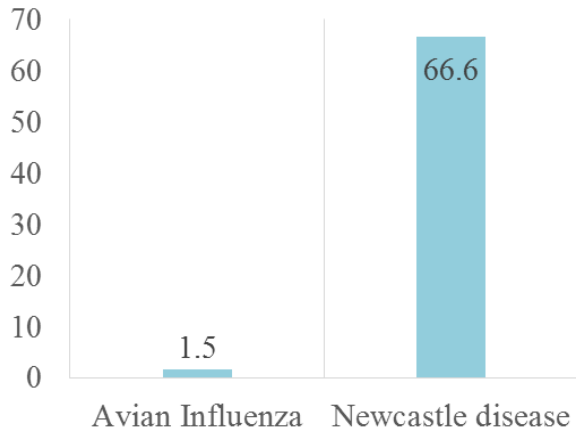

Figure 1. *Percentage seroprevalece of AI and ND HI Titres in the experimental quails.*

4. Discussion

The result of this experiment revealed seroprevalence (1.5%) of AI (H5N2) and ND (66.6%) maternal antibodies in the embryonating eggs of the experimental quails as the embryonating eggs were positive to AI and ND. The quails used in the study had no history of vaccination against NDV and AI. Consequently, the antibodies demonstrated were most likely to be as a result of passive immunity derived from their infested hens. All the 79 quail blood filter paper extracts were negative to both AI and NDV.

Maternal antibodies in an offspring may be as a result of vaccination or natural infection. In this experiment, the modified methods of HI determination [2] was employed to ascertain the type of antibody (passive) that was present in the egg yolk extracts [1].

Consequently, the titre values obtained were as a result of maternal antibodies in the embryonating eggs, thus suggesting that *Coturnix coturnix japonica* could be a potential source of AI and ND in the epidemiology (perpetuation) of these viruses in an environment.

Quails serve as an intermediate host in the transmission and perpetuation of influenza viruses crossing the species barrier. They have been proposed to be the "route modulator" that alters the mode of transmission from that of faecal-oral to aerosol [9]. Quails may have a role in the history of influenza because of the ability of influenza viruses to replicate in the trachea of quails and the quail's susceptibility to multiple subtypes.

Newcastle Disease (ND) is an important viral infection in birds that could cause huge economic losses for the poultry industry [4]. In this context, *Coturnix coturnix japonica*, is considered an important potential carrier for the ND virus [13]. This study suggests that besides the carrier role, the Japanese quail could play an ultimate host role in the epidemiology of ND and HI viruses.

5. Conclusion

The results obtained from this investigation showed that there is sero-prevalence of AI (H5N2) and ND maternal antibodies in the experimental Japanese quails thus establishing the potential of Japanese quails in the epidemiology of these two viral diseases.

6. Recommendations

(1) There is the need to routinely vaccinate local (domestic) chickens and quails against ND.

(2) The National policy of zero-vaccination of poultry birds against AI should be sustained in order to minimize the problems of antigenic shift and drift commonly encountered in the case of Avian Influenza Viruses.

(3) The National Herd Health Programme should incorporate regular flock profiling as a way of routine sero-surveillance to checkmate the outbreak of the two diseases with indepth phylogenetic studies.

Abbreviations

AI	Avian Influenzas
ND	Newcastle Disease
AIV	Avian Influenza Virus
HI	Haemagglutination Inhibition
FAO	Food and Agriculture Organisation

Conflicts of Interest

The authors declare no conflicts of interest.

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