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DETECTION OF NEWCASTLE DISEASES ANTIBODIES IN BIRDS IN JOS, BUKURU AND ENVIRONS USING AGAR-GEL PRECIPITATION TEST

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ABSTRACT

Newcastle Disease Virus (NDV lasota) antibodies were surveyed. A serological study of 500 sera from local chicken, turkey, ducks and guinea fowl was carried out to determine the prevalence of Newcastle disease using Agar-gel precipitation technique. Samples were obtained from chickens slaughtered in Jos, Bukuru and environs. The samples were made up of 40 ducks, 50 turkeys, 20 guinea fowl and 390 local chickens. The precipitin antibodies were detected in ducks 7 (17.5%), turkey 12 (24.0%), guinea fowl 3 (15.0%), and local chickens 54 (13.9%). Of the 500 samples screened, a total of 76 (15.2%) were positive for NDV antibodies. Quantitative analysis of sero-positive samples in the four avian species tested yielded a range of antibody titres of 2-16. The importance of using cost effective technique for the detection of NDV carrier state in local birds has been highlighted.

Keywords: NDV antibodies, Agar-gel precipitation technique (AGPT), NDV carrier state

INTRODUCTION

Newcastle disease (ND) is an acute, rapidly spreading, contagious, nervous and respiratory disease of birds of all ages caused by the avian Paramyxovirus serotype 1 (APMV-1) (Okeke and Lamorde, 1988). Transmission of the virus is most common through bird to bird contacts (Hugh-Jones *et al.,* 1973), faecal exposure, respiratory discharges or other discharges from infected birds and through contact with contaminated feeds, water, equipment, poultry attendants and clothing, leading to high morbidity and mortality (Oladele *et al*., 2003; Saidu *et al.,* 2006). ND is a major viral disease of economic significance in poultry (Anosa and Adene, 2007) and ranked as one of the chief constraints to the development of rural poultry production in Nigeria and in most developing countries, triggering serious dangers and concerns (Shamaki *et al.,* 1989; Oladele *et al*., 2003). The disease is most common during harmattan period of the year (Alexander, 1997) and occurs in domestic fowls, exotic birds, turkeys, ducks, geese, pigeons and wild birds (Roy and Chamham, 2007; Echeonwu *et al.,* 1993). It is characterized by nasal and ocular discharge, yellowish to greenish diarrhea, sneezing, coughing, drooping of wings, twisting of head, drop in egg production and thin shelled eggs, complete paralysis, weakness and sudden death (Chansiripomchai and Sasipreyayan, 2006; Roy and Chamham, 2007). Domestic chicken have been known to be sources of the spread of ND virus (Lancaster, 1963; Roy *et al.,* 1998). Alexander *et al*. (1984) reported that the spread of ND virus to chickens has occurred in several countries, including Great Britain, where 20 out breaks in unvaccinated chickens occurred in 1984 through feed contaminated by faeces of infected pigeons. In rural Nigeria, it is common sight to find a combination of different poultry species and breeds together in the same compound (Ibrahim and Abdu, 1992), including chickens, turkeys, ducks and pigeons. Presently, it is accustomed to find ostriches, peacocks, geese and ducks in the same compound in cities and in some poultry farms. ND was reported in Nigeria in guinea fowls and a highly velogenic strain of ND virus was isolated from seemingly healthy ducks (Echeonwu *et al.,* 1993). Outbreak of ND in young ostrich was also reported by (Saidu *et al.,* 1999). There is only one serological evidence of ND infection in pigeons in Nigeria (Oladele *et al.,* 1996). Therefore, validating a simple, cost effective technique for the detection of NDV carrier states in field bird in a limited resource setting cannot be underscored.

MATERIALS AND METHODS

Collection of blood samples

A total of five hundred (500) blood samples between April, 2005 and August, 2005 were collected from local birds such as local chickens, guinea fowls, turkey and ducks from Jos, Bukuru and environs slaughter houses into sterile McCarthney bottles. None of the birds were vaccinated against ND. The blood samples were made up of 390 local chickens, 20 guinea fowl, 40 ducks and 50 turkeys as shown in Table 1. The bottles were kept in a slanting position for the blood to clot and transported immediately to the laboratory.

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The samples were left overnight at 4°C, the serum were neatly collected by aspirating into bijou bottles and cryovials, labeled and stored in a freezer at -20°C until required.

Antigen (NDV Lasota)

The NDV lasota antigen was used as a positive control for this research in the form of freeze-dried vaccine supplied by the National Veterinary Research Institute (NVRI) in Vom, Nigeria.

Preparation of 0.7% Agar-gel

The purified agar was used, it was prepared by weighing 8g of Nacl and Agar 0.7g into sterile 250ml Scott bottle and the volume was made to 100ml with distilled water and mixed properly. The preparation was autoclaved at 121°C for fifteen (15) minutes to dissolve the agar. The agar was allowed to cool to about 45°C and then poured into sterile petri dishes, arranged on a flat surface; plates were covered and left at room temperature for agar to set. The agar plate were stored at 4°C refrigerator and used within seven days of preparation.

Agar-gel precipitation test (AGPT)

The Qualitative and Quantitative agar-gel precipitin test has been used for the detection of antibodies in poultry virus (Witter, 1972; Baron, 1991). Two fold serial dilution of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256 of all test sera were prepared using sterile phosphate buffered saline (PBS) as diluent.

RESULTS AND DISCUSSION

Out of 500 samples, 76 (15.2%) were positive and 424 (84.8%) were negative for Newcastle disease virus antibodies. The highest overall prevalence of ND was found in turkey (24.0%) (Table 2).

Table 1. Number of blood samples used in this study

Sources	Chicken	G/F	Ducks	Turkey	Total
Bukuru slaughter market	230	13	27	30	300
Jos slaughter market	160	87	13	20	20
Total	390	20	40	50	500

Table 2. Distribution of NDV (lasota) antibodies detected in the 4 avian species sampled

Types of Birds	Number sampled	Number positive	Prevalence (%)
Ducks	40	7	17.5
Turkeys	50	12	24.0
Guinea fowl	20	3	15.0
Local chicken	390	54	13.9
Total	500	76	15.2

With recent surge of incidence of different strains of avian influenza virus, the quest for developing and validating already existing methods that are cost effective, fast turnaround time and easy to handle cannot be underscore. From the result of qualitative analysis, a total prevalence of 15.2% (76) positive agar-gel precipitin antibody reaction to newcastle disease virus (lasota) was recorded among unvaccinated local birds (duck, turkey, guinea fowl (G/F) and chicken), this therefore, suggested that the presence of antibodies to Newcastle disease virus were due to exposure to the ND virus. The results of this present study as shown in table 2 indicate that there are significant activities of NDV (lasota) in local birds in Bukuru, Jos and environs. Since there is no vaccination programme for these local birds, presence of antibody in these apparently healthy local birds is an indication of sub-clinical infection and could act as a carriers of ND virus (Bell and Mouloudi, 1988; Olabode *et al.,* 1992). Table 3 showed the significant varying titre reactivity of each avian species with turkey and local chicken having the highest titre of 8 and 16 respectively, while Table 4-7 showed the breakdown of the titration for each of the avian species that showed significant titre reactivity with local chicken and turkey showing the highest percentage of the number of the positive sera screened.

Table 3. Quantitative analysis of sero-positive samples antibody range

Type of Birds	Number of positive	Line of precipitin	No line of precipitin	Titre
Ducks	7	2	5	2-4
Turkeys	12	4	8	2-8
Guinea fowl	3	1	2	2-4
Local chicken	54	12	42	2-16

Table 4. Quantitative analysis of sero-positive samples: NDV antibody titres in ducks

Antibody titre (log2)	Number of positive	(%) positive
4	0	0.0
3	0	0.0
2	1	50.0
1	1	50.0

Table 5. Analysis of sero-positive samples NDV antibody titre in turkeys

Antibody titre (log2)	Number of positive	(%) positive
4	0	0.0
3	2	50.0
2	1	25.0
1	1	25.0

Table 6. Quantitative analysis sero-positive samples: NDV antibody in guinea fowl (G/F)

Antibody titre (log2)	Number of positive	(%) positive
4	0	0
3	0	0
2	0	0
1	1	100

Table 7. Quantitative analysis sero-positive samples: NDV antibody in local chickens

Antibody titre (log2)	Number of positive	(%) positive
4	1	8.3
3	5	41.7
2	4	33.3
1	2	16.7

The findings of this study are similar to results of similar studies carried out by other authors (Iroegbu and Echeonwu, 1997; Mai *et al.,* 2004; Musa *et al*., 2009; Nwanta *et al.,* 2006). The qualitative analyses of the distribution of NDV antibodies in the 4 local birds gave a cogent breakdown of the prevalence of the ND virus amongst the 4 avian species with turkey having the highest prevalence.

The NDV Agar-gel precipitation test (AGPT), will be greatly valuable in areas of the world where limited facilities or technical know-how is a challenge for using serological procedures. The susceptibility of local birds to ND virus in Nigeria is somewhat high and no matter the age of the birds if it remains unvaccinated it is still susceptible.

CONCLUSION

The AGPT has been found to be simple, inexpensive and faster in assessing ND infection in birds, though there are more sensitive techniques for the detecting of NDV but the AGPT method used for this research was easily available and affordable at the time and location. Education of farmers on good practice of poultry keeping should still be encourage to prevent spread of ND infection among other flock and this include proper handling and disposal of used litters, construction of bird and vermin prof houses and proper disinfection of poultry house time to time with suitable disinfectant as the virus can persist in the environment for a very long time.

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