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Newcastle Disease Antibodies in Apparently Healthy Wild Birds in Kogi State, Nigeria

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ABSTRACT

A survey was carried out to determine the presence of Newcastle disease (ND) antibodies in wild birds from 12 Local Government Areas (LGAs) of Kogi State, Nigeria. The families of wild birds that were captured alive and blood obtained from them include *Apodidae, Hirundinidae, Columbidae, Psittacidae, Sylviidae, Muscicapidae, Ciconiidae* and *Accipitridae*. The haemagglutination inhibition (HI) test and enzyme link immunosorbent assay (ELISA) were used to detect ND antibodies from sera of the wild birds. The overall seroprevalence of ND in wild birds by HI test was 29.2% with mean antibody titre of 2.69±0.51 $\log_2$ while the seroprevalence was highest in Ankpa, Ofu and Olamaboro LGAs each with 50.0% and lowest in Adavi, Mopamuro and Okene LGAs with 0.0%. *Hirundinidae* had the highest ND seroprevalence of 50.0% by HI test, followed by *Apodidae* with 33.3%, *Columbidae* with 23.1% and lowest in *Accipitridae*, *Psittacidae*, *Muscicapidae*, *Ciconiidae* and *Sylvidae* with 0.0% while 18.9% of the wild birds' sera had antibody titre ≥7 $\log_2$. Using ELISA, the same sera from wild birds were analyzed with the overall seroprevalence of ND in free flying wild birds being 27.1% with mean antibody titre of 11.06±1.28 ELISA Units. The seroprevalence of ND using ELISA in wild birds was highest in Ankpa LGA with 42.9% and lowest in Mopamuro and Okene LGAs with 0.0%. Also using ELISA, the seroprevalence of ND among wild bird families was highest in *Accipitridae* with 66.7%, followed by *Hirundinidae* with 38.9%, *Apodidae* with 33.3%, *Columbidae* with 15.4% and lowest in *Psittacidae*, *Muscicapidae*, *Ciconiidae* as well as *Sylvidae* with 0.0%. The presence of ND antibodies in wild birds was an indication of natural exposure and the presence of wild type ND viruses in wild birds in Kogi State. It is recommended that vaccination of rural poultry should be encouraged, backyard poultry should be screened from wild birds and scavenging rural poultry.

Key words: Newcastle disease, antibodies, wild birds, HI test, ELISA, Kogi State

INTRODUCTION

Wild birds are known to be liable for the wide intercontinental spread of Newcastle disease (ND) and other pathogens (Jourdain *et al*., 2007; Swayne and Halvorson, 2008). In most parts of Africa and Nigeria, ND is endemic often causing outbreak in backyard and commercial poultry (Ezeokoli *et al*., 1984; Adene, 1996). The impact of ND is more in Nigeria where over 90% of the poultry are rural poultry that are left to roam freely to scavenge for food and water bringing them into close activity space with wild birds (Adene and Oguntade, 2006).

Although, ND is mostly pathogenic for the domestic chickens, turkeys and guinea fowls; other poultry and wild birds may not show clinical disease but do develop antibodies to the virus (Orajaka *et al*., 1999; Mohammed *et al*., 1998; Sa'idu *et al*., 2004). These birds act to maintain ND viruses which serve as sources of frequent outbreaks in rural and backyard poultry (Alexander, 2001; Sa'idu *et al*., 2004). Outbreak of ND had been caused by contamination of poultry feed with faeces of pigeon in the UK (Alexander, 2011).

Wild birds and semi-domestic birds are known to be susceptible and develop antibodies to ND hence, play major roles in the spread of ND viruses especially in Nigeria as well as other African countries with poor poultry husbandry practice (Ezeokoli *et al*., 1984; Oladele *et al*., 1996; Sa'idu *et al*., 2004). The circulation of ND viruses in wild birds and maintenance in the environment were responsible for most outbreaks in backyard and commercial poultry even after vaccination (Sa'idu *et al*., 2008).

Reports on the status of ND in wild birds in Nigeria are scanty as a result of limited surveillance in wild birds (Assam, 2014). The present study was designed to determine the seroprevalence of ND in wild birds from twelve LGAs of Kogi State. To the best of our knowledge, this is the first report of ND antibodies in wild birds in Kogi State, Nigeria.

MATERIALS AND METHODS

Study area: The study area is Kogi State which lies between Latitude 6°44'-7°36' N and Longitude 7°49'-8°27' E situated at a height of about 789 km above sea level and covering a land area of 29,833 km^2. The State is bordered by FCT and Niger State on the north, Benue and Nasarawa States on the east, Ekiti and Kwara States on the west, Edo, Anambra and Enugu States on the south.

The vegetation of Kogi State is guinea savannah on the north and a belt of rain forest on the southern fringe with rivers Niger and Benue passing through the State, which later converged at a point to form a confluence. The annual rainfall ranges from 1100-1250 mm starting from April to October (KGSADP., 2009).

Kogi State has a total of twenty-one Local Government Areas (LGAs). The human population of the state is 2,099,046 with the major economic activities of the people being farming, fishing and trading (KGSADP., 2009). The poultry population is estimated to be 3,685,211 with 91.5% being rural or backyard poultry and rest being commercial poultry (Adene and Oguntade, 2006).

Sample size for wild birds: Data on population size of wild bird species and prevalence of ND antibodies in wild birds are scanty in Nigeria, a problem adjudged to be a major challenge for designing disease surveys in wild birds in most countries (Wilking *et al*., 2009). Hence, the sample size was not predetermined but was thought reasonable to limit the sample size to available resources and number of wild birds captured as done by other workers (Teru *et al*., 2012; Assam, 2014).

Sampling method for wild birds: Wild birds kept in households and those sold in the LBMs were sampled. Also, free flying wild birds seen around poultry farms and wetlands were baited and caught alive for samples collection. A total of 48 species from 8 families of wild birds were captured alive using mist net and glue traps from September, 2012 to February, 2013. The families of wild birds that were captured alive include *Accipitridae*, *Muscicapidae*, *Apodidae*, *Hirundinidae*, *Psittacidae*, *Ciconidae*, *Sylvidae* and *Columbidae*.

Collection of blood sample: Blood was collected from 48 species of apparently healthy wild birds after proper restraint with a 23 or 24 gauge needle (depending on the size of bird) attached to a 2 mL syringe to withdraw 0.5-1 mL of blood. The blood collected was allowed to stand for 2 h for clotting to occur and the serum decanted into a serum bottle, stored under ice, transported to the laboratory and kept at -20°C until used.

Detection of newcastle disease antibodies

Haemagglutination inhibition test: The antigen used was La Sota strain of ND Vaccine batch 09/2012 and antiserum obtained from the Veterinary Research Institute (NVRI), Vom, Nigeria. A 1% suspension of chicken red blood cells (RBC) was prepared and used as indicator in the haemagglutination (HA) and haemagglutination inhibition (HI) tests. The HA titre of the La Sota antigen was determined as prescribed by WOAH (2009) and diluted to contain 4 HA units. This concentration of antigen was used for the HI test. The HI antibody titre for each test serum was determined and expressed in $\log_2$ and the mean titre was also calculated.

Enzyme link immunosorbent assay: The ND antibody ELISA test kit used was manufactured by Affinitech Ltd, Bentonville, AR 72712, USA. The reagents supplied included antigen coated microtitre plates, sample diluent, wash solution, conjugate (IgG alkaline phosphate), substrate (p-Nitrophenyl phosphate), stop solution (3.0 M NaOH), positive and negative controls which were prepared and used according to manufacturer's instructions. The ELISA plates were read using dual wavelength microtitre plate ELISA reader with 405 nm primary filter and 630 nm reference filter blanked on air. Serum with 15 ELISA Units (EU) or more were taken as positive.

RESULTS

Newcastle disease antibodies detection by haemagglutination test and enzyme link immunosorbent assay: A total of 48 sera of wild bird species from 8 families were analyzed for antibodies to ND (Table 1). Fourteen (29.2%) of the sera were positive for antibodies to ND by HI test with overall mean antibody titre of 2.69±0.51 and the seroprevalence in the LGAs were 50.0% each for Ankpa, Ofu and Olamaboro; 33.3% for Ajaokuta; 22.2% for Lokoja; 20.0% for Dekina while Adavi, Mopamuro and Okene LGAs had 0.0%, respectively (Table 1). Based on families, the seroprevalence of ND by HI test were 50.0% for *Hirundinidae*, 33.3% for *Apodidae* and 23.1% for *Columbidae* while the other families of wild birds had 0.0%, respectively (Table 2). Based on species, the seroprevalence of ND by HI test were 58.3% for *Hirundo rustica*, 50.0% for *Apus cafer*,

Table 1: Seroprevalence of newcastle disease detected by haemagglutination test in sera of wild birds from the surveyed local government areas of Kogi State, Nigeria

Local government area	No. tested	No. positive (%)	Antibody titre ≤3 $\log_2$	Antibody titre ≥7 $\log_2$	Mean antibody titre±SEM
Adavi	4	0 (0.0)	4 (100.0)	0 (0.0)	0.00
Ajaokuta	3	1 (33.3)	2 (66.7)	0 (0.0)	2.00±2.00
Ankpa	6	3 (50.0)	3 (50.0)	2 (33.3)	3.17±1.52
Dekina	5	1 (20.0)	4 (80.0)	0 (0.0)	1.60±1.03
Lokoja	9	2 (22.2)	7 (77.8)	3 (33.3)	2.78±1.34
Mopamuro	2	0 (0.0)	2 (100.0)	0 (0.0)	0.00
Ofu	6	3 (50.0)	3 (50.0)	2 (33.3)	4.33±1.87
Okene	5	0 (0.0)	5 (100.0)	0 (0.0)	0.00
Olamaboro	8	4 (50.0)	4 (50.0)	3 (37.5)	4.63±1.41
Overall	48	14 (29.2)	34 (70.9)	9 (18.9)	2.69±0.51

Table 2: Seroprevalence of newcastle disease detected by haemagglutination inhibition test in the different families and species of wild birds surveyed in Kogi State, Nigeria

Family of wild bird	Species of wild bird	Species seroprevalence (%)	Family seroprevalence (%)
Apodidae	*Apus barbatus*	0/1 (0.0)	2/6 (33.3)
	Apus affinis	1/3 (33.3)	
	Apus caffer	1/2 (50.0)	
Hirundinidae	*Hirundo rustica*	7/12 (58.3)	9/18 (50.0)
	Hirundo aethiopica	2/6 (33.3)	
Columbidae	*Columbia livia*	0/4 (0.0)	3/13 (23.1)
	Columbia guinea	0/2 (0.0)	
	Streptopelia senegalensis	3/7 (42.9)	
Psittacidae	*Psittacula krameri*	0/2 (0.0)	0/2 (0.0)
Sylvidae	*Hippolais polyglotta*	0/2 (0.0)	0/2 (0.0)
Ciconiidae	*Ciconia nigra*	0/2 (0.0)	0/2 (0.0)
Accipitridae	*Milvus migrans*	0/3 (0.0)	0/3 (0.0)
Muscicipidae	*Muscicapa infuscata*	0/1 (0.0)	0/1 (0.0)
Total		14/48 (29.1)	14/48 (29.1)

Table 3: Seroprevalence of Newcastle disease detected by haemagglutination test in sera of wild bird from different families in Kogi State, Nigeria

Family of wild bird	No. tested	No. positive (%)	Antibody titre ≤ 3 $\log_2$ (%)	Antibody titre ≥ 7 $\log_2$ (%)	Mean antibody titre±SEM
Accipitridae	3	0 (0.0)	3 (100.0)	0 (0.0)	0.00
Columbidae	13	3 (23.1)	10 (76.9)	1 (7.7)	2.31±0.79
Muscicapidae	1	0 (0.0)	1 (100.0)	0 (0.0)	0.00
Psittacidae	2	0 (0.0)	2 (100.0)	0 (0.0)	0.00
Ciconiidae	2	0 (0.0)	2 (100.0)	0 (0.0)	0.00
Hirundinidae	18	9 (50.0)	9 (50.0)	8 (44.4)	4.44±1.02
Apodidae	6	2 (33.3)	4 (66.7)	0 (0.0)	2.67±0.96
Sylvidae	2	0 (0.0)	3 (100.0)	0 (0.0)	0.00
Overall	48	14 (29.2)	34 (70.9)	9 (18.9)	2.69±0.51

Table 4: Seroprevalence of Newcastle disease in sera of wild birds detected by enzyme link immunosorbent assay from the surveyed local government areas of Kogi State, Nigeria

Local government area	No. tested	No. positive (%)	Mean antibody titre±SEM
Adavi	3	1 (33.3)	11.77±6.96
Ajaokuta	3	1 (33.3)	15.53±3.39
Ankpa	7	3 (42.9)	15.79±3.35
Dekina	6	2 (33.3)	14.38±2.62
Lokoja	9	3 (33.3)	9.08±3.34
Mopamuro	2	0 (0.0)	0.00
Ofu	6	1 (16.7)	6.78±3.82
Okene	5	0 (0.0)	0.00
Olamaboro	7	2 (28.6)	12.24±3.94
Overall	48	13 (27.1)	11.06±1.28

42.9% for *Streptopelia senegalensis*, 33.3% each for *Hirundo aethiopica* and *Apus affinis* while the other species had 0.0%, respectively (Table 2). Thirty-four (70.9%) of the wild bird species had antibody titre ≤ 3 $\log_2$ while 9 (18.9%) had antibody titre ≥ 7 $\log_2$ (Table 3).

Using ND ELISA, 13 (27.1%) of the sera of wild birds were positive for antibodies to ND with overall mean antibody titre of 11.06±1.28 (Table 4). On the basis of LGAs, the seroprevalence of ND using ELISA were 42.9% for Ankpa; 33.3% each for Adavi, Ajaokuta, Dekina and Lokoja; 28.6% for Olamaboro and 16.7% for Ofu while Mopamuro and Okene LGAs had 0.0%, respectively (Table 4). Based on families the seroprevalence of ND using ELISA were 66.7% for *Accipitridae,* 38.9% for *Hirundinidae*, 33.3% for *Apodidae* and 15.4% for *Columbidae* while the rest families of wild birds had 0.0%, respectively (Table 5). Based on species the seroprevalence of ND using ELISA were 66.7% for *Milvus migran,* 50.0% each for *Hirundo rustica* and *Apus caffer,* 33.3% for *Apus affinis* and 28.6% for *Streptopelia senegalensis* while the other species had 0.0%, respectively (Table 6).

Table 5: Seroprevalence of Newcastle disease in sera of wild bird from different families detected by enzyme link immunosorbent assay from Kogi State, Nigeria

Family of wild bird	No. tested	No. positive (%)	Mean antibody titre± SEM
Accipitridae	3	2 (66.7)	16.57±2.68
Columbidae	13	2 (15.4)	9.88±2.06
Muscicapidae	2	0 (0.0)	0.00
Psittacidae	1	0 (0.0)	0.00
Ciconiidae	2	0 (0.0)	0.00
Hirundinidae	18	7 (38.9)	13.39±2.46
Apodidae	6	2 (33.3)	11.90±4.02
Sylvidae	3	0 (0.0)	0.00
Overall	48	13 (27.1)	11.06±1.28

Table 6: Seroprevalence of Newcastle disease detected by enzyme link immunosorbent assay in the different families and species of wild birds surveyed in Kogi State, Nigeria

Family of wild bird	Species of wild bird	Species seroprevalence (%)	Family seroprevalence (%)
Apodidae	*Apus barbatus*	0/1 (0.0)	2/6 (33.3)
	Apus affinis	1/3 (33.3)	
	Apus caffer	1/2 (50.0)	
Hirundinidae	*Hirundo rustica*	6/12 (50.0)	7/18 (38.9)
	Hirundo aethiopica	1/6 (16.7)	
Columbidae	*Columbia livia*	0/4 (0.0)	2/13 (15.4)
	Columbia guinea	0/2 (0.0)	
	Streptopelia senegalensis	2/7 (28.6)	
Psittacidae	*Psittacula krameri*	0/2 (0.0)	0/2 (0.0)
Sylvidae	*Hippolais polyglotta*	0/2 (0.0)	0/2 (0.0)
Ciconiidae	*Ciconia nigra*	0/2 (0.0)	0/2 (0.0)
Accipitridae	*Milvus migrans*	2/3 (66.7)	2/3 (66.7)
Muscicipidae	*Muscicapa infuscata*	0/1 (0.0)	0/1 (0.0)
Total		13/48 (27.1)	13/48 (27.1)

Table 7: Distribution of positive and negative sera to Newcastle disease antibodies by enzyme link immunosorbent assay and haemagglutination test in the wild birds surveyed in Kogi State, Nigeria

	ND HI test			
ND ELISA	Positive	Negative	Total	%
Positive	4	9	13	27.1
Negative	10	25	35	72.9
Overall (%)	14 (29.1)	34 ((70.8)	48	100.0

Table 8: Odd of reactors of wild bird sera to Newcastle disease antibodies by enzyme link immunosorbent assay and haemagglutination tests in the surveyed Local Government Areas of Kogi State

Local government area	ELISA positive	HI test positive	OR (95%CI)	p-value
Ajaokuta	1 (7.7)	1 (7.2)	1.00 (0.034-29.807)	1.000
Ankpa	3 (23.1)	3 (21.4)	1.33 (0.149-11.929)	0.797
Dekina	2 (15.4)	1 (7.2)	0.50 (0.031-7.794)	0.621
Lokoja	3 (23.1)	2 (14.3)	0.57 (0.070-4.644)	0.599
Ofu	1 (7.7)	3 (21.4)	5.00 (0.344-72.767)	0.221
Olamaboro	2 (15.4)	4 (28.6)	2.500 (0.164-9.538	0.398

OR: Odd ratio, CI: Confidence interval

The ND HI test and ELISA were compared to see the number of samples that were positive and negative to both tests. Four (8.3%) sera were positive and 25 (52.1%) sera were negative with both tests (Table 7). The odd of reactors to the two tests were calculated but were not significant across the LGAs (Table 8).

DISCUSSION

The detection of antibodies to ND in free flying wild birds in this study confirms reports of the prevalence in nature of ND virus strains among free range poultry, apparently healthy ducks and other wild birds in Nigeria (Ibu *et al*., 2009; Sa'idu *et al*., 2004). The overall ND seroprevalence of 29.2% determined in this study is higher than that reported by Schelling *et al*. (1999) who determined a seroprevalence of ND in wild birds using tissue fluids from dead birds. Detectable antibody levels are higher during active infection than in convalescent and morbid birds.

Newcastle disease antibodies were not detected in wild birds in Mopamuro and Okene LGAs probably due to the organization of the poultry industry and ecological structures of these areas. In the two LGAs where ND antibodies were not detected, the backyard poultry is more organized to avoid contact with free range poultry and wild birds. This may suggest the likely source of the ND viruses (NDV) to be from vaccinated poultry as the LGAs where ND antibodies were detected in wild birds had rural and some backyard poultry on free range that interact with wild birds in scavenging for food around dump sites, farms and open fields.

The association of ND infected but apparently healthy free flying wild birds with free range and commercial poultry may be responsible for vaccine failure often seen in the field. Maminiaina *et al*. (2010) reported the existence of divergence of up to 15-25% between the wild type NDV strains (genotype VII) that are endemic in Africa, Asia and Europe in comparison to the La Sota vaccine strain (genotype II) resulting in variable immunological responses.

On the basis of species, swift (*Apus* spp.), swallow (*Hirundo* spp.) and laughing dove (*Streptopelia senegalensis*) had detectable levels of ND antibodies. The source of the infection is not known for certainty but ND antibodies have been detected elsewhere in these species which are found in association with rural and commercial poultry (Schelling *et al*., 1999; Ibu *et al*., 2009; Oladele *et al*., 2012; Assam, 2014). The occurrence of ND antibodies in these species was an indication of their susceptibility to ND probably due to their close interactions with vaccinated poultry and or other wild birds that might be carriers of ND viruses. Laughing dove (*Streptopelia senegalensis*), swallow (*Hirundo* spp.) and swifts (*Apus* spp.) are insects and seed eaters which are likely to pick ND virus when they scavenge for spillage feed around backyard farms and from maggots in poultry manure which are often disposed undiscriminatingly in refuse dump (Assam, 2014). Airborne spread of NDV within short distance is also established (Sa'idu *et al*., 2004; Abdu, 2007). Hence, these species of wild birds could acquire ND viruses from infected or vaccinated flocks as a result of their close association via air dispersal during the cold, dry months when the wind speed is high. Swallow (*Hirundo* spp.) and swifts (*Apus* spp.) are also known migrants making them important species in the introduction and spread of ND to areas earlier thought to be free of the disease (Schelling *et al*., 1999).

Schelling *et al*. (1999) reported an ND seroprevalence of 10.2% in Switzerland while Dimitrov *et al*. (2008) reported a seroprevalence of 14.8% among wild birds in Bulgarian zoos. In Nigeria, Sa'idu *et al*. (2004) reported high ND seroprevalence of between 4.0-52.0% in semi domestic and wild birds which included anseriformes, columbiformes and struthioformes while Ibu *et al*. (2009) reported antigen prevalence of 7.0% among wild and captive birds that included passeriformes, falconiformes and strigiformes. The difference of these reported seroprevalence of ND from the present study may be due to difference in test procedures, time and method of sampling as well as geographic and ecological conditions.

The overall seroprevalence of ND in wild birds by ELISA was slightly lower than the value obtained by HI test in Ofu and Olamaboro LGAs. This may be due to the fact that ELISA detects IgG which is produced at the early stage of infection and mostly by young birds than HI test which detects IgM that is produced at a later stage of infection and in older birds. The sensitivity of ELISA is reported to be higher than HI test with good agreement hence, sera negative to the HI test may not be false positive to ELISA (Schelling *et al*., 1999; De Sousa *et al*., 2000; Faraz *et al*., 2010).

The seroprevalence of ND by ELISA were lower in laughing dove (*Streptopelia senegalensis*) and swallow (*Hirundo* spp.) may be for the same reason of the species not being able to produce enough IgG precipitating antibodies, later stage of infection as well as the age of the birds sampled. The ELISA however, detected ND antibodies in black kite (*Milvus migrans*), a species in which HI test failed to detect antibodies. Black kite (*Milvus migrans*) may not be able to produce agglutinating antibodies (IgM) which are detected by HI test. Black kite (*Milvus migrans*) is a migrant species and a diurnal raptor that eats other birds and may contract the NDV when it feeds on NDV infected birds. There have been reports of the isolation of NDV from raptors such as eagles, owls, hawks, buzzards and vultures elsewhere (Schelling *et al*., 1999; Ibu *et al*., 2009).

The absence of ND antibodies in fly catcher (*Muscicapa infuscata*), parakeet (*Psittacula krameri*), black stork (*Ciconia nigra*) and melodious warbler (*Hipploais polyglotta)* either by HI test or ELISA probably showed the non susceptibility of these species of wild birds to NDV. However, psittacine and most passerine birds are susceptible to ND and the absence of the antibodies in these species may be relative to the few number of birds sampled which might not have represented the true status of the population.

The effects of the limitation of the small sample size on the results of the study did not permit strong conclusion on the status of ND in wild bird species in the study area.

However, the detection of ND antibodies in some of the species of wild birds surveyed was an indication of the susceptibility of these species to ND and the presence of wild type NDV in the study area which may negatively affect the course of ND and its control in Kogi State, Nigeria. Backyard and commercial poultry farmers should screen their birds from these species of wild birds so identified. Equally, there is need to determine the pathotype of the strains of the NDV in wild birds in Kogi State, Nigeria.

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