



# Incidence of Newcastle Disease Virus in Apparently Healthy Domestic and Wild Birds: A Concern for Newcastle Disease Control in Nigeria

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## Abstract

The greatest impact of Newcastle disease (ND) is in the over 65% developing nation's poultry population that is made of village or backyard poultry and which lacks practically based sustainable biosecurity measures. This cross-sectional study was conducted from November 2015 to July 2016 by testing cloacal and tracheal swabs to detect whether domestic poultry and wild birds were exposed to ND in two states of Nigeria. Commercial and rural backyard poultry, captive wild birds (CWB) and free flying wild birds (FFWB) were sampled in this study. Molecular detection using polymerase chain reaction gave an overall 18% and an equivalent 8.5% ND detection rates in poultry and wild birds in Bauchi and Gombe States respectively. Newcastle disease virus was detected at significant levels in apparently healthy domestic and wild birds. Strategies for effective ND control measures should duly consider ND routine and sustainable surveillance in domestic and wild birds held in households, commercial farms and live bird markets.

**Keywords:** Newcastle; Virus; Detection; Birds; Nigeria.

## 1. Introduction

Poultry production holds a key position in the Nigerian economy by contributing about 10% to agriculture and more significantly to immediate family income and employment opportunity [1]. The rural poultry is held in almost every household in rural communities and usually forms an integral part of life with important social implications such as emergency source of funds, traditional and religious celebrations, as source of cheap protein requirement and can be given as gift to visitors and friends [2, 3]. Poultry in rural areas are mainly kept on free range that lacks considerations to biosecurity measures. Birds are thus characterized by poor nutritional status and high susceptibility to diseases [4]. Unfortunately, Newcastle disease (ND) continues to limit the growth of the poultry industry in most developing nations with its greatest impact on village or backyard poultry that forms over 65% of the poultry population [5, 6]. Disease control appears impossible in developing nations due to inadequate cost effective and rapid diagnostic facilities, problems of reliable surveillance system and effective implementation of biosecurity measures [5].

The first documented case of ND in Nigeria was in Ibadan in 1952 and later in other parts of the country [7]. In Nigeria, ND had been reported to be the most important poultry disease, with variations in mortality rates and seasonal influence [3, 8]. According to Nwanta, *et al.* [9], variable prevalence rates were reported in different parts of Nigeria with the highest (74%) in the north east and lowest (38%) in the south west. More recently [10] reported an ND prevalence rate of 56% with a seasonal influence revealing a 3.4 times likelihood of ND occurrence in the pre-dry season in Gombe state. A lower prevalence rate of 17% was however reported in the Federal Capital Territory in local chickens [11]. Emergence of new antigenic lineage of Newcastle disease virus (NDV) in Africa has been reported [12].

Also, antigenic differences between vaccines and field strains have contributed to continuous ND outbreaks in vaccinated and unvaccinated flocks in Nigeria for a long time [7]. Newcastle disease virus was shown to circulate continuously and homogeneously in a large range of wild birds' species [13]. Different NDV genotypes were also reported to circulate concurrently in different birds species which were phylogenetically related to strains circulating in domestic poultry, suggesting the roles of wild birds in the epidemiology of ND in Africa [14]. Wild birds can feed; water, roost or nest in or around domestic poultry in open feeding sites. Furthermore, human agro-forestry activities have altered the ecosystems which continues to attract and create contacts between wild and domestic birds thereby increasing the risk of disease exposure [15, 16].

This study was conducted with a view to finding whether some species of wild birds could contribute to the maintenance and spread of ND in the wild bird population as well as serving as bridge species in terms of ND transmission between wild birds and poultry. Organisation International de epizootics (OIE) recommends ND to be

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effectively controlled or kept in check in order to prevent its devastating effects. Therefore, the control strategies must include routine and sustainable surveillance of ND in domestic and wild birds kept in households, commercial farms and live bird markets and in the wild habitats.

## 2. Materials and Methods

### 2.1. Study Area

The study was carried out in randomly selected villages of Bauchi and Gombe States, Nigeria Bauchi located between latitudes 10° 10' to 10° 33' N and longitudes 9° 40' to 10° 13' E in the Sudan savannah in the south and Sahel savannah in the central and northern regions. The state has the Yankari Game Reserve that harbours wild animal and many species of wild birds. Its poultry population is over a million made of mainly rural poultry which are often bought and sold in LBMs [6]. Gombe state borders Bauchi state with similar livestock and poultry activities. It is located in the Sudan Savannah that lies between longitude 10° 45' to 11° 45' N and latitude 11° 15' to 9° 30' E. Free animal and human movements and trade in live birds exist between the two states.

#### 2.1.1. Sample Size Determination and Sample Types Collected

Sample size was determined using the formula by Thrusfield [17].

$N = Z^2 pq/d^2$  Where

$q = 1 - P$ ;

$N$  = sample size;

$d$  = desired precision = 0.05 (95% CI);

$p$  = anticipated prevalence of (13.4%) obtained by Wakawa [18].

$z$  = appropriate value from normal for desired confidence;  $N = 1.95^2 (13.4(1-13.4)/0.5^2) = 255$ . Therefore, 500 cloacal and tracheal swabs were collected from domestic poultry and wild birds in each state based on convenient sampling, that is those households and LBMs that obliged to our request had their birds sampled.

### 2.2. Cloacal and Tracheal Swabs Collection

Swabs were collected using commercially sourced sterile polyester swab sticks with plastic handles. The domestic poultry and wild birds were properly restrained by an assistant and swab sticks of various insert according to the size of the birds and rubbed on the mucosa of the trachea after opening the birds' mouth by gentle application of digital pressure at the commissure of the mouth. Another sterile swab was inserted into the cloaca of each bird and by gentle pressure rotated two or three times against the wall of the cloaca. After gentle removal of the swab if any large pieces of faeces were present these were shaken off and the plastic handles of the swabs cut into tubes containing viral transport medium (VTM) and stored in liquid nitrogen until processed.

### 2.3. Sampling Units and Types of Birds Sampled

Samples collected were from commercial poultry farms (layers and broilers), backyard farms (rural poultry, pigeons, free ranging commercial layers and broilers), Live bird markets (rural poultry, commercial layers and broilers, pigeons and quails), households (where captive wild birds were kept and were accessible), trapped free flying wild birds (using locally made baited traps) several species were trapped, sampled and released into the wild.

### 2.4. Detection of Newcastle Disease Viruses

#### 2.4.1. Processing of Swabs

Analyses were conducted in DNA Laboratory of Katuru road Ungwan Sarki Kaduna. Surfaces of swab sample tubes were first decontaminated using methanol and dried with sterile gauze. Five tracheal swab samples from five birds of the same species kept in the same place were pooled into one sterile tube and labeled. Same was done for cloacal swabs [19].

#### 2.4.2. Viral Nucleic Acid (NA) Extraction and Detection

The RNA virus extraction kit for ND was obtained from DNALand and Scientific miTotal® RNA Extraction Miniprep kit (DNALand Scientific, [www.DNALandSci.com](http://www.DNALandSci.com) USA).

### 2.5. Primers for ND Virus

The primers for ND used was synthesized by the DNALand Scientific, USA.

The sequences for ND forward and reverse primers used were: NDVF 5'GCAGCTG-CAGGGATTGTGGT-3'; NDVR 5'TCTTTGAGCAGGAG-GATGTTG-3' targeting M-gene at 280 bp. Viral ribonucleic acid (RNA) was extracted from each of the pooled samples using DNALand Scientific miTotal® RNA Extraction Miniprep kit by spin protocol according to the manufacturers' instructions. The eluted RNA was stored at -70 °C until used.

### 2.6. Reverse Transcriptase-Polymerase Chain Reaction

One step RT-PCR was used to amplify sample in final reaction mixture volume of 25 µl. The RT-PCR mixture contained nuclease free H<sub>2</sub>O, 10 p/mole PCR dilution buffer, 50 Mm MgCl<sub>2</sub>, 10 mM deoxynucleoside triphosphate (dNTP), 20 mM dithiothreitol (DTT), 10 µl each of the oligonucleotide (M52 and M253- forward and reverse

primers), 20 U of reverse transcriptase, 5 U of ribonuclease inhibitor, 5 U of Ampli-Tag DNA polymerase (DNALand Sceintific, [www.DNALandSci.com](http://www.DNALandSci.com) USA) and 5 µl of RNA extract. Thermocycline was done in GeneAmp® PCR System 9700, Applied Biosystems (USA) with the following cycling conditions: Activation at 42°C for 1 hour, initial denaturation at 95°C for 15 sec, annealing at 53°C for 40 sec, elongation at 72°C for 5 minutes and held at 4°C.

### 2.7. Preparation of Tris-Borate Ethylenediaminetetraacetic Acid Buffer and 1.5% Gel

The TBE 1x buffer used in the 1.5% gel and agarose electrophoresis preparation was done by: adding 100 ml of TBE 10x to 900 ml distilled water. 1.5 g of agarose was added to 100 ml of TBE 1x and mixed by swirling. The solution was microwaved (Biorad power® PAC 300) under medium heat for 10 minutes and allowed to cool to 45°C. 5 µl of ethidium bromide was added as dye and mixed by gentle shaking in an Erlenmeyer flask.

### 2.8. Agarose Gel Electrophoresis of the Amplicon

The amplicons were mixed with a loading dye (blue/green, Promega®) on a paraffin paper in the ratio of 2:8. The gel was then placed in the electrophoresis tank (BIORAD®) submerged by the TBE buffer and loaded in the wells of the gel. One Kb plus DNA ladder was used as band maker with each lane of the wells having positive and negative (distilled water) controls. The electrophoresis voltage regulator (MJ Research Inc® USA) was set at 100 V for 60 min after which the bands were read in a UV box, viewed on a computer monitor and snapped.

## 3. Results

Result of RT-PCR on swabs of birds from Bauchi and Gombe States showing samples 5 and 10 to be positive for NDV is shown in Plate I. It is indicated by the presence of specific bands in agarose gel at M-molecular weight marker of 280bp. The coordinates of sites where swab samples were collected as well as the RT-PCR results of tested pooled swabs obtained from 3 LGAs of Bauchi State are as shown in Table 1. An overall NDV detection rate was 18%. 10% in Bauchi, 4% in Katagum, and 4% in Misau LGAs respectively. Of the 9 ND detection rate in Bauchi state, 5 (56%) was in Bauchi LGA of which 2 (40%) was in local chickens. Local poultry accounted for 12% and commercial poultry 4%, wild birds 2%. Table 2 shows the coordinates of sites and the polymerase chain reaction results of tested pooled swabs obtained from 3 LGAs of Gombe State. The state also had an overall 18% NDV detection with Gombe (8%), Kaltungo (6%) and Yamaltu-Deba (4%) LGAs. Of the 9 ND detection rate in Gombe state, 4 (44%) was in Gombe LGA of which 3 (75%) was also seen in local chicken. Local poultry had 14% NDV detection rate, 2% each for commercial and wild birds.

## 4. Discussion

Newcastle disease virus was detected in apparently healthy wild birds, commercial and local poultry using RT-PCR molecular technique in this study. Reverse transcriptase polymerase reaction technique used in this study to detect AIV had earlier been reported to be a sensitive, reliable and rapid molecular test used by Medical and Veterinary diagnosticians [20]. Reports showed that almost all species of birds are affected by NDV with varying outcomes [21, 22]. Virulent forms of ND have been isolated from all forms of commercial poultry and sometimes from wild birds in other parts of the world [21, 22]. In Nigeria, virulent forms of ND were isolated in parrots, healthy free ranging chickens and guinea fowls in the late 90s [23, 24]. With our findings, ND situation in wild and domestic birds has most likely remained unchanged for several years. Furthermore, wild birds have also been blamed for the introduction of virulent NDV into poultry populations and virulent NDVs were also isolated from captive caged birds, thus supporting their roles in possible virulent ND transmission and outbreaks in Nigeria and other parts of the world [9, 21].

In this study, apparently healthy chickens, turkeys, ducks, guinea fowl, dove and crowned crane were found positive for NDV. Report showed that gallinaceous birds, pheasants, psittacines, ratites, pigeons and doves are most susceptible to ND, nocturnal and diurnal raptors, storks, penguins and passerines are of intermediate susceptibility while waterfowls, pelicans, shags, coots, gulls and cranes are least susceptible to ND [14]. Detection of NDV in turkeys, guinea fowls, ducks, dove and crowned crane are of concern as these birds are not or rarely vaccinated against ND, and are mainly managed extensively. Turkeys, ducks, guinea fowls and crowned crane are seen reared together with commercial chickens in Nigeria and many developing nations [5, 25]. Doves fly long distances to various households and poultry farms and could disseminate ND, if infected. This is why many nations have restriction in the importation of companion birds especially psittacines as they were found to disseminate NDV without obvious clinical signs [26]. It was further speculated that apathogenic viruses endemic in wildlife populations could undergo increased virulence through mutations when introduced into commercial chicken populations [26]. Therefore, studies of the true prevalence of ND are required for an effective ND control and this means evaluating the status of ND in all susceptible birds' species. Unfortunately, use of live vaccines in ND control, lack of reporting and misdiagnosis of ND outbreaks in backyard flocks, local chickens, and wild birds make the true prevalence of ND difficult or almost impossible to obtain [5, 27]. Wild birds pose a potential risk to biosecurity because they can transfer pathogens including ND viruses to poultry farms. Disease surveillance has thus become pertinent in early detection of signals to outbreak occurrence in many farms and regions of the world as free living and captive wild birds' exposure to virulent ND virus in many countries of the world alerted attention to possible threat and reservoir roles of some wild bird species that may serve as bridge species between wild birds and domestic birds [7, 26, 28-31]. Crowned cranes are

endangered wild bird species due to habitat loss, illegal trade and are affected by deadly poultry pathogens like ND viruses [29]. In the wild, crowned cranes undergo seasonal movements and congregate at periphery of water bodies [29], in Nigeria they are kept as pets by rich individuals [25]. These make crowned cranes potential source of ND transmission at aquatic habitats and to poultry farms. In most rural communities, poultry are kept extensively, intermingle freely with wild birds and commonly eat materials likely to be contaminated with NDV infected faeces from other infected poultry or wild birds. Live infected chickens are reported to be the most likely means of introduction of NDV into village poultry populations with the LBMs playing a central role as a major source of infection [27, 32].

Because ND can cause severe economic losses, and it is a notifiable disease of birds of almost all types to World Organization for Animal Health [33]. To keep ND under control, OIE recommends prophylactic vaccination to be applied on a large scale all over the world [13] which seems to be practically impossible in developing nations, where ND epidemiology is much complicated by the poultry management systems as well as free fling wild birds. What seems more practically impossible in rural communities is the OIE further recommendation that in case of ND outbreak, confirmed infected birds are killed in order to eradicate the disease and control measures should be taken in suspected flocks and in areas around the outbreaks [33]. In these areas a protection zone of 3 km is maintained for at least 21 days, and a surveillance zone of 10 km is kept for at least 30 days, as required by the Council Directive 92/66/EEC [34].

Pigeons and doves particularly fly far and wide to various households and poultry farms and could disseminate ND if infected. Alexander [21], reported outbreaks of NDV in Great Britain due to contamination of poultry feed with faeces of ND infected feral pigeons. Pigeons and doves were grouped among others as the most susceptible birds species to ND virus [14], thus infected free flying wild birds especially pigeons and doves could serve as a possible source of NDV into flocks. Speckled pigeon positive for NDV in this study is a free living wild bird, is frequently seen around human habitat and also co-exist with other wild birds thereby serving as “bridge” specie with high chance of ND cross transmission. In this regards, many nations have now imposed regulations to quarantine companion birds especially psittacines as they were found to disseminate NDV without obvious clinical signs [26]. It was further speculated that apathogenic viruses endemic in wildlife populations could undergo increased virulence through mutations when introduced into commercial chicken populations [26].

In the recent past, two genetic clades of NDV termed classes I and II were reported [12]. Class I was of low virulence and isolated from LBMs in the US and waterfowl worldwide, while the virulent class II was isolated from poultry and wild birds. Virulent strains of NDV were reported to be endemic in many tropical and subtropical countries [5]. Equally, velogenic strains of NDV were reported to be prevalent in village poultry in Nigeria [7, 35]. Based on reports from other countries it is believed that viscerotropic velogenic NDV is responsible for majority of ND disease outbreaks in village poultry throughout the developing nations [36]. Overall, presence of carrier chickens, introduction of susceptible birds, other poultry species, wild birds and environmental factors contribute to the maintenance of NDV [36].

The NDV detection rate was highest in Bauchi 5 (56%) and Gombe 4 (44%) LGAs respectively as compared to other LGAs within the two states. Also, ND virus was detected highest in local chickens in Bauch (56%) and Gombe (75%) LGAs. Incidentally, the two towns are the state capitals of the respective states with each having many daily LBMs unlike other towns reported that have a single each of LBM in this study. For instance, 3 each of the LBMs form part of the sample units in Bauchi and Gombe metropolises. Over 65% of the poultry population in Nigeria is local poultry which are often bought and sold in LBMs [6]. Live bird markets are undoubtedly a mixing avenue for healthy and sick birds of various species from various places through different live bird marketing chains [29]. This might have influenced the rate of ND detection in this study and most importantly emphasizes the need to incorporate LBMs in ND control strategies. However, the big concern is that these local poultry are often bought from same LBMs and reared alongside commercial poultry in many places in Nigeria.

## 5. Conclusion

Various species of poultry and wild birds sampled in this study were found to be sub clinically infected with ND to considerable levels thereby posing threat to effective ND control efforts.

## Recommendations

Effective ND control in a given community should include routine ND surveillance in domestic and wild birds so that a more comprehensive control measure targeting susceptible birds' species can be implemented. Minimizing wild birds' access to poultry farms may reduce incidence of Newcastle disease in farms.

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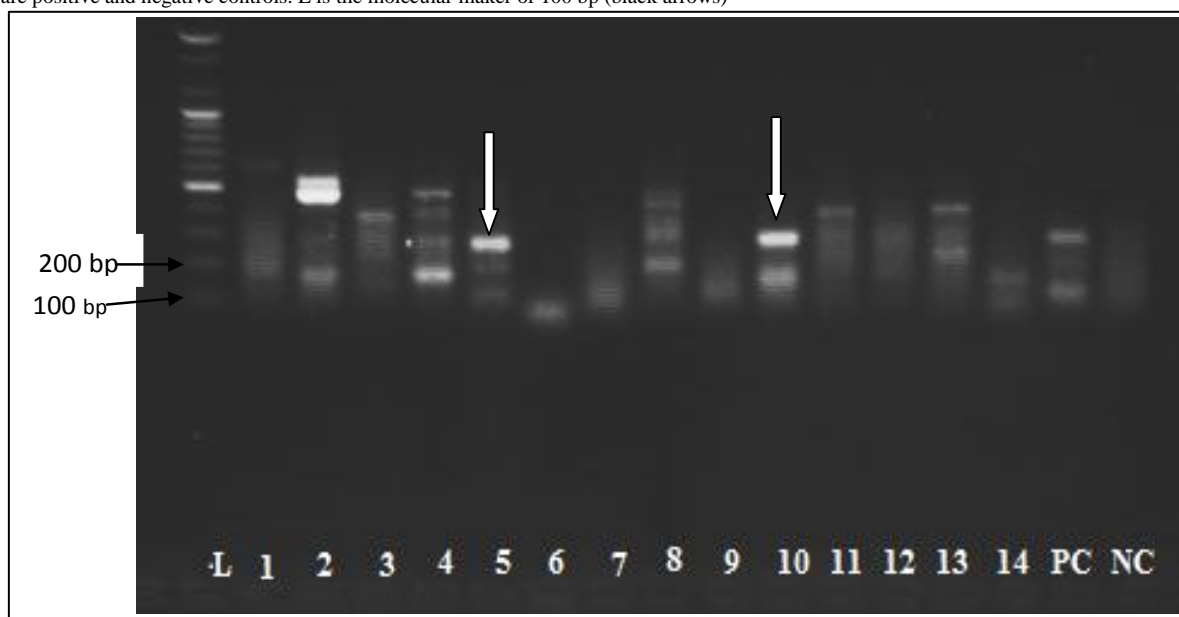
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**Plate-I.** Reverse transcriptase polymerase chain reaction results on agarose gel of pooled swabs from birds in Bauchi and Gombe states. Samples 5 and 10 were positive (indicated by the presence of bands) for the presence of ND virus at M = molecular weight markers (280 bp). Wells 15 and 16 are positive and negative controls. L is the molecular maker of 100 bp (black arrows)



**Table-1.** Global positioning, reverse transcriptase polymerase chain reaction results of tested swabs for Newcastle disease virus in birds from 15 study sites within three Local Government Areas of Bauchi State, Nigeria

Local Govt. Area	Site	Longitude and Latitude	Altitude in metre (m)	Number of pooled swabs tested	RT-PCR Positive ND	Bird specie
Bauchi	Federal Low cost	N 10° 19' 13.1" E 09° 48' 45.8"	630	3	1	Com. chicken
	Fadaman-mada	N 10° 19' 13.0" E 09° 48' 55.8"	626	4	1	Captive wild bird
	Muda-lawal	N 10° 19' 00.0" E 09° 50' 35.8"	642	5	2	Local chicken
	Yelwa	N 10° 18' 40.7" E 09° 45' 41.4"	646	3	1	Turkey
	Wunti	N 10° 19' 05.3" E 09° 56' 07.0"	630	3	0	0
Katagum	Kasuwan kaji	N 11° 40' 20.6" E 01° 10' 55.6"	412	4	1	Local chicken
	Kakimari	N 11° 40' 29.7" E 01° 11' 20.0"	409	3	0	0
	Madangala	N 11° 40' 22.7" E 01° 11' 21.0"	413	3	0	0
	Govt. residential area	N 11° 39' 30.3" E 01° 10' 27.6"	408	3	0	0
	Central	N 11° 42' 25.7" E 01° 11' 31.0"	411	3	1	Guinea fowl

<b>Misau</b>	Fada	N 11°16'50.3" E 01°28'38.1"	439	3	1	Commercial chicken
	Govt. residential area	N 11°44'36.1" E 01°23'33.6"	449	3	0	0
	Legal	N 11°17'39.5" E 01°28'37.4"	453	3	0	0
	Kasuwan kaji	N 11°18'50.1" E 01°27'38.2"	441	4	1	Local chicken
	Luggudi	N 11°20'44.4" E 01°27'54.0"	454	3	0	0
<b>Total (Detection rate %)</b>				50	9 (18)	

**Table-2.** Global positioning, reverse transcriptase polymerase chain reaction result of tested swabs for Newcastle disease virus in birds from 15 sites within three Local Government Areas of Gombe State, Nigeria

<b>Local Govt. Area</b>	<b>Site</b>	<b>Longitude and Latitude</b>	<b>Altitude in meter (m)</b>	<b>Number of pooled swabs tested</b>	<b>RT-PCR Positive ND</b>	<b>Bird specie</b>
<b>Gombe</b>	Jekadafari	N 10° 16'13.8" E 011°13' 05.3"	463	3	1	Local chicken
	Pantami	N 10°26' 12.9" E 011°09' 37.8"	463	3	1	Local chicken
	Malam-inna	N 10°17' 11.9" E 011°11' 05.4"	436	3	1	duck
	Tashan-dukku	N 10°134'40.7" E 011°159'5.9"	488	4	1	Local chicken
	Riyal	N 10°16'05.3" E 011°52'37.0"	452	3	0	0
<b>Kaltungo</b>	Kalaring	N 09°48'53.4" E 011°18'50.5"	505	4	1	Local chicken
	Judo	N 09°42'49.7" E 011°15'49.0"	605	4	0	0
	Ture-mai	N 09°40'22.7" E 011°16'41.5"	636	4	1	Turkey
	Ture-kwe	N 09°39'50.3" E 011°40'30.6"	636	3	0	0
	Ture-fulani	N 09°42'25.7" E 011°41'31.8"	506	4	1	Local chicken
<b>Yamaltu-deba</b>	Wuro-shehu	N 10°16'13.4" E 011°13'06.3"	398	3	0	0
	Kunuyel	N 10°10'29.2" E 011°12'50.9"	399	3	0	0
	Kwadon	N 10°16'36.4" E 011°232'6.1"	348	3	1 1	FFWB Com. Chicken
	Deba	N 10°18'50.1" E 011°27'38.2"	439	3	0	0
	Liji	N 10°16'37.1" E 011°13'44.8"	388	3	0	0
<b>Total (Detection rate %)</b>				50	9 (18)	

**Key:** FFWB-free flying wild bird