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Research Article

Survey of Seroprevalence of Newcastle Disease Virus in the Domestic Pigeons of Kanyakumari District (Tamilnadu), India

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ABSTRACT

Newcastle disease virus (NDV) is always circulating in the pigeon flocks through reservoir birds that look more like healthy pigeons being maintained in the lofts. In Kanyakumari district, seroprevalence of NDV was found in all the months of the year 2015, which was found to be high during the monsoon seasons and low during the summer. During the southwest monsoon (May-July) 17.9±5.8% of birds had titer of 2^1 -2^3 while 14.6±4.0% of birds had titer of 2^4 – 2^7, during the northeast monsoon (Sep-Nov) 12.5±8.8% of birds had titer of 2^1 -2^3 while 8.7±3.70% of birds had titer of 2^4 – 2^7, and in the summer months10.0±3.4% of birds had titer of 2^1 -2^3 while the remaining 6.7±3.2% of birds had titer of 2^4 – 2^7. The NDV affected pigeons showed reduced level of PCV, RBC and haemoglobin, and elevated level of TLC, heterophils, basophils, eosinophils, monocytes and lymphocytes compared to normal healthy pigeons. HI titer value of NDV-seropositive pigeons ranged between $\log_2 1$ and $\log_2 7$ in unvaccinated flocks. The HI titer below $\log_2 4$ indicates that the suspected bird is a reservoir of NDV and the titers above that denotes that the suspected bird has enough protective antibodies against the NDV. If pigeons negative for anti-NDV antibodies are chosen and purchased for keeping farms, there would be least chance for the entry of a carrier bird into a farm. This study recommends that pigeon keepers should subject the birds to HI titer assay before purchasing from or selling to other growers to prevent the spreading of NDV through infected birds that look like healthy birds.

Key words: Newcastle disease virus (NDV), seroprevalence, Pigeon, Haematological parameters

INTRODUCTION

Newcastle disease (NCD) is a highly communicable disease of pigeon (Alexander,2000) that causes a rapid high-mortality characterized by loss of appetite, lethargy, gastrointestinal regurgitation and diarrhea, neurological signs like head-shaking and torticollis, and respiratory signs like cough and sneezing (Ballouh *et al*., 1985). Newcastle disease of pigeon is caused by Avian Paramyxovirus serotype 1 (APMV-1), which together with viruses of the other eight APMV serotypes (APMV-2 to APMV-9) has been placed in the genus *Rubulavirus* of the sub-family *Paramyxovirinae* of the family *Paramyxoviridae*. Since the pigeon APMV-1 has retained some antigenic differences from the other serotypes of NDVs, it is known as pigeon paramyxovirus type 1 (PPMV-1). The PPMV-1 is highly pigeon specific and causes Newcastle disease only in pigeons, but when chicken are experimentally inoculated with this virus strain, only sub-clinical symptoms appear in them and the chicken seem to be carriers of this virus strain. However, it is believed that PPMV-1 had evolved from APMV-1 of chicken by mutation, which is confirmed by the presence of the sequence ^{112}G-R-Q-K-R-F^{117} in the C-terminus of F2 protein of pigeon instead of 1 ^{112}G/E-K/R-Q-G/E-R-L^{117}in chicken (Collins *et al*., 1992). This serotype was first reported in racing pigeons of Italy in 1981 (Biancifiori and Fioroni, 1983) and afterward produced a true panzootic by spreading in domestic and wild pigeons in all parts of the world (Alexander, 1997).

Based on their virulence, PPMV-1 strains are divided into three pathotypes: (1) Lentogenic strains which have least virulence, (2) mesogenic strains which are moderately virulent, and (3) velogenic strains which are the most virulent to cause Newcastle disease in pigeon (Nanthakumar *et al*., 2010). Most pathotypes of PPMV-1 are intermediates between the potent velogenic strains and least virulent lentogenic strains (Geering *et al*., 1995; Collins *et al*., 1996). Seasonal outbreaks of Newcastle disease in pigeon has been reported from South Africa (Pfitzer *et al*., 2000), Australia (Westbury, 1983), Indonesia, East Timor, South-east Asia, Queensland

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(Simmons 1967), Spain, Nigeria (Snoeck *et al*., 2009), Scotland (Blaxland, 1951), Canada (Lancaster, 1966; Wobeser *et al*., 1993), the USA (Walker *et al*., 1973), China, Japan (Arias-Ibarrondo *et al*., 1978), Egypt (Ayman *et al*., 2012), India (Nanthakumar *et al*., 2000) and Pakistan (Mubarak *et al*., 2001). The virulent strains of PPMV-1 may produce morbidity as high as 80% and mortality rates approach up to 100%.in some flocks (SQ, 2012).

Both the NDV-free pigeons and NDV-reservoirs seem to be healthy birds, and hence they cannot be distinguished from one another by any of the visual examinations, but quantification of HI titer would provide a suitable alternative to diagnose NDV-infected pigeons in the lofts. Presence of anti-NDV antibodies in birds is an indication of previous exposure of the birds to NDV, which reveals that the suspected birds are, though free from apparent Newcastle disease, effective reservoirs for this virus. In poultry, it is demonstrated that the HI titer below $\log_2 4$ is a sign of birds surviving as the reservoirs of NDV and the titer of $\log_2 4$ - $\log_2 7$ is a sign of the birds having enough protective antibodies against the NDV (Olabode *et al*., 1992; Abraham-O *et al*., 2014). If the entry of NDV-seropositive healthy reservoirs into commercial flocks can be effectively restrained by adopting quarantine measures, then there would be least chance for unexpected outbreaks of Newcastle disease in the flocks. Even if seroprevalence of NDV has been analyzed in poultry, there is no report of that in pigeons in India.

Unforeseen outbreak of Newcastle disease in pigeon lofts is a serious menace that leads to heavy mortality of pigeons and loss to growers. Since many birds seem to be carriers for the NDV and live as normal birds, this disease cannot be diagnosed by visual symptoms until there has been any outbreak. If the carrier birds are diagnosed and disposed safely, it would be possible to eradicate Newcastle Disease that appears in the lofts unexpectedly especially during the cold seasons. The present study was carried out to find out the seroprevalence of NDV in pigeons of Kanyakumari district and haematological conditions of the pigeons.

MATERIALS AND METHODS

Study area

Kanyakumari district- the southernmost district of Tamilnadu state in India- lies between 77º 15' and 77º 36' of the eastern longitudes and between 8º 03' and 8º 35' of the northern latitudes. This district is bound by Tirunelveli district on the North and East sides, the Gulf of Mannar on the South-east side, the Indian Ocean and the Arabian Sea on the South and South-west sides, and by Trivandrum district of Kerala on the West and North-west sides. It has a total area of 1672.4 square kilometers. This province accommodates about more than 250 species of birds, of which 53 species are migratory birds and 12 species are endemic (Balachandran, 1998). According to Balachandran (1998), nearly 55,000 fancy pigeons, 64,000 rock pigeons and about 1800 racing pigeons have been grown in houses, temples and churches while wild pigeons are relatively fewer in number.

Survey of pigeons

Field visits were conducted in all the four taluks - Agasteeswaram, Kalkulam, Vilavankodu and Thovalai- of Kanyakumari district every month of the year 2015. Pigeon lofts maintained by rural and urban growers were surveyed. A total of 200 pigeons (50/ taluk) were randomly selected every month to test the birds for seroprevalence of NDV by HI titer assay.

Collection of blood

The lower surface of the wing was surface sterilized by wiping with cotton soaked with surgical spirit and blood sample was taken from the wing vein through vein puncture using 23 G sterile hypodermic needle of Dispovan Insulin syringe. About 2 ml of blood was taken in from a pigeon, as done by Oladele *et al*. (2008), on the day of surveying. Of this, 1 ml is stored in labeled Bijou bottles containing ethylene diamine tetra acetic acid (EDTA) at the concentration of 2mg/ml as anti-coagulant for the study of haematological parameters and the remaining 1 ml blood was stored in labeled bottle without any anti-coagulant for the preparation of serum for HI assay.

HI titer assay for NDV

To confirm the infections with PPMV-1, sera of all the birds were subjected to HA and HI titer assay according to the standard methods described by Hanson (1975). Pigeons, whose HI titer value was above zero, were considered to be NCD sero-positive pigeons.

Haematological analysis

Hematological parameters like packed cell volume (PCV), red blood cells (RBC) count, haemoglobin (Hb) concentration, total leukocytic count (TLC) and differential count for heterophilis, basophils, eosinophils, monocytes and lymphocytes were done using the standard techniques described by Rehman *et al.* (2003).

Statistical analysis

All the data obtained from this experiment were subjected to one-way ANOVA, using SPSS (1997) computer software. The significant differences among the means values were analyzed with the Duncan multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Table 1 shows the seroprevalence of Newcastle disease virus in the domestic pigeons of Kanyakumari district in the year 2015. In January, out of 200 pigeons, 152 birds were negative for HI titer, 25 birds had titer of 2^1 -2^3 while titers of 2^4 - 2^7 were found in 21 birds, so that the NDV prevalence was 34%. In the next month, 157 birds were negative for HI titer, 27 birds had the titer of 2^1-2^3while titers of 2^4 - 2^7 were found in 18 birds, which had shown the NDV prevalence of 21.5%. During the month of March, 165 samples were negative for anti-NDV antibodies, 25 samples had titers of 2^1 - 2^3 while 18 samples had the titers of 2^4 to 2^7, so that the NDV prevalence was found to be 17.5%. In the following month (April), the NDV prevalence was estimated to be 15.5% since 169 samples were negative for anti-NDV

Table 1: Occurrence of NDV-seropositive pigeons in Kanyakumari district in 2015

Month	HI titer values (Log_2)							
	2^0	2^1	2^2	2^3	2^4	2^5	2^6	2^7
January	152	8	10	9	9	7	3	2
February	157	8	9	8	8	6	3	1
March	165	8	7	7	5	4	3	1
April	169	7	5	5	6	4	3	1
May	113	9	20	14	13	9	5	3
June	128	10	19	15	10	9	5	4
July	144	8	6	6	13	11	8	5
August	169	4	5	6	6	6	4	2
September	171	4	6	5	4	3	3	1
October	165	8	7	7	5	4	3	1
November	136	9	18	11	10	9	4	2
December	112	9	21	16	11	9	5	3

antibodies, 17 samples had titers of 2^1 - 2^3 and remaining 15 samples had the titers of 2^4 to 2^7. On the other hand, there was a slight rise in the prevalence rate of NDV in May - June: in the former the prevalence was 43.5% since about 113 samples were negative for anti-NDV antibodies, 43 samples had titers of 2^1 - 2^3 and the remaining 30 samples had the titers of 2^4 to 2^7, and in the latter the NDV prevalence was 36.0% because 128 samples were negative for anti-NDV antibodies, 44 samples had titers of 2^1 - 2^3 and the other 30 samples had the titers of 2^4 to 2^7. In the month of July, the prevalence of anti-NDV antibodies was 28% (20 samples with titers 21 -23 and 28 samples with titer of 24-27) and 144 samples were negative for NVD-antibodies. In the subsequent month (August), about 15 samples had titers of 2^1 -2^3 and 18 samples had titer of 2^4-2^7 whilst 144 samples were negative for NDV-antibodies; total prevalence of NDV virus antibodies was 15.5%. In September, 171 birds were negative for anti-NDV antibodies, 15 birds had titers of 2^1 - 2^3 and the rest (11) had titers of 2^4 to 2^7 (NDV prevalence 14.5%). In the subsequent month (October), as much as 165 birds were negative for anti-NDV antibodies, 22 birds had titers of 2^1 - 2^3 and 13 had shown titers of 2^4 to 2^7; the overall NDV prevalence was 17.5%. In November, about 136 pigeons were negative for anti-NDV antibodies, 38 pigeons had titers of 2^1 - 2^3 and 25 pigeons had the titers of 2^4 to 2^7; therefore, the overall NDV prevalence was 32.0%. In the last month of this survey (December), 112 birds were negative for anti-NDV antibodies, 36 birds had titers of 2^1 - 2^3 while 28 had shown the titers of 2^4 to 2^7, which indicated the NDV prevalence of 44.0%.

Haemagglutination inhibition (HI) titre value of NDV positive birds is greater than 1 forever and that in the serum of NDV-free pigeons it is always zero (Alexander, 1997; Mishra *et al.,* 2000; Wakamatsu *et al.,* 2006; Saidu *et al,* 2006; Ricarda *et al.,* 1992; Abraham *et al.,* 2014). More like the previous reports, in the present study the HI titer values were higher than zero in NDV-positive pigeons. According to Salihu *et al.* (2012), who had investigated the HI antibody titer of healthy chicken, fowl are protective against NDV if their HI antibody titer was above $log_2 4$ level in the serum. Higher titer values in the birds further convey the fact that they might have survived clinical or subclinical NDV infection which might have induced the birds to produce neutralizing antibodies against the NDV (Nwanta, 2003). Many workers have already demonstrated that 14-68% of fowls had detectable levels of NDV antibodies, of which only 14.1% had HI titer above $log_2 4$ to offer adequate protection against the viral infection (Musa *et al*., 2009) and the rest would possibly serve as the reservoirs of the NDV (Olabode *et al*., 1992; Orajaka *et al*., 1999; Salihu *et al*., 2012).

Salihu *et al* (2012) had proved that the prevalence of NDV in wild birds is ranged from 24% to 31.2% in Nigeria, but in domestic fowls it ranged from 34.6% to 68.4% (Abdu *et al*., 1985; Saidu *et al*., 2006; Nwanta, 2003). In Kanyakumari district of India, seroprevalence of NDV in pigeons is ranged from 14.5% during the summer to 43.5% during the monsoon season, of which only 10 -15% had adequate amount of protective HI antibodies. This range of NDV seroprevalence coincided with the earlier reports (Blaxland, 1951; Lancaster, 1966; Simmons, 1967; Walker *et al*., 1973; Arias-Ibarrondo *et al*., 1978; Westbury, 1983; Abdu et al., 1985; Wobeser *et al*., 1993; Pfitzer *et al*., 2000; Nanthakumar *et al*., 2000; Mubarak *et al*., 2001; Snoeck *et al*., 2009; Ayman *et al*., 2012). This study further confirms that nearly 28% of domestic pigeons being grown in this district are reservoirs of NDV and it may produce disease outbreaks at any time when the climate is suited for epidemics and local immunity fails to work.

During the southwest monsoon (May-July), 64.1 ±6.7 birds were negative for anti-NDV antibodies, 17.9±5.8% of birds had titer of 2^1 -2^3 while the remaining 14.6±4.0% of birds had titer of 2^4 – 2^7 (Table-2). Likewise, during the northeast monsoon (Sep-Nov), 78.8 ±7.1% of birds were negative for anti-NDV antibodies, 12.5±8.8% of birds had titer of 2^1 -2^3 while the remaining 8.7±3.70 % of birds had titer of 2^4 – 2^7. In the summer months, 83.3 ±9.1% of birds were negative for anti-NDV antibodies, 10.0±3.4% of birds had titer of 2^1 -2^3 while the remaining 6.7±3.2% of birds had titer of 2^4 – 2^7. Results of this study reveal that prevalence of NDV was the maximum during the south-east monsoon season and was found to be in low level during the summer.

Hot summer was unsuitable for the existence of Paramyxovirus-1 in contaminated things and its transmission from infected birds to healthy birds (Abraham *et al*., 2014) since high heat was capable of dehydrating the surface of hosts and ultraviolet radiation in the sunlight had destroyed the virus (Alexander, 2000). In pigeon lofts, PPMV-1 transmission occurs by exposure to fecal matters and other excretions from infected birds, and through contact with contaminated feed, water, equipment and clothing (Olabode *et al*., 2006). Moist weather of monsoon season has assisted for the rapid multiplication of PPMV-1 in the bird's feather and skin, and for the dispersal of the virus particles from infected bird to healthy birds being grown in the same loft. Further, it also reduces the immunity of birds (Alexander, 2001). Therefore, prevalence of Newcastle disease in pigeons was more during the monsoon season than in the preceding summer as has been generalized by Olabode *et al*., (2006), Salihu *et al*. (2012), Abdu *et al*. (1985) and Saidu *et al*., (2006) in chicken.

Differences in haematological parameters in the blood samples taken from healthy birds and NDV seropositive pigeons are given in table 3. Normal value of PCV in healthy birds was 46.5±0.68 but in NDV attacked

Table 2: Relative percentage of individuals and their HI titers during different seasons

Season	HI titer values (Log_2)							
	2^0	2^1	2^2	2^3	2^4	2^5	2^6	2^7
South-west Monsoon (May-July)	64.1±6.7[b]	4.5±1.1[a]	7.5±2.3[a]	5.9±1.7[a]	6.8±1.8[b]	4. 8±1.2[a]	3.0±0.6[a]	2.0±0.4[b]
North-east Monsoon (Sep –Nov)	78.8±7.1[b]	3.3±0.8[a]	5.4±2.4[a]	3.8±1.6[b]	3.4±1.3	2.7±1.1[a]	1.7±0.8[b]	0.9±0.5[a]
Summer (Mar-Apr)	83.3±9.1[a]	4.0±1.0[a]	3.0±1.3[a]	3. 0±1.1[a]	2.7±1.2[a]	2.0±1.2[b]	1.5±0. 5[a]	0.5±0.3[a]

Figure after ± denote standard deviation; [a] significance (P<0.05); [b] significance (P<0.01)

Table -3: Haematological parameters of healthy pigeons and NCD seropositive pigeons

Parameter	NCD negative pigeon		NCD virus positive pigeons	
	Range	Mean value	Range	Mean value
PCV (%)	43.9 – 49.6	46.5±0.68[a]	40.5 – 46.4	43.8±1.41 [a]
RBC (x 10^6/µL)	2.76 – 4.38	3.2±0.54 [a]	2.34 – 3.89	2.88±0.42 [a]
Haemoglobin (g/dL)	9.92 – 12.21	11.83±1.12 [b]	6.84 – 9.87	8.12±0.31 [b]
TLC (x 10^3/µL)	21.65 – 27.28	24.54±2.12 [b]	28.58 – 34.76	32.45±2.12 [a]
Heterophils (x 10^3/µL)	5.45 – 7.87	6.82± 0.86 [b]	6.58 – 8.73	7.20±0.54 [a]
Basophils (x 10^3/µL)	0.43 – 0.62	0.55±0.01 [a]	0.53 – 0.79	0.72±0.01 [a]
Eosionophils (x 10^3/µL)	0.35 – 0.52	0.43±0.14 [a]	0.32 – 0. 62	0.52±0.10 [a]
Monocytes (x 10^3/µL)	0.81 – 1.69	1.22±0.05 [a]	1.33 – 1.83	1.66±0.03 [b]
Lymphocytes (x 10^3/µL)	11.85 – 14.25	13.46±1.12 [b]	14.21 – 16.24	15.52±1.21 [b]

Figure after ± denote standard deviation; [a] significance level (P<0.05); [b] significance (P<0.01); n =20 birds.

birds the PCV was 43.8±1.41%. The PCV of healthy birds was lower than the reference values set by Fudge (2000) but little higher than the values measured by Saleem *et al* (2008). Further, the PCV had decreased in diseased birds as pointed out by Oladele *et al* (2008). RBC count in healthy pigeons was 3.2±0.54 x 10^6/µL whereas in NDV positive pigeons it was 2.88±0.42 x 10^6/µL. The RBC level coincided with the reports of Mubarak and Rizvi (2002) and Basit *et al* (2006). Infection with PPMV-1 had decreased the RBC count in pigeons (Basit *et al.,* 2006). In the meantime, haemoglobin level in healthy pigeons was 11.83±1.12 g/dL while in NDV-positive pigeons the value was 8.12±0.31 g/dL. Investigations of Barton *et al* (1992), Haag-Wackernagel and Moch (2004) and Saleem *et al* (2008) show that haemoglobin content in the NDV attacked pigeons is comparatively lower than that in the disease free birds. Usual TLC in healthy birds was 24.54±2.12 x 10^3/µL, but in NDV attacked birds the TLC was 32.45±2.12 x 10^3/µL. The TLC value in healthy birds agreed with the TLC count recorded by Saleem *et al* (2008), but in NDV attacked birds the TLC count was higher as has been investigated by Mubarak and Rizvi (2002). The heterophils count in healthy birds was 6.82± 0.86 x 10^3/µL but in NDV attacked birds it was 7.20±0.54 x 10^3/µL. The heterophils count in the present observation coincided with Saleem *et al* (2008). The normal value of basophils count in healthy birds was 0.55±0.01 x 10^3/µL but in NDV attacked birds the basophils count was 0.72±0.01 x 10^3/µL. The basophils count in NDV-affected pigeons was slightly higher than that in healthy pigeons as pointed out by Rehman *et al* (2003). Normal eosinophils count in healthy pigeons was 0.43±0.14 x 10^3/µL but in NDV attacked birds the eosinophils count was 0.52 ± 0.10 x 10^3/µL. As suggested by (Saleem *et al*., 2008), there was a slight increase in eosinophils count of pigeons attacked by NDV. The monocytes count in healthy birds was 1.22±0.05 x 10^3/µL but in NDV attacked birds it was 1.66±0.03 x 10^3/µL. Monocytes which are necessary for phagocytosis (Morrissey *et al*., 1989) were more in NDV-attacked pigeons than in NDV-free pigeons (Saleem *et al,* 2008). Normal lymphocytes count in healthy pigeons was 13.46±1.12 x 10^3/µL, but in NDV attacked birds it was 15.52±1.21 x 10^3/µL. Lymphocyte level was higher in NDV-positive pigeons than in healthy pigeons because of the immune triggering activity of NDV in the host (Ritchie *et al,* 1994; Saleem *et al,* 2008; Oladele *et al*., 2008).

Conclusions

From the results of present study, it can be concluded that, as has been demonstrated in chickens, Newcastle disease virus of pigeons has been always in circulation through diseased birds as well as the healthy reservoirs that seem to be more like healthy uninfected birds in the flocks. Both the NDV-free pigeons and NDV-reservoirs seem to be healthy birds and they cannot be distinguished from one another by any of the visual examinations, but quantification of HI titer would provide a suitable alternative to diagnose NDV-infected pigeons in any flock. The HI titer below $log_2 4$ indicates that the suspected bird is a reservoir of NDV and the titers above that denotes that the suspected bird has enough protective antibodies against the NDV. If pigeons negative for anti-NDV antibodies are chosen and purchased for keeping farms, there would be least chance for the entry of a carrier bird into a farm. This study recommends that pigeon keepers should subject the birds to HI titer assay before purchasing from or selling to other growers to prevent the spreading of NDV through infected birds that look like healthy birds.

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