Susceptibility of Three Phenotypes of Village Chickens To Newcastle Disease In Adamawa State

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**Key words: Phenotypes, Frizzle, Naked neck, Smooth chickens, Newcastle disease virus.**

**ABSTRACT:**

The susceptibility of three phenotypes of Village chickens to Newcastle Disease Virus (NDV) (Hertz 33/56) was investigated at Modibbo Adama University poultry farm, Yola. Forty five (45) village chickens, made up of fifteen (15) each of Frizzle, Naked neck and Smooth feathered types were infected with NDV (Hertz 33/56) through intraocular and intranasal routes and subsequently bled on day 0, 3, 7, 14 and 28 post infection. A morbidity rate of 100% and mortality rates of 58.3%, 41.7% and 75% for frizzle, naked neck and smooth feathered types was observed respectively. The mean antibody titre of all the phenotypes before the infection was zero and naked neck started developing protective antibodies on day 7 post infection (PI). All the phenotypes, with the exception of the naked necks in the intraocular group, exhibited highest Geometric mean titre (GMT) of NDV HI antibodies on day 28 PI through both routes. The GMT in the control group remained low throughout the experiment. It could therefore be concluded that, the naked neck group and their crosses were the most resistant phenotype, followed by frizzle feathered and their crosses and the smooth feathered and their crosses were the least resistant and poor sero-converts to NDV infection. It is therefore recommended that naked neck group and it crosses should be adapted into the rearing programmes.

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1. INTRODUCTION

Newcastle disease (ND) is caused by viruses of avian paramyxovirus type 1 (APMV 1) serotype of the genus Avulavirus belonging to the subfamily paramyxovirinae, family paramyxoviridae. The paramyxoviruses isolated from avian species have been classified by serological testing into nine serotypes designated as APMV-1 to APMV-9 (Alexander, 2003). One of the most characteristic properties of different strains of NDV has been the great variation in pathogenicity for chickens. Strains of NDV have been grouped into five pathotypes on the basis of clinical signs seen in infected chickens (Beard and Hanson, 1981).

Newcastle disease is the most important limiting factor in rural chicken farming in most developing countries of the world and a serious threat to intensively reared chickens (Echeonwu et al., 2008). The disease is endemic in Nigeria and continues to be responsible for high mortality and morbidity among village and exotics poultry (Saidu et al., 1998; Baba et al., 1998). It is currently been ranked as the most economically important disease of chickens in Nigeria and elsewhere and currently controlled by routine vaccination (Ezeokoli, 1984). High prevalence rate of 60.8% in local fowls, 57.2% in layers and 37.7% in broilers were
reported by (Sadiq et al., 2011). Similarly Njagi et al. (2010) reported sex seroprevalence of 11.5% and 8.3% for adult female and adult male respectively. Furthermore he also reported seroprevalence of 6.5% and 3.6% in grower females and males respectively. Village chickens are generally believed to be resistant to endemic diseases and stressful environment. This ability to endure with disease and stress is what is responsible for their higher survival under village condition when compared to other strains (Msoffe, 2003). Several research findings indicated that village chickens are not resistant to all endemic diseases (Minga et al., 1989; Lin and Lee, 1996; Okoye and Aba-Adulugba, 1998). Natural resistance to Newcastle and infectious bursal disease has been reported in Egyptian breeds of native chickens and also differences within and between ecotypes in immunocompetence for endemic disease like ND has also been investigated (Hassan et al., 2004). Similarly, natural resistance to Salmonellae infections have been reported in some lines of White Leghorn chickens (Bumstead and Barrow, 1993). Resistance to ectoparasites infestation such as lice has also been reported elsewhere among village poultry (Aini et al., 1989). Village chickens are believed to be resistant to many common diseases, partly because little attention is paid to disease control measures among this group of poultry (Melewas, 1989; Chrysostome et al., 1995). Previous studies have shown that local chickens appear to be more susceptible to some diseases than exotic commercial types (Okoye and Aba-Adulugba, 1998). Testing of the disease resistance potential can be by infecting the host directly with the virulent strain (Okoye and Aba-Adulugba, 1998) or by contact. So far there are very limited research findings and dearth of information on the disease resistance potentials of different phenotypes of village chickens in Nigeria. It is important to determine the disease resistance potentials of our native chickens as a means of using their genetic potentials to control endemic infectious diseases. This study is strictly designed to compare the susceptibility of three phenotypes of village chickens to Newcastle disease virus (Hertz 33/56)

2. MATERIAL AND METHODS
2.1 Study Area
The study was carried out in the Teaching and Research Farm of the Federal University of Technology, Yola. Three phenotypes of village chickens were used for the study. Yola is situated at Latitude 9 and 11 North and Longitude 11 and 14 East. The climate is tropical with distinct dry and wet season. The rainfall starts in April and ends in October while Dry season starts in November to March. The state has an annual rainfall of about 700mm-1600mm and relative humidity ranges from 5%-42% with the average temperature of 39° (Adebayo and Tukur, 1999).

2.2 Experimental Birds
Forty five village chickens, made up of fifteen (15) each of frizzle, naked neck and smooth feathered types were hatched and raised up to twenty weeks of age.

2.3 Experimental Design
Forty five village chickens comprising fifteen (15) each of frizzes, naked necks and smooth feathered types were used in this study. Thirty (30) chickens comprising 10 of each phenotype were assigned to two groups A and B and 9 chickens comprising 3 of each phenotype out of the remaining 15 were assigned to group C. The remaining 6 chickens, two (2) of each phenotype were introduced to groups A and B to serve as in-contact. Group A and B were challenged with Newcastle disease virus (Hertz 33/56) of chicken origin using intraocular (i/o) and intranasal (i/n) routes respectively. While group C served as uninfected control group. 0.2mls of the diluted virus were instilled into the eyes and nostril of each chicken.

2.4 Serum Samples
The experimental birds were bled on days 0, 3, 7, 14, 21 and 28 post infections. 3 ml of blood was collected from each experimental bird through the wing vein using sterile 21G
needle into sterile vacutainer tubes. The blood samples collected in the vacutainer tubes were kept at room temperature to clot. Serum samples were harvested after centrifuging the vacutainer tubes at 1,500 rpm for 10 minutes, into sterile cryotubes and kept at -20 °C until tested.

2.5 Chicken Red Blood Cells
4 ml of chicken red blood cells was drawn onto 1 ml of Alsever’s solution (anticoagulant) in a sterile syringe. The RBC solution was washed 3 times first with Alsever’s solution and subsequently with Phosphate Buffered Saline (PBS), Ph 7.4 and centrifuged at 1,000 rpm for 5 minutes. A 1% suspension of the chicken RBC in PBS was prepared for use in Haemagglutination (HA) and Haemagglutination inhibition (HI) tests as described by Allan and Gough (1974).

2.6 Antigen
Newcastle disease vaccine ‘LaSota’ obtained from National Veterinary Research Institute (NVRI) Diagnostic Laboratory Yola, was used as an antigen for the HA and HI tests. The HA titre of the ND vaccine LaSota was determined as described by Allan and Gough (1974) and appropriately diluted to obtain the 4 HAUs computed from the result of the HA titration. Briefly, 25µl of PBS was added to all the wells of a microtitre plate and 25µl of the test serum was added into the first well (A1) and diluted across the plate by transferring 25µl from the first well to the second well and up to the last well (A12). Twenty five microlitres (25µl) of appropriately diluted antigen (4HAU) was added to all the wells across the plate. Finally, 25µl of 1% chicken RBC was added to all the wells and the plate incubated at room temperature for 30 minutes. Positive and negative NDV sera were also included on the plate. The titre was taken as the reciprocal dilutions where there was complete inhibition of agglutination of the chicken RBC.

2.9 Data Analysis
All the data generated were calculated using simple percentage and the Geometric mean titre of Newcastle disease antibodies was calculated using the formula below (CDC 1998). Briefly, the geometric mean of a sample of n positive observations was defined as the nth root of the product of the n numbers:

\[ X_{geo} = \sqrt[n]{x_1 \times x_2 \ldots \times x_n} \]

Thus, the geometric mean was calculated as

\[ X_{geo} = \text{antilog}_{10} \left( \frac{1}{n} \sum \log_{10} x_i \right) \]

where \( x_i \) = the reciprocal of dilution.

3. RESULTS
The results of experimental infection of three phenotypes of village chickens with Hertz 33/56 NDV indicated that all the infected birds came down with the disease as from day three (3) after infection. The onset of mortality started from day 4 PI for Frizzles and day 5 PI for naked necks and smooth feathered. A percentage mortality of 58.3%, 41.7% and 75% was observed for frizzle, naked neck and smooth feathered birds respectively. Morbidity rate of 100% was demonstrated for all the phenotypes except the in-contact chickens. The duration of the disease was 7, 9 and 10 days for Frizzle, Naked neck and Smooth feathered types respectively (Table 1). The males were more
susceptible than the females in all the three phenotypes studied. Furthermore the naked and smooth feathered are more susceptible to intraocular routes of infection while frizzle feathered are susceptible to intranasal routes of infection (Table 2). The geometric mean antibody titer for experimental infection through intraocular route indicated that most of the phenotypes started developing protective antibody titer as from Day 7 post infection (Table 3). Similarly most of the phenotypes inoculated intranasal started developing antibodies as from day 3 except the in contact chickens (Table 4). None of the chickens in the control group developed protective HI antibody throughout the experiment (Table 5).

Table 1: Results of the morbidity and mortality rates among different phenotypes of village chickens challenged with Hertz 33/56 NDV

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Frizzle</th>
<th>Naked neck</th>
<th>Smooth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of clinical signs (days)</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Onset of mortality (days)</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Morbidity (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>58.3</td>
<td>41.7</td>
<td>75</td>
</tr>
<tr>
<td>Duration of the disease (days)</td>
<td>7</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 2: Sex and route susceptibility of infected and non infected local chickens

<table>
<thead>
<tr>
<th>S/N</th>
<th>P/Type</th>
<th>Total No.</th>
<th>m</th>
<th>f</th>
<th>i/o</th>
<th>i/n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>10</td>
<td>5(100)</td>
<td>2(40)</td>
<td>3(60)</td>
<td>4(80)</td>
</tr>
<tr>
<td>2</td>
<td>NK</td>
<td>10</td>
<td>1(50)</td>
<td>3(37.5)</td>
<td>3(60)</td>
<td>1(20)</td>
</tr>
<tr>
<td>3</td>
<td>S</td>
<td>10</td>
<td>4(100)</td>
<td>3(50)</td>
<td>4(80)</td>
<td>3(60)</td>
</tr>
<tr>
<td>4</td>
<td>MF</td>
<td>2</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>5</td>
<td>MNK</td>
<td>2</td>
<td>1(100)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>1(100)</td>
</tr>
<tr>
<td>6</td>
<td>MS</td>
<td>2</td>
<td>2(100)</td>
<td>0(0)</td>
<td>1(100)</td>
<td>1(100)</td>
</tr>
</tbody>
</table>

Key: M = Male, F = Female, i/O = intraocular, i/N = intranasal, P/type = phenotype
Table 3: Geometric mean titer (GMT) of NDV HI antibodies of three phenotypes of village chickens infected with Hertz 33/56 NDV using intraocular route

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phenotype</th>
<th>Total No Tested</th>
<th>GMT of HI Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>D₀</td>
</tr>
<tr>
<td>1</td>
<td>NK</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>N</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

HI = Haemagglutination inhibition Test, F = Frizzle, NK = Naked Neck, N = Smooth, M = In-contact chicken NDV = Newcastle diseases virus

Table 4: Geometric mean titer (GMT) of NDV HI antibodies of three phenotypes of village chickens infected with Hertz 33/56 NDV using intranasal route

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phenotype</th>
<th>Total No Tested</th>
<th>GMT OF H1 Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>D₀</td>
</tr>
<tr>
<td>1</td>
<td>NK</td>
<td>5</td>
<td>0.26</td>
</tr>
<tr>
<td>2</td>
<td>N</td>
<td>5</td>
<td>2.3</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>3</td>
<td>0.0</td>
</tr>
</tbody>
</table>

HI = Haemagglutination inhibition, F = Frizzle, NK = Naked neck S = Smooth, M = In-contact chicken NDV = Newcastle diseases virus
Table 5: Geometric mean titer (GMT) of NDV HI antibodies of control group

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phenotype</th>
<th>Total No tested</th>
<th>GMT OF HI Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>D₀</td>
</tr>
<tr>
<td>1</td>
<td>CNK</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>CN</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>CF</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

KEY: HI = Haemagglutination inhibition test, CNK = Control Naked Neck, CN = Control Smooth, CF = Control Frizzle.

4. DISCUSSION

The onset of clinical signs on the third and fourth day post infection recorded in this study agreed with the findings of Msoffe et al. (2002) and Fayeye et al. (2011). Similarly the onset of mortality recorded in this study concurs with the report of Msoffe et al. (2002). The variable mortality rates obtained among the three phenotypes tend to agree with Akinoluwa et al. (2012) who obtained 40%, 30% and 70% for Yoruba frizzle, Yoruba naked neck and Yoruba smooth feathered respectively. The findings disagreed with the report of Fayeye et al. (2011) who recorded 100% and 91% respectively for some Nigerian Fulani and Ilorin ecotypes. Similarly the research findings also contradicted Msoffe et al. (2002) on four ecotype chickens in Tanzania. The relatively higher mortality observed in this study for smooth feathered chickens compared to other phenotypes compared favorably with earlier report by Akinoluwa et al. (2012). The least mortality observed among the naked neck chickens is in harmony with El-Safty et al. (2006) who reported that naked neck have a better ability to secret Acute Phase Protein (APP) by liver cells, which gives protection to the birds against infection or any invasion. Furthermore, lower incidence of pathology was also observed in naked neck compared to other phenotypes and these suggest greater disease resistance associated with Na gene (Fraga et al. 1999; Gonzales et al. 1998). The duration of the disease among the three phenotypes observed in this study is within the range reported by Saidu et al. (2006) for three breeds of local chickens in Nigeria.

Higher mortality in males compared to female chickens recorded among the three phenotypes in this study contradicts Njagi et al. (2010) who reported that female chickens were more affected compared to males, but agrees with Kutubuddin (1973) who reported that male chickens were more affected with NDV than females. Ezeokole et al. (1984) suggested that sex of birds may influence their morbidity and mortality. The differences could be due to the fact that in the present study all the phenotypes were infected using different routes of infection. Similarly this study also showed that mingling and housing NDV infected chickens and non infected can result to infection, the findings is in agreement with Akinoluwa et al. (2012) on Nigerian local chickens Ecotypes elsewhere. The 80% mortality observed among the smooth feathered chickens in the intraocular route, which is further confirmed by GMT of HI antibodies and failure to develop protective immunity in the first seven (7) days of infection is in agreement with OIE (2000) that antibody titre less than Log₂ 2² may not be protective and this probably the reason why this phenotype recorded highest mortality. The HI antibody titre recorded in this study with all the three phenotypes and through both routes of infection recorded except in smooth feathered group inoculated through intraocular routes is far higher than
that reported by Akinoluwa et al., (2012) in three Yoruba ecotypes. The reasons adduced for this difference is that, in this study all the chickens were infected with NDV isolates and this triggers more antibody response and also in his study all the chickens had residual antibody pre contact, although it was not protective. This is probably interfering with the production of solid immunity.

5. CONCLUSIONS
It could therefore be concluded that the naked neck group and their crosses were the most resistant phenotype, followed by frizzle feathered and their crosses and the smooth feathered and their crosses were the least resistant and poor seroconverts to NDV infection.

6. RECOMMENDATIONS
Naked neck and its crosses should be adapted in rearing programmes in rural areas. Further studies should be carried out on why male chickens died more than the female ones.

7. ACKNOWLEDGEMENT
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