Seroprevalence of Newcastle disease virus antibodies in village chickens in the three senatorial zones of Plateau State, Nigeria

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Introduction
Newcastle disease (ND) is one of the most common poultry diseases worldwide, and it has been identified as one of the leading causes of death among village chicken (Rinle et al., 2019; Egbuji et al., 2017; Lawal et al., 2016). Newcastle disease affects domestic poultry, aviaries, and wild birds and is extremely contagious. The Newcastle diseases virus strains were divided into three pathotypes by Aldous & Alexander (2010): i.e highly virulent (velogenic), intermediate (mesogenic), and non-virulent (lentogenic), with clinical symptoms ranging from neurologic to pulmonary signs (Alexander et al., 1985). Since its first
report in Ibadan, Nigeria in 1953, ND has grown to become the most important viral disease of chickens, widely recognized by both village chickens and commercial poultry farmers (Lawal et al., 2015), and is widely spread throughout Nigeria, with annual epidemics in highly susceptible poultry flocks (Aliyu et al., 2015).

Village poultry species, especially when grown together or in close vicinity, could play a key role in the epidemiology and spread of the virus to more vulnerable commercial exotic chickens or immune deficient village poultry species (Egbuji et al., 2017). Since Nigeria has a poultry population of 140 million, with backyard poultry production accounting for more than 60% of the total flock and an asset value of more than 5.75 billion dollars (Nnadi & George, 2010), the disease could pose a threat to both successful village poultry and commercial exotic chicken production (Lawal et al., 2016; Egbuji et al., 2017).

Although the presence of specific antibodies to ND virus in bird's serum provides little information about the infecting strain of the virus and hence has limited diagnostic value regarding strain identification, the evidence of infection may be sufficient for immediate intervention and control measures (Musa et al., 2009). Several authors studied the seroprevalence of ND in Plateau State (Musa et al., 2009; Egbuji et al., 2017) with no information on the seroprevalence in village chickens in the three Senatorial zones of the state. As a result of these, the aim of this study was to determine the seroprevalence of ND in the three Senatorial zones of Plateau State, Nigeria.

Materials and Methods

Study location

The research was conducted in Plateau State, Nigeria, which is situated between the latitudes of 8° and 10° North and the longitudes of 7° and 11° East. The State has borders with Bauchi State to the east, Taraba State to the South east, Nasarawa State to the South and Kaduna State to the North. It has an average annual rainfall of 1,317 mm in the Southern part and 1460 mm on the high plateau, with an average ambient temperature of 24°C and a relative humidity of 50%. The state's population is estimated to be around 3.5 million people, including more than 30 ethnic groups. Plateau State is divided into three Senatorial zones (NPC 2006; NRSC 2008; NFDP 2009).

Study design

The sampling areas were chosen using a multi-stage sampling technique based on the State's political division into three senatorial zones (Figure 1). Three LGAs were chosen in each senatorial zone using a non-probability sampling technique, and two districts were chosen in each LGA based on the convenience and availability of the live bird market. The LGAs used in this study were Jos North, Jos South, and Jos East in the northern zone, Bokkos, Kanke, and Mangu in the central zone, and Langtang North, Shandam, and Qua'an Pan in the southern zone. In total, 389 blood samples were collected from village chickens across the three Senatorial zones.

Sample size determination

Using the formula $N = \frac{Z^2 pq}{l^2}$, the sample size was calculated (Thrusfield, 2007); where $Z = 1.96$; $P = 36.4\%$ prevalence in chickens in Zaria (Sule et al., 2019); $q = 1 - p$; $l = 5.0\%$ allowable error. There were 198 samples in all. But, 389 village chickens from the nearby area were sampled.

Blood sample collection

Blood sample was collected from each of the village chickens, irrespective of age and sex with history of no vaccination against ND. A 21 G needle attached on a 5ml syringe was used to collect two millilitres of blood from the wing vein and allowed to coagulate in the syringe. For complete serum separation, the clotted blood was left at room temperature overnight. The separated serum was then decanted into cryovials, immediately delivered to the laboratory and stored at -20°C until it was analysed for ND antibodies.
**Serology**
The harvested serum samples were tested using haemagglutination and haemagglutination inhibition tests. According to the method of the Office International des Epizootics (OIE, 2010), 1% suspension of chicken red blood cells (RBCs) was prepared as an indicator for the haemagglutination (HA) and haemagglutination inhibition (HI) tests (OIE, 2010). According to the method of Allan and Gough (1974), HA titre of a standard NDV antigen was determined, which was diluted to contain 4HA units. In an HI test, ND antibody titres were determined in harvested sera using reconstituted antigen containing the 4HA units. The agglutination was assessed by tilting the plates. Only those wells in which the RBCs streamed at the same rate as the control wells were considered to show HI.

**Data analysis**
Data were stored in Microsoft excel spread sheet. Descriptive statistics was carried out using Microsoft excel and proportions were obtained using open Epi version 2.3.1 statistical tool (Open-source epidemiological statistic for public health calculation). Chi square was used to measure the association between location and sex and the prevalence of ND antibodies. Value of $p<0.05$ were considered significant. Results were presented in tables and graphs.

**Results**
The result of this study revealed an overall Seroprevalence of 36.8% (CI: 32.1-41.6) in the three Senatorial zones under the study. The Seroprevalence of ND virus antibodies in the village chickens in the selected LGA's in the three Senatorial zones showed the highest prevalence of 8.7% (CI: 6.3 – 11.9) in Bokkos, (Central Zone). This was followed by 7.9% (CI: 5.7 – 11.1) in Jos-South (Northern Zone) and 1.5% (CI: 0.7–3.3) in Qua’an Pan (Southern zone) with statistically significant differences $p<0.05$ (Table 1).

<table>
<thead>
<tr>
<th>LGA</th>
<th>Northern Senatorial Zone</th>
<th>Proportion %</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jos South</td>
<td>48</td>
<td>31</td>
<td>17</td>
</tr>
<tr>
<td>Jos East</td>
<td>44</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>Jos North</td>
<td>44</td>
<td>13</td>
<td>31</td>
</tr>
<tr>
<td>Bokkos</td>
<td>48</td>
<td>34</td>
<td>14</td>
</tr>
<tr>
<td>Kanke</td>
<td>24</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>Mangu</td>
<td>43</td>
<td>10</td>
<td>33</td>
</tr>
<tr>
<td>Langtang N</td>
<td>47</td>
<td>13</td>
<td>34</td>
</tr>
<tr>
<td>Shendam</td>
<td>45</td>
<td>7</td>
<td>38</td>
</tr>
<tr>
<td>Qua’an Pan</td>
<td>46</td>
<td>6</td>
<td>40</td>
</tr>
</tbody>
</table>

**Table 1**: Seroprevalence of Newcastle disease virus antibodies in three Senatorial zones of Plateau State based on Local Government Areas

Chi square =74.43, P value = <0.0000001 (P<0.05), Degree of freedom =8
Overall prevalence =36.8% (CI: 32.1-41.6)

<table>
<thead>
<tr>
<th>Zone (Z)</th>
<th>Number Examined</th>
<th>Number Positive</th>
<th>Number Negative</th>
<th>Proportion %</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern Z.</td>
<td>136</td>
<td>68</td>
<td>68</td>
<td>17.5</td>
<td>14.0 - 21.6</td>
</tr>
<tr>
<td>Central Z</td>
<td>115</td>
<td>49</td>
<td>66</td>
<td>12.6</td>
<td>9.7 - 16.3</td>
</tr>
<tr>
<td>Southern Z.</td>
<td>138</td>
<td>26</td>
<td>112</td>
<td>6.7</td>
<td>4.6 - 9.6</td>
</tr>
<tr>
<td>Total</td>
<td>389</td>
<td>143</td>
<td>246</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chi square =31.01, P value = 0.000000185 (P<0.05), Degree of freedom =2

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antibodies between the sexes of the village chickens. A Seroprevalence of 18.5% (CI: 14.7 – 22.4) was obtained for females while the value for males was 18.3% (CI: 14.7 – 22.4) (Table 3).

Discussion
The overall Seroprevalence rate of 36.8% recorded in this study poses a significant danger to effective village chicken production (Lawal et al., 2016) and even more so to Plateau state’s commercial poultry industry. If not appropriately controlled, the 36.8% Sero-positive chickens could serve as a source of infection for a vast population of village and commercial poultry. This is because most infected birds might shed substantial amounts of ND virus in their faeces (Alexander et al., 1985), which may be spread by people, equipment, vehicles, contaminated poultry and poultry products, contaminated feed and water, and other animals (Capua & Alexander, 2009). The overall seroprevalence recorded in this study is in contrast to the report of Musa et al. (2009) who determined a seroprevalence of 51% in Plateau State. The reduction may be attributed to the constant awareness created by veterinarians on the need to vaccinate village chickens with the ND I2 vaccine, which is readily available at an affordable price.

The ND antibodies detected in the village chickens might have been as a result of natural exposure to the ND virus, since the chickens had no history of previous vaccination against ND.

Although, Musa et al. (2009) based their data on only four LGAs (2 each from the North and Southern zones of Plateau State), the total seroprevalence rate of NDV in village hens observed in this study does not correspond with the 51% seroprevalence rate reported by Musa et al. (2009). This investigation also differs from that of Lawal et al. (2016), who found 62.7% seroprevalence to ND virus antibodies in village hens in Gombe State, and Nwanta et al. (2008), who found 32.2% in Kaduna State. Other studies, such as those by Ameji et al. (2011) and Chollum et al. (2013), Jibril et al. (2014), and Eze & Ike (2015), revealed seroprevalence rates of 96%, 35.8%, 25.5% and 65.1% respectively.

The differences between the results of this study and those of Ameji et al. (2011), Chollum et al. (2013), Jibril et al. (2014), and Eze & Ike (2015) was that the latter collected samples from village chickens in live bird markets, where birds were known to be brought from various locations, including neighbouring states and congregate for sales.

The significant difference between the LGAs could be due to ecological differences in the ND virus’s activity, the effect of the environment on the virus’s viability and spread (Orajaka et al., 1999; Eze & Ike 2015), or the high concentration of commercial poultry farms in the LGAs sampled in Plateau state’s Northern and Central zones. The large volume of manure produced in concentrated poultry production areas can contribute to the contamination of ground and surface waters (Mallin & Cahoon, 2003).

Manure from poultry litter is generally used as fertilizer and applied to fields for growing agricultural commodities (USPEA, 1998). Jos South and Bokkos LGAs, which are in the State’s northern and central zones, are known to have a large concentration of maize and Irish potato farmers. These agricultural products are grown using organic manure from poultry litters, which could be another reason for the significantly higher seroprevalence rate observed among the village chickens sampled in the Northern and Central zones (where high concentration of commercial poultry farms are located), when compared with the Southern zone of the State. This is because ND viruses are frequently discharged in high amounts in poultry faeces (Alexander et al., 1985). Village chickens are known to scavenge the environment for food, and ingestion of faeces during such activity could be a major route of transmission of ND virus among them (Sa’idu et al., 2006).

The insignificant difference observed in the seroprevalence of ND virus between sexes showed that ND virus has no sex specificity and therefore can infect both sexes of village chickens and can serve as reservoir to ND virus. This result agrees with that of Elifuraha & Emmanuel (2021), who also reported no significant difference in the seroprevalence of ND virus between both sexes of village chickens in

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number Examined</th>
<th>Number Positive</th>
<th>Number Negative</th>
<th>Proportion %</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>194</td>
<td>71</td>
<td>123</td>
<td>18.3</td>
<td>14.7 - 22.4</td>
</tr>
<tr>
<td>Females</td>
<td>195</td>
<td>72</td>
<td>123</td>
<td>18.5</td>
<td>14.9 - 22.7</td>
</tr>
<tr>
<td>Total</td>
<td>389</td>
<td>143</td>
<td>246</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chi square =0.0044, P value =0.95, (P<0.05), Degree of freedom =1
Njombe and Bahi Districts in Tanzania. But however, they differ from the reports of Unigwe et al. (2020), who reported a higher seroprevalence of ND virus in males than in females in Ido and Atiba LGA’s of Oyo State, Nigeria, and Conteh et al. (2020), who showed a significantly higher seroprevalence of ND virus in females than in males in Sierra Leone.

In conclusion, the study found a prevalence of ND virus antibodies in village chickens. As a result, it is recommended that more awareness be raised about the importance of vaccinating village chickens to protect them against ND virus.

Conflict of Interest
The authors declare that there is no conflict of interest.

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