Research Article

Velogenic Viscerotropic Newcastle Disease Virus Produces Variable Pathogenicity in Two Chicken Breeds

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ARTICLE HISTORY


ABSTRACT

Newcastle disease has a high prevalence in Pakistan and outbreaks are reported even in vaccinated chickens. Disease resistance of various breeds to Newcastle disease needs to be studied for better husbandry and breeding decisions. The aim of the present study was to compare resistance of two chicken breeds to Velogenic Viscerotropic Newcastle Disease Virus (VVNDV) infection. It was carried by using different pathogenicity tests like mean death time (MDT) of chicken embryos, intracerebral pathogenicity index (ICPI) in day old chicks and intravenous pathogenicity (IVPI) in 6–week old chickens. MDT in VVNDV inoculated embryonated eggs of Fayoumi and White Leghorn breeds was 41.5±16 and 42.1±10.3 hours, respectively but difference was not statistically significant. In embryonated eggs of Fayoumi 0.6 log₅₀ higher EID₅₀ and significantly higher (0.8 log₂) hemagglutination titers of VVND virus than White Leghorn breed was observed. The ICPI in Fayoumi and White Leghorn, was found to be 0.93 and 0.71 while IVPI in Fayoumi and White Leghorn was found to be 2.31 and 2.65, respectively. Average lesion scores for intravenously infected chickens were 4.8 and 4.9 while in contact exposed chickens scores were 6.6 and 8.4 for Fayoumi and White Leghorn breeds, respectively. Embryos and day–old chicks of Fayoumi were found more susceptible to VVNDV than White Leghorn as they showed lower MDT along with higher HA / EID₅₀ tiers and ICPI, scores than White Leghorn. Both intravenously inoculated as well as contact–exposed chickens of Fayoumi breed were found more resistant as they had lower IVPI and postmortem lesion scores.

INTRODUCTION

Newcastle disease (ND) is regarded as one of the most important diseases in the poultry industry of the world. Among the viral diseases affecting poultry, Newcastle disease (ND) has evolved into a greater challenge due to emergence of novel strains and ultimately vaccine failures. Since 2010, various outbreaks of Velogenic Viscerotropic Newcastle Disease (VVND) have been reported throughout Pakistan. Moreover, a failure of previously effective live vaccines in protecting the birds from current field isolates has also been reported. The velogenic form of ND continues to appear in both vaccinated flocks and Marek’s disease has been discovered (Cheng, 2010). Moreover, some chickens can survive even challenged with NDV deserves further attention. Various breeds of poultry may vary in terms of resistance to infectious diseases like VVND. However effective quantifiable tests measures are needed.

In present study, pathogenicity of VVNDV has been evaluated in Fayoumi and White Leghorn (WLH) breeds through embryonic mean death time (MDT), intracerebral pathogenicity index (ICPI), intravenous pathogenicity index (IVPI), VVNDV production and contact–exposure to the virus.

MATERIALS AND METHODS

Virus Isolation and Culture

The VVND isolate, APMV–1/chicken/Multan /–19–06/2012, was propagated in the allantoic cavity of nine–day old embryonated chicken eggs. The amnioallantoic fluid (AAF) were obtained after the death of embryos (72 hours post infection). The AAF harvested from embryonated eggs was pooled, aliquoted and frozen at ~80°C. This virus stock solution was used for all experimental trials.

Embryo Lethal Dose (ELD₅₀), Embryo Infectious Dose (EID₅₀) and Mean Death Time (MDT) Determination

Embryonic Lethal Dose (ELD₅₀) and Infectious Dose (EID₅₀) were determined in 09 days old embryonated eggs of two chicken breeds by the method as described by Alexander and Senne, 2008. Briefly, fresh AAF having VVNDV was serially (10 fold) diluted in sterile normal saline to give dilutions ranging from 10⁻¹ to
10^{-25}. A volume of 0.1 ml of each dilution (10^{-7} to 10^{-25}) was inoculated through chorio-allantoic sac (CAS) route in embryonated chicken eggs (5 eggs were used for each dilution). The eggs were incubated at 37°C, were candelled every eight hours daily for next 7 days and the time of embryo mortality was noted. The embryonic mean death time was determined as described by Alexander and Senne, 2008. All eggs showing embryo mortality were chilled for three hours, AAFs were harvested and tested by both the micro and rapid HA assay. Embryos not showing mortality up to 7 days post-inoculation were also opened and tested for VVNDV by HA test. Calculation EID_{50} was performed by using Reed and Muench method (Reed and Muench, 1938).

**Rapid and Micro Hemagglutination (HA) Assay:**
The micro HA titration was performed in 96-well micro titer plates while the rapid HA was performed on a marble stone as is already described (Alexander and Senne 2008).

**Intracerebral Pathogenicity Index (ICPI)**
ICPI was determined in day-old chicks by intracerebral injection of VVNDV as per the standard procedure described in OIE manual (OIE 2012). Briefly, freshly obtained AAF having VVNDV with an HA titer 1/16 was tenfold diluted in normal saline solution. A volume of 30 µl was intra-cerebrally inoculated into 10 day-old chicks of each breed. The sterile normal saline solution was also intra-cerebrally inoculated into ten chicks (of both breeds), which served as negative controls. The inoculated chicks were then monitored daily and scored as normal (0), sick or paralyzed (1), and dead (2), to compile an index for the 8 days observation period.

**Intravenous Pathogenicity Index (IVPI)**
Chickens of both breeds were raised at SPVC Karachi for up to six weeks of age. The standard vaccination schedule was followed except that these were not vaccinated against ND. Briefly, ten 6-week old chickens of each breed were inoculated with 0.1 ml of 10-fold diluted freshly obtained AAF containing VVNDV in normal saline solution. Moreover, control groups comprising eight birds of each breed were also injected with sterile normal saline solution. The birds were examined daily for 10 days and observation for each bird was recorded and scored accordingly (0 if normal 1 if sick, 2 if paralyzed, and 3 if dead). The IVPI is the mean score per bird per observation over the 10-day period. An index of 3.00 means that all birds died within 24 hours, and an index of 0.00 means that no bird showed any clinical sign during the 10-day observation period (Alexander and Senne 2008).

**Study of VVNDV Induced Pathology in Two Breeds of Chickens**
Ten 6-week old birds of both breeds were intravenously infected with VVNDV for determination of IVPI. While another group of ten birds of each breed with contact-exposed by intermixing with already intravenously infected chickens. The birds were observed daily for morbidity and mortality. The postmortem examination was performed of dead birds to study the gross lesions produced during the course of disease. On necropsy, any lesions on trachea, brain, bursa of Fabricious, eyelids, proventriculus, small intestine and caecal tonsils were examined and lesions were scored as none (0), mild (1) or severe (2). Scores per bird (0–14) were calculated by adding scores of all seven organs. Scores of all birds in each group were combined and means were determined.

**Statistical Analysis**
Analysis of variance (One way ANOVA) was performed to find out the significant differences among the data obtained for MDT and HA / HI titration, using the computer package Student Edition of Statistics (SXW), version. 8.1 (copy right 2005, Analytical Software, USA). The least significant differences of mean (LSD = 0.05) test was used to compare the significant differences between the groups.

**RESULTS**
**VVNDV Inoculation of Embryonated Eggs of Two Breeds Produces Variable EID_{50} and HA Titers**
EID_{50} / ml of virus stock solution in in Fayoumi embryonated eggs was 10^{4.2} while as in WLH embryonated eggs it was 10^{2.5} (Figure 1). The embryos of Fayoumi breed produced 06 times more VVNDV virus particles than WLH breed. The mean HA titers of VVNDV produced in embryonated eggs of Fayoumi and WLH were 7.9±1.5 and 7.1±1.6, respectively (Figure 2). The differences of HA titers of two breeds were analyzed and were found statistically significant.

**Figure 1:**
![Figure 1](http://nexusacademicpublishers.com/journal/11)

**Figure 2:**
![Figure 2](http://nexusacademicpublishers.com/journal/11)

Mean Death Time (MDT)
The MDT of embryos of VVNDV infected Fayoumi and WLH was determined in 9–days old embryonated eggs. MDT in VVNDV inoculated embryonated eggs of Fayoumi and WLH breeds was 41.5±16 and 42.1 ±10.3 hours, respectively (Figure 3). MDT was higher in WLH while it was lower in Fayoumi but the difference between breeds was not statistically significant.

Intracerebral Pathogenicity Index (ICPI)
Intracerebral pathogenicity index (ICPI) in day–old chicks of Fayoumi and WLH breeds was found to be 0.93, and 0.71, respectively (Table 1). No morbidity or mortality was found in the control groups of two breeds when sterile PBS was injected intracerebrally. On day 8 post inoculation, all surviving chicks were euthanized and blood samples were taken for determination of HI titers to check whether surviving birds are seroconverted or not. HI titers of the surviving birds were 8.25 and 9.2, in Fayoumi and WLH breeds, respectively (Figure 4). The difference in HI titers was not statistically significant.

Intravenous Pathogenicity Index (IVPI)
Intravenous pathogenicity index in Fayoumi and WLH was determined in 6 week–old chickens intravenously inoculated with VVNDV. The IVPI in Fayoumi and WLH was found to be 2.51 and 2.65 respectively (Table 2). No morbidity or mortality was observed in the control groups of 4–week old chickens of two breeds intravenously injected with sterile PBS.

Table 1: Intracerebral pathogenicity index for VVND virus infected day–old chicks

<table>
<thead>
<tr>
<th>Groups</th>
<th>Clinical signs</th>
<th>Days</th>
<th>Weight</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fayoumi</td>
<td>Normal</td>
<td>10</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sick</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Dead</td>
<td>2</td>
<td>35</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>74/80= 0.93</td>
<td></td>
</tr>
<tr>
<td>White Leghorn</td>
<td>Normal</td>
<td>10</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sick</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Dead</td>
<td>0</td>
<td>27</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>57/80= 0.71</td>
<td></td>
</tr>
</tbody>
</table>

Morbidity and Mortality Rates Post–Intravenous Inoculation and Contact–Exposure to VVNDV
In intravenously inoculated chickens of both breeds, first clinical signs appeared day–2 post–intravenous inoculation of VVNDV while in sentinel chickens of both breeds the signs appeared on day–3 post–exposure. Mortality in both breeds started on day–2 post–intravenous infection and was 20% and 60% in Fayoumi and WLH. By day–3 post–intravenous inoculation, mortality rate reached 100% in both breeds. Mortality in sentinel birds of both breeds started on day–4 post exposure VVND and by day–6 mortality rate reached 100% (Figure 5). Mortality rates in sentinel birds of both breeds were 40, 30, and 30 % in Fayoumi and 30, 30 and 20 % in WLH on day 4, 5 and 6, respectively.
Necropsy Findings

On necropsy, the lesions included inflammation and petechial hemorrhages on trachea, brain, bursa of Fabricious and eyelids. Proventriculus, small intestine and caecal tonsils had multifocal, necrotic and hemorrhagic areas. The lesions in major organs including brain, bursa of Fabricious, eye lids, trachea, proventriculus and small intestine were scored as none (0), mild (1), moderate (2) and severe (3). Lesion scores per bird (0–18) were calculated by adding scores of all six organs. Scores of all birds in each group were combined and means were determined. Average lesion scores for intravenously infected chickens were recorded as 4.8, and 4.9 for Fayoumi and WLH breeds, respectively. Whereas, mean lesion scores for sentinel chickens were 6.6 and 8.4 for Fayoumi and WLH, respectively (Figure 6).

Intravenous pathogenicity index (IVPI) of VVNDV was measured in six weeks old chicken two breeds. Our study shows a variation in intravenous pathogenicity of VVND virus in two breeds which is evident from variation in IVPI which were 2.51 and 2.65, for Fayoumi, and WLH breeds, respectively (Table 2). Velogenic strains possess IVPI ranging from 1.5 to 2.0 (Parimal et al., 1997, Namita et al., 1995). According to this criterion, the VVND virus used in...
CONCLUSION

Present study concludes that conventional virus pathogenicity tests like MDT, ICPI and IVPI may give variable results due to breed variation and therefore may be used evaluate breed resistance to ND. Embryos and day-old chicks of Fayoumi are more susceptible to VVNDV than White Leghorn as they show lower MDT along with higher HA / EID<sub>50</sub> tiers and ICPI, scores than White Leghorn. Both intravenously inoculated as well as contact-exposed 6-week old chickens of Fayoumi breed are more resistant as they have lower IVPI and postmortem lesion scores.

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REFERENCES


