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Mini Review

# Newcastle Disease Virus Respiratory and Digestive Viral Load Distribution Comparison

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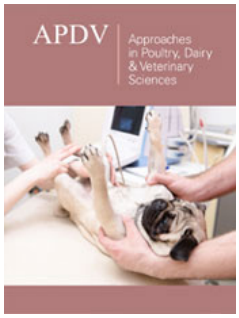


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## Abstract

Newcastle disease (ND) is one of most devastating disease of domestic and wild bird. Brazilian active and passive epidemiological surveillance have been implemented to avoid the Newcastle Disease Virus (NDV) infection risk. The present study was performed comparing the respiratory and digestive viral load distribution from apathogenic positive samples obtained from active surveillance and with the frequency through qPCR positive results from these swabs. The results show a higher load of NDV detected in respiratory samples and some swabs were positive only for cloaca demonstrating as both sampling as the molecular screening corroborate to improve the knowledge the NDV infection characteristics in the nature.

**Keywords:** Newcastle disease virus; Diagnosis; Active surveillance; qPCR

## Introduction

Newcastle disease virus (NDV) belongs to Orthoavulavirus genus of the Paramyxoviridae family [1]. The NDV is responsible for a highly contagious and widespread avian disease that affects all bird species, and it is one of the most economically relevant diseases to the poultry industry. The active surveillance collecting samples from poultry slaughtered, migratory birds and imported genetic material and production with ≥10% mortality. The passive surveillance based on virus screening in suspicious samples. The samples collected for active surveillance were mainly cloacal and tracheal swabs, that arrived at laboratory in aliquots that varies in number (2,3 to 6 for each tissue) which were analyzed by reverse Real-time PCR (qPCR) to detect the NDV- M gene. The comparing the respiratory and digestive viral load distribution of apathogenic ND from positive samples and the frequency through qPCR positive results from these swabs were performed. The medium values of cycle threshold (Ct) obtained were also compared.

## Material and Methods

### Samples processing

The swabs have maintained in MEM and BHI culture mediums with antibiotics until centrifuged at 8,000rpm for 10min. An aliquot of 100µL of each supernatant from the 31 samples. The extraction of total nucleic acid using equipment Magna Pure LC. The reverse real-time PCR (qPCR) was carried on with AgPath ID™ One-Step kit at Applied Biosystems 7500 RT-PCR cycler. The protocol of the National Veterinary Services Laboratories (NVSL) [2] was applied. The primer sequence has consisted of 120 base pairs detected by fluorescence probe. The Ct was calculated automatically by the apparatus at the time that the amplification of viral cDNA. Analyzing the relationship between Ct and the proportion of positive results for each matrix (tracheal and cloacal swabs) noticed that this proportion decreases as the Ct value increases until it reaches its limit (Ct>40). Statistical analysis: The comparison of the Ct values for the paired samples since the samples have been processed simultaneously

considering the null hypothesis is no difference between the results of PCR from different swabs. Hypothesis Ho: pt≤pc vs Ha: pt>pc.

## Result

The median Ct found and its tolerance interval of 95% probability to the trachea was 36.89 (35.48 to 37.70) and the cloaca 36.76 (32.27 to 38.00) to fit relationship by probit analysis (SAS, 2000). However, considering that the two arrays are from the same record, there were a greater number of samples in positives tracheal than in cloacal swabs. It was confirmed by the comparison test of proportions [3], under the hypothesis Ho: pt ≤pc vs Ha: pt>pc, in which Ho was rejected (p=0.003), emphasizing the greater positivity in tracheal swabs than in the cloaca. The estimated difference between proportions and confidence intervals of 95% was 0.12 (0.03 to 0.22), showing that from respiratory system (tracheal) is most probable the detection of the virus. In the Figure 1 showing the difference between proportion of results in swabs of trachea and cloaca, by registration. The Figure 2. Proportion of positive results in swabs of trachea and cloaca on same samples, by registration.

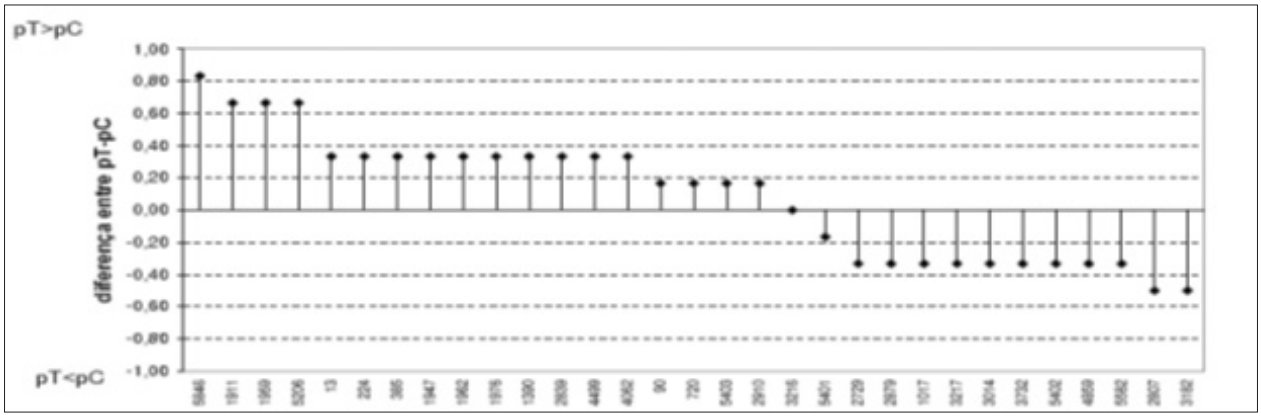


**Figure 1:** Difference between proportion of positive results in swabs of trachea and cloaca, by registration.

pT= Proportion of Positive Results in Trachea Swab; pC=Proportion of Positive in Cloaca Swab

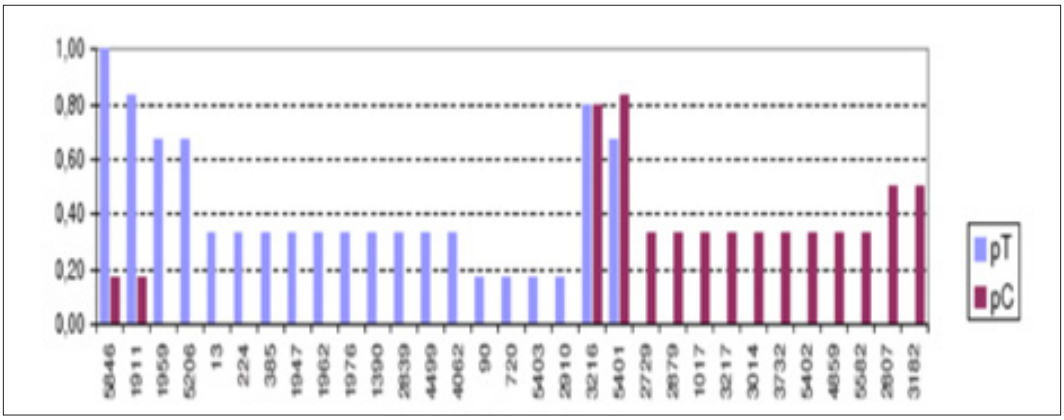


**Figure 2:** Proportion of positive results in swabs of trachea and cloaca on same samples, by registration.

pT=Proportion of Positive Results in Trachea Swab; pC=Proportion of Positive in Cloaca Swab

## Discussion

This study explores the distribution of respiratory and digestive viral loads in birds identified as infected with NDV in active surveillance by LDFA-SP. The results have demonstrated the detection of virus predominance in respiratory system (trachea). Molecular techniques allow the amplification of the viral genome directly from infected tissues, avoiding the need for isolation of the virus. However, its performance is hindered by the presence of PCR inhibitors in various organs and tissues, especially in the feces [4] which may explain the difference observed in the detections made from swabs. Gohm & Hofmann [5] had reported success in expanding a 182bp product, including the cleavage site, directly from infected chicken tissues. The main disadvantage is that no tissue was always positive during a time evolution study, implying the need to test a wide variety of tissues and organs.

## Conclusion

These results have showed a higher load of NDV detected in respiratory tissue (trachea) and that, the higher frequency of positive results is in this tissue. Nevertheless, some swabs were

positive only for cloaca. Such results demonstrate the importance of the type of sample collected and used for the molecular diagnosis of screening for ND, emphasizing the need for concomitant collection of both swabs to increase the chances of NDV detection.

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