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# Insights into Newcastle disease: Isolation and propagation of non-virulent strain for antigen production

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**Abstract**
The Newcastle disease virus (NDV) strains are categorized into three groups: virulent (velogenic), moderately virulent (mesogenic), and non-virulent strains (lentogenic). The non-virulent LaSota strain is commonly utilized as a live vaccine, offering effective immunity against the virulent strains. This study aimed to cultivate and propagate the Newcastle disease virus in the laboratory, assess cytopathic effects in chicken embryos, and confirm virus growth through a hemagglutination test. The non-virulent LaSota strain, utilized as a live vaccine, was employed for this purpose. The chicken embryonated eggs were inoculated with the NDV, and incubated at 37 °C for 48 hours, and the collected allantoic fluids underwent further processing. Distinct petechial hemorrhages were observed in infected embryos, while un-inoculated eggs displayed normal embryos without any lesions. To validate virus growth, the presence of the virus in the allantoic fluid was confirmed through a hemagglutination test. This discovery serves as a foundational step for preparing Newcastle disease virus antigen, crucial for various laboratory techniques.

**Keywords:** Newcastle disease virus (NDV), Allantoic fluid, haemagglutination test

## Introduction
Newcastle disease (ND) is one of the most pathogenic viral diseases of avians and it is economically significant due to the high death and morbidity rates. NDV is classified as a member of the genus Avulavirus in the subfamily Paramyxovirinae of the family Paramyxoviridae, according to Lamb [1]. According to pathogenicity, the Newcastle disease virus (NDV) strains are categorized into three groups: virulent (velogenic), moderately virulent (mesogenic), and non-virulent strains (lentogenic). The trade constraints and sanctions in the areas where the disease is spreading may be significantly impacted by velogenic ND, which can cause 100% mortality in chickens [2]. The symptoms of bird infection can vary greatly according to the specific type of virus, the breed of bird, underlying medical conditions, and prior immunity [3].
Globally, the low-virulence lentogenic (LaSota vaccine) strains are widely utilized and, if feasibly given to healthy birds, can offer immunity to virulent strains. The downstream processing of virus particles, such as Newcastle disease virus (NDV), involves various techniques for the concentration and purification of the virus. These techniques include precipitation, centrifugation, filtration, and chromatography. For example, ultracentrifugation, ultrafiltration, and different chromatography methods are used to achieve the required purity and concentration of the virus. The purification and concentration of the virus are essential to remove host contaminants and obtain the high-titer ultrapure virus, which is necessary for systemic intravenous delivery. While propagation in cell culture is possible, virus yields are lower compared to propagation in embryonated chicken eggs. Therefore, efficient purification and concentration techniques are employed to obtain high-quality virus products for various applications, such as intra-tumoral inoculation, vaccination, or systemic intravenous delivery.

The most popular passive technique for measuring the quantity of virus is the hemagglutination test (HA), which is produced from the allantoic fluid extracted from chicken eggs. Because many viruses have protein antigens that can attach or agglutinate the red blood cells, this test is based on this observation [4].

The objective of this research was to cultivate a less pathogenic strain of Newcastle disease virus, exemplified by the LaSota vaccine, within embryonated chicken eggs. This served as the initial step for the preparation of virus antigens.

## Materials and Methods

The viruses employed in this research were cultured in embryonated chicken eggs. The operational virus concentration was established by diluting the initial virus stock (vaccine) at a ratio of 1:1000 in phosphate-buffered saline (PBS) supplemented with 1% antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin). The total 10 fertilized eggs were taken and kept for a 9-day incubation period at 37 ℃ before being subjected to virus inoculation.

### Egg candling

Upon completion of the incubation period, the embryos were examined using an egg-candling box in the dark area. The air sacs were delineated with a pencil to identify the injection site of live chick eggs. The eggs lacking developing embryos were removed.

### Egg inoculation with marking the site of inoculation

Firstly, the blunt end of the egg is placed against the candle box and an indication of the location for inoculation has been done by using a marker. The egg is cleaned at the designated inoculation site using cotton wool and 70% alcohol. An opening is created at the inoculation site. The inoculum is extracted using a 1 ml syringe and 0.1 ml of the inoculum is introduced into the egg through the puncture. The shell opening is sealed with tape or gum. The inoculated eggs are transferred into an incubator.

### Harvesting of allantoic fluid

At 48 hours post-inoculation, allantoic fluid was harvested from the eggs. Thoroughly cleanse the egg's upper surface with 70% ethanol. Beginning at the injection site, employ sterile forceps and surgical scissors to carefully break and open the apical side of the egg, where the air sac is situated, revealing the chorioallantoic membrane. With the aid of forceps, gently push the embryo downward and meticulously gather the allantoic fluid using a 10 mL serological pipette, transferring it into a 50 mL centrifuge tube. Embryos were placed in sterile Petri dishes to observe the cytopathic effects, and subsequent documentation was carried out through the capture of photographs.

### Identification and detection of virus by Hemagglutination assay:

Detection of the virus through hemagglutination involved processing the allantoic fluid using a hemagglutination assay. Blood samples were obtained from chickens and deposited in tubes with an anticoagulant layer. After an initial centrifugation step where the serum was removed, red blood cells (RBCs) were purified using Alsever's buffer, resulting in a 5% pure RBC solution after a series of centrifugations. Confirmation of the presence of viruses in the allantoic fluid was achieved through the hemagglutination assay. Concisely, a glass slide was cleaned and readied for the experiment. A drop of allantoic fluid was put on the slide with another drop of red blood cells (RBCs) in each well. The negative control involved mixing a drop of RBCs with another drop of Phosphate Buffer Saline (PBS). The reaction was allowed to proceed for a few minutes. Agglutination of RBC has been seen by the naked eye.

## Results

The embryopathy was observed in a 10-day-old chicken embryo that underwent inoculation with the Newcastle disease virus vaccine strain, displaying petechial hemorrhages supported by a previous study [5]. Conversely, uninfected embryos exhibited no discernible lesions. Virus detection through hemagglutination yielded positive results in the hemagglutination test of the allantoic fluid obtained from inoculated eggs, confirming the presence and growth of the virus. In contrast, the control group exhibited a negative outcome. The presence of the virus in the allantoic fluid indicates successful virus growth and isolation.

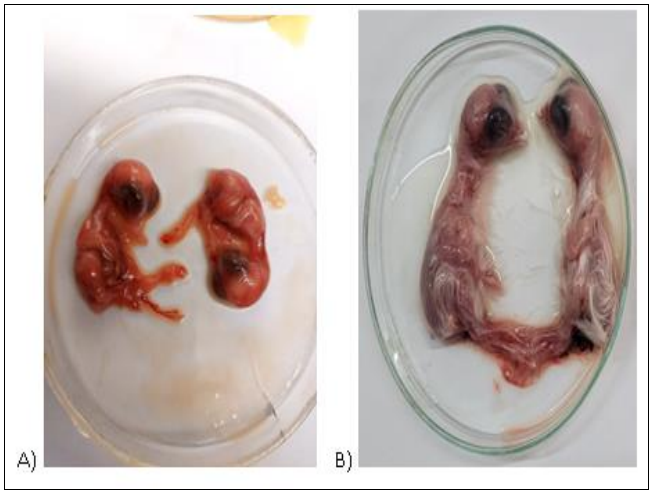


**Fig 1:** Petechial haemorrhages present on chicken embryos after inoculation of NDV and control without inoculation

## Discussion

The Newcastle disease virus (NDV) serves as an ideal viral system for understanding the factors influencing viral pathogenicity. Certain strains of this virus pose significant threats leading to high poultry mortality, while others, such as the LaSota strain, are avirulent and utilized as vaccines [6]. Effective methods for preparing and titrating virus stocks are crucial for these diverse applications. The utilization of embryonated chicken eggs for virus growth is regarded as a superior method for virus isolation [7].

The successful isolation and propagation of the non-virulent strain (LaSota strain) of Newcastle disease virus in embryonated chicken eggs is a crucial step in preparing viral antigens essential for various laboratory techniques. The study contributes to the broader knowledge of Newcastle disease and supports ongoing efforts to manage and prevent its spread. The isolated virus can be used for the preparation of bulk viruses, which are important in performing various laboratory techniques such as ELISA, hemagglutination, hemagglutination inhibition, and neutralization tests. The study provides initials for further research and the preparation of a diverse range of antigens is essential for various laboratory techniques, emphasizing the need for further studies in this area.

## Conclusion

This research successfully isolated and propagated the non-virulent of Newcastle disease virus in embryonated chicken eggs. The study contributes valuable insights into viral pathogenicity, offering a foundation for preparing crucial viral antigens for diverse laboratory techniques. The findings support ongoing efforts in managing and preventing Newcastle disease, with potential applications in bulk virus preparation for various tests. Emphasizing the efficacy of using embryonated chicken eggs for virus isolation, this study provides a platform for future research in virology and Newcastle disease control.

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