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ISOLATION OF NEWCASTLE DISEASE VIRUS (NDV) IN EMBRYONATED CHICKEN EGGS

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ABSTRACT

Newcastle disease virus (NDV) strains have been divided into three groups: virulent (velogenic), moderately virulent (mesogenic), and non-virulent strains (lentogenic). The non-virulent virus strain (LaSota strain) has been used as a live vaccine, which gives good immunity against the virulent strains. The aim of the study was to grow and propagate Newcastle disease virus in the lab, determination ofcytopathic effects in chicken embryos, and confirmation of virus growth by hemagglutination test. Non-virulent strain (LaSota strain)represented by live vaccine was used for this purpose. Embryonated eggs were inoculated with the virus and incubated for 48 hours; and theallantoic fluids were then collected for further processing. Petichial hemorrhages were clearly observed in the embryos following infection while in the un-inoculated eggs; the embryos appeared normal and did not show any lesions. For further virus growth confirmation, the presence of virus in the allantoic fluid was determined by hemagglutiation test. This finding is considered as a starting point for Newastle disease virus antigen preparation, which is essential for the applications of several laboratory techniques.

INTRODUCTION

Newcastle disease (ND) is one of the most significant diseases of poultry worldwide. It is caused by Avian Paramyxoviruses type-1 (APMV-1) which is classified with the other avian paramyxovirusesin the genus Avularius, family Paramyxoviridae(1). Newcastle disease virus (NDV) strains have been divided into three groups: virulent (Velogenic), moderately virulent

(Mesogenic) and non-virulent (Lentogenic), which differ in the number of basic amino acids at the cleavage site of the fusion protein(2,3). Signs of infected birds are very variable depending on the strain of virus,species of bird,concurrent disease and preexisting immunity(4,5).

The lentogenic(LaSota vaccine) strains of low virulence are commonly used worldwide, and can provide protection against virulent strains if the vaccines are viable, administered correctly to healthy birds (6).

The disease can be diagnosed in the laboratory using some techniques such as enzyme linked immunosorbent assay(ELISA), polymerase chain reaction (PCR) and gene sequencing.The most convenient method of propagating Newcastle disease virus in the laboratory is by the inoculation of the allantoic cavity of embryonated eggs with the clinical samples. This method is also important to grow the virus for other purposes such as preparation of viral antigens and vaccine production(7).

The hemagglutination assay (HA) is a most common indirect method to quantify amounts of virus particles, generated from cell culture supernatant or allantoic fluid harvested from chicken eggs. This assay relies on the fact that many viruses contain proteins that can bind and agglutinate red blood cells (8).

The aim of this study was to propagate a low virulence strain of Newcastle disease virus (represented byLaSota vaccine) in embryonated chicken eggs as a starting point for virus antigen preparation.

MATERIALS AND METHODS

Virus propagation and harvesting

Viruses used in this study were propagated in fertile chicken's eggs. Working virus concentration was prepared by diluting the stock virus (vaccine) 1:2000 in phosphate buffer saline (PBS) containing 1% antibiotics (100 U/ mL penicillin and 100 ug/ mL streptomycin).
The fertilized eggs were incubated for 9 days at 37.5˚C and then inoculated with the virus. At the end of this period of incubation, the embryos were candled using an egg-candling box in a dark room and the air sacs were outlined with a pencil in order to determine the site for injection. Eggs without developing embryos were discarded. After wiping the egg surface with ethanol, a small hole was made in the shell at the site of injection with a specific drill without

damaging the shell membranes. A hypodermic syringe (1 mL) fitted with a fine needle was used for virus inoculation. The needle was passed through the hole in the egg shell, through chorioallantoic membrane, and the virus (0.1 mL) was injected in the allantoic cavity, which is filled with allantoic fluid. The hole was carefully sealed with wax or tape, and the eggs were placed at 37.5˚C for 48 hr.

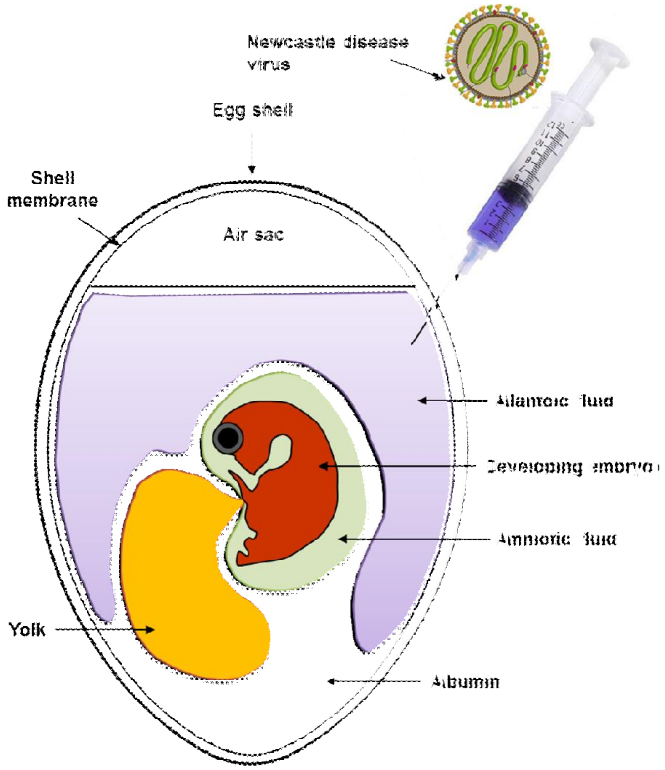

Figure 1: Inoculation of Newcastle disease virus into a chicken embryo.

A healthy 8 day old embryo is represented schematically; 0.1 ml of virus was inoculated through the air sac into the allantoic cavity as indicated with a syringe.

Allanoic fluid was collected from eggs, 48 hours post inoculation. Embryos were put in clean Petri dishes to observe the cytopathic effects. Photos were then taken for documentation.

Virus detection by hemagglutination

The allantoic fluid was processed for hemagglutination assay to detect the viruses.

Blood samples were collected from chicken and placed in tubes coated with anticoagulant layer. Samples were first centrifuged and the serum was discarded. RBCs were purified using Alsever'sbuffer to obtain 5% pure RBCs following series of centrifugations.

The presence of viruses in the allantoic fluid was confirmed using the hemagglutination assay. Briefly, a ceramic agglutination plate which contains six wells were cleaned and prepared for this experiment. A drop of allantoic fluid was mixed with another drop of RBC in the well. The negative control was represented by mixing a drop of RBC with another drop of Phosphate Buffer Saline (PBS). The reaction was left for a few minutes and photos were taken for documentation.

RESULTS

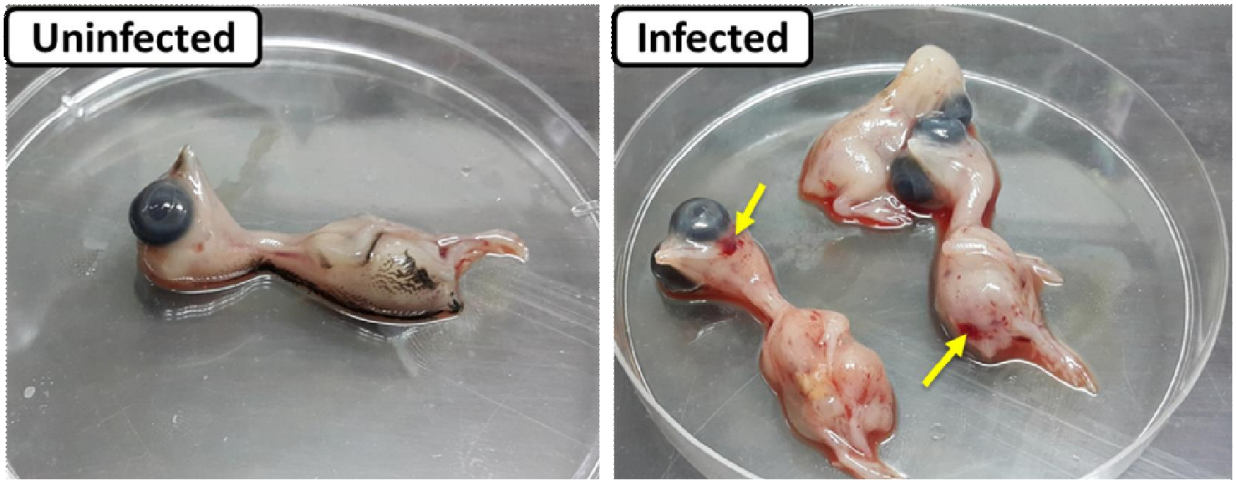

Figure 2 Chicken embryos inoculated with Newcastle disease virus.
Embryopathy in a 10 day old chicken embryo inoculated with Newcastle disease virus vaccine strain showing petechial hemorrhages (indicated by arrows). Uninfected embryos showed no any lesions.

Detection of viruses by hemagglutination

Results of hemagglutinationtestofallantoic fluid,which has been collected from inoculated eggs were positive, which confirms the growth of the virus. Negative result was shown in the control group**(Figure 3)**.

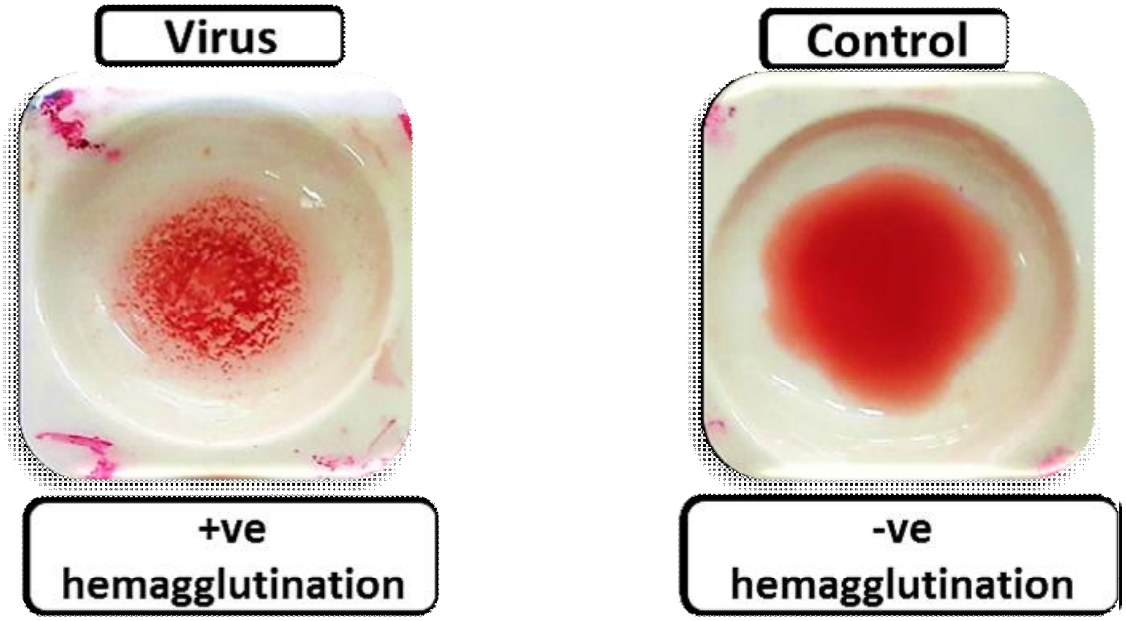

Figure 3: Detection of virus growth by hemagglutination test.

The positive hemagglutination result is clearly observed in the allantoic fluid of inoculated eggs while in the non-inoculated, there is no evidence of hemagglutination.

DISCUSSION

Newcastle disease virus (NDV) is an ideal virus system for elucidating determinants of viral pathogenicity. Some strains of this virus are important agricultural pathogens that cause disease in poultry with a high mortality while other strains are avirulent (such sLaSota strain) and used as vaccines. Methods for the preparation and titration of virus stocks are essential for all of these purposes. The use of embryonated chicken eggs to grow the virus is considered as a superior method for virus isolation (9).

In this project, an avirulent virus vaccine strain was successfully isolatedinembryonated chicken eggs to produce a high titer of virus stock which is essential for virus antigen preparation. Such virus antigens are important in performing various laboratory techniques such as ELISA, hemagglutination, heamagglutination inhibition and neutralization tests.

The Lasota virus strainhas been grown in embryonated eggs for the purpose of vaccine production (7). In comparison, in the present study, this strain was grown for another purpose which is a local antigen preparation. In this regard, it is strongly recommended to prepare antigens from the isolated viruses using different laboratory methods. Furthermore, it is crucial

to decide which viral antigen (protein) should be isolated(e.g the type of antigen used in ELISA test is different from that used for hemagglutination test).

The kinds of approaches outlined above may lead to the potentially new findings, which will help to prepare different kinds of antigens necessary for a variety of laboratory techniques, and hence warrants further studies.

عزل فايرس مرض النيوكاسل من أجنة الدجاج

فراس طعمه منصور ،حازم طالب ثويني ،خديجه مهدي ،سرى خميس

فرع الاحياء المجهريه ،كلية الطب البيطري وجامعه البصره ، البصره ،العراق

الخلاصة

تقسم سلالات فيروس مرض نيوكاسل إلى ثلاثة مجموعات: سلالات عالية الضراوة، معتدلة الضراوة، وغيرضارية. للحد من الاصابة بمرض النيوكاسل يستخدم لقاح LaSotaوالذي يمثل السلالة غير الضارة ولكنها بنفس الوقت تعطي مناعة ضد السلالات عالية الضراوة. الهدف من هذه الدراسة هو تنمية فيروس النيوكاسل في المختبر، وتحديد الافات المرضية المصاحبة للاصابة بالفيروس في أجنة الدجاج، وتأكيد نمو الفيروس عن طريق اختبار التلازن الدموي. تم استخدام سلالة غير ضارية (سلالة LaSota) لهذا الغرض متمثلة باللقاح الحي الذي يستخدم في التحصين الحقلي ضد المرض. تم حقن بيض الدجاج المخصب بالفيروس ومن ثم حضنها لمدة 48 ساعة، بعدها تم جمع السوائل الموجودة في داخل البيضة. لوحظ نزيف نقطي بوضوح في الأجنة بعد الإصابة في حين أن الاجنة في البيض غيرالمحقون بالفيروس كانت طبيعية ولم تظهر فيها اي افات مرضية. لمزيد من التأكيد لنمو الفيروس، تم تحديد وجود الفيروس في سوائل البيضة عن طريق اختبار التلازن الدموي. وتعتبر هذه النتيجة بمثابة نقطة انطلاق لإعداد مستضدات لفيروس النيوكاسل والذي يعد أمر ضروري لتطبيق عدة تقنيات في مختبر علم الفيروسات.

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