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Thermostability Profile of *Newcastle* Disease Virus (*Dongola* strain) Following Serial Passages

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Abstract: *Dongola* strain is a lentogenic *Newcastle* disease virus. A series of selection procedures was carried out at 56˚C to enhance heat resistance. The study was therefore conducted to determine the effect of serial passage on thermostability of the strain. The virus was passaged and at intervals samples were subjected to heat treatment by two methods; stepwise method in which the virus was exposed to increasing temperatures for 15min and extreme heat at 56˚C for increasing time for 10 passages. After selection, recovered virions from the last passage were propagated and tested for thermostability. The results showed that extreme heat selection was better since infectivity titre of the 10^{th} passages decreased by one logarithmic order within 15min of incubation at 56˚C, while the titre decreased by two logarithmic orders in stepwise method.

Keywords: Newcastle disease , *Dongola* strain , Serial passage

Introduction

Newcastle disease (ND) is one of the most important viral diseases of poultry worldwide. The disease can cause up to 100% mortality in susceptible chickens (Alexander., 2003). It is caused by a virus of the avian paramyxovirus type- 1 (APMV-1) of the genus *Avulavirus* belonging to the family Paramyxoviridae (Alexander., 2003). The disease has been reported from all provinces of the Sudan (Eisa., 1979). In the commercial poultry sector there are quite a number of conventional vaccines available for the control of the disease. It is important to note here that most, if not all, of these vaccines are heat labile and hence cannot be used in the rural areas since the provision of cold-chain facilities is practically impossible, coupled with the behavior of the rural scavenging chicken (Chen and Wang., 2002; Adwar and Lukesova., 2008). Isolates of the virus were obtained from apparently healthy chickens in Dongola, Northern Sudan. They were considered to be lentogenic strains (Khalafalla.,1994). Azzam. (2007) reported that *Dongola* strains could provoke comparable immunogenicity and replace lasota strain in vaccination of chickens. Unfortunately the strains were found thermolabile.

The vaccine stability is defined by the length of time the vaccine retains an infectivity titre sufficient to induce a protective immune response at a particular temperature (Simi *et al*., 1970; Young *et al*, 2002). The development of thermostable vaccine might offer an opportunity to improve ND vaccination strategy for poultry in tropical countries (Alders and Spradbrow., 2000). Previous studies on thermostability of various strains of *Newcastle* disease virus (NDV) indicated that most strains lost their infectivity on exposure to 50–55^{0}C for 30min (Lomniczi. 1975). Since then several studies have shown that it is possible to select virus subpopulations with heat resistance for production of a robust vaccine that can be taken into the field with minimum dependence on cold chains (Spradbrow., 1992). Several techniques have been used to select heat resistant strains of NDV (Lomniczi.,

1975; Spradbrow., 1992; Varadarajan *et al*., 2000; King., 2001).

The main objective of the present study was to determine the effect of serial passages on the thermostability of *Dongola* strain.

Materials and Methods

***Newcastle* disease virus:**

Newcastle disease virus (*Dongola* strain) is a lentogenic field strain isolated from apparently healthy chickens in Dongola, Northen Sudan (Khalafalla., 1994).

Bacterial and fungal sterility tests

Three vials of the virus ampoules were randomly selected and subjected to bacterial and fungal sterility tests in Thioglycolate broth and Sabouraud's agar, respectively.

Virus propagation

The virus was propagated in 10-days old embryonated chicken eggs as described by Spradbrow *et al*. (1995) and Alexander. (1998). Non-infected allantoic fluid was used as a negative control.

Haemagglutination (HA) test

To detect the presence of HA, 50µl of allantoic fluid was placed in a well of a V-bottomed 96-well microtitre plate. A volume of 25µl of 1% washed chicken red blood cells (RBC) was added to the well. The plate was incubated at room temperature for 45min before the results were read. Negative control wells contained diluent (Phosphate Buffered Saline) and RBCs.

Thermostability testing

Two vials of the allantoic fluids containing the virus were selected one of them was left unheated and the other one was placed in a water bath at 56^0C for 15min. Recovered viruses were then titrated according to the method described by Reed and Muench (1938).

Improving thermostability

Stepwise method

A portion (1ml) of the virus was taken and passaged serially for 10 passages. At each passage, 10 embryonated eggs at 10 days old were inoculated with 0.1ml and incubated at 37^0C for 4 days before being harvested and used for the subsequent passage. Virus at each passage interval was exposed to gradually increased temperatures of 40, 44, 48, 52, 56, 60, 64, 68, 72 and 76^0C for 15min. Each vial was thawed only once for use in this experiment.

Extreme heat exposure

A portion (1ml) of the virus was taken and passaged serially for 10 passages. At each passage, 10 embryonated eggs at 10 days old were inoculated with 0.1ml and incubated at 37^0C for 4 days before being harvested and used for the subsequent passage. Virus at each passage interval was exposed to 56^0C for 10, 15, 20, 25, 30, 35, 40, 45, 50 and 55 min. Each vial was thawed only once for use in this experiment.

Thermostability testing

Viral propagation:

Allantoic fluids harvested from the final passages were propagated in embryonated eggs as described by Spradbrow *et al*. (1995) and Alexander. (1998).

Thermostability testing

Allantoic fluids containing the recovered viruses from the last passages were exposed to 56^0C for 15min.

Viral titration

Viral titration of the allantoic fluids harvested from the last passages were perfo-rmed according to the method described by Reed and Muench (1938).

Results

Bacterial and fungal sterility tests

No evidence for bacterial and fungal contamination was found.

Thermostability testing

The loss of infectivity among *Dongola* strain is shown in figure 1. The infectivity decreased, where the titres decreased by two logarithmic orders within 15min of incubation at 56^0C. The Effective $Dose_{50}$ (ED_{50}/ml) was found to be $10^{9.63}$/ml for unheated and $10^{7.5}$/ml for heated virus.

Improving heat stability
Stepwise method

The loss of infectivity among passages of *Dongola* strain is shown in figure 1. The infectivity decreased at 10^{th} passages, where the titres decreased by two logarithmic orders within 15min of incubation at 56^0C. The ED_{50}/ml was found to be $10^{10.5}$/ml for unheated and $10^{8.5}$/ml for heated virus.

Extreme heat exposure

The loss of infectivity among passages of *Dongola* strain is shown in figure 1. The infectivity decreased at 10^{th} passages, where the titres decreased by one logarithmic order within 15min of incubation at 56^0C. The ED_{50}/ml was found to be $10^{10.5}$/ml for unheated and $10^{9.5}$/ml for heated virus.

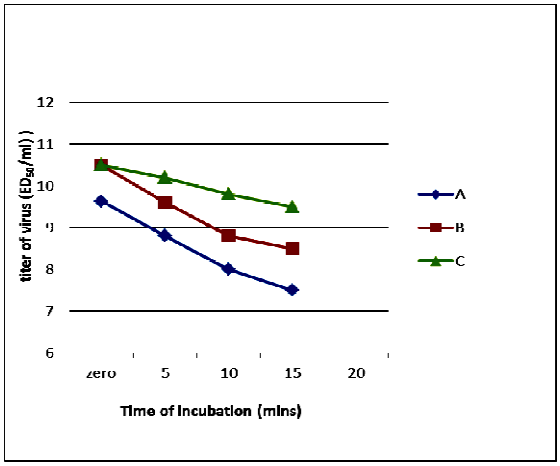

Figure 1: Thermostability testing of the original (A) and the passaged viruses by stepwise (B) and extreme heat exposure (C).

Discussion

Thermostable ND vaccines have proved useful for controlling the disease in developping countries. The commercial vaccines are relatively thermolabile and require maintenance of a cold chain. Any break in this chain removes any guarantee of effective protection against the disease (Aini *et al*., 1990; Bensink and Spradbrow., 1999). Thermostable vaccine can be produced by simple allantoic cavity inoculation and then harvesting of allantoic fluid. Sophisticated laboratories could freeze dry the vaccine, but other laboratories could simply store diluted allantoic fluid at 4^0C, and dispatch it in quantities determined by the estimated titre and the number of chickens to be vaccinated. Refrigeration would not be essential for transport or for short-term storage in developing countries (Bensink and Spradbrow., 1999). The main objective of the present study was to evaluate the effect of serial passage with heat selection on thermostability of the *Dongola* strain. Thermostability testing showed that infectivity of the virus was decreased by about 2log10, following exposure at 56^0C for 15minutes. Azzam. (2007) declared that *Dongola* strain was thermolabile. A series of selection procedures of relatively thermostable progeny viruses was carried out by stepwise method involving exposure of the harvested allantoic fluid to gradually

increased temperature (40 to 72^{0}C) for 15min viruses was also attempted by extreme heating, which included exposing allantoic fluid to 56^{0}C for 10 to 55min for 10 passages. After selection recovered virions from high passage were propagated and tested for thermostability. The results showed that extreme heat selection seemed to be better than stepwise method since infectivity titre of the final passage was decreased by about 1 log10 and 2 log10 respectively, following exposure to 56^{0}C for 15min. King. (2001) declared that extreme selection at 56^{0}C of few longest-surviving virus particles is an effective way of obtaining the seed for the next generation. Spradbrow. (1992) reported that extreme heat selection gives rapid results and it is assumed that virions that survive at 56^{0}C will certainly for 10 passages. Selection of thermo-stable survive well at ambient temperatures in tropical countries. These findings seem to agree with Lomniczi. (1975) and Hanson and Spalatin. (1978), who reported that heat stable haemagg-lutinins were derivable through selection and subsequent passage of strain Bn11 heterogenous NDV subpopulations. In the present study a NDV variant was selected which is relatively thermostable than the parent virus. It can be concluded that a degree of improvement concerning thermo-stability was attained in comparison with the original virus. Further studies are required to evaluate the effect of thermal selection on thermostability and other viral characteristics, particularly those essential for maintenance of the viral immunogenicity.

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الثبات الحراري لفيروس مرض سمير (عترة دنقلا) بعد التمريرات التسلسلية

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المستخلص:

العترة الفيروسية المعروفة بإسم دنقلا هي عترة ضعيفة طبيعيا تتبع للفيروس المسبب لمرض سمير. أجريت الدراسة لتحديد مدي تأثير تمرير الفيروس علي مقاومته للحرارة. تم تمرير الفيروس في جنين البيض لعشرة تمريرات وتعريضه للحرارة بطريقتين بين كل تمريرة، الطريقة التدريجية وفيها تم تعريض الفيروس لدرجات حرارة ترتفع تدريجيا (40–76^{0}م) لمدة 15 دقيقة، في الطريقة الحرارية المطلقة تم تعريض الفيروس لدرجة حرارة عالية (56^{0}م) لفترات زمنية متزايدة (10–55 دقيقة). بعد الإنتخاب الحراري تم إكثار الفيروسات وإختبار مدي تأثرها للحرارة. أظهرت النتائج أن طريقة الإنتخاب الحراري المطلق أعطت نتائج أفضل عند تعريض الفيروس لحرارة 56^{0}م لمدة 15 دقيقة حيث إنخفض المعيار بلوغريثم واحد بالمقارنة بإنخفاضه بلوغريثمين في الطريقة التدريجية.