NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY

APPLIED SCIENCES

DEPARTMENT OF BIOLOGY AND BIOCHEMISTRY

Project: DEVELOPING A MOLECULAR BASED TEST FOR THE DIAGNOSIS AND CHARACTERISATION OF NEWCASTLSE DISEASE VIRUS

RUJEKO NJIKIZANA
N990117A
2002/2003

ABSTRACT

Virus isolation was performed on 30 samples which had been stored at -80°C as antibiotic- treated supernatants of homogenised NDV infected organs. Virus isolation and growth was carried out in 9-10 day old embryonated chicken eggs, obtained from Irvine's Day Old Chicks. Haemagglutination (HA) and Haemagglutination Inhibition (HAI) tests were carried out on allantoic fluid that had been harvested from the infected eggs. Of the thirty samples, 19 were found to be HA positive. Of the 19 that were HA positive, 16 were tested for HAI and 13 were found to be HAI positive. As the HAI is a confirmatory test for NDV, it was concluded that 13 out of 30 samples were infected by NDV. For the pathogenicity test, of the six samples that were tested, 5 were found to be infected by velogenic NDV.

The reverse transciptase polymerase chain reaction (RT-PCR) was carried out based on the fusion protein cleavage site (FCS), of NDV. Primers were designed for this target sequence. The forward primer was **5'-ccagaccttctaccaagaac-3'** and the reverse primer was **5'-caatgaggctgtgcacgag- 3'**. However before these primers were delivered preliminary RT-PCR reactions were carried using primers obtained from the University of Zimbabwe (U.Z).

Reverse transcription was carried using MuLV reverse transciptase at either 42°C or 39°C for a period of 90 minutes. PCR was carried out using Taq DNA polymerase at varying cycle temperatures and periods.

In all cases except once, the FCS was not amplified. Characterisation by restriction fragment length polymorphism (RFLP) could therefore not be done.