NEWCASTLE DISEASE VIRUS IN THE INTESTINAL CONTENTS OF BROILERS AND LAYERS

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ABSTRACT

Two hundred intestines pieces (100 each of broilers and layers) of about 8 cm length were collected from the poultry sale shops in Faisalabad city. These pieces were opened, scratched and vigorously shaken into sterilized normal saline, the suspension was centrifuged and supernatants were subjected to spot haemagglutination with 2% chicken RBC's. Out of 200 samples, 95% samples of layers and 75% of the broilers showed positive spot haemagglutination. Micro haemagglutination inhibition with Newcastle disease (ND) antiserum revealed, 85 and 66 samples positive in layers and broilers respectively. A total of 10% samples of the layers and 9% of the broilers were not inhibited by ND antiserum suggesting other HA viruses. A total of 20 samples were used to isolate the virus in embryonated eggs (allantoic route). These isolates were confirmed as NDV by haemagglutination inhibition test. Five isolates were tested for intracerebral pathogenicity index (ICPI) in day old chicks. The ICPI values obtained were 0.28, 0.31, 0.37, 0.38 and 0.46. The isolates were found to be lentogenic.

Key words: Newcastle disease virus, intestinal contents, broilers, layers, ICPI.

INTRODUCTION

Newcastle disease (ND) is one of the highly contagious viral diseases of poultry which causes huge economic losses to the poultry industry. The disease is complicated due to different pathotypes and strains of the virus that may induce enormous variation in the severity of disease. The virus has been isolated from the trachea, spleen, intestine (duodenum, caecum and cloaca) and droppings of different avian species (Parimal *et al.*, 1997).

A large number of viruses are shed in droppings of birds. Vaccinated flocks shed virulent field strain for more than four months (Utterbach and Schwartz, 1973) and as the virus can remain infectious for 42 to 53 days in poultry litter, there is a considerable potential for contamination of objects coming into contact with such material. Mechanical transfer of infected faeces by rodents, fleas, insects, dogs or scavenging animals may occur in some circumstances. There is now good evidence that avain paramixovirus-1 viruses may become virulent by mutating after introduction into chickens. Some virulent viruses emerge perhaps, requiring as few as two point mutation (Alexander, 2001).

Phylogenetic studies have shown that virulent viruses are closely related to the endemic viruses of low virulence, suggesting their emergence by mutation. The mechanism by which virulent viruses arise, is a worrying development, not least, because of vast quantities of live vaccines are used (Alexander *et al.,* 1992).

The present study reports the occurrence of Newcastle disease virus (NDV) in the intestinal contents of slaughtered birds (6-7 weeks of age) and pathogenicity of isolates using intracerebral pathogenicity index (ICPI) in day-old chicks.

MATERIALS AND METHODS

Collection of samples

Two hundred small pieces of jejunum (about 8 cm in size) were taken from the broilers and layers (100 each) slaughtered at poultry shops in Faisalabad city. Each piece was opened, the mucosal surface was scratched and shaken vigorously in 25 ml sterilized normal saline containing penicillin (2000 IU/ml), streptomycin (2 mg/ml), and nystatin (100 units/ml). The suspension was centrifuged at 1000 rpm for 5 minutes and supernatant (4 ml) was collected.

Spot haemagglutination

The supernatants were examined for haemagglutination (HA) activity with 2% chicken RBCs on a glass slide.

Micro haemagglutination

Haemagglutination was performed in a 96-well microtitration plate. The supernatant was diluted two fold with sterilized normal saline and HA activity was observed by adding 0.5% chicken RBCs.

Confirmation of NDV

For confirmation of NDV, beta procedure of HI was adopted (Beard and Wilks, 1973). For this purpose, the known ND antiserum was diluted two fold with sterilized normal saline. Then 4 HA units of the supernatant was added in each dilution. After incubation for 30 minutes, 0.5% chicken RBCs were added and HI titre was determined.

Isolation of NDV

A 0.1 ml of supernatant from intestinal samples was inoculated into 9-11 days embryonated chicken eggs via allantoic route. The eggs were incubated at 37°C for 48 hours. The allantoic fluid of egg containing dead or dying embryos was tested for HA activity.

Intracerebral pathogenicity index

The intracerebral pathogenicity index of the 5 positive isolates was assessed in day-old chicks (Pearson *et al.*, 1987).

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RESULTS

Screening of virus in broilers

One hundred intestinal samples of broilers were processed for HA activity. Among these, 75% gave spot haemagglutinatoin. These samples were subjected to micro-haemagglutination test and HA titre was raised from 1:4 to 1:128. In most samples HA titre fell into 1:8 (32%), 1:4 (28%), 1:16 (39.3%), 1:32 (5.3%), 1:64 (2.6%) and 1:128 (2.6%).

Haemagglutination inhibition of all the HA positive 75 gut samples from broilers were performed using ND-antiserum. Out of these, 66 samples were inhibited by the ND antiserum, 9 (12%) samples were not inhibited.

Screening of virus in layers

Of the 100 samples collected from layers, 95 showed spot haemagglutination activity and 5 samples were negative. These 95 positive samples were checked further using micro haemagglutination. HA titre was determined which ranged from 1:4 to 1:128. HA titres fell in 1:16 (28.4%), 1:4 (13.6%), 1:8 (17.8%) 1:32 (25.3%), 1:64 (11.6%) and 1:128 (3.2%). Haemagglutination inhibition of all the 95 gut samples were performed using ND-antiserum. Out of these, 85 samples were inhibited by ND antiserum, only 10 samples were not inhibited by the ND antiserum.

Intracerebral Pathogenicity Index (ICPI)

After intracerebral injections of virus isolates the observation were made for eight days. The clinical symptoms in the birds were also recorded which were mostly nervous in nature i.e., paralysis of legs (one or both) and wings (one or both). On post mortem examination no specific lesions were observed. The virus was re-isolated from the spleen of dead chicks. The ICPI values were 0.31, 0.37, 0.46, 0.38 and 0.28 for isolates 1, 2, 3, 4 and 5, respectively.

DISCUSSION

With spot haemagglutination test, 95% samples of layers were positive, while 5% were negative. Out of 95 samples, 85 samples were inhibited using known NDV antiserum and 10 samples were not inhibited. In case of broilers 75% samples were positive to spot haemagglutination and 25% showed no haemagglutination activity. Out of these 75 samples, 66 were inhibited by haemagglutination inhibition test with known NDV antiserum, while 9 samples were not inhibited. Such a high amount of NDV may interfere with other enteric viruses and vast quantities of other vaccines become useless.

Parimal *et al.* (1997) also reported more than 60% of the intestinal samples positive for Newcastle disease virus. A number of workers from different countries, reported the presence of Newcastle disease virus in the faeces of healthy broilers and layers. According to Ramadass *et al.* (1996), 46.3% were positive by ELISA and 44.7% by IFA. The findings of the present study are higher than the results of Eisa and Omer (1984). This may be due to lavish use of vaccination.

In the present study, 9% samples in layers and 10% samples in broilers were not inhibited by ND antiserum, although these were HA positive which could be due to other haemagglutinating viruses like avian influenza or infectious laryngotracheitis virus. These findings are also supported by the results of Azam *et al.* (1984), Reynolds *et al.* (1987) and Guy (1998).

The intracerebral pathogenicity index (ICPI) of all the isolates ranged from 0.28 to 0.46. These values are in accordance with results of Azam *et al.* (1984), Zhuang *et al.* (2000) and Stanislawek *et al.* (2002), who reported that ICPI value of lentogenic strains rainged from 0.25 to 0.7.

Among the consequences of the epidemics of ND in Italy during 2000, virological investigation in affected poultry flocks and backyard flocks yielded virulent isolates of NDV which produced ICPI ranging from 1.6 to 2.0. It is well known that high concentration of virus occurs in faeces that can contaminate the whole environment (Capua *et al.*, 2002).

According to Alexander (2001), virulent viruses emerge perhaps requiring as few as two point mutation. Westbury (2001) and Huovilainen *et al.* (2001), have stated that asymptomatic strains which are able to

induce mild respiratory signs equivalent to that induced by vaccine strains such as LaSota may evolve pathogenic strains so called precursor of the virulent virus. It leads to outbreaks in vaccinated flocks. Such high presence of lentogenic NDV in healthy broiler and layers is suggestive of mutation and consequently outbreak of Newcastle disease among broiler and layers. So lavish use of oral LaSota vaccine must be discouraged.

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