Archives of Razi Institute, Vol. 62, No. 1, Winter (2007) 39-44

Short Communication

Isolation and pathogenicity identification of avian paramyxovirus serotype 1 (Newcastle disease) virus from a Japanese quail flock in Iran

Momayez[1*], R., Gharahkhani[2], P., Pourbakhsh[1], S.A., Toroghi[3], R., Shoushtari[1], A.H., Banani[1] M.

1. Department of Avian Diseases Research and Diagnostic, Razi Vaccine and Serum Research Institute, Karaj. Iran
2. Faculty of Veterinary Medicine, Azad University of Karaj, Karaj, Iran
3. Department of Veterinary Research and Biotechnology, Razi Vaccine and Serum Research Institute, Mashhad. Iran

Received 14 Sep 2006; accepted 26 Jan 2007

ABSTRACT

Newcastle disease virus (NDV) was isolated and identified for the first time from a flock of Japanese quail suspected to ND in Iran. Birds of the flock aged variously, and some of them died from the disease. Loss of appetite, weakness and decrease in egg production, diarrhea and nervous symptoms were the main clinical signs in the birds. Hemorrhagic lesions of the intestinal tracts and proventriculus were found in gross pathology investigations. Samples were taken for bacteriology, parasitology and virology. The results of parasitology and bacteriology examinations were negative. The virus was isolated only from brain samples and was identified as Avian Paramyxovirus type 1 (APMV-1) by HI test using NDV-Specific antibody. The pathogenicity of the virus was identified by mean death time (MDT) in embryonated chicken eggs, intracerebral pathogenicity index (ICPI) and intravenous pathogenicity index (IVPI) in chickens. Values 40, 1.62 and 2.31 were obtained for MDT, ICPI and IVPI respectively. These results indicate that isolated APMV-1 belongs to NDV velogenic strains with severe pathogenicity. According to these findings vaccination against NDV in quail farms of Iran is highly recommended.

***Keywords*:** Isolation, Identification, Pathogenicity, Newcastle disease virus, Quails, Iran

INTRODUCTION

Newcastle disease (ND) is caused by specified viruses of the Avian Paramyxovirus serotype 1 (PMV-1), the serotype of the genus *Rubulavirus*, be - longing to the family *Paramyxoviridae* (Alexander 2000). There are nine serotypes of avian paramyxoviruses designated APMV-1 to APMV-9 (Jorgensen *et al.*, Rima *et al.*, 1995 and Alexander 2003). The first outbreaks of ND caused by virulent strains of virus occurred in 1926 in Java, Indonesia and in Newcastle –upon-Tyne, England (Doyle

* Author for correspondence E-mail: r.momayez@rvsri.ir

1927). Kaleta and Baldauf (1988) concluded that at least 241 species of birds from 27 of the 50 orders of birds are susceptible to ND virus (NDV). The disease occurs worldwide and has a considerable economic impact on the world poultry industry. Viscerotropic velogenic ND (VVND) has been reported in Brazil in ducks, pigeons, quail *(Coturnix)*, turkeys, teal and guan (Cubas 1993). The virulence of NDV strains varies greatly with the host (Higgins 1971), But breed or genetic stock does not appear to have a significant effect on the susceptibility of chickens to the disease (Cole and Hutt 1961). Although Japanese quails are more resistant to NDV than chickens, the severity of the disease may increase under stress conditions. For appropriate developing of quail industry it is necessary to consider prevention of ND in this bird. Abshar *et al* (2000) reported respiratory signs and high mortality of a Japanese quail flock of Iran caused by quail bronchitis virus (QBV) or Serotype 1 of avian adenovirus. In that report they did not isolate other agents such as VVND virus.

There is a wide variation in the infectivity of the disease produced by NDV in birds (Alexander *et al*, 2002 and Alexander 2003). The pathogenicity of various NDV strains varies from apathogenic strains to velogenic highly virulent strains. NDVs have been placed into five pathotypes based on disease produced in susceptible chickens under laboratory conditions: 1) viscerotropic velogenic NDVS cause a highly virulent form of disease in which hemorrhagic lesions are characteristically present in the intestinal tract; 2) neurotropic velogenic NDVS cause high mortality following respiratory and nervous signs; 3) mesogenic NDVs cause respiratory and sometimes nervous signs with low mortality; 4) lentogenic respiratory NDVs cause mild or inapparent respiratory infection; 5) asymptomatic enteric NDVs cause inapparent enteric infection (Alexander *et al*, 2002 and CEC, 1992). In the laboratory, the pathogenicity of a NDV strains can be estimated by different means of which the most widely used is the mean death time (MDT) in embryonated eggs, the intracerebral pathogenicity index (ICPI) and the intravenous pathogenicity index (IVPI) in chickens (Alexander and Allan 1974 and Alexander 2003). ICPI values may vary between 0 (apathogenic) and 2 (highly virulent). ICPI values above 0.7 are considered as NDV infections for which control legislation will be imposed within the European Union (CEC, 1992). The IVPI indices for virulent strains will approach 3.0., whereas lentogenic and some mesogenic strains will have values of 0. The MDT has been used to classify NDV strains by determining the mean time in hours for the minimum lethal dose to kill all the inoculated embryos. The MDTs are under 60 for velogenic, between 60-90 for mesogenic and more than 90 for lentogenic strains. The pathogenicity of APMV-1 is influenced by amino acid sequences at the cleavage site of the F0 proteins. Basic amino acids forming two pairs at the cleavage site are associated with high pathogenicity, while low pathogenicity APMV-1 strains possesses single basic amino acids separated by two others at the cleavage site (Alexander 2003 and Olav *et al*, 2003). In addition to variation in pathogenicity of different NDVs, the severity of disease is highly dependent on the avian host species. For instance waterfowls, although susceptible to infection, rarely show severe disease, while domestic fowls are highly susceptible to both infection and disease (Alexander 1995).

There were no reports on the occurrence of APMV-1 in Japanese quails in Iran. In the recent years quail industry has developed in the world and also in Iran. Therefore, it is necessary to study more about isolation and identification of ND in this bird. The present paper describes the pathogenicity identification of NDV isolated from a Japanese quail in Tehran province in Iran.

MATERIALS AND METHODS

Population. This study was performed on the 32

sick and 17 dead quails which were submitted to Razi institute for diagnosis. The flock was located in Tehran province with 1500 quails aged variously between 1 day, 3 weeks, 6 weeks, and 3-6 months, without vaccination against NDV. Morbidity was more than 10% and mortality was about 5%. The sick birds were suspected to infection with NDV showing the signs of loss of appetite, weakness, decrease in egg production, diarrhea, and nervous symptoms including paralysis of wings and legs and waving movements of the heads and the necks. There was a small native chicken flock without vaccination against NDV in the neighbor of this flock that had signs suspected to ND with some mortality before the onset of the disease in the quail flock.

Gross pathology and sampling. Postmortem examinations were performed on the dead birds. Liver, bone marrow, and trachea samples from 10 sick and 10 dead quails were taken for bacteriology. Brain, trachea, and spleen samples were used for virology. Feces samples from 5 suspected quails were examined for the presence of Eimeria and cryptosporidia.

Virus isolation and detection. Brain, tracheas, and spleen samples from 10 sick and 10 dead quails with typical signs were homogenized to make a 10% *w/v* suspension in phosphate buffer saline (PBS) pH 7.0-7.2 containing 10,000 IU/ml penicillin, 10,000 µg/ml streptomycin, and 250 IU amphotericin-B/ml. Homogenized materials then were centrifuged at 1000 ×g for 15 minutes. The supernatant fluid was inoculated at 0.2 ml into 10 days old SPF embryonated chicken eggs (Valo, Lohmann, and Cuxhaven, Germany) via chorioallantoic cavity. Inoculated eggs were incubated at 37 °C. The embryos that died within 24 hours post inoculation were discarded. Allantoic fluids were harvested from dead or live embryos during 6 days post inoculation, and then were examined for hemagglutination activity. At least two serial blind passages were made. Harvested allantoic fluids were tested according to the procedure described for HA and HI tests (CEC 1992) using SPF chicken red blood cells.

Pathogenicity determination. The pathogenicity of the isolate was tested using Mean Death Time (MDT) in SPF chicken embryonated eggs, Intracerebral Pathogenicity Index (ICPI), and Intravenous Pathogenicity Index (IVPI). Briefly, for MDT, fresh infected allantoic fluid was diluted in a tenfold series (10^{-5} to 10^{-9}) in sterile saline. Five 9 to 10 days old SPF chicken embryonated eggs were inoculated with 0.1 ml of each dilution, and placed at 37 ºC. Eight to ten hours later a further five eggs were inoculated with 0.1 ml of each dilution. Embryos were examined twice daily for seven days. The minimum lethal dose is the highest dilution at which all embryos die and MDT is the mean time in hours for the minimum lethal dose to kill those embryos. For ICPI, The fresh infected allantoic fluid were diluted to 1/10 in sterile isotonic saline and 0.05 were injected intracerebrally into each of 10 one-day old chicks hatched from SPF chicken embryonated eggs. The birds were examined every 24 hours for eight days. At each observation each bird was scored 0 if normal, 1 if sick and 2 if dead. The ICPI is the mean score per bird over the eight day period. For IVPI, 0.1 ml of fresh infected allantoic fluid diluted 1/10 in sterile isotonic saline was inoculated intravenously into each of 10 six-week-old SPF chickens. The birds were then examined every 24 hours and scored 0 if normal, 1 if sick and 2 if paralyzed and 3 if dead at each observation. The IVPI is the mean score per bird for observation over the 10 days period (Alexander & Allan 1974, Alexander 1998 & 2003).

Serology examinations. Serum samples from seven sick quails which were submitted to Razi Institute were examined with HI test. The HI test was carried out according to standard method (Allan & Gough 1974, Beard & Wilkes 1985, CEC 1992).

RESULTS AND DISCUSSION

Pathology, Bacteriology and Parasitology. Hemorrhagic lesions of the intestinal tract and proventriculus were the most important finding of postmortem examination. Bacteriology and parasitology results of the samples were negative.

Virus isolation and detection. Virus was isolated only from 10 pooled brain samples and was identified to be AMPV-1 by the results obtained from HI test with NDV-specific antibodies.

Pathogenicity determination. MDT, ICPI, and IVPI results of the isolated virus are shown in table 1. The values 40, 1.62 and 2.31 were obtained for MDT, ICPI and IVPI, respectively. In comparison with the value of different pathotypes which are shown in the table, the isolated virus can be classified in the Velogenic NDV pathotypes..

Table 1. Pathogenicity indices of NDV pathotypes and isolated AMPV-1.

Pathotype	MDT	ICPI	IVPI
VVNDV[1]	<60	1.5 - 2.0	2.0-3.0
NVNDV[2]	<60	1.5 - 2.0	2.0-3.0
Isolated virus	40	1.62	2.31
Mesogenic	60-90	1.0 - 1.5	0.0-0.5
Lentogenic	>90	0.2 - 0.5	0.0
Asymptomatic	>90	0.0 - 0.2	0.0

[1] viserotropic velogenic NDV
[2] neurotropic velogenic NDV

Serologic examination. Titers of HI antibodies against NDV were 2 and 3 (log2) in two quails, but HI antibody was not detected in the sera of 5 birds.

As the gross lesions and clinical signs produced by NDV are not pathognomonic, virus isolation and detection methods must be used for diagnosis. This study is the first report of NDV isolation in quails in Iran. Hemorrhagic lesions of intestinal tracts and proventriculus were the important gross lesions in the birds died from the disease. Clinical signs included loss of appetite, weight loss, weakness, diarrhea and nervous signs. In related to viral diseases of quail in Iran, Abshar *et al* reported only respiratory signs causing by an adenovirus (QBV), whereas in this report there is no respiratory signs. We isolated the virus only from the brain samples and it was identified as APMV-1 by HI using NDV-specific antibodies.

We could detect NDV-specific antibodies using HI test with titers of 2 and 3 (log2) in the serum of 2 sick birds, but HI antibody was not detected in the sera of 5 birds. The low titers and the absence of antibody in examined birds in the initial steps of the disease, and high morbidity and mortality in the flock, showed that the birds were sensitive to NDV. Therefore, it seems that vaccination program against NDV is necessary for the prevention of the disease in commercial quail flocks of Iran.

In order to identify the pathogenicity of the virus, *in vivo* well-established and reliable tests including MDT, ICIP, and IVIP were used. One of the advantages of these tests is that they overcome the problems that may be experienced with the sequencing technology if mixture of virulent and avirulent viruses is isolated (Alexander & Jone, 2002). MDT, ICIP, IVIP values in this study were 40, 1.62 and 2.31 respectively, showing that this virus can be placed in velogenic NDV pathotypes.

The nervous signs usually occur in the advanced steps of the disease following respiratory signs in domestic fowls, but gastrointestinal and nervous signs were predominant in our report, and were seen without respiratory signs in quails. These data indicate that the pathogenicity of AMPV-1 in quails might be different from domestic chickens.

It is important to know whether or not the APMV-1 we isolated in quails, is indistinguishable from those infecting different species of poultry. Therefore, further studies are required to investigate the relationship between the virus we have isolated and other viruses isolated from different species of poultry in Iran.

The results obtained from this study showed that

velogenic NDV is present in quails in Iran. Japanese quails are more resistant to NDV rather than chickens, but the severity of the disease may be increased under stress conditions. On the other hand, as ICPI values above 0.7 are considered as NDV infections for which control legislation will be imposed within the European Union (CEC 1992), controlling factors like isolation of the farms, applying bio-security, decreasing stress conditions, certification of quail movement and particularly enforcement of vaccination program ect., must be considered to improve disease security and reduce the danger of spreading of infection.

References

Abshar, N., Aghakhan, S.N., Marounesi, Ch., Sami. Z., Rasoul Nejad Fereidouni, S., Khodashenas, M. and Pourbakhsh, S.A. (2000). Study of quail bronchitis virus (serotype 1, avian adenovirus) isolated from quails. *Pajouhesh & Sazandegi* 48: 128-134. (In Persian).

Alexander, D.J. and Allan, W.H. (1974). Newcastle disease virus pathotypes. *Avian Pathology* 3: 269-278.

Alexander, D.J. (1995). The epidemiology and control of avian influenza and Newcastle disease. *Journal of Comparative Pathology* 112: 105-126.

Alexander, D.J. (1998). Newcastle disease virus and other avian Paramyxoviruses. In: D.E. Swayne, J.R. Glisson, M.W. Jackowod, J.E. Pearson and W.M. Reed, (Eds.), *A Laboratory Manual for the Isolation and Identification of Avian Pathogens*. Pp: 156-163. Kennet Square, PA: American Association of Avian Pathologists, USA.

Alexander, D.J. (2000). Newcastle disease in ostriches (*Struthio camelus*) - a review. *Avian Pathology* 29: 95-100.

Alexander, D.J. and Jones, R.C. (2002). Paramyxoviridae. In: F., Jordan, M., Pattison, D., Alexander and T. Faragher (Eds.), *Poultry Diseases* (5th edn.). Pp: 257-280. W.B. Saunders, UK.

Alexander, D.J. (2003). Newcastle disease In: Y. M. Saif., J.R. Barnes, A.M. Glisson, L.R. McDougald Fadly and D.E. Swayn (Eds.), *Diseases of Poultry.* (11th edn.). Pp: 101-119. Iowa State University Press: Ames.

Allan,W.H. and Gough, R.E. (1974). A standard haemagglutination inhibition test for Newcastle disease. A comparison of macro and micro methods. *Veterinary Record* 95: 120-123.

Beard, C.W. and Wilkes, W.J. (1985). A comparison of Newcastle disease haemagglutination-inhibition test results from diagnostic laboratories in the southeastern United States. *Avian Ddiseases* 29: 1048-1056.

CEC (1992). Council Directive 92/66/EEC of 14 July 1992 introducing Community measures for the control of Newcastle disease. *Office Journal of the European Commission*, L260, Pp: 1-20. European Union (EU).

Cole, R.K. and Huts, F.B. (1961). Genetic differences in resistance to Newcastle disease. *Avian Diseases* 5: 205-214.

Cubas, Z.S. (1993). Natural diseases of free- ranging birds in South America. In: M.E. Fowler (Ed.), *Zoo and wild animal medicine, current therapy 3.* Pp: 166-172. W.B. Saunders Company, USA

Doyle, T.M. (1927). A hitherto unrecorded disease of fowls due to a filter-passing virus. *Journal of comparative Pathology and Therapeutics* 48: 1-20.

Higgins, D.A. (1971). Nine disease outbreaks associated with myxoviruses among ducks in Hong Kong. *Tropical Animmal Health and Production* 3: 232-240.

Jorgensen, P.H., Lomniczi, B., Manvell, R. J., Holm, E. and Alexander, D.J. (1998). Isolation of avian paramyxovirus type 1 (Newcastle disease) viruses from a flock of ostriches (Struthio camelus) and emus (Dromaius novaehollandiae) in Europe with inconsistent serology. *Avian Pathology* 27: 352-358.

Kaleta, E.F. and Baldauf, C. (1998). Newcastle disease in free-living and pet birds. In: D.J. Alexander (Ed.), *Newcastle Disease.* Pp: 197-246. Kluwer Academia Publishers, Boston, USA.

OIE (1996). Newcastle disease. *Office Internationale des Epizooties Manual of Standards for Diagnostic Tests and Vaccines*. Pp: 161-169. Office Internationale des Epizooties. Paris.

Olav, S.L., Hartog, L., Koch, G., and Peeters, B.P.H. (2003). Effect of fusion protein cleavage site mutations on virulence of Newcastle disease virus: non-virulent cleavage site mutants revert to virulence after one passage in chicken brain. *Journal of General Virology* 84: 475-484.

Rima, B., Alexander, D.J., Billeter, M.A., Collins, P.L., Kingsbury, D.W., Lipkind M.A., Nagai, Y., Orvell, C., Pringle, C.R., and TerMeulen, V. (1995). Paramyxoviridae. In: F.A. Murphy, C.M. Fauquet, D.H.L. Bishop, S.A. Ghabrial, A.W. Jarvis, G.P.

Martelli, M.A. Mayo and M.D. Summers (Eds.), *Virus Taxonomy, Sixth Report of the International Committee on Taxonomy of Viruses*. Pp: 268-274. Springer-Verlag, Wien.