

**Original Article**

# **Experimental Infection of Turkeys with A Virulent Newcastle Disease Virus Isolated from Broiler Chickens**

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## **ABSTRACT**

Newcastle disease (ND) is a highly contagious infection of many avian species, mainly chickens and turkeys, with a devastating impact on worldwide poultry production. The ND accounts for heavy losses in Iranian poultry flocks. There are some reports regarding the epidemiology of this infection in Iran. This study was performed to investigate the infection of turkeys with a Newcastle disease virus (NDV) isolated from a broiler chicken flock in southwestern Iran during 2013. For the purpose of the study, 70 day-old Wishard bronze poult were allocated into two groups of control (n=25) and infected (n=45). At 32 days of age, each bird in the infected group was inoculated with 0.1 mL (50 µL per eye) of NDV-infected allantoic fluid through an ocular route and received 10<sup>5</sup> EID<sub>50</sub> of viral inoculum. On the other hand, the birds in the control group were inoculated with phosphate buffered saline by the same route. Swab samples were taken from both groups at different time points, namely from 1 to 21 days postinoculation, and verified for NDV infection by using reverse transcription-polymerase chain reaction (RT-PCR). Both groups were also examined serologically by haemagglutination inhibition test. Clinically, the infected turkeys exhibited anorexia, severe depression, sitting on the hock joint, white to greenish (sometimes bloody) diarrhea, neurological disorders, and mild respiratory problems. Out of 45 inoculated birds, 9 (20%) cases died. Based on RT-PCR, virus shedding was observed in the challenged birds 3-8 days postinoculation. The NDV was detected more in tracheal swabs (50%) than in cloacal swabs (12.5%). The infected birds showed a high seroconversion. Therefore, the NDV circulating in Iranian chicken flocks has the potential to cause a serious illness in commercial turkeys. The vaccination of turkeys, as well as biosecurity, should be considered carefully to prevent the ND outbreaks in the future.

**Keywords:** Newcastle disease, Turkeys, Immune response, Pathogenicity, RT-PCR

## **Infection expérimentale des dindes avec un virus virulent de la maladie de Newcastle isolé chez des poulets de chair**

**Résumé:** La maladie de Newcastle (MN) est une infection très contagieuse touchant de nombreuses espèces d'oiseaux, principalement les poulets et les dindes, avec un impact dévastateur sur la production de volaille dans le monde. La MN est responsable de lourdes pertes dans les troupeaux de volailles iraniens. Il y a quelques rapports concernant l'épidémiologie de cette infection en Iran. Cette étude a été réalisée dans le but d'analyser l'infection de dindons par le virus de la maladie de Newcastle (VMN) isolé d'un troupeau de poulets de chair dans le sud-ouest de l'Iran en 2013. Dans le cadre de cette étude, des dindes bronze Wishard âgées de 70 jours ont été réparties en deux groupes de contrôle (n = 25) et infecté (n = 45). À l'âge de 32 jours, chaque oiseau du groupe infecté a été inoculé avec 0,1 ml (50 µL par œil) de liquide allantoïque infecté par le VMN par voie oculaire et a reçu l'équivalent de 10<sup>5</sup> EID<sub>50</sub> d'inoculum viral. Par ailleurs, les oiseaux du groupe témoin ont été

inoculés avec une solution saline tamponnée au phosphate par une voie similaire. Des échantillons d'écouvillon ont été prélevés dans les deux groupes à différents moments, à savoir de 1 à 21 jours après l'inoculation, et ont été vérifiés pour l'infection par le VMN en utilisant une réaction en chaîne de transcription-polymérase inverse (RT-PCR). Les deux groupes ont également été examinés sérologiquement par un test d'inhibition de l'hémagglutination. Sur le plan clinique, les dindes infectées présentaient une anorexie, une dépression grave, une assise sur le jarret, une diarrhée blanche à verdâtre (parfois sanglante), des troubles neurologiques et de légers problèmes respiratoires. Sur les 45 oiseaux traités, 9 (20%) des cas sont morts. Sur la base de la RT-PCR, des excréments de virus ont été observés chez les oiseaux exposés entre 3 et 8 jours après l'inoculation. Le VMN a été détecté davantage dans les écouvillons trachéaux (50%) comparé aux écouvillons cloacaux (12,5%). Les oiseaux infectés ont présenté une séroconversion élevée. Le virus de la maladie de Newcastle qui circule dans les troupeaux de poulets iraniens est susceptible de causer de graves infections chez les dindes commerciales. Par conséquent, la vaccination des dindes, ainsi que la biosécurité, doivent être sérieusement prise en compte afin de prévenir les épidémies de MN.

**Mots-clés:** Maladie de newcastle, Dindons, Réponse immunitaire, Pouvoir Pathogène, RT-PCR

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## INTRODUCTION

Newcastle disease (ND) is a highly contagious and fatal disease affecting at least 241 species of domestic and wild birds of both genders and all age groups (Kaleta and Baldauf, 1988). The causative agent of this disease is a virulent virus of the avian paramyxovirus serotype I of *Avulavirus* genus belonging to the family Paramyxoviridae (Manual, 2008). Velogenic Newcastle disease virus (NDV) is endemic in many countries of the Middle East, Africa, and Asia (Miller and Koch, 2013). The inclusion of ND in the list of notifiable diseases by the World Organization for Animal Health (2008) is indicative of the paramount economic impact of this disease on the worldwide poultry industry. Among poultry, chickens are the most susceptible species, followed by turkeys, pigeons, and ducks. In 1926, the first outbreaks of ND were observed in chickens in Java, located in Indonesia, and Newcastle-upon-Tyne in England (Miller and Koch, 2013). In addition, many outbreaks have occurred in turkeys (Gale et al., 1961; Graham et al., 1996; Alexander et al., 1998; Capua et al., 2002). Recently, an outbreak with high morbidity and mortality has been reported among the turkeys and Japanese quails housed along with chickens in a multi-species poultry farm in India (Gowthaman et al., 2013). In Iran, ND is endemic in different parts of the

country. Accordingly, this disease is one of the most important diseases in poultry industry causing enormous losses since it is associated with high mortality, slaughterhouse condemnation of carcasses, suboptimal production, and great prevention and treatment expenses. In recent years, ND outbreaks have been sporadically observed in different avian species (Ghiamirad et al., 2010; Madadgar et al., 2013; Mehrabanpour et al., 2014; Ahmadi et al., 2016). During 2012-2013, some outbreaks with heavy losses occurred among the commercial broiler chicken flocks located in southwestern Iran. The isolation of the virulent viruses accounting for this great loss resulted in their classification into genotype VII and subclassification into subgenotype VIIId (Boroomand et al., 2016). Turkey rearing is rapidly developing in Iran to the extent that this industry has obtained the second rank after chicken industry. The NDV can be easily transmitted from one species to another; accordingly, some outbreaks in turkeys were epidemiologically related to the spread of the virus from chickens (Capua et al., 2002) and wild birds (Heckert et al., 1996). The viruses emerging from the field strains may appear with relatively new features. Regarding this, the present study was conducted to investigate the clinical manifestations, virus shedding, and serological response in commercial turkeys experimentally infected with isolated NDV.

## MATERIAL AND METHODS

**Newcastle disease virus.** The applied viruses were isolated from a broiler chicken flock infected with NDV during an outbreak occurring in 2013 in southwestern Iran. The virus was assigned an accession number of NDa:KP347437 (Boroomand et al., 2016). The virus was propagated twice in 9-day-old embryonated chicken eggs inoculated through the chorioallantoic membrane route. The 50% embryo infective dose (EID<sub>50</sub>) was calculated for the second passage following the method adopted by Reed and Muench (Villegas, 2008). Furthermore, the harvested allantoic fluid was used as inoculum as specified in the experimental design.

**Experimental Design.** Seventy day-old commercial Wishard bronze unsexed poults were purchased and randomly divided into two groups of control (n=25) and infected (n=45). They were housed in cages separately in the Animal Research Unit of Shahid Chamran University of Ahvaz, Iran, and received feed and water *ad libitum* during the experiment. At 32 days of age, each bird in the infected group was inoculated with 0.1 mL (50 µL/eye) of allantoic fluid harvested from infected embryonated eggs through the ocular route, and received 10<sup>5</sup> EID<sub>50</sub> of viral inoculum. The birds in the control group were inoculated with phosphate buffered saline (PBS) by the same route. All birds were visually monitored for clinical signs and mortality daily. Tracheal and cloacal swabs were taken from two birds per group before inoculation and 1, 2, 3, 4, 6, 8, 14, and 21 days postinoculation. They were examined for NDV infection by using reverse-transcription polymerase chain reaction (RT-PCR). All birds which died during the experiment were subjected to virological examination. For serological assay, 10 birds from each group were bled via brachial vein before inoculation and 14 and 28 days postinoculation. The blood samples were left to coagulate at room temperature for 8 h, and then centrifuged at 2000 rpm for 5 min. The collected sera were stored at -70 °C until

examined by conventional haemagglutination-inhibition (HI) test.

**Reverse-transcription polymerase chain reaction.** The swab samples were individually placed in microtubes, containing 250 µl PBS. After removing the swabs from the microtubes, the RNA extraction was performed using the RNXTM-Plus Kit (CinaGen, Tehran, Iran) according to the manufacturer's protocol. Isolated RNAs were directly used for the RT-PCR or stored at -70 °C. The partial F gene, including the cleavage site sequence, was amplified using a pair of specific primers. The primer sequences were TT GAT GGC AGG CCT CTT GC and GG AGG ATG TTG GCA GCA TT (Mehrabanpour et al., 2014). The complementary DNA (cDNA) was synthesized using BioNer RT PreMix kit (BioNer Corporation, South Korea) following the manufacturer's instruction. The PCR assay was carried out in a 20 µL reaction volume consisting of 2 µL 10x PCR buffer, 0.2 µL of 10 mM deoxynucleotide, 1 µL of each primer (20 pmol/ml), 0.2 µL Taq DNA polymerase (5 U/ml), 0.6 µL of 50 mM magnesium chloride, 10 µL distilled water, and 5 µL cDNA dilution. The PCR conditions included initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 sec, 55 °C for 60 sec, 72 °C for 60 sec, and a final extension at 72 °C for 10 min. The PCR products (330 base pairs) were subjected to electrophoresis using 1.5% agarose gel. The NDV-infected allantoic fluid from our previous work was used as positive control, and distilled water was employed as negative control. In addition, a 100-bp DNA marker was used in all electrophoreses for determining the PCR product size.

**Hemagglutination inhibition test.** Sera obtained by the centrifugation of the samples were first left in a water bath at 56 °C for 30 min. Subsequently, they were assessed for haemagglutination-inhibiting antibody using 4 HA units of NDV antigen and two-fold serum dilutions as recommended by Thayer and Beard (2008). The results were expressed as Log<sub>2</sub>.

**Statistical analysis.** All analyses were carried out in SPSS software (Version 20.0., Armonk, NY: IBM Corp.). The t-test and Tukey's test were used to analyze the HI titers. P-value less than 0.05 was considered statistically significant.

## RESULTS

**Clinical signs and mortality.** No morbidity or mortality was observed in the noninfected turkeys, whereas the infected birds developed the first clinical symptoms 5 days after inoculation. The affected turkeys presented anorexia, severe depression, somnolence, poor growth, sitting on the hock joint, white to greenish (sometimes bloody) diarrhea, incoordination, head tremor, head dropping, and leg paralysis (figures 1 and 2). They were reluctant to move and hypothermic just before death. In addition, few birds developed mild dyspnea. Almost one third of the birds became morbid and showed clinical signs; however, 9 (20%) cases out of 45 turkeys died. Mortality commenced 6 days postinoculation, reached the peak (i.e., 3 deaths) 8 days after inoculation, and continued by 10 days (Table 1). The survived birds returned to a normal condition 14 days after the onset

of the disease.

**Virus shedding.** An attempt to detect the virus was made for a period of 3 weeks, and the results of RT-PCR are presented in Figure 3. Based on the RT-PCR, 50% of the infected turkeys shed the virus through respiratory and/or intestinal tracts. All tracheal swabs collected from the infected birds were virus-positive 3-8 days postinoculation, whereas the cloacal swabs were positive only 8 days after inoculation. In addition, NDV was detected in all swab samples taken from the dead birds. However, virus was not detected in any swab samples obtained from the noninfected control birds.

**Serological examination.** Table 2 summarizes the results of HI examination. The results revealed that the serum samples of both groups were negative (i.e.,  $< \text{Log}_2 4$ ) before inoculation. This status continued in the control group by 28 days postinoculation, whereas a significant seroconversion was detected in the infected group 14 and 28 days after inoculation ( $P < 0.05$ ). However, there was no significant difference between the two time points in this regard ( $P > 0.05$ ).

## DISCUSSION

The NDV is a very important pathogen in poultry

**Table 1.** Daily mortality in turkeys experimentally infected\* with a velogenic chicken isolate of Newcastle disease virus

Group	Days postinoculation													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Infected (n=45)	-	-	-	-	-	1	2	3	2	1	-	-	-	-
Control (n=25)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

\*At 32 days of age, each bird in infected group was inoculated intaconjunctivally with 0.1 mL (50  $\mu\text{L}/\text{eye}$ ) of infected allantoic fluid, and received  $10^5$  EID<sub>50</sub> of viral inoculum.

**Table 2.** Mean hemagglutination-inhibition titers ( $\text{Log}_2 \pm \text{SE}$ )\* in turkeys after experimental infection with a velogenic chicken isolate of Newcastle disease virus

Group	Days postinoculation		
	0	14	28
Infected (n=45)	1.5 $\pm$ 0.21 <sup>b</sup>	8.1 $\pm$ 0.34 <sup>a</sup>	8.5 $\pm$ 0.45 <sup>a</sup>
Control (n=25)	1.5 $\pm$ 0.21 <sup>b</sup>	1.5 $\pm$ 0.34 <sup>b</sup>	1.6 $\pm$ 0.30 <sup>b</sup>

\*Ten birds/group were bled at each time point after inoculation at 32 days of age.

ab Different superscripts show statistical significance ( $P < 0.05$ ).

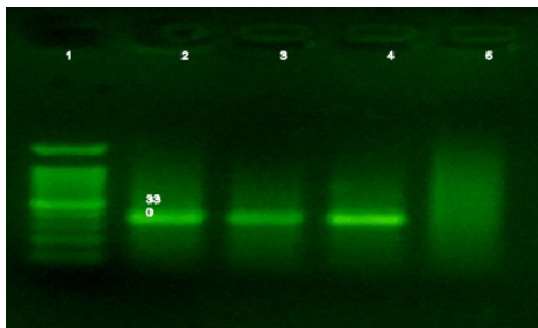
industry. Velogenic NDV is so virulent; accordingly,



**Figure 1.** White diarrhea in a poult infected with a velogenic chicken isolate of Newcastle disease virus 6 days postinoculation



**Figure 2.** Severe depression and leg paralysis in poult infected with a velogenic chicken isolate of Newcastle disease virus 8 days postinoculation



**Figure 3.** Electrophoresis of reverse transcription -polymerase chain reaction product of F gene in turkeys infected with Newcastle disease virus; lane 1: ladder (100 bp); lane 2 (330 bp): positive control, lanes 3 and 4: positive cloacal and tracheal samples 8 days postinoculation, respectively, lane 5: negative control.

unvaccinated birds may die suddenly with no clinical signs. This disease has a mortality rate of 100% (Miller and Koch, 2013); nonetheless, the severity of the disease with any given virus varies depending on the host parameters, including species, breed, age, and immune status, as well as coinfection with other organisms, environmental and nutritional conditions, and route of exposure (Kaleta and Baldauf, 1988; Wakamatsu et al., 2006). The NDVs used in this study were isolated from a vaccinated chicken flock infected with the virus in an outbreak with a mortality rate of over 30%. The isolated viruses were characterized as velogenic strains based on the sequencing of the F protein cleavage site. However, in spite of lacking immunity, 20% of the turkeys infected in this experiment died (Table 1), and the morbid birds recovered rather rapidly. Accordingly, the disease was of lower severity in turkeys than in chickens, which is in line with the previous reports (Alexander et al., 1999; Capua et al., 2002; Piacenti et al., 2006). In a similar study performed by Wakamatsu et al. (2006), commercial turkeys infected with velogenic NDV manifested clinical signs 6 days postinoculation with no mortality, whereas chickens developed the clinical signs earlier (i.e., 2 days after inoculation) and had a mortality rate of 100%. The general signs, including diarrhea and central nervous system disorders (as the most prominent signs), such as head tremor, incoordination, and muscle paralysis, observed in the current study are among the typical signs of the disease. These clinical signs have been also reported previously in both natural (Capua et al., 2002; Gowthaman et al., 2013) and experimental (Piacenti et al., 2006; Diel et al., 2012) NDV infections. In our study, turkeys exhibited mild respiratory distress with no overt nasal discharge or conjunctivitis, which is in contrast with the results of the previous studies (e.g., Piacenti et al., 2006; Wakamatsu et al., 2006; Gowthaman et al., 2013). These variations in clinical signs are ordinary, even in infection with velogenic NDV, and are related to the viral strain. In the same vein, in a study carried

out by Piacenti et al. (2006), clinical observations in commercial turkeys, experimentally infected with three velogenic NDV isolates from natural outbreaks, were greatly dependent on the viral strain. These clinical signs varied from only body tremor by one isolate to severe respiratory nervous disorders by the two other isolates. The RT-PCR is one of the reliable laboratory techniques facilitating a rapid diagnosis by detecting virus in clinical specimens (Manual, 2008). In the current study, NDV was first detected 3 days postinoculation before the manifestation of the clinical signs (i.e., 5 days after inoculation). In a similar study, Wakamatsu et al. (2006) isolated NDV from the swab samples of the infected turkeys 2 days after inoculation, while the onset of the disease was 6 days postinoculation. Furthermore, in the current study, tracheal swabs had a higher virus detection rate (8/16), compared to the cloacal swabs (2/16). This is relatively consistent with the results reported by the previous studies (Piacenti et al., 2006; Wakamatsu et al., 2006; Haque et al., 2010), in which NDV was more frequently isolated from oral swabs than from cloacal swabs. The higher detection or isolation rate of NDV from tracheal or oral swabs may be influenced by the tropism of the virus. Nonetheless, some studies have demonstrated the lack of sensitivity in detecting the virus in fecal samples, because they contain more extraneous organic materials that can interfere with RNA recovery and amplification by PCR, suggesting that tracheal or oropharyngeal swabs are often the specimens of choice (Manual, 2008). As the serologic results indicated (Table 2), HI titer in both groups was lower than  $\log_2 4$  before inoculation, and remained unchanged in the control group during the experiment, which can be regarded as being nonspecific (Manual, 2008). However, the drastic seroconversion observed in the infected group 14 and 28 days postinoculation implies the stimulation of immune response by challenging virus. The HI titers of  $2^3$ - $2^6$  (tested with 8 HAU of antigen) were found in a breeder turkey flock suffering from a natural infection with extremely low virulent NDVs with no clinical signs (Graham et al.,

1996). Moreover, in a report performed by Piacenti et al. (2006), seroconversion in commercial turkeys infected with velogenic NDV started 10 days postinoculation. These findings indicate that serum antibody alteration in turkeys following NDV infection is rapid and very similar to that in chickens. In NDV-infected chickens, antibodies are detectable in the blood after 6 days and peaks after 21-28 days (Miller and Koch, 2013).

In conclusion, the results indicated that the velogenic NDV circulating in Iranian chicken flocks has the potential to cause a serious illness in commercial turkeys. Regarding this, it is required to adopt a stringent vaccination program while considering biosecurity to prevent ND outbreaks among turkeys in the future.

#### **Ethics**

We hereby declare all ethical standards have been respected in preparation of the submitted article.

#### **Conflict of Interest**

The authors declare that they have no conflict of interest.

#### **References**

- Ahmadi, E., Pourbakhsh, S.A., Ahmadi, M., Mardani, K., Talebi, A., 2016. Phylogenetic characterization of virulent Newcastle disease viruses isolated during outbreaks in northwestern Iran in 2010. *Arch Virol* 161, 3151-3160.
- Alexander, D.J., Manvell, R.J., Banks, J., Collins, M.S., Parsons, G., Cox, B., *et al.*, 1999. Experimental assessment of the pathogenicity of the Newcastle disease viruses from outbreaks in Great Britain in 1997 for chickens and turkeys, and the protection afforded by vaccination. *Avian Pathol* 28, 501-511.
- Alexander, D.J., Morris, H.T., Pollitt, W.J., Sharpe, C.E., Eckford, R.L., Sainsbury, R.M., *et al.*, 1998. Newcastle disease outbreaks in domestic fowl and turkeys in Great Britain during 1997. *Vet Record* 143, 212-209.
- Boroomand, Z., Jafari, R.A., Mayahi, M., 2016. Molecular characterization and phylogenetic study of the fusion genes of Newcastle disease virus from the recent outbreaks in Ahvaz, Iran. *Virus Dis* 27, 102-105.

- Capua, I., Dalla, P.M., Mutinelli, F., Marangon, S., Terregino, C., 2002. Newcastle disease outbreaks in Italy during 2000. *Vet Record* 150, 565-568.
- Diel, D.G., Susta, L., Cardenas Garcia, S., Killian, M.L., Brown, C.C., Miller, P.J., et al., 2012. Complete genome and clinicopathological characterization of a virulent Newcastle disease virus isolate from South America. *J Clin Microbiol* 50, 378-387.
- Gale, C., Mc, C.M., Sanger, V.L., 1961. Newcastle disease in turkeys. *J Am Vet Med Assoc* 139, 462-465.
- Ghiamirad, M., Pourbakhsh, A., Keyvanfar, H., Momayaz, R., Charkhkar, S., Ashtari, A., 2010. Isolation and characterization of Newcastle disease virus from ostriches in Iran. *Afr J Microbiol Res* 4, 2492-2497.
- Gowthaman, V., Singh, S.D., Barathidasan, R., Ayanur, A., Dhama, K., 2013. Natural Outbreak of Newcastle Disease in Turkeys and Japanese Quails Housed Along With Chicken in a Multi-Species Poultry Farm in Northern India. *Advanc Anim Vet Sci* 1, 17-20.
- Graham, D.A., Connor, T.J., McCullough, S.J., McKillop, E.R., Alexander, D.J., Manvell, R.J., et al., 1996. Isolation and characterisation of an avian paramyxovirus type 1 from turkeys in Northern Ireland. *Vet Record* 138, 416-417
- Haque, M., Hossain, M., Islam, M., Zinnah, M., Khan, M., Islam, M., 2010. Isolation and detection of Newcastle disease virus from field outbreaks in broiler and layer chickens by reverse transcription-polymerase chain reaction. *J Vet Med* 8, 87-92.
- Heckert, R.A., Collins, M.S., Manvell, R.J., Strong, I., Pearson, J.E., Alexander, D.J., 1996. Comparison of Newcastle disease viruses isolated from cormorants in Canada and the USA in 1975, 1990 and 1992. *Can J Vet Res* 60, 50-54.
- Kaleta, E.F., Baldauf, C., 1988. Newcastle disease in free-living and pet birds. *Newcastle disease*, Springer, pp. 197-246.
- Madadgar, O., Karimi, V., Nazaktabar, A., Kazemimanesh, M., Ghafari, M.M., Azimi Dezfouli, S.M., et al., 2013. A study of Newcastle disease virus obtained from exotic caged birds in Tehran between 2009 and 2010. *Avian pathology: journal of the W.V.P.A* 42, 27-31.
- Manual, O.T., 2008. World organisation for animal health manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees). Volume 2, 2008.
- Mehrabanpour, M.J., Khoobyar, S., Rahimian, A., Nazari, M.B., Keshtkar, M.R., 2014. Phylogenetic characterization of the fusion genes of the Newcastle disease viruses isolated in Fars province poultry farms during 2009-2011. *Veterinary research forum: an international quarterly journal* 5, 187-191.
- Miller, P.J., Koch, G., 2013. Newcastle disease. *Diseases of poultry* 13, 89-138.
- Piacenti, A.M., King, D.J., Seal, B.S., Zhang, J., Brown, C.C., 2006. Pathogenesis of Newcastle disease in commercial and specific pathogen-free turkeys experimentally infected with isolates of different virulence. *Veterinary pathology* 43, 168-178.
- Villegas, P., 2008. Titration of biological suspensions, p 217-221. *A laboratory manual for the isolation, identification, and characterization of avian pathogens*. American Association of Avian Pathologists, Kennett Square, PA.
- Wakamatsu, N., King, D.J., Kapczynski, D.R., Seal, B.S., Brown, C.C., 2006. Experimental pathogenesis for chickens, turkeys, and pigeons of exotic Newcastle disease virus from an outbreak in California during 2002-2003. *Veterinary pathology* 43, 925-933.