GMPC-Thesis and Opinions Platform

eISSN:2747-5735

Short Communication

Interference between low pathogenic avian influenza H9N2 and avirulent Newcastle diseases viruses in embryonated Specific Pathogen-Free chicken eggs

Amal Essalah-Bennani[1*], Asma Fagrach[2], Abderrazak El Khantour[3], Ouafaa Fassi Fihri[4], Moncef Bouzouaia[5], and Saâdia Nassik[6]

[1] CEVA Animal Health, Z. I. Oulad Saleh, lot 132-BP 3920180 Bouskoura, Morocco
[2] Department of Microbiology and Contagious Diseases, Agronomy and Veterinary Institute Hassan II, BP 6202, Rabat-Institutes, Rabat, Morocco
[3] Division of Pharmacy and Veterinary Inputs, ONSSA, Avenue Hadj Ahmed Cherkaoui, Agdal, 10090-Rabat, Morocco
[4] Department of Microbiology and Contagious Diseases, Agronomy and Veterinary Institute Hassan II, BP 6202, Rabat-Institutes, Rabat, Morocco
[5] Department of Poultry Diseases and Management, National Veterinary School, Sidi Thabet, Tunisia
[6] Avian Pathology Unit, Department of Pathology and Veterinary Public Health, Agronomy and Veterinary Institute Hassan II, BP 6202, Rabat- Institutes, Rabat, Morocco

Abstract

Co-infection with low pathogenic avian influenza virus (LPAIV) H9N2 and Newcastle disease virus (NDV) has become a worrying concern for the poultry industry. The problem arises when the hidden virus influences the replication of another suspected virus. Subsequently, misdiagnosis of the actual cause may be ended up as a source of contamination for the other healthy flocks by the spread of the covered-up virus. In this preliminary study, we determined the potential impact of concurrent infection with H9N2 and avirulent NDV (Lasota) on the virus replication in Specific Pathogen-Free embryonated chicken egg (SPF-ECE) model. Assessment of the potential interference phenomena was carried out based on embryonic lesions, mortalities, and virus replication using real-time PCR. Our results showed that H9N2 interferes with LaSota growth, regardless of which infection occurred first. Our obtained preliminary results are a call for scientists to study the interference between LPAIV H9N2 and NDV both *in-vivo* and *in-vitro* in more detail.

Keywords: LPAI H9N2 virus, avNDV LaSota virus, SPF-ECEs, Interference

Citation: Essalah-Bennani, A., Fagrach, A., El Khantour, A., Fihri, O. F., Bouzouaia, M., and Nassik, S. 2021. Interference between low pathogenic avian influenza H9N2 and avirulent Newcastle diseases viruses in embryonated Specific Pathogen-Free chicken eggs. GMPC TOP (2): 7-10. https://doi.org/10.51585/gtop.2021.2.0007

Article History:
Received: 22-May-2021
Accepted: 09-June-2021

***Corresponding author:**
Amal Essalah-Bennani
E-mail:amal.essalah-bennani@ceva.com

Introduction

Avian influenza virus (AIV) and Newcastle disease virus (NDV) are two major viral diseases that cause serious losses to the poultry industry (Capua and Alexander, 2009) due to increased mortality, impaired growth, and reduced egg and meat productions. Both AIV and NDV are RNA avian viral diseases caused by type A orthomyxoviruses and type 1 avian paramyxoviruses, respectively. Both viruses affect chicken's respiratory systems and result in a reduction in body weight gain and a life-long decrease in bird's performance (Ge et al., 2012). Although many vaccination programs to control the two viruses were adopted, NDV continues to cause serious problems and high economic losses in the Moroccan poultry industry. The outbreak of AIV H9N2 in Morocco in 2016 has complicated this situation (El Houadfi et al., 2016).

Co-infection of chickens with NDV and AIV H9N2 has become a widespread condition among chicken flocks (Essalah-Bennani et al., 2020). Thus, it becomes important to study the effect of LPAI H9N2 virus in SPF eggs and evaluate the interactions between this virus and avNDV LaSota strain during dual infection. This will be a point toward assessing the effectiveness of the control measures applied such as vaccination regarding the importance of both viruses and their impact on the poultry industry.

The "Interference phenomenon" or "cell blockade" is produced by the interference of one strain or species of a virus with the pathogenic activi-

Table 1: Experimental design for testing the potential interference between H9N2 and avNDV Lasota in embryonated chicken eggs.

Group	Number of eggs	Inoculation[1]	Mortalities (%)	Embryonic lesions
G1	5	200l H9N2	80	Hemorrhagic head, Congestion, Dwarfism
G2	5	200l avNDV Lasota	40	No lesions
G3	8	Sequentially infected with 200l avNDV Lasota followed by 200l H9N2 after 24h	100	Congestion
G4	8	Sequentially infected with 200l H9N2 followed by 200l avNDV Lasota after 24h	100	Congestion
G5	8	Simultaneously infected with 200l avNDV Lasota and 200l H9N2	87.5	Congestion, Dwarfism
G6	5	Negative control	0	No lesions

[1] The dose was 10^6 EID_{50} in 0.2 ml, H9N2= A/chicken/Morocco/01/2016, acc. no. KU947112, avNDV= LaSota strain (Ceva PHYLAXIA, Budapest, HUNGARY).

ties of another (Bang, 1949). This study aimed to evaluate the interaction between LPAI H9N2 (strain A/chicken/Morocco/SF1/2016) and avNDV LaSota during simultaneous and sequential infections *in-ovo* compared to the results of single inoculation using real-time RT-PCR (rRT-PCR).

Study Methods

In this study, the potential interference between H9N2 Influenza A virus (A/chicken/Morocco/01/2016, acc. no. KU947112) and live NDV LaSota vaccine strain designated as avNDV LaSota (Ceva PHYLAXIA, Budapest, HUNGARY) was tested in 10-day-old embryonated SPF-ECE (Division of Pharmacy and Veterinary Inputs, ONSSA, Morocco). The viruses were inoculated alone, sequentially, or simultaneously at a dose of 10^6 EID_{50} in 0.2 ml into the allantoic cavity of SPF-ECE (Table 1) according to the OIE guidelines (OIE, 2015). After viral inoculations, SPF-ECEs were incubated at 37°C and candled daily for 4 successive days. At each candling, allantoic fluids were harvested from eggs with dead embryos. However, the allantoic fluid of survived SPF-ECEs after the AIV H9N2 and avNDV LaSota inoculations was harvested after 96h of incubation. Bacteria-free allantoic fluid was aliquoted and stored at 80°C until tested.

The virus replication was assessed based on embryonic lesions, mortalities, and virus replication using real-time PCR (rRT-PCR). For rRT-PCR, RNA was extracted using ID Gene® Spin Universal Extraction Kit. To detect H9N2 and ND viruses, PCR reactions were performed on Agilent cycler, using ID Gene® Influenza A Duplex and ID Gene® Newcastle Disease Duplex (IDvet, https://www.id-vet.com) according to the manufacturer's instructions. PCR results and cycle quantification value (Cq) were recorded on the provided software. For viral growth quantification, positive Cq values for each analyzed sample were determined using the corresponding standard curves derived from AIV H9N2 and avNDV LaSota standard plasmid template. Consequently, the number of viral copies present in each sample was computed.

Data analyses were performed using IBM SPSS statistics version 19 (IBM, Chicago, US). Group's means were compared with a two-tailed Student t-test. The differences were considered statistically significant at $p<0.05$.

Results and Discussion

Results showed that both mixed and superinfected groups had 100% embryo mortality (Table 1). Embryos in G1 showed hemorrhages at 96h post-inoculation, while Embryos in G4 showed congestion and dwarfism of embryos at 96h post-inoculation (Figure 1).

The quantitative rRT-PCR quantification of AIV H9N2 and avNDV when pre-, post-, and simultaneously inoculated to SPF-ECEs showed replication interference by AIV H9N2 strain on avNDV LaSota strain (Figure 2). The LaSota strain yield in simultaneously inoculated SPF-ECEs with AIV H9N2 was significantly lower ($p<0.05$) than those from singly infected eggs. When H9N2 was inoculated 24h earlier or later than avNDV LaSota strain (group 3 and 4, respectively), there was a significant inhibition of the growth of avNDV. In contrast, avNDV LaSota strain failed to inhibit the growth of AIV H9N2. Though the SPF-ECEs inoculated simultaneously with avNDV LaSota strain and AIV H9N2, and those infected first with avNDV LaSota strain and reinoculated 24h later with AIV H9N2 showed lower AIV H9N2 virus yield; however, the interference degree of avNDV was non-significant ($p>0.05$).

The results of this experiment clearly demonstrate that AIV H9N2 partially inhibited the growth of avNDV. The study also indicates that avNDV LaSota can't affect the replication of AIV H9N2 even when the avNDV LaSota was given a replication advantage by being inoculated 24h before AIV H9N2. In general, these findings are in accordance with those reported in previous studies reporting that AIV interferes with

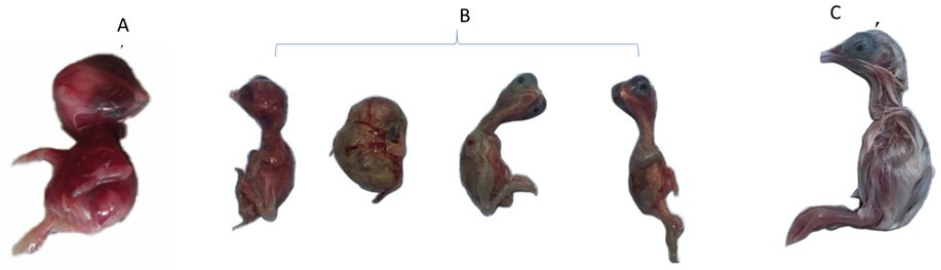

Figure 1: (**A**) Embryo inoculated with H9N2 (200µl containing 106 EID50) showing hemorrhages at 96h post-inoculation. (**B**) Embryos sequentially infected with 200µl H9N2 followed by 200µl avNDV Lasota after 24h and 96h post-inoculation showing congestion and dwarfism of embryos. (**C**) Negative control.

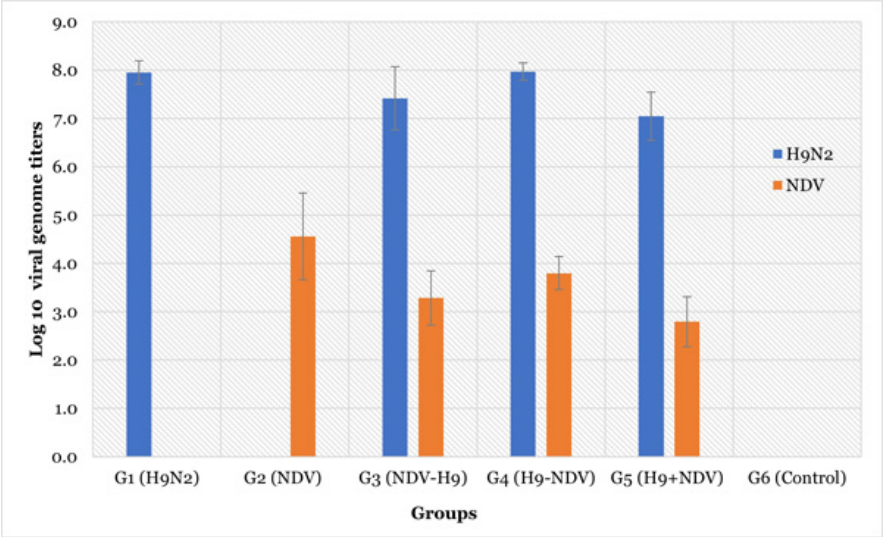

Figure 2: Quantitative rRT-PCR of AIV H9N2 and avNDV LaSota strains in SPF-ECEs in single infection (G1 and G2), simultaneous infection (G5), and the 24h interval sequential infections (G3 and G4).

the NDV replication (Bang, 1949; Shortridge and King, 1983; Liu et al., 2003; Ge et al., 2012). The interference between the two viruses is mainly attributed to the competition for the same viral receptors containing sialic acid (Costa-Hurtado et al., 2014), cell attachment blockage by the pre-inoculated virus (Ziegler et al., 1944; Bratt and Rubin, 1968), or due to interferon produced by the first inoculated virus (Sonnenfeld and Merigan, 1979).

In the current study, although avNDV LaSota was pre-inoculated, its growth was inhibited by AIV H9N2. This can be explained by the fact that NDV replication and interferon induction are closely related to the virulence of the virus (Durand, 1961; Dortmans et al., 2010). Additionally, Ge et al. (2012), reported a remarkable difference between the avirulent and virulent strains of NDV in terms of interfering with AIV H5N1 virus growth in ECE where virulent NDV was more potent than avirulent LaSota in resisting interference by AIV. Moreover, 24h interval in the simultaneously inoculated group is probably not sufficient for avNDV LaSota to induce inhibiting interferon levels to suppress the replication of AIV H9N2 (Fazekas De St Groth et al., 1952). The dwarfism observed in embryos inoculated by AIV H9N2 alone or in embryos simultaneously infected with avNDV LaSota could be attributed to the extensive viral replication and/or cytokine induction that severely affected the embryo development and growth (Sharma et al., 2020).

Conclusions

The current study opened an active discussion around the potential interference of the two viruses during a simultaneous application of vaccines against ND and H9N2 diseases. Our study is a call to further investigating this potential interference phenomenon and its impact on vaccines efficacy under field conditions.

Article Information

Funding. This research received no funds.

Conflict of Interest. The authors declare no conflict of interest.

References

Bang, F.B., 1949. Cell blockade in Newcastle disease of chickens and chicken embryos. The Journal of Experimental Medicine 89, 141–154. 10.1084/jem.89.2.141.

Bratt, M.A., Rubin, H., 1968. Specific interference among strains of Newcastle disease virus. 3. Mechanisms of interference. Virology 35, 395–407. 10.1016/0042-6822(68)90218-3.

Capua, I., Alexander, D.J. (Eds.), 2009. Avian influenza and Newcastle disease. Springer Milan, Milano. 10.1007/978-88-470-0826-7.

Costa-Hurtado, M., Afonso, C.L., Miller, P.J., Spackman, E., Kapczynski, D.R., Swayne, D.E., Shepherd, E., Smith, D., Zsak, A., Pantin-Jackwood, M., 2014. Virus interference between H7N2 low pathogenic avian influenza virus and lentogenic Newcastle disease virus in experimental co-infections in chickens and turkeys. Veterinary Research 45, 1. 10.1186/1297-9716-45-1.

Dortmans, J.C.F.M., Rottier, P.J.M., Koch, G., Peeters, B.P.H., 2010. The viral replication complex is associated with the virulence of Newcastle disease virus. Journal of Virology 84, 10113–10120. 10.1128/JVI.00097-10.

Durand, D.P., 1961. Interference between viable strains of Newcastle disease virus. Journal of Bacteriology 82, 979–983. 10.1128/jb.82.6.979-983.1961.

El Houadfi, M., Fellahi, S., Nassik, S., Guérin, J.L., Ducatez, M.F., 2016. First outbreaks and phylogenetic analyses of avian influenza H9N2 viruses isolated from poultry flocks in Morocco. Virology Journal 13, 140. 10.1186/s12985-016-0596-1.

Essalah-Bennani, A., Nassik, S., Fagrach, A., Bouzouaia, M., Fassi Fihri, O., 2020. Characterization and phylogenetic analysis of the hemagglutinin gene in H9 influenza viruses from chickens in Morocco from 2017 to 2019. Avian Diseases 64. 10.1637/aviandiseases-D-20-00009.

Fazekas De St Groth, S., Isaacs, A., Edney, M., 1952. Multiplication of influenza virus under conditions of interference. Nature 170, 573. 10.1038/170573a0.

Ge, S., Zheng, D., Zhao, Y., Liu, H., Liu, W., Sun, Q., Li, J., Yu, S., Zuo, Y., Han, X., Li, L., Lv, Y., Wang, Y., Liu, X., Wang, Z., 2012. Evaluating viral interference between influenza virus and Newcastle disease virus using real-time reverse transcription-polymerase chain reaction in chicken eggs. Virology Journal 9, 128. 10.1186/1743-422X-9-128.

Liu, W., Ding, W., Kong, J., Wang, H., Hu, S., He, H., Zhang, R., Liu, X., 2003. The interference in virus propagation in chicken embryo and in the HI test between the Newcastle disease virus and the H9 subtype influenza virus. Chinese Poultry Science 7.

OIE, 2015. Infection with Avian influenza viruses. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.{04_AI}.pdf.

Sharma, B.K., Kakker, N.K., Bhadouriya, S., Chhabra, R., 2020. Effect of TLR agonist on infections bronchitis virus replication and cytokine expression in embryonated chicken eggs. Molecular Immunology 120, 52–60. 10.1016/j.molimm.2020.02.001.

Shortridge, K.F., King, A.P., 1983. Cocultivation of avian orthomyxoviruses and paramyxoviruses in embryonated eggs: implications for surveillance studies. Applied and Environmental Microbiology 45, 463–467. 10.1128/aem.45.2.463-467.1983.

Sonnenfeld, G., Merigan, T., 1979. The role of interferon in viral infections. Springer Seminars in Immunopathology 2. 10.1007/BF00198722.

Ziegler, J.E., Lavin, G.I., Horsfall, F.L., 1944. Interference between the influenza viruses : II. the effect of virus rendered non-infective by ultraviolet radiation upon the multiplication of influenza viruses in the chick embryo. The Journal of Experimental Medicine 79, 379–400. 10.1084/jem.79.4.379.