Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine,
Zagazig University, 44511, Egypt.
Volume 50, Number 4, p. 276-286, December 2022
DOI: 10.21608/zvjz.2022.179646.1196

RESEARCH ARTICLE

Rapid Reliable EID_{50} Determination for live Newcastle Disease Viruses and Vaccines

Amal A M Eid [1 *], Abd El-Shakour N. Ismail [1] , Hagar F. Gouda 2 and Reham M ElBakrey 1
[1]Avian and Rabbit Medicine Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt
[2]Animal Wealth Development Department (Biostatistics subdivision), Faculty of veterinary medicine, Zagazig University, 44511, Egypt
*Corresponding author: amalaeidvet@gmail.com

Article History: Received: 11/12/2022 Received in revised form: 22/12/2022 Accepted: 26/12/2022

Abstract

Newcastle disease (ND) was first recognized more than nine decades ago and continues to be a problem for poultry producers besides being enzootic in many countries including Egypt. Diagnostic and ND virus/vaccine titration are core objects in virus evaluation. A rapid assay based on micro plate hemagglutination (HA) activity was applied to investigate its reliability as alternative method for virus titration. A comparative determination of EID_{50} /0.1ml was carried out via chicken embryo (CE) inoculation for 35 Newcastle disease virus (NDV) strains. They are previously identified by pathogenicity indices and revealed variable virulence (lentogenic-mesogenic and velogenic). The data for both methods were analyzed using SPSS version 25. A Wilcoxon signed-rank test showed that HA titer method did not elicit a statistically significant change in median of reading Virus titer of samples with the median of standard embryonated chicken egg (ECE) method ($Z = -0.197$, $p = 0.844$). Spearman's correlation coefficient ($r= 0.42$, $p =0.01$) showed a noteworthy moderate correlation between two methods. Heat map showed the differences between each pair of methods and the relationship between them. Bland-Altman plot revealed difference which fit normality distribution $W=0.96$, $p=0.24$. Accordingly, the use of HA activity assay for NDV/vaccine titration is a rapid easy and reliable, especially when needed for primary evaluation.

Key words: NDV, ECE, HAU, EID_{50}, live viral vaccines

Introduction

Newcastle disease virus (NDV), also known as avian *orthoavulavirus 1*, is the furthermost lethal viral pathogens in many avian species [1]. The NDV often causes severe disease in young and susceptible birds, depending on its virulence as well as bird age and its immune status [2]. Since the first identification of Egyptian NDV in 1948, the virus outbreaks have been recounted throughout the country, posing a significant economic burden [3].

NDV belongs to the family *Paramyxoviridae*, Subfamily *Avulavirinae*, and the lately is renamed Avian *Orthoavulavirus-1* (AOaV-1) [4]. It is an enveloped single-stranded, negative sense, non-segmented RNA virus. The genome structured as; nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase (HN) and large polymerase protein (L). Furthermore, V and W nonstructural proteins can be detected in the NDV infected cells which coded through P

protein mRNA editing [5]. The F and HN are the integral glycoproteins of NDV. The F protein is one of the main defensive antigenic targets, facilitates virus entry, inter-cellular spreading, and is also a chief determinant for virulence [6-8]. Even though, the Fusion protein has a chief role in the antigenicity and pathogenicity of NDV, the HN protein is also included in the virulence and immunostimulatory impact of virus which can bind the red blood cells (RBCs)' surface receptors creating hemagglutination activity [9,10].

According to its pathogenicity in chickens, NDV is categorized into velogenic, mesogenic, and lentogenic pathotypes [11,12]. Virulent NDV (VNDV) isolates continue to be an ongoing threat to the poultry industry and are notifiable to the World Organization for Animal Health (OIE) because of threat of spread between countries [12]. Despite regular vaccination programs, Newcastle disease (ND) outbreaks are to be reported in vaccinated flocks in many countries including Egypt [13-15].

Newcastle disease virus vaccines are widely available and could protect from virulent NDVs [16]. Both live attenuated and inactivated vaccines are applied, however broiler chicken sector consume a huge quantity of live attenuated vaccines which have the potential advantage of inducing a strong humoral immune response with an early immunity onset of both cellular and mucosal immunity components [17]. Natural live Lentogenic and mesogenic viruses are usually used for the vaccine production either with or without modification. The type of vaccine applied in poultry is depending on the disease circumstances and national necessities. Conventional marketable live NDV vaccines are two clusters: the first is Lentogenic including Hitchner-B1, V4, LaSota, I2 and the second is mesogenic as Komarov, Mukteswar and Roakin [12]. Low-virulence strains such as Hitchner B1 and LaSota used in vaccination program can provide sufficient protection against high-virulence strains, since NDV isolates are thought of as one serotype [18].

There are different methods of live NDV vaccines which may be administered in the drinking water, instilled intranasally/ ocularly or supplied as a coarse spray (aerosol). There are factors responsible for these breaks; Errors in vaccine production such as virus particle contents, Lyophilization, stabilizers, and storage are included [19,20] as well as in such factors among vaccinated birds. Moreover, the administered NDV vaccine particles (dose) for each bird play important roles in the induction of optimum immune response and consequent successful protection [21,22]. The Embryo infectious dose 50 (EID50) calculations is very important to justify the virus particles contents in any vial and is considered the numerical method for virus quantity determination [12,23].

The objective of the existing study was to investigate an attempt to facilitate EID50 evaluation and preliminary titration stemming from the HA titer of studied live field ND viruses and vaccines as a rapid initial and/or alternative method. A comparison with the traditional embryo inoculation is carried out to validate the results.

Material and methods

Ethical statement

This research work was governed by rules set by the Animal Welfare and Research Ethics Committee, Faculty of Veterinary Medicine, Zagazig University,

Egypt. All the methods relating to handling of virus were done in agreement with the applicable guidelines and rules. The biological wastes were disposed as per applicable guidelines.

Viruses and vaccines

A total of thirty-five NDVs were investigated in the current study. Thirty-three field NDV isolates were previously isolated from different chicken farms at Sharkia Governorate and identified in Avian and Rabbit Medicine Department, Faculty of Veterinary Medicine, Zagazig University. About 18 out of 33 NDV isolates were identified as velogenic strains using biological indices; intracerebral pathogenicity index (ICPI) and intravenous pathogenicity index (IVPI) which ranged from 2-3.7 and 1.5-1.8, respectively. The other eleven NDV strains were classified as mesogenic strains, in which ICPI ranged from 0.4-1.84 and IVPI was 0.7-0.81. While four NDV strains were lentogenic and the score of ICPI was zero.

Two live NDV vaccinal strains were submitted and included ND CEVA® NEW L (Live Freeze-dried vaccine, LaSota strain) and MEVACTM ND HB1 (lympholized live attenuated Newcastle disease vaccine).

Chicken embryo inoculation

Embryonated chicken eggs (ECEs) were purchased from commercial breeding flocks at Heria, Sharkia Governorate and were used for virus preparation, titration, and calculation of mean death time. For propagation, about 0.2ml of allantoic fluid (AF) of 33 field NDV isolates were inoculated into the allantoic cavity of incubated ECEs at days 9-11 of age. Inoculated eggs were incubated at 37°C with moisture source for 5 days; dead embryos at 24 hours post-inoculation (PI) were eliminated. After 5 days PI, all live and dead embryos were examined and their allantoic fluids were collected and checked by rapid hemagglutination test with 10% chicken RBCs [12].

Haemagglutination (HA) test

The titer of virus was determined for all 35 NDVs according to the protocol of OIE [12] using a plastic 96-well microtiter plate (U-bottomed wells). Twofold dilutions of the viruses were made across the plate with initial dilution 1/10 and chicken RBCs were added in concentration 1% to each well. Afterwards they were incubated for 40 minutes at room temperature, the HA unit (HAU) was determined by the highest dilution giving complete HA.

Virus titration and calculation of Embryo Infectious Dose50 (EID_{50})

Tenfold serial dilution were prepared from the fresh AF of 33 field NDV isolates and suspension of two live NDV vaccinal strains using sterile phosphate buffered saline with antibiotics (Penstrept, Lonza). From each dilution a volume of 100µL was inoculated through allantoic sac route in 5 ECEs at 9-11 days old. After incubation of inoculated ECEs, the allantoic fluid of eggs showing embryo mortality and not showing mortality up to 5 days PI were harvested and tested by both the micro and rapid HA assay. Calculation EID50 was performed by using Reed and Muench method [23].

Formula of virus particle calculation using HAU

The formula for calculation or correlation the HA titer to EID50 by conversion one HA unit from Log base 2 into Log base 10, then add the value of

virus particle of the HA unit according to Anon [24] and Tolba & Eskarous [25].

Correlation the HA titer to EID_{50}= (value of HAU with Log 10) + (value of virus particle of HAU)

Subsequently, this formula was used for all NDVs, and the results of EID50 using ECEs were statistically compared with those using the formula.

Statistical analysis

The statistical analysis of the obtained data was carried out using SPSS version 25 (Armonk, NY: IBM Corp), Graph Pad prism 8.0.2 (GraphPad Software, Inc), and MedCalc version 15.6 - © 1993-2015 MedCalc Software bvba. The data screened for normality by Shapiro-Wilk (W) test. Wilcoxon matched pairs signed rank test [26] was used to assess differences between the two methods in detecting EID50 and assessing effectiveness of pairing between two methods. Spearman's correlation was performed to test relationship between two methods [27]. The agreement between ECE and HA and the limits of agreements were determined by Bland-Altman analysis and plot [28] and defined as the mean difference ±1.96 standard deviation (SD) of differences. The level of statistical significance was set < 0.05.

Results

The hemagglutination test and pathotyping

The hemagglutinating titer Log base 2 with initial dilution of 1/10 of different field NDVs was ranged from 640-10240 (7- 11 Log2), which the vaccine strains were 5120 (10 log2) for the LaSota vaccine and 10240 (11 log2) for the Hitchner B1. The pathotyping using biological assay (ICPI and IVPI) was classified the viral strains into velogenic (18/33; 54.5%), mesogenic (12/33; 36.4%) or lentogenic (3/33; 9.1%) as shown in Table (1). For example the isolate 1, EID50 using HA assay was 107.31 which was equivalent to 107.80 using ECE method.

Table 1: Hemagglutinating titer Log 2 of different NDV pathotype

Isolate No	*HA titer	Patho-typing	Isolate No	*HA titer	Patho-typing	Isolate No	*HA titer	Patho-typing	Isolate No	*HA titer	Patho-typing
1	640	M	**10**	10240	M	**19**	10240	V	**28**	640	M
2	1280	L	**11**	1280	M	**20**	10240	V	**29(LS)**	5120	L
3	640	V	**12**	10240	M	**21**	10240	L	**30(B1)**	10240	L
4	2560	M	**13**	10240	V	**22**	10240	V	**31**	1280	M
5	1280	V	**14**	1280	M	**23**	1280	M	**32**	1280	M
6	640	V	**15**	1280	L	**24**	640	M	**33**	5120	V
7	10240	V	**16**	10240	V	**25**	2560	V	**34**	2560	V
8	1280	V	**17**	10240	V	**26**	1280	V	**35**	80	V
9	10240	V	**18**	640	V	**27**	10240	M			

***** Serial two fold with initial tenfold dilution was carried out for each virus sample.
HA: Hemagglutination, **V:** Velogenic, **M:** Mesogenic, **L:** Lentogenic, **LS:** LaSota, **B1:** Hitchner B1

The calculation of EID50 based on HAU quantity

The results of HA titer Log base 10, and EID50 of the tested field NDV isolates and vaccinal strains were recorded which ranged from 107.25 to 109.40. The calculation of EID50 based on HAU quantity was carried out according to Formula of virus particle calculation using HAU. Also, the comparison between the two methods is shown in Figure (1).

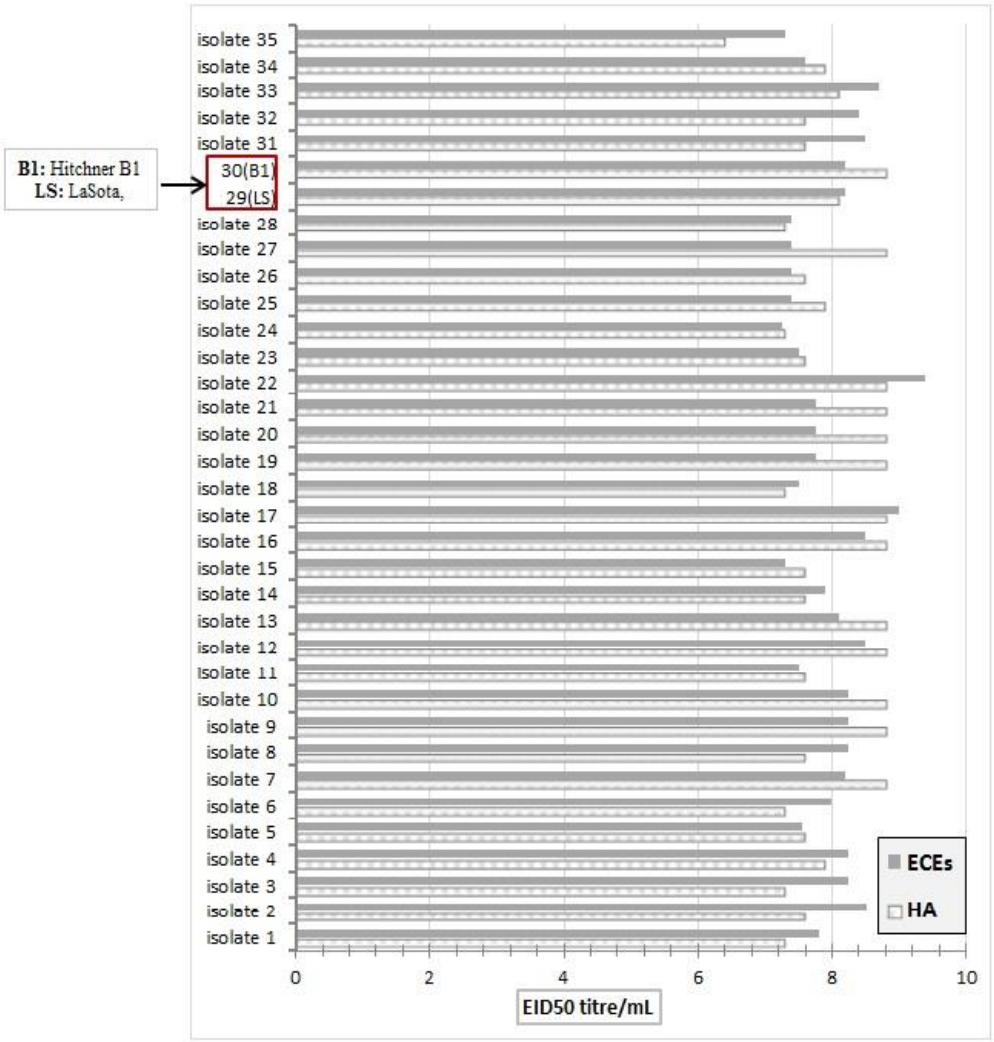

Figure 1: Comparative estimation of EID_{50}/0.1mL of different NDVs based on HA activity titer Log base 10 and ECEs inoculation findings [23]

HAU measurement and NDVs EID50 titer correlation using A Wilcoxon signed-rank test and Spearman's correlation coefficient

The correlation between the HAU measurement and NDVs EID50 titer was indicated that HA titer did not elicit a statistically significant change in median

of reading titer of samples with the median of standard ECE method (Z = -0.197, p = 0.844) using A Wilcoxon signed-rank test. Additionally, Spearman's correlation coefficient (r= 0.42, p=0.01) showed a significant moderate correlation between two methods. Heat map (Figure 2) showed the differences between each pair of methods and the relationship between them. To assess the agreement between two methods Bland-Altman plot was used. The difference was fit normality distribution W=0.96, p=0.24.

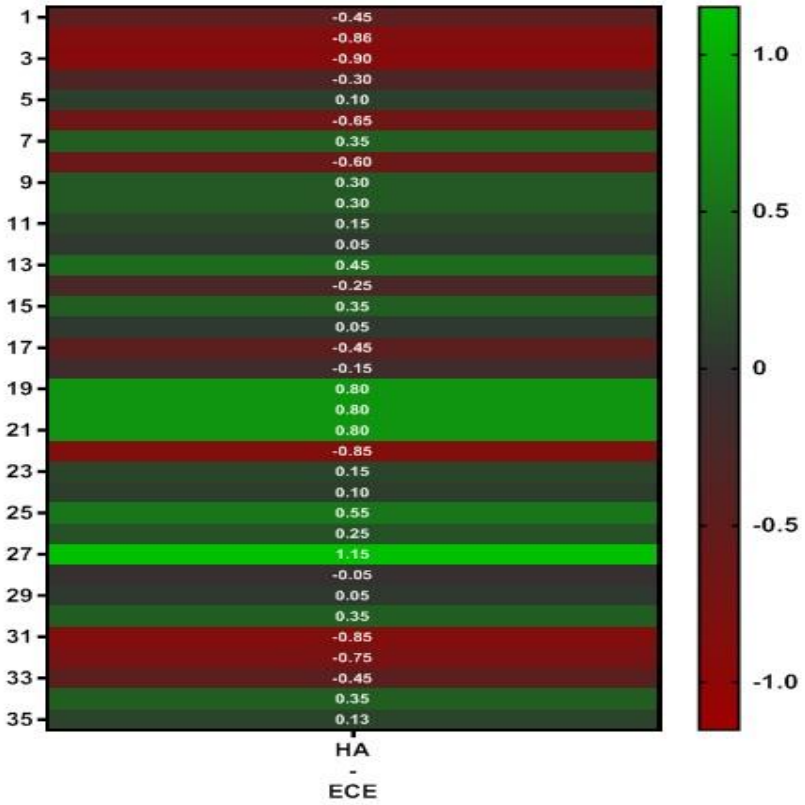

Figure 2: Correlation heat-map using the 35 selected sample codes for both ECE and HA methods. The degree of relationship of the difference of row of data that represents two points of the two methods assessed based on the color scale on the right. The closer the color to +1 or -1 the higher the correlation is, and the closer to 0 the lower the correlation.

Difference between the HAU measurement and NDVs EID50 titer using Bland-Altman analysis and plot

The scatter diagram of difference between the two methods as shown in Figure 3 detected that the bias (mean difference) is 0.04. The agreement limits are from -1.01 to 1.09. These limits of agreement do not surpass the maximum allowable difference between the two methods. Thus, the two approaches are reflected to be in covenant and may be applied interchangeably. The graph also enables us to detect a moderate positive trend of differences.

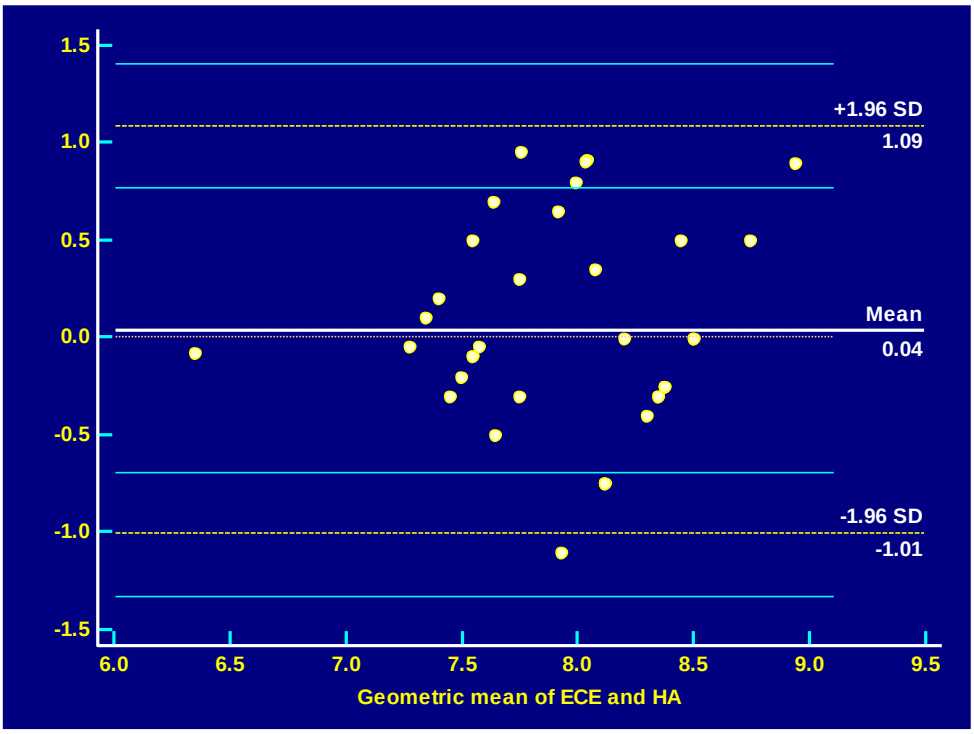

Figure 3: Bland-Altman plot of differences between ECE method and HA method, expressed as discrepancy of the values on the *Y*-axis [(ECE – HA)], *vs*. the geometric mean of the two measurements on *X*-axis. Shaded areas present confidence interval limits for mean and agreement limits.

Discussion

Newcastle Disease is still being one of the greatest problems threatening the poultry industry. Next its reporting in 1926, Newcastle disease (ND) is stared as being enzootic in numerous countries including Egypt [12,14,15]. Prophylactic vaccination is practiced in all but still; there are continuous devastating outbreaks that necessitate enhancement in both diagnostic and monitoring methods particularly those related to virus titration and virus particle contents either in infected suspensions or vaccine vials for time saving and effort of experimental animal use. Thus, in order to facilitate EID50 evaluation and preliminary titration based on the HA titer of examined live field ND virus and vaccinations, this study aimed to further explore this approach as a quick initial and/or an alternative method. Consequently, we used the inoculation in embryonated chicken eggs as a prevailing method for calculation of virus titration. Which EID50 of the tested field NDV isolates and vaccinal strains was ranged from 107.25 to 109.40. Also, the haemagglutination test was used in this investigation as a method used to quantify amounts of virus particles. NDVs were quantified by the HA test and ranged from 7- 11 Log2 of virus-RBC union displayed as a positive result and was a typical haemagglutination mesh pattern of chicken red blood cells.

Virus isolation in ECEs is the reference and a superior method [29], but then again is laborious and time-consuming, utilized chiefly for the

preliminary diagnosis among any outbreak and to get viral isolates for additional laboratory examination. However, the haemagglutination (HA) test is the most common indirect method to quantify amounts of virus particles [30], The HA activity of NDV in bacterial free harvested allantoic fluids from infected chicken eggs or vaccine vials is a gift that must be utilized to pardon the costs, time and effort in a preliminary investigation. So, according to Anon [24], and Tolba and Eskarous [25] that previously estimated the virus particle load in HA unit, we equipped a formula for conversion or/ Correlation the HA titer to EID50. The current formula could be used to help in rapid evaluation and monitoring the virus particles in vaccine patches, and any infectious suspensions. Where, there are several problems facing poultry farms related to vaccines.

In spite of the regular use of NDV vaccine in poultry farms for the ND control, repeated infections have been reported in vaccinated flocks. Vaccine letdown may be accountable for these outbreaks [31]. Consequently, regular monitoring of vaccine patches along the commercial chain to be sure from the viral contents in each patch is within the protective mass (potency). Richard et al. [32] evaluated five commonly used products of LaSota ND live vaccines using HA test and EID50. HA test revealed viral titers of 7- 9 log2 for vaccines with egg infectivity dose (EID50) of 107.00 to 108.49. Moreover, Liljebjelke et al. [33] concluded that the linearity of correlation between the hemagglutination (HA) assay measurement and the 50% embryo infectious dose titer of NDV Hitchner B_1 vaccine virus was determined. Whereas, the titration of virus particles in the vaccinal strains (LaSota and HB1) used in this study was 10 and 11 log 2, respectively by using the HA test and 108.20 EID50 for both strains using ECEs. By converting log base 2 of the HA test into Log base 10 the outcome of calculation equivalent to 108.11 and 108.81, respectively. By digital calibration, the results of converting the HA titer into log base 10 were very close to that calculated by inoculation in embryonated chickens (EID50), and the same for the results of 33 NDV isolates.

For more certainty of the similarity and convergence of the two methods in calibrating the concentration of NDVs, we used statistical analysis. The results came in consistence with the above-mentioned findings, as the correlation heat map showed a higher correlation between the ECEs and HA test results. Furthermore, the scattered diagram of the difference between the two tested methods revealed that the mean difference is 0.04 and this is an acceptable result in comparison with the agreement limits (-1.01 to 1.09). So, these limits of the agreement did not exceed the maximum allowed difference between the two methods.

Conclusion

From the obtained results, it could be concluded that NDV particles number in any preparation may be approximated on the bases of HA activity and could be used interchangeably with embryo inoculation for virus titration, being cheap, not time-consuming, and easy to carry out. The two methods are considered to be in agreement with particular utilization as a rapid spotlight HA method. As it can be relied upon with the application of the mentioned formula as a way to reach a quick conclusion about the content of virus particles, which can be utilized in a quick judgment to

reduce the corruption of the content inside the vaccine vials in the different stages of their circulation among market and also to reduce the number of ECEs up to 50% that used when needed.

Conflict of Interest

There is no conflict of interest to declare.

References

[1] Dortmans, J. C.; Koch, G.; Rottier, P. J. and Peeters, B. P. (2011): Virulence of Newcastle disease virus: what is known so far?. Veterinary research, 42(1): 1-11.

[2] Miller, P. J. and Koch, G. (2013): Newcastle disease. Diseases of poultry, 13: 89-138.

[3] Daubney, R. and Mansy, W. (1948): The occurrence of Newcastle disease in Egypt. Journal of Comparative Pathology and Therapeutics, 58: 189-200.

[4] Dimitrov, K.M.; Abolnik, C.; Afonso, C.L.; Albina, E., Bahl, J.; Berg, M.; Briand, F.X.; Brown, I.H.; Choi, K. S.; Chvala, I. and Diel, D.G. (2019): Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. Infection, Genetics and Evolution, 74: 103917.

[5] Czeglédi, A.; Ujvári, D.; Somogyi, E.; Wehmann, E.; Werner, O. and Lomniczi, B. (2006): Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications. Virus research, 120(1-2): 36-48.

[6] Fields, B.N.; Knipe, D.M. and Howley, P.M. (2013): Paramyxoviridae in Fields virology/editors-in-chief, David M. Knipe, Peter M. Howley, 957–95.

[7] Waltzek, T.B.; Kelley, G.O.; Alfaro, M.E.; Kurobe, T.; Davison, A.J. and Hedrick, R.P. (2009): Phylogenetic relationships in the family Alloherpesviridae. Diseases of aquatic organisms, 84(3): 179-194.

[8] Mao, Q.; Ma, S.; Schrickel, P.L.; Zhao, P.; Wang, J.; Zhang, Y.; Li, S. and Wang, C. (2022): Review detection of Newcastle disease virus. Frontiers in Veterinary Science, 9.

[9] Huang, Z.; Panda, A.; Elankumaran, S.; Govindarajan, D.; Rockemann, D.D. and Samal, S.K. (2004): The hemagglutinin-neuraminidase protein of Newcastle disease virus determines tropism and virulence. Journal of virology, 78(8): 4176-4184.

[10] de Leeuw, O.S.; Koch, G.; Hartog, L.; Ravenshorst, N. and Peeters, B.P. (2005): Virulence of Newcastle disease virus is determined by the cleavage site of the fusion protein and by both the stem region and globular head of the haemagglutinin–neuraminidase protein. Journal of General Virology, 86(6): 1759-1769.

[11] Alexander, D.J. (2003): Newcastle disease. In Saif, Y.M.; Barnes, H.J.; Lisson, J.R.; Fadly, A.M.; McDougald, L.R. and Swayne, D.E. editors. Disease of Poultry, Iowa State University Press. 64-87.

[12] OIE Terrestrial Manual (2021): Newcastle Diseases (infection with Newcastle disease virus) (Chapter 3.3.14). In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. [Online]. [Accessed September 20, 2022]. Available:http://www.oie.int/standard-setting/ter-restrial-manual/access-online/.

[13] Ali, A.; Abdallah, F.; Farag, G.K. and Sameh, K. (2022): A Mini-Review on Newcastle Disease Virus in Egypt, With Particular References to Common Vaccines and Their Development. Zagazig Veterinary Journal, 50(1): 19-36.

[14] Megahed, M.M.; Eid, A.A.; Mohamed, W. and Hassanin, O. (2018): Genetic characterization of Egyptian Newcastle disease virus strains isolated from flocks vaccinated against Newcastle disease virus, 2014-2015. Slov Vet Res., 55(20): 17-29.

[15] Mansour, S.M.; ElBakrey, R.M.; Mohamed, F.F.; Hamouda, E.E.; Abdallah, M.S.; Elbestawy, A.R.; Ismail, M.M.; Abdien, H.M. and Eid, A.A. (2021): Avian Paramyxovirus Type 1 in Egypt: Epidemiology, Evolutionary Perspective, and Vaccine Approach. Frontiers in Veterinary Science, 8.

[16] Ferreira, H.L.; Reilley, A.M.; Goldenberg, D.; Ortiz, I.R.; Gallardo, R.A. and Suarez, D.L. (2020): Protection conferred by commercial NDV live attenuated and double recombinant HVT vaccines against virulent California 2018 Newcastle disease virus (NDV) in chickens. Vaccine, 38(34): 5507-5515.

[17] Grimes, S.E. (2002): Newcastle disease vaccines: an overview. In Grimes, S.E. editor. A Basic Laboratory Manual for the Small-Scale Production and Testing of I-2 Newcastle Disease Vaccine. Bangkok: FAO- RAP publication. 22; 200.

[18] Goddard, R.D.; Nicholas, R.A.J. and Luff, P.R. (1988): Serology-based potency test for inactivated Newcastle disease vaccines. Vaccine, 6(6): 530-532.

[19] Adams, G. (2007): The principles of freeze-drying. Cryopreservation and freeze-drying protocols, 15-38.

[20] Latif, M.Z.; Muhammad, K.; Hussain, R.; Siddique, F.; Altaf, I.; Anees, M. and Farooq, M. (2018): Effect of Stabilizers on Infectivity Titer of Freeze Dried Peste Des Petits Ruminants Virus Vaccine. Pakistan Veterinary Journal, 38(2).

[21] Leidner, A.J.; Fisun, H.; Trimble, S.; Lucas, P.; Noblit, C. and Stevenson, J.M. (2020): Evaluation of temperature stability among different types and grades of vaccine storage units: Data from continuous temperature monitoring devices. Vaccine, 38(14): 3008-301.

[22] Dimitrov, K.M.; Afonso, C.L.; Yu, Q. and Miller, P.J. (2017): Newcastle disease vaccines—A solved problem or a continuous challenge?. Veterinary microbiology, 206: 126-136

[23] Reed, L.J. and Muench, H. (1938): A simple method of estimating fifty percent endpoints. American journal of epidemiology, 27(3): 493-497.

[24] Anon (1963): Methods for examination of poultry biologics. National academy of science, National research council Washington D.C publications705 (2nd eds.)

[25] Tolba, M.k. and Eskarous, J.K. (1962): In diseases of poultry 8th eds (Hofstad et al eds. Iowa state, Univ. press, Ames USA.

[26] Wilcoxon, F. (1992): Individual Comparisons by Ranking Methods. In Kotz, S. and Johnson, N.L. editors. Breakthroughs in Statistics: Methodology and Distribution. New York, NY, Springer New York. 196-202.

[27] Myers, J.L.; Well, A.D. and Lorch, R.F. (2013): Research design and statistical analysis, Routledge.

[28] Bland, J.M. and Altman, D.G. (1986): "Statistical methods for assessing agreement between two methods of clinical measurement." Lancet, 1(8476): 307-310.

[29] McGinnes, L.W.; Pantua, H. and Reitter, J. (2006): "Newcastle disease virus: propagation, quantification, and storage." Curr Protoc Microbiol, Chapter 15: Unit 15F 12.

[30] Al Ahmad, M.; Mustafa, F.; Ali, L.M. and Rizvi, T.A. (2014): "Virus detection and

quantification using electrical parameters." Sci Rep4: 6831.

[31] Roy, P.; Venugopalan, A.T. and Manvell, R. (2000): Characterization of Newcastle disease viruses isolated from chickens and ducks in Tamilnadu, India. Veterinary Research Communications, 24(2): 135-142.

[32] Richard, A.Y.; Mirabeau, T.Y.; Tony, O.I.; Solomon, C.C.; Samsom, E.S. and Ayodeji, O.O. (2014): Evaluation of the efficacy of Newcastle disease (LaSota) live vaccines sold in jos, plateau state, Nigeria. European Scientific Journal, 10(27).

[33] Liljebjelke, K.A.; King, D.J. and Kapczynski, D.R. (2008): Determination of minimum hemagglutinin units in an inactivated Newcastle disease virus vaccine for clinical protection of chickens from exotic Newcastle disease virus challenge. Avian Dis., 52(2): 260-268.

الملخص العربى

تحديد الجرعة النصف معدية (EID_{50}) لفيروسات واللقاحات الحية لمرض النيوكاسل باستخدام طريقة سريعة وموثوقة

أمال عيد 1 *، عبد الشكور إسماعيل 1، هاجر جودة 2، رهام محمد البكري1

1- قسم طب الطيور والأرانب ، كلية الطب البيطري ، جامعة الزقازيق ، 44511 ، الزقازيق ، مصر

2- قسم تنمية الثروة الحيوانية (قسم الإحصاء الحيوي) ، كلية الطب البيطري ، جامعة الزقازيق ، 44511 ، مصر

تم التعرف على مرض النيوكاسل لأول مرة منذ أكثر من تسعة عقود ولا يزال يمثل مشكلة لمنتجي الدواجن إلى جانب كونه مستوطنًا بين العديد من البلدان بما في ذلك مصر. يعتبر التشخيص لفيروس النيوكاسل معايرة اللقاح مهام أساسية في تقييم الفيروس. تم تطبيق اختبار سريع يعتمد على نشاط التلازن الدموي لكرات الدم الحمراء للتحقق من موثوقيتها كطريقة بديلة لمعايرة الفيروس. وعليه تم إجراء تحديد مقارن لـ (EID50 / 0.1ml) عن طريق حقن اجنة الدجاج لعدد 35 سلالة من فيروس مرض النيوكاسل والتى تم تحديدها مسبقًا من خلال مؤشرات الإمراضية وكشفت عن ضراوة متغيرة (lentogenic-mesogenic and velogenic). ثم تم تحليل بيانات كلتا الطريقتين باستخدام SPSS الإصدار 25 والذى أظهر اختبار تصنيف موقع Wilcoxon أن طريقة المعايرة بنشاط التلازن الدموي لكرات الدم الحمراء لم ينتج عنها تغيير مهم إحصائيًا مقارنة بمتوسط قراءة معايرة الفيروس للعينات عن طريق حقن اجنة الدجاج (Z = -0.197، p = 0.844). أظهر معامل ارتباط سبيرمان (r = 0.42، p = 0.01) إرتباطًا معتدلاً جديرًا بالملاحظة بين الطريقتين. ولقد أظهرت الخريطة الحرارية الاختلافات بين كل زوج من الطرق والعلاقة بينهما. كشفت طريقة بلاند-ألتمان اختلافًا يتناسب مع التوزيع الطبيعي W = 0.96، p = 0.24. ووفقًا لذلك ، يعد استخدام اختبار نشاط التلازن الدموي لكرات الدم الحمراء لمعايرة لفيروس /للقاح مرض النيوكاسل سريعًا وسهلاً وموثوقًا ، خاصة عند الحاجة للتقييم الأولي.