IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF VELOGENIC NEUROTROPIC NEWCASTLE DISEASE VIRUS AFFECTING CHICKENS IN EASTERN REGION OF SAUDI ARABIA

Maged Gomaa Hemida[1, 2]

1. Department of Microbiology and Parasitology, College of Veterinary Medicine, King Faisal University, Saudi Arabia. 2. Department of Virology, Faculty of Veterinary Medicine, Kafr elsheikh University, Egypt. Gomaa55@gmail.com, mhemida@kfu.edu.sa

A B S T R A C T

Newcastle disease virus (NDV) is one of the major threats of the poultry industry worldwide. NDV causes high morbidity, mortality among the affected chicken. The main goals of the current study were first; isolation and identification of the circulating NDV strains in the Eastern region of Saudi Arabia (ESA), second; utilization of the state of-Art-molecular based diagnostic techniques for the early detection of NDV, third; conducting seroprevalence of NDV among farms in Eastern Saudi Arabia, fourth; comparing the sensitivity of different techniques in early diagnosis of NDV. To achieve these goals, we collected oronasal swabs, lung, cecal tonsils, and brain from farms in Al-Ahsa, Dammam, and Abqaiq. Isolation of NDV was carried out by using 9-11 days old non NDV vaccinated baladi chicken eggs. Identification of the isolated strains was done by hemagglutination, RT-PCR and Real Time PCR. Our results showing high prevalence of velogenic neurotropic and velogenic visrotropic strains of NDV. Seroprevalence is showing high antibody titers against NDV in the sera of chickens using hemagglutination inhibition (HI) and Enzyme Linked Immunosorbent Assay (ELISA). The velogenic NDV-F gene strains specific probes revealed the presence of these strains in the Eastern region. Real time PCR is more superior to Reverse Transcriptase (RT-PCR) which is more superior to HA and ELISA in NDV detection. In conclusion, several velogenic NDV strains are currently circulating in the ESA. These strains require more molecular characterization. To the best of our knowledge this is the first study in ESA comparing the sensitivity of various techniques in the detection of NDV.

Keywords: Newcastle diseases virus, molecular, Velogenic, Neuorotropic, PCR, Real time PCR, RT-PCR

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1-INTRODUCTION

Newcastle disease virus is one of the most highly contagious infectious viral diseases of poultry (1). It is responsible for high economic losses to the poultry industry due to the high mortality rates among the affected flocks furthermore; it will result in sharp decline in the meat and egg production (1). Despite various control measures such as regular vaccination, slaughter and compensation of the affected farms, NDV continue to cause considerable losses in different poultry population (1). This virus was first identified in UK then after it spreads all over the world including Asia, Australia, Africa and Europe. By 1962, almost 103 countries out of 149 reported the presence of NDV (2). It has been also reported in many neighboring countries to the Saudi Arabia including Indonesia, Pakistan, Palestine, and Kuwait (3). NDV belongs to the family paramyxoviridae and genus Avula virus. The viral genome is single strand negative sense RNA and about 15

Kb in length (4). NDV genome usually encodes six proteins including the RNA-dependant RNA polymerase (L), fusion (F) protein, the hemagglutinin neuramindase (HN), the phosphoprotein (P), and the nucleoprotein (N) (5). NDV had been classified into different pathotypes based on their virulence (6). This includes the highly pathogenic (velogenic) which is further classified according to the tropism into neurotropic and viserotropic (7), the moderately pathogenic (mesogenic) and the mild pathogenic (lentogenic) strains which are (highly virulent, moderately virulent and less virulent respectively) (8). NDV can also infect many other species of birds including pigeons and doves (9). The virulence of NDV strains is recently found to be associated with presence of certain amino acid sequence at the cleavage of the NDV-F protein (10). This cleavage occurs by some host cell proteases Many conventional diagnostic assays have been used for detection of NDV infection such as HA, HI, AGIDT, and SNT (11, 12). These techniques are time consuming and lack the sensitivity and specificity in most of the cases (13). Molecular techniques have been recently adopted for the diagnosis of NDV. These techniques include Enzyme linked immunosorbent assay, Dot-ELISA, Reverse Transcriptase polymerase chain reaction (RT-PCR) and real time PCR (14). The real time PCR was found to be more sensitive in the detection of NDV pathotypes than that the other techniques. The sensitivity of that technique is 100 times than the conventional PCR (15). These techniques are rapid sensitive and specific techniques used for the rapid detection of NDV in different samples (16). Moreover, the Real time PCR has been recently used to classify NDV strains according to their virulence. This mainly depends on the section of certain conserved motives among the velogenic NDV strains (17). There are few number of studies have been carried out to investigate the prevalence of NDV in Saudi Arabia (18). The main goal of the current study was to identify the most circulating NDV strains in Eastern Saudi Arabi. Meanwhile, comparing the sensitivity of the conventional and modern molecular based techniques in the diagnosis of NDV was another goal.

2-MATERIALS AND METHODS

2.1. Samples

Samples were collected from several chicken farms in the Eastern region of Saudi Arabia including Al-Ahsa, Dammam, and Abqaiq. Several samples had been collected from baladi chickens submitted to our laboratory. Different samples were collected from birds received in the morbid stage of the diseases as well as from apparently healthy birds. Oral, cloacal swabs and blood were collected from live birds. Birds were euthanized to conduct the necropsy examination. Five birds were collected per each farm. Pooling of the organs of these birds was done. For examples, the brain of the five birds were collected in one tube and counted as one sample. The collected organs were stored at -80°C till further use. The following table is showing the types, number and localities of the collected samples.

2.2. Tissues and Organs

Tissues and organs (brain, trachea, lung, cecal tonsils) were collected from both apparently healthy and diseased chickens showing nervous, respiratory, or enteric manifestations. Samples were collected from different chicken farms around Al-Ahsa as well as from clinical cases admitted to the poultry clinic at the College of Veterinary Medicine, King Faisal University. The 10 % tissue suspensions were prepared from the collected organs as described earlier (17).

2.3. Swabs

Both oronasal and cloacal swabs were collected from birds. The swabs were collected on DMEM viral transport media

Table1: Types, numbers and localities of the collected samples

Sample/locality	Tissues	Sera	Oronasal Swabs
Al-Ahsa	20	50	20
Dammam	15	25	15
Abqaiq	10	25	10
Total	45	100	45

Table 2: Oligonucleotides used in the gel based PCR reactions

Primer Name	Target gene	Size of the fragment (bp)	Primer Sequence	Ref
NDV-F1	Fusion	101	5'-TCCGGAGGATACAAGGGTCT-3'	(21)
NDV-R1	Fusion	101	5'-AGCTGTTGCAACCCCAAG-3'	(21)

Table 3: Oligonucleotides and probes used in the Real Time based PCR

Primer Name	Target gene	Size (bp)	Sequence	Ref
NDV-VN-F1	Fusion	121	5'-TCCGGAGGATACAAGGGTCT-3'	(17)
NDV-VN-F2	Fusion	121	5'-AGCTGTTGCAACCCCAAG-3'	(17)
NDV-F-Probe	Fusion	101	5'FAM-AAGCGTTTCTGTCTCCTTCCTCCA-BHQ-3'	(17)

Table 4: Identification of NDV by Hemagglutination (HA) test

Sample/ locality	Tissues									Oronasal Swabs							
		0	**1:2**	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**		0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Al-Ahsa	20	2	5	2	3	3	2	2	1	20	1	7	5	2	2	0	1	2
Dammam	15	1	7	2	2	1	2	0	0	15	0	4	4	2	1	1	0	1
Abqaiq	10	0	3	3	2	1	1	0	0	10	0	2	2	2	0	1	2	1
Total	45	3	15	7	7	5	5	2	1	45	1	14	11	6	2	2	3	4

containing antibiotics cocktails and 1% fetal bovine serum.

2.4. Whole Blood Samples

The whole blood samples were collected without anticoagulants by vena puncture. Samples then left in the refrigerator for overnight at 4^{O}C. Centrifugation of the blood samples was done at 3000 rpm for 10 min.

2.5. Preparation of 0.5 % washed chicken RBCs

The sediment RBCs will be subjected to 3 times of washing by PBS. The packed RBCs will be diluted in PBS to prepare (.5%) suspension and used for conducting the Hemagglutination and Hemagglutination inhibition tests as discussed below.

2.6. Serum Samples

A total of 100 serum samples were collected from several farms in the Eastern region of Saudi Arabia (Table 1). Blood was withdrawn from the wing vein of birds. Sera were separated as previously described. The collected serum samples were heat inactivated at 56 ºC for 30 min then stored at -20^{O}C till use.

2.7. Virus isolation

Virus isolation was done from different samples such as swabs and tissue suspension by using 9-11-day-old native breed (non NDV vaccinated) embryonated chicken egg as previously described (19).

2.8. Hemagglutination (HA) and Hemagglutination inhibition (HI) test

The HA test was performed as previously described (20). Briefly, serial two fold dilution of the NDV antigen (LASota strain) was prepared (6). After that 25 µl of the egg fluids contains the virus were added to the first well then serial two fold dilutions will be carried out. Then 25 µl of 0.5 % chicken RBCs were added to each well. Plates were incubated at room temperature for 1 hr then reading the results was done. The HI test was done as previously described by the manufactures instructions (20).

2.9. Enzyme linked immunosorbent assay (ELSA)

A commercial enzyme linked immunosorbent assay was obtained from (ID Screen® Newcastle Disease Competition, ID.Vet innovative Diagnostics, USA) and be carried out according to the instructions of the kits..

2.10. RNA extraction

Viral RNAs were extracted from various samples including different kinds of swabs, serum, and tissues. Extraction was carried out according to the instruction of the RNA extraction kits QIA amp Viral RNA mini kits (Qiagen, Inc., Valencia, CA). Briefly, about 140 µl from the samples was transferred to 580 µl of the Qiagen lysis buffer. Negative control extraction from the suspending transport medium will run in parallel to each sample. RNAs were eluted and stored at -80 ºC till use.

2.11. Oligonucleotides used in the gel based PCR technique

The following primers were used to amplify the NDV-F gene from the collected samples. The following table is showing these oligonucleotides and their sequences

Two sets of primers and probes were used to identify the velogenic neurotropic NDV. These oligonucleotides were targeting the fusion gene of NDV (Table 2)

2.12. RT-PCR

The RT-PCR was carried out using the antisense stranded oligonucleotides for NDV-F gene as listed in Table 2. The reactions were carried out as previously described (22).

2.13. PCR

PCR was performed according to the method described by (13) in 50 µl of a reaction mixture containing a final concentration of 10 mM Tris (pH 9.0), 50 mM KCl, 0.01% gelatin, 1.9 mM MgCl2, 5% (wt/vol) glycerol, 0.2 mM deoxynucleotide triphosphate , 0.1 M of each primer listed in Table 2, 10 U of Taq polymerase per µl and 5 µl of extracted

NDV-RNA. The PCR mixtures were subjected to 38 repeated cycles of amplification in a DNA thermal cycler. The cycling conditions was as follows: denaturation 95ºC for 1 min, primer annealing at 60ºC for 1 min and extension at 72ºC for 1 min. Negative and positive control reactions was included

2.14. Gel electrophoresis

Ten µl of PCR products was resolved by horizontal 1% electrophoresis in agarose gels containing SYBR® Safe DNA Gel Stain (Life Technologies). Amplified DNA fragments was visualized under ultraviolet light and photographed using a gel documentation system (Bio-Rad Laboratories, Inc., Hercules, California, USA).

2.15. Real Time PCR

The Real Time PCR reactions for NDV- M gene was carried out in (the Applied Biosystem 7500 machine) the reaction was done according to (2, 14) with some modifications. Briefly, we prepared the reaction mixtures of 20 µl including the following (1 µl of 10 pmol of each primer strand, 5 µl of cDNA, 5 µl of the master mix, containing SYBR Green I and completed the reaction to 20 µl using nuclease free water. We adjusted the conditions of Real Time PCR to be as follow, the initial denaturation at 95 ºC for 15 min then 94 ºC for 10 seconds (this step repeated 45 cycles), then 52 ºC for 5 seconds, and finally 72 ºC for 10 seconds.

3-RESULTS

3.1. Clinical profiles of NDV infected chickens

Statistically representative samples from each farm from different localities in the Eastern region of Saudi Arabia including (AL-Ahsa, Dammam, and Abqaiq) were received. Physical examination of the tested birds was done in our laboratory. Birds showed various clinical signs including respiratory (mouth breathing, gasping and rales) Figure 1A, nervous (abnormal posture, ataxia and torticollis) Figure 1 B, and enteric (diarrhea and soiled vent). Necropsy examination was carried out and revealed typical NDV lesions in many birds in the form of (petechial hemorrhage in the gizzard proventriculus junction as well as cecal tonsils) Figure 1C.

3.2. Isolation of NDV via ECE inoculatio

We used the non NDV-vaccinated baladi chicken eggs for the isolation and propagation of the circulating NDV strains in the Eastern Saudi Arabia. Swabs and tissue suspensions from different organs were used to isolate various NDV strains. Our results indicated that many virulent (velogenic) strains of NDV are currently circulating in this area of the Kingdom. The inoculated embryos were hemorrhagic and smaller in size (right) in comparison to the sham phosphate buffer saline (PBS) (left) inoculated eggs starting 3 days post inoculation (3dpi) as shown in (Figure 2).

3.3. Detection of NDV antigen in different samples by Hemagglutination (HA) test

Both the tissue suspensions and the swabs were subjected to the HA test using 0.5% chicken RBCs to detect NDV in these samples (Figure 3A and Table 4).

3.4. Seroprevalence of NDV in the Eastern Saudi Arabia using Hemagglutination inhibition (HI) test

Testing the collected sera from different localities was done initially by the HI test. Our results are showing high seroprevalence of NDV as shown in (Figure 3B and Table 5).

3.5. Seroprevalence of NDV in the Eastern Saudi Arabia using commercial ELISA kits

The commercial available ELISA kits were used to evaluate the immune status of chicken flocks at the indicated localities in the Eastern Saudi Arabia. Our results are

Table 5: Seroprevalence of NDV in the Eastern Saudi Arabia using Hemagglutination inhibition (HI) test

Sample/ locality	Total	HI titers								
		0	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Al-Ahsa	50	2	10	8	3	7	8	2	7	3
Dammam	25	1	7	4	6	2	1	2	1	1
Abqaiq	25	0	2	3	7	6	2	2	3	0
Total	100	3	19	15	16	15	11	6	11	4

Table 6: Seroprevalence of NDV in the Eastern Saudi Arabia using commercial ELISA kits

Locality	No of tested samples	(+Ve)	(-Ve)
Al-Ahsa	50	43	7
Dammam	25	19	6
Abqaiq	25	22	3
Total	100	84	16

Table 7: Comparison of HI and ELISA in the detection of NDV antibodies

Locality	No of tested samples	HI	ELISA
Al-Ahsa	50	33	43
Dammam	25	13	19
Abqaiq	25	17	22
Total	100	63	84

Table 8: Pathotyping of NDV by Real Time PCR

Type of Sample	Amplification	T_m (M gene)
Pooled organs	+	86.46
Pooled organs	+	86.66
Pooled organs	+	79.38
Pooled organs	+	78.81
Pooled organs	+	79.50
Positive control (LaSota vaccine)	+	85.46
Negative control (Avian Inf. H9N2 allantoic fluid)	-	-
Negative control Nuclease free water	-	-

Table 9: Comparison of the sensitivity of different techniques in detection of NDV

Technique	HA	PCR	Real Time PCR
% of total (+Ve)	43	77	92

Figure 1. Clinical picture and Necropsy findings of NDV affected chickens. NDV infected chicken showing typical respiratory manifestation in the form of opening the mouth and gasping. B. NDV infected chicken showing typical torticollis appearance. C. Necropsy findings showing peticeal hemorrhage at the cecal tonsils. D. petechial hemorrhage at the tips of the gizzard-proventriculus junction. E. Petechial hemorrhage in the brain.

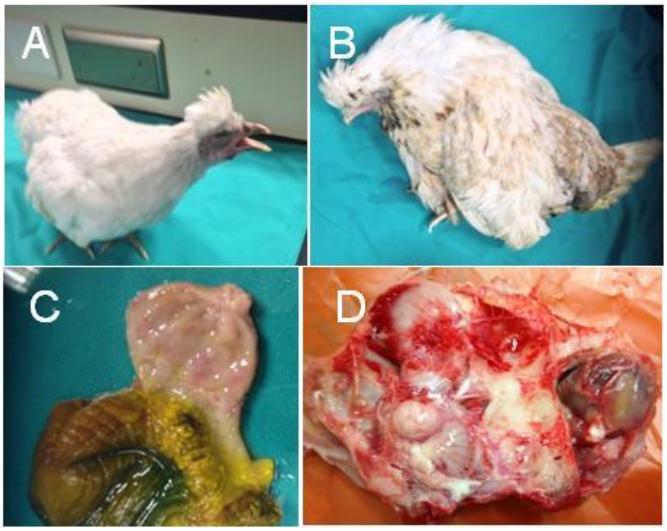


Figure 2. Effects of NDV on the Embryonated Chicken Eggs. Thirteen days old embryonated eggs non infected showing typical embryonated egg of this age (left), the NDV infected eggs showing dwarfing and hemorrhage (right), 3 days post infection.

Figure 3. Hemagglutination and Hemagglutination inhibition of NDV. A- Detection of NDV by Hemagglutination test in the collected samples. B- Hemagglutination inhibition of the chicken sera against NDV. Figure 3: (A) Hemagglutination (HA) test. (B) Hemagglutination inhibition (HI) test

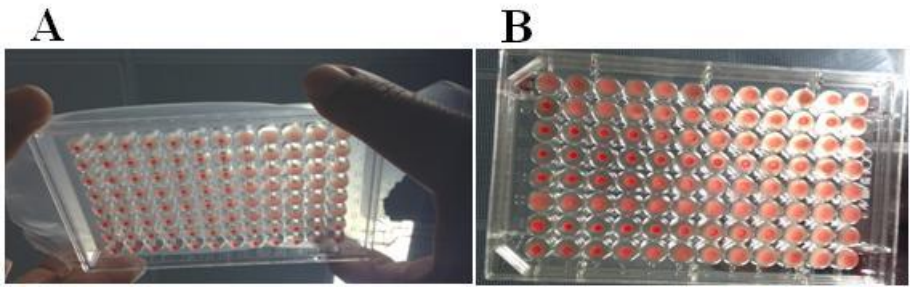


Figure 4. RT-PCR of NDV in the clinical specimens. Results of the RT-PCR of the tested chicken samples to NDV using the F gene primers. Lane (M) is for ladder, lane 1-6 are positive bands for the NDV-M gene. Lane 7 is negative control non-template cDNA sample.

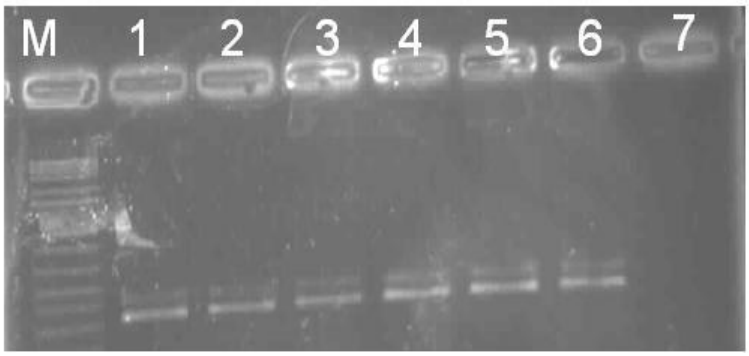
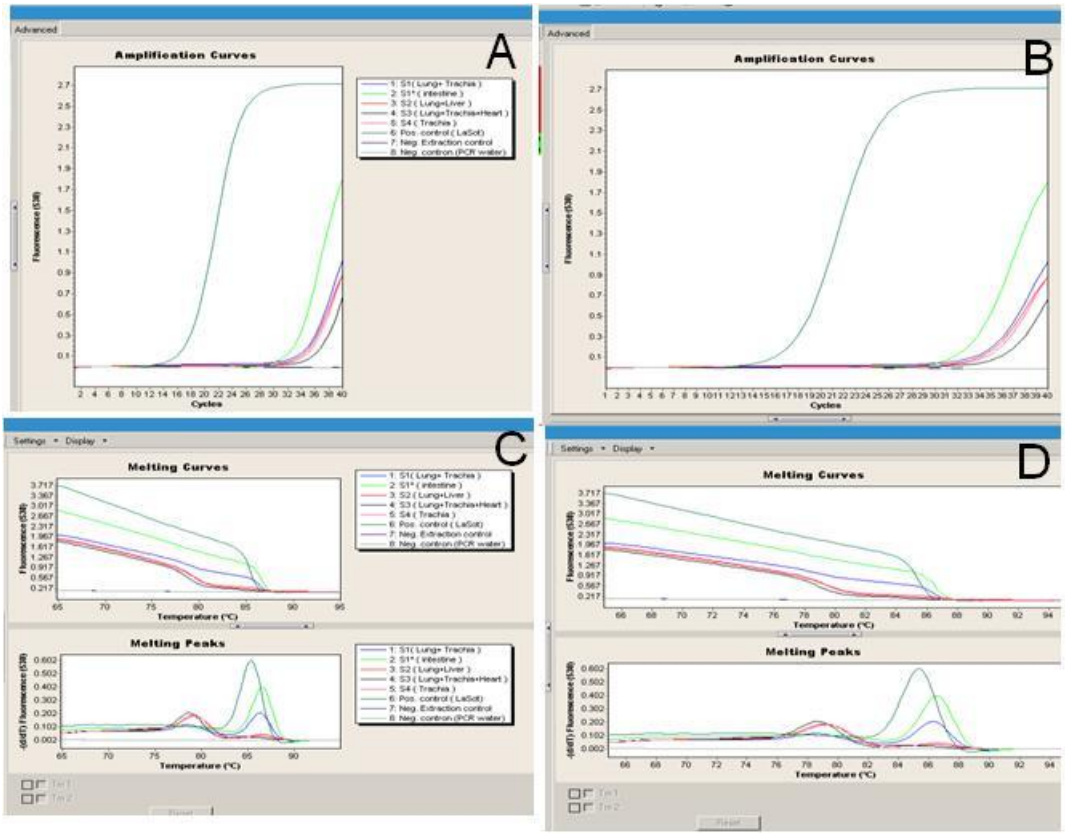


Figure 5. Real Time PCR of NDV in the clinical specimens. Results of the Real Time PCR of the tested samples from chickens using the designed F gene oligonuclotiesds. (A) The amplification curve of the tested samples, (B) the melting curve of the tested samples and (C, D) the melting peaks of the tested samples.



showing high seroprevalence of NDV as shown in (Table 6).

3.6. Comparison of HI and ELISA in the detection of NDV antibodies

We conducted a comparison between the HI and ELISA tests in sensitivity of detection of NDV specific antibodies. These results are listed in Table 7

3.7. Molecular characterization of NDV

A- Gel based PCR

A conserved fragment of NDV-F gene (101 bp) was used for the early detection of NDV in the collected tissues and swabs. Figure 4 showing the amplified products of several samples using the indicated primers (Table 2). We used LAsota vaccine strain as a positive control

B- Real Time PCR

We used the SybrGreen I real time PCR along with the melting curve analysis for the early detection and pathotyping of NDV Figure 5. In this approach we used the most conserved fragments of the M of velogenic NDV. The melting temperatures (TM) of the velogenic strains circulated in Eastern Saudi Arabia are listed in (Table 8).

3.8. Comparison of the sensitivity of conventional and molecular based techniques in diagnosis of NDV

Based on our data, we compared the sensitivity of various techniques for the detection of NDV as listed in Table 9. The real time PCR showed the highest sensitivity (92%) in the detection limits of NDV compared to HA and gel based PCR, which were (43% and 77%) respectively.

4. DISCUSSION

Great efforts had been done globally to combat NDV in the poultry industry including intensive vaccination programs, application of hygienic measures and quarantine measures (23). However, many outbreaks of NDV still reported so frequently. This is may be due to many reasons including the random use of intensive vaccines, frequent mutations of the NDV, and emerging of new pathotypes of NDV (9). The science based control measures are mainly depend on the accurate diagnosis of the circulating strain, the evaluation of the immune status of the flocks against NDV plus adoption of good management programs. Since its discovery, many techniques have been developed to diagnose NDV. Comparing the sensitivity of some conventional techniques such as (HA, isolation via ECE, and HI) to some new techniques such as (PCR, Real Time PCR and ELISA) for the detection of NDV. Our results are showing that Real time PCR (Table 9 and Figure 5) is more superior than the gel based RT-PCR in the detection of NDV in different samples (Table 9, Figure 4) which in turn more sensitive to HA (Table 9, Figure 3) in the detection of NDV in different clinical specimens. Our results are consistent with other studies which found that PCR is more sensitive than HA in the early detection of NDV (24). In similar trend, ELISA was found to be more sensitive in the detection of NDV antibodies in sera of chicken than does HI test 63 % and 84 % respectively (Table 7). This is in agreement with other studies (25) developed recombinant based NDV-N based ELISA using the baculovirus expression system. This study also found the c-ELISA was able to detect the reactive animals to NDV in HI negative tested samples (25). One critical point is the identification of the causative NDV in certain outbreak. Previously, pathotyping of NDV was mainly dependant of the Pathogenicity index either the intracerebral (ICPI) or intravenous pathogenicity (IVPI) index or the mean death time (MDT) (26). Although these techniques are specific and were acceptable for many years however they are less specific, labor intense and

time consuming (27). One problem hinder the molecular basotyping approaches of NDV in Saudi Arabia is the lack of any reported NDV complete genome sequence reported in the gene bank (NCBI) so far. Nowadays, the presence of certain amino acids at the cleavage sites of NDV-F proteins used as a virulence markers of NDV pathotypes. (28). It is now accepted that, the NDV virulent strains have two pairs of basic amino acid (R/K-R-O-K/K-R-F) while the less virulent strains are characterized by only two single basic amino acids (G/E-K/R-Q-G/E-R-L) at NDV-F protein cleavage sites (29). These basic amino acids are cleaved by host cell proteases as in cans of the virulent strains however these concencess are cleaved by trypsin like enzymes in case of the lentogenic strains (30). In the current study, we applied the SYBER Green I technique using the NDV-F specific primers and probes for the pathotyping of the circulated strains of NDV in the Eastern Saudi Arabia. This technique is coupled with melting curve analysis. Our results are showing that the melting temperature (Mt) of the tested samples were (86.46), (Table 8 and Figure5). This was in contrast to the lentogenic LASota vaccine strains used as negative control, their melting temperature was 85.46 (Table 8 , Figure 5). This approach was feasible in many other studies that used similar strategy for pathotyping of NDV (15). Only few studies have been done about the pathotyping of NDV in the Eastern Saudi Arabia (18) however to the best of our knowledge this is the first study reporting the circulation of virulent NDV strains in Eastern Saudi Arabia. Seroprevalence study using both HI and ELISA techniques reveals high seroprevalence of IBV in the Eastern Saudi Arabia (Tables 5-7). Either this is suggesting the application of NDV vaccines in these birds or those birds were exposed to the NDV infection at certain point of their life. In conclusion, many velogenic strains are currently circulating in the Eastern Saudi Arabia. Molecular based techniques are more superior to the conventional ones in the early rapid and sensitive detection of NDV in the affected chicken flocks. Establishing he complete genome sequence of these strains will open several avenues for the development of novel specific diagnostic assays specific to these Saudi isolates. Meanwhile, it is going to be the platform for local vaccine preparation.

Conclusions: Several virulent NDV strains are currently circulating in the Eastern region of Saudi Arabia. Further molecular characterization is required to develop specific diagnostic assays as well as vaccine representing the local field strains.

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6. REFERENCES

1. Alexander, D.J., Wilson, G.W., Russell, P.H., Lister, S.A., Parsons, G. 1985. Newcastle disease outbreaks in fowl in Great Britain during 1984. Vet Rec. 117(17):429-34.
2. Choi, K.S., Lee, E.K., Jeon, W.J., Kwon, J.H. 2010. Antigenic and immunogenic investigation of the virulence motif of the Newcastle disease virus fusion protein. J Vet Sci. 11(3):205-11.
3. Siddique, N., Naeem, K., Abbas, M.A., Malik, A., Rashid, F., Rafique, S., 2013. Sequence and phylogenetic analysis of virulent Newcastle disease virus isolates from Pakistan during 2009-2013 reveals circulation of new sub genotype. Virology. 444(1-2):37-40.

4. De Leeuw, O., Peeters, B. 1999. Complete nucleotide sequence of Newcastle disease virus: evidence for the existence of a new genus within the subfamily Paramyxovirinae. J Gen Virol. 80 (1):131-6.
5. Munir, M., Abbas, M,, Khan, M.T., Zohari, S., Berg, M. 2012. Genomic and biological characterization of a velogenic Newcastle disease virus isolated from a healthy backyard poultry flock in 2010. Virol J. 9:46.
6. Adi, A.A., Astawa, N.M., Putra, K.S., Hayashi, Y., Matsumoto, Y. 2010. Isolation and characterization of a pathogenic Newcastle disease virus from a natural case in indonesia. J Vet Med Sci. 72(3):313-9.
7. 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. Vet Pathol. 43(2):168-78.
8. Ecco, R., Susta, L., Afonso, C.L., Miller, P.J., Brown, C. 2011. Neurological lesions in chickens experimentally infected with virulent Newcastle disease virus isolates. Avian Pathol. 40(2):145-52.
9. Pchelkina, I.P., Manin, T.B., Kolosov, S.N., Starov, S.K., Andriyasov, A.V., Chvala IA, 2013. Characteristics of pigeon paramyxovirus serotype-1 isolates (PPMV-1) from the Russian Federation from 2001 to 2009. Avian Dis. 57(1):2-7.
10. Yuasa, K., Futamatsu, G., Kawano, T., Muroshita, M., Kageyama, Y., Taichi, H. 2012. Subtilisin-like proprotein convertase paired basic amino acid-cleaving enzyme 4 is required for chondrogenic differentiation in ATDC5 cells. FEBS J. 279(21):3997-4009.
11. Cho, S.H., Kim, S.J., Kwon, H.J. 2007. Genomic sequence of an antigenic variant Newcastle disease virus isolated in Korea. Virus Genes. 35(2):293-302.
12. He, Y.T., Gong, Y.Y., Zhao, P., Cui, Z.Z. 2012. [Antigenic comparative analysis of Newcastle disease viruses with evolutional mutations in HN and F genes under antibody immune pressures]. Bing Du Xue Bao. 28(5):489-95.
13. Gopinath, V.P., Raj, G.D., Raja, A., Kumanan, K., Elankumaran, S. 2011. Rapid detection of Newcastle disease virus replication in embryonated chicken eggs using quantitative real time polymerase chain reaction. J Virol Methods. 171(1):98-101.
14. Li, X., Chai, T., Wang Z, Song, C., Cao, H., Liu, J., et al. 2009. Occurrence and transmission of Newcastle disease virus aerosol originating from infected chickens under experimental conditions. Vet Microbiol. 12;136(3-4):226-32.
15. Pham, H.M., Konnai, S., Usui, T., Chang, K.S., Murata, S., Mase, M., et al. 2005. Rapid detection and differentiation of Newcastle disease virus by real-time PCR with melting-curve analysis. Arch Virol. 150(12):2429-38.
16. Zhang, Y., Fu, D., Chen, H., Zhang, Z., Shi, Q., Elsayed, A.K. 2013. Partial Antiviral Activities Detection of Chicken Mx Jointing with Neuraminidase Gene (NA) against Newcastle Disease Virus. PLoS One. 8(8):e71688.
17. Wise, M.G., Suarez, D.L., Seal, B.S., Pedersen. J.C., Senne, D.A., King, D.J. 2004. Development of a real-time reverse-transcription PCR for detection of newcastle disease virus RNA in clinical samples. J Clin Microbiol. 42(1):329-38.
18. el-Zein, A. 1986. Characterization of a velogenic Newcastle disease virus isolated from broilers in Saudi Arabia. Avian Dis. 30(4):825-8.

19. Radwan, M.M., Darwish, S.F., El-Sabagh, I.M., El-Sanousi, A.A., Shalaby, M.A. 2013. Isolation and molecular characterization of Newcastle disease virus genotypes II and VIId in Egypt between 2011 and 2012. Virus Genes. 47(2):311-6.
20. Ke, G.M., Chuang, K.P., Chang, C.D., Lin, M.Y., Liu, H.J. 2010. Analysis of sequence and haemagglutinin activity of the HN glycoprotein of Newcastle disease virus. Avian Pathol. 39(3):235-44.
21. Tiwari, A.K., Kataria, R.S., Nanthakumar, T., Dash, B.B., Desai, G. 2004. Differential detection of Newcastle disease virus strains by degenerate primers based RT-PCR. Comp Immunol Microbiol Infect Dis. 27(3):163-9.
22. Gomaa, M.H., Barta, J.R., Ojkic, D., Yoo, D. 2008. Complete genomic sequence of turkey coronavirus. Virus Res. 135(2):237-46.
23. Claassen, I., Maas, R., Oei, H., Daas, A., Milne, C. 2004. Validation study to evaluate the reproducibility of a candidate in vitro potency assay of newcastle disease vaccines and to establish the suitability of a candidate biological reference preparation. Pharmeuropa Bio. 2004(1):1-15.
24. Mohammed, M.H., Zahid, A.H., Kadhim, L.I., Hasoon, M.F. 2013. Conventional and Molecular Detection of Newcastle Disease and Infectious Bursal Disease in Chickens J World's Poult Res. 3(1):05-12.
25. Phan, L.V., Park, M.J., Kye, S.J., Kim, J.Y., Lee, H.S., Choi, K.S. 2013 Development and field application of a competitive enzyme-linked immuno-sorbent assay for detection of Newcastle disease virus antibodies in chickens and ducks. Poult Sci. 92(8):2034-43.
26. Mishra, S., Kataria, J.M., Sah, R.L., Verma, K.C., Mishra, J.P. 2001. Studies on the pathogenicity of Newcastle disease virus isolates in guinea fowl. Trop Anim Health Prod. 33(4):313-20.
27. Chen, S., Hao, H., Wang, X., Du, E., Liu, H., Yang, T., et al. 2013. Genomic characterisation of a lentogenic Newcastle disease virus strain HX01 isolated from sick pigs in China. Virus Genes. 46(2):264-70.
28. Collins, M.S., Bashiruddin, J.B., Alexander, D.J. 1993. Deduced amino acid sequences at the fusion protein cleavage site of Newcastle disease viruses showing variation in antigenicity and pathogenicity. Arch Virol. 128(3-4):363-70.
29. Collins ,M.S., Strong, I, Alexander, D.J. 1994. Evaluation of the molecular basis of pathogenicity of the variant Newcastle disease viruses termed "pigeon PMV-1 viruses". Arch Virol. 134(3-4):403-11.
30. Samal, S., Kumar, S., Khattar, S.K,, Samal, S.K. 2011. A single amino acid change, Q114R, in the cleavage-site sequence of Newcastle disease virus fusion protein attenuates viral replication and pathogenicity. J Gen Virol. 92(10):2333-8.

كلية معتمدة 2013

التوصيف البيولوجي الجزيئي لبعض العترات الضارية من فيروس النيوكاسل في المنطقة الشرقية من المملكة العربية السعودية

ماجد جمعة حميدة[1-2]

1- قسم اللاحياء الدقيقة و الطفيليات-كلية الطب البيطري و الثروة الحيوانية – جامعة الملك فيصل-المملكة العربية السعودية. 2- قسم الفيرولوجي كلية الطب البيطري جامعة كفر الشيخ-جمهورية مصر العربية

الملخص العربي

يعتبر فيروس النيوكاسل من اهم المشاكل صناعة الدواجن في العالم لما يتسبب فية من معدلات اصابة ونفوق عالية. صممنا الدراسة الحالية لكي تهدف الي (1) عزل وتوصيف العترات الموجودة في المنطقة الشرقية (2) استخدام الطرق البيولوجيا الجزيئية الحديثة في الكشف عن هذة العترات (3) تقيم المستوي المناعي لقطعان الدواجن ضد الفيروس. (4) مقارنة التقنيات العادية والتقنيات البيولوجية الحديثة في الكشف عن الفيروس. لتحقيق اهداف الدراسة الحالية جمعنا عينات من العديد من المزارع في المنطقة الشرقية وتحديدا من الاحساء والدمام وابقيق شملت اجزاء من الاحشاء الداخلية ومسحات من القصبة الهوائية والسيرم. لقد استخدمنا بيض بلدي مخصب عمر 9–11 يوم غير محصن ضد النيوكاسل في عزل العترات. نتج عن ذلك نزيف وتقزم في الاجنة المحقونة ووفاة العديد من الاجنة بعد 3–5 ايام. قمنا بالكشف عن العترات المختلفة باستخدام اختبار تلزن الدم. وللتأكيد قمنا باستخدام تقنيات البيولوجيا الجزيئية الحديثة مثل الاجار جيل بي سي ار والريال تايم بي سي ار. ولقد توصلنا الي وجود العديد من العترات الضارية من الفيروس في المنطقة الشرقية. خلصت الدراسة ان التقنيات البيولوجيا الحديثة أكثر دقة وحساسية في التعرف على وتصنيف العترات المختلفة من الفيروس. وأثبتت ايضا وجود الاجسام المضادة للفيروس في امصال الطيور باستخدام اختبار مانع التلزن والاليزا. الخلاصة ان الدراسة اثبتت وجود العديد من العترات الضارية من فيروس النيوكاسل في الشرقية. وتوصي بإجراء المزيد من الابحاث هذة العترات باستخدام تقنيات البيولوجيا الحديثة وذلك لابتكار اختبارات معملية جديدة لتشخيص فيروس النيوكاسل وامكانية تحضير لقاحات محلية الصنع مستقبلا.

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