Case Report

Emergence of Genotype VIIg of Velogenic Newcastle Disease Virus in Iran, 2018: The First Report

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Abstract

Newcastle disease virus (NDV) is a prototype member of avian paramyxovirus serotype 1 (APMV-1), which causes severe and contagious disease in the commercial poultry and wild birds. In this study, we report the results of phylogenetic analyses of recent NDVs isolated from Markazi province of Iran during a recent outbreak in the commercial broiler with respiratory signs and digestive system lesions. Phylogenetic analysis revealed that isolate was clustered within class II NDV, in sub-genotype VII-g. This NDV isolates shared high homology with the prevalent genotype NDV strains that circulate in China and Taiwan (95.39%-84.89%). Overall, our results confirmed the presence of genotype VII-g in Iran (The first report) and indicated that different genotypes of NDV could circulate simultaneously among poultry (VII-d & VII-i). The phylogenetic characterization of these isolates helps to characterize the evolution of NDV and may help with the development of vaccines specific to our regional necessities.

Keywords: Newcastle disease; Iran; Phylogenetic analysis; Genotyping, Genotype VII-g.

Introduction

ewcastle disease (ND) is one of the most contagious and devastating diseases to poultry in the world and was originally detected in Java Indonesia and Newcastle- 'On-Tyne, England (1). The causative agent of NDV is enveloped and belongs to the genus Avulavirus, sub-family Paramyxovirinae, family Paramyxoviridae. The viral RNA genome is negative sense, nonsegmented, single-stranded and encodes six

major virus proteins, which F protein is considered to play a vital role in the virulence of NDV strains. The virulence of NDV depends upon the amino acid sequence at the cleavage site of the F protein (2, 3). It is widely accepted that cleavage of the F protein precursor (F0) is the primary determinant of virulence. Virulent (velogenic or mesogenic) NDV strains contain a multi-basic amino acid-rich region (112R/K-R-X-R/K-R-F117) in the cleavage site whereas avirulent (lentogenic) strains possess (112G/E-K/R-X-G/E-R-L117) motif cleaved by secreted trypsin-like protease into active F protein, which consists of disulfide-linked F1 and F2 polypeptides (4). Based on the complete genomic length and phylogenetic relationship, NDV strains are divided into class I and class

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II. Class, I NDV strains mainly consist of avirulent strains and are mostly isolated from wild birds. On the other hand, class II includes both virulent and avirulent strains isolated from both domestic and wild birds. NDV is classified based on nucleotide sequence of the F gene into 19 genotypes, class I has single, and class II includes 18 genotypes (I-XVIII)(5). Different genotypes of NDV have been circulating worldwide (6). Genotypes VII and VIII were responsible for ND outbreaks in Asia, including Pakistan, and in Europe since 1984 or earlier (7, 8).

There were four panzootic caused by NDV different genotypes in the world since the 1920s, and the genotype VII viruses were responsible for the fourth panzootic began southeast Asia in 1985, which continues today, has spread from Asia to Africa, Europe and has even been detected in South America (6, 9-11). In Iran, first NDV outbreak was reported in the year 1926. Virulent NDV is endemic in Iran and in the past few decades, implementation of extensive vaccination programs in commercial poultry farms, and to some extent in small rural poultry farms, has reduced the number of epizootic outbreaks of ND in Iran (12). Sporadic cases of ND, however, are reported every year. In recent years, the frequent occurrence of ND outbreaks in vaccinated flocks with high hemagglutination inhibition (HI) titer antibody levels raises questions about the causative agent (12, 13). In the present report, we detected and identified the new velogenic strain of NDV in commercial broiler chicken in Iran and finally doing phylogenetic analysis on it.

Methods

In the present study, an outbreak with respiratory signs and digestive system lesions were recorded in Markazi province during the September 2017. Trachea tissue samples were collected from broilers flocks and transferred with ice to the virology laboratory of veterinary medicine faculty of Tehran University.

The NDV positive samples were collected from both ailing and dead birds reported with

pathological signs and symptoms. NDV isolates were recovered by inoculating homogenized trachea tissue samples into specific pathogen-free 9-days-old SPF embryonated chicken eggs following standard procedure.

Final confirmation of the positive lung samples was done by haemagglutination assay (HA) and stored at -80 °C for further use.

Total RNA extraction from trachea samples was carried out through Qiagen RNeasy Mini extraction kit (Valencia, CA, USA) followed the standard protocol.

RNA was stored at -70°C until RT-PCR was performed. RNA reverse-transcribed to cDNA with random hexamer and RevertAidTM M-MuLV Reverse Transcriptase (Fermentas, Canada) according to the manufacturer's protocols. cDNA was amplified using Kant's primer sets (A: 5'-TTG ATG GCA GGC CTC TTG C-3' B: 5'-GGA GGA TGT TGG CAG CAT T-3') (14).

The PCR condition was 94°C for 2 minutes denaturation, and 35 cycles of 94°C for 10 seconds, 58°C for 20 seconds (annealing), and 72°C for 20 seconds, followed by 70°C for 10 minutes final extension. The product was purified and cleaned using AccuPrep® PCR Purification kit (Bioneer Co, South Korea).

For sequencing, the sample was sent to Source BioScience Company (UK). Percent nucleotide identity, sequence editing and prediction of amino acid sequences, and primary alignments were carried out using CLC sequence viewer (Ver. 6.0.2). Nucleotide analysis was performed by Molecular Evolutionary Genetics Analysis, version 7.01 (MEGA 7) (15).

Multiple nucleotides and amino acid sequence alignment for the studied gene were performed using Clustal W model.

Phylogenetic trees were drawn from amino acid based on the partial segment of F gene segments using minimum evolution analysis with neighbor joining.

The neighbor-joining algorithm was implemented with the Kimura2 parameter model (16). 1000 bootstrap trees were created to evaluate the reliability of the ancestral location.

Results and Discussion

After working on the sequences, from the pathotype prediction based on the cleavage site of the F protein, all the isolates were placed into the velogenic group with the motif 112RRQKRF 117. A phylogenetic tree (Fig. 1) was constructed based on the nucleotide sequences of the F gene hypervariable region all field isolates of NDV and the corresponding regions of the other NDV strains retrieved from GenBank. A total two

field isolates isolated in recent outbreaks were classified into genotype VII-g. These isolates shared high homology with the prevalent genotype NDV strains that circulate in China (XD/Shandong/08 China genotype VII(g) (GQ) 994433.1)), Japan ((Miyadera) F0 genotype III (M18456.1)) and Taiwan (CH-A7/96 genotype $_{\text{VII}(f)}(\text{AY}028995.1))$ (95.39%-84.89%). The data have been shown in table 1.

New genotypes of NDV are continuously described worldwide. To date, 18 class II genotypes of NDV have been

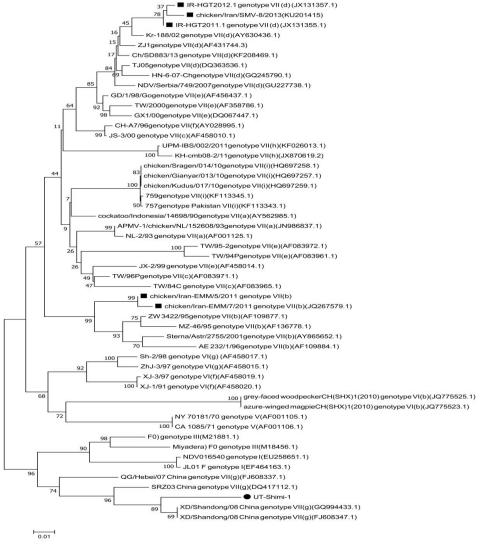


Fig. 1. Nucleotide acid-base phylogenetic relationships of fusion gene of Newcastle disease virus detected from Iran. The phylogenetic tree was generated using neighboring joining model with MEGA (version 7.0.14). Numbers below branches indicate bootstrap value from 1000 replicates, bootstrap values. Horizontal distances are proportional to the minimum number of nucleic acid differences required to join nodes. The vertical lines are for spacing branches and labels. The analysis was based on complete open reading frames of all gene segments. The scale bar represents the distance unit between sequence pairs. The virus genome characterized in this report is indicated as black circle • . Iranian NDV are indicated black square circle

	Newcastle disease v	iruses	· (NID	Table 1. Percent identity of partial nucleotide sequences of the Fusion glycoprotein genes of new subgenotype VII-g														
	Newcastle disease viruses (NDV) to those of NDV reference strains.																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
1	$XD/Shandong/08_China_genotype_VII(g)(FJ608347.1)$															l		
2	UT-Shimi-1	95.39																
3	$IR\text{-}HGT2012.1_genotype_VII_(d)_(JX131357.1) \text{iran}$	77.01	78.53															
4	chicken/Sragen/014/10genotype_VII(i)(HQ697258.1) indonesia	86.44	80.31	91.48														
5	UPM-IBS/002/2011genotype_VII(h)(KF026013.1) Malaysia	86.58	81.89	89.43	92.32					Ì								
6	QG/Hebei/07_China_genotype_VII(g)(FJ608337.1)	93.97	84.16	81.03	89.77	89.00												
7	CH-A7/96genotype_VII(f)(AY028995.1)	89.24	84.89	92.85	92.94	93.22	91.29											
8	APMV-1/chicken/NL/152608/93genotype_VII(a)(JN986837.1)	87.28	83.25	93.13	94.68	93.94	89.76	94.48										
9	TW/95-2genotype_VII(e)(AF083972.1)	73.63	80.34	91.95	90.27	86.52	79.91	91.49	92.04									
10	F0)_genotype_III(M21881.1)	86.03	84.83	84.57	87.33	86.83	89.28	87.87	87.15	84.44								
11	Miyadera)_F0_genotype_III(M18456.1)	84.88	86.98	81.75	86.07	85.80	88.07	86.78	86.44	82.73	95.47							
12	NDV016540_genotype_I(EU258651.1)china	86.38	84.16	81.03	86.40	84.96	92.55	85.64	85.68	79.16	93.06	91.84						
13	ZhJ-3/97_genotype_VI(g)(AF458015.1)china2003	84.74	82.57	86.12	89.53	88.29	87.47	90.05	89.87	85.22	88.72	87.33	87.26					
14	CA_1085/71_genotype_V(AF001106.1)	80.24	78.74	85.55	86.92	84.36	82.81	88.64	88.55	85.79	87.84	85.53	83.53	91.12				
15	chicken/Iran-EMM/5/2011_genotype_VII(b)	86.46	84.01	89.34	91.76	90.99	88.52	92.11	92.78	88.40	87.20	86.72	86.12	89.93	88.93			

described, and most diversity was identified in genotype VII viruses which are undertaking ND outbreak in the Middle East and Asia. Genotype VII viruses likely originated from the Far East in the 1990s and finally spread to another continent such as Asia, Europe, South Africa and South America. According to the reviewed amino acid motifs and pathotyping, all genotype VII viruses are velogenic (17). Among class II, highly diverse genotype VII was separated into eight sub-genotypes (VII ah). VII-a isolates had been identified in Poultry in Western Europe in the 1990s, but likely they had Indonesian ancestors from 1980s (18). Viruses of VII-b had frequently been isolated from poultry in China during 1998-2014 (19-23) and other Asian countries such as Vietnam in 2007 and Israel in 211-2014 (24). Viruses recovered from chickens and pigeons from China and Taiwan during 1996-2000 belonged to VII-c and VII-d sub-genotypes (11). VII-d viruses rapidly spread all over the world and became one of the most prevalent subgenotypes circulating since the first of 21st century. Also, this sub-genotype is dominant in Iran (12). Isolates from this sub-genotypes have been identified from poultry in China in

1998-2013 (20-23, 25, 26), South Korea (2000-2005) (27, 28), Colombia (2006-2010) (24, 29), Israel, South Africa, Ukraine and Venezuela (2004-2009) (10, 24, 30). Viruses of VII-e were identified in China and other Asian countries such as Japan, Taiwan, and Vietnam from chickens and domestic waterfowl during 1997–2014 (11, 21, 25, 31-34). Viruses of VIIf had been isolated from domestic poultry and pigeons in China during 1996-2008 (9, 11, 21, 25). Viruses of VII-g likely all represent recombinant strains (5). This sub-genotype virus was isolated from poultry in China, Nigeria, Indonesia, and Island during 2003-2010. With a few number of isolates, VII-h had been reported in Cambodia, China, Indonesia, Malaysia, Mozambique, and South Africa during 2007-2014 (17, 24, 35). VII-i was another contemporary sub-genotype recently emerged from chickens and pet birds in Indonesia, Israel, and Pakistan since 2010 (36, 37). The increasing genomic diversity of NDV poses several problems for the control and surveillance of ND. In fact, genetically distant NDV strains might have the potential for increased virulence, increased host range, or vaccine escape (38). ND is one of the most serious infections of poultry and is endemic in

Iranian poultry industries. VII-d is currently the dominant genotypes of NDV in Iran, and it is now endemic sub-genotype in this country (12, 39). Overall, our results confirm the presence of genotype VII-g in Iran (The first report), The virulence of the new isolate was confirmed by a sequence of the predicted cleavage site of the F protein, which indicated that these two isolates carried the 112-RRQKRF-117 107 motif corresponding to the cleavage site of virulent NDV.

Accordance to the high percentage of identity of the present isolate (genotype VII-g) to current Chinese isolates (genotype VII-g from Shandong province in 2008 from chickens), they originated from a common ancestor. Owing to pass of flyways of migratory birds over Iran and poor hygienic measures in poultry farms of this country, the interaction between migratory birds and rural chicken might have occurred.

Consequently, recombination was took placed and commer-cial poultry have infected by the new sub-genotype VII-g (40, 41). High identity (95.39%) of nucleotide sequence of F0 proteins between studied isolate and Chinese isolates 2008, suggesting the rapid worldwide expansion of this new sub-genotype in poultry and further isolation of this sub-genotype viruses from the other countries such as Nigeria, Indonesia and Island suggest that viruses of genotype VII-g may have the potential to start a new panzootic (17).

This isolate didn't show a high percentage of identity with previous Iranian isolates which were assumed originated from Russian NDV (42, 43). We first time report the presence of a new NDV sub genotype VII-g circulating in poultry in Iran, which has caused enormous losses in vaccinated poultry as compared to other subgenotypes of a virulent type such as VII-d. More studies about genome sequencing of Iranian ND viruses need to measure relatedness among NDVs from different geographic regions of the world (44).

Overall continuous and accurate monitoring of other species of birds would help to obtain an insight into the evolution of NDVs and control of panzootic viruses in future.

References

- 1. Doyle T. A hithero unrecorded disease of fowls due to a filter-passing virus. J Comp Pathol Therap. 1927;48:1-20.
- 2. Nath B, Barman NN, Kumar S. Molecular characterization of Newcastle disease virus strains isolated from different outbreaks in Northeast India during 2014–15. Microb Pathog. 2016;91:85-91.
- 3. Zhu J, Hu S, Xu H, Liu J, Zhao Z, Wang X, et al. Characterization of virulent Newcastle disease viruses from vaccinated chicken flocks in Eastern China. BMC Vet Res. 2016;12(1):113.
- 4. Ren S, Xie X, Wang Y, Tong L, Gao X, Jia Y, et al. Molecular characterization of a Class I Newcastle disease virus strain isolated from a pigeon in China. Avian Pathol. 2016;45(4):408-17.
- 5. Morla S, Shah M, Kaore M, Kurkure NV, Kumar S. Molecular characterization of genotype XIIIb Newcastle disease virus from central India during 2006–2012: Evidence of its panzootic potential. Microb Pathog. 2016;99:83-6.
- 6. Miller, P.J., E.L. Decanini, and C.L. Afonso, Newcastle disease: evolution of genotypes and the related diagnostic challenges. Infect Genet Evol. 2010;10(1):26-35.
- 7. Diel DG, da Silva LHA, Liu H, Wang Z, Miller P, Afonso CL. Genetic diversity of avian paramy-xovirus type 1: proposal for a unified nomenclature and classification system of Newcastle disease virus genotypes. Infect Genet Evol. 2012;12(8): 1770-79.
- 8. Shabbir MZ, Zohari S, Yaqub T, Nazir J, Shabbir MAB, Mukhtar N, et al. Genetic diversity of Newcastle disease virus in Pakistan: a country-wide perspective. Virol J. 2013;10(1):170.
- 9. Herczeg J, Wehmann E, Bragg RR, Travassos Dias PM, Hadjiev G, Werner O, et al. Two novel genetic groups (VIIb and VIII) responsible for recent Newcastle disease outbreaks in Southern Africa, one (VIIb) of which reached Southern Europe. Arch Virol. 1999; 144(11):2087-99.
- 10. Perozo F, Marcano R, Afonso CL. Biological and phylogenetic characterization of a genotype VII Newcastle disease virus from Venezuela: efficacy of field vaccination. J Clin Microbiol. 2012;50(4):1204-8.
- 11.Yu L, Wang Z, Jiang Y, Chang L, Kwang J. Characterization of newly emerging Newcastle disease virus isolates from the People's Republic of China and Taiwan. J Clin Microbiol. 2001;39(10): 3512-9.
- 12. Hosseini H, Ghalyanchilangeroudi A, Torabi R. Molecular characterization and phylogenetic study

- of Newcastle disease viruses isolated in Iran, 2010–2012. Avian Dis. 2014;58(3):373-376.
- 13. Sohrab, V., Newcastle disease in Iran. Bulletin de L'Office International des Epizooties, 1974;81: 899–903.
- 14. Kant A, Koch G, Van Roozelaar DJ, Balk F, Huurne AT. Differentiation of virulent and non-virulent strains of Newcastle disease virus within 24 hours by polymerase chain reaction. Avian Pathol. 1997;26(4):837-849.
- 15. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7): 1870-4
- 16. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28(10): 2731-9.
- 17. Miller PJ, Haddas R, Simanov L, Lublin A, Rehmani SF, Wajid A, et al. Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features. Infect Genet Evol. 2015;29:216-29.
- 18. Lomniczi B, Wehmann E, Herczeng J, Ballagi-Pordany A, Kaleta EF, Werner O, et al., Newcastle disease outbreaks in recent years in western Europe were caused by an old (VI) and a novel genotype (VII). Arch Virol. 1998;143(1):49-64.
- 19. Duan Z, Ji X, Xu H, Zhao J, Ruan Y, Chen J. Identification of a genotype VIId Newcastle disease virus isolated from Sansui Sheldrake ducks in Guizhou Province, China. Genome Announc. 2015; 3(2):e00161-15.
- 20. Liu HL, Wang YK, Zhu GQ. Studies on biological characterization of fusion gene of chicken's paramyxovirus. Zhongguo Jiaqi. 2002; 23:119–122.
- 21. Liu X, Wan HQ, Ni XX, Wu YT, Liu WB. Pathotypical and genotypical characterization of strains of Newcastle disease virus isolated from outbreaks in chicken and goose flocks in some regions of China during 1985–2001. Arch Virol. 2003;148(7):1387-1403.
- 22. Rui Z, Juan P, Jingling S, Jixun Z, Xiaoting W, Shouping Z, et al., Phylogenetic characterization of Newcastle disease virus isolated in the mainland of China during 2001–2009. Vet Microbiol. 2010; 141 (3-4):246-257.
- 23. Zhang YY, Shao MY, Yu XH, Zhao J, Zhang GZ. Molecular characterization of chicken-derived genotype VIId Newcastle disease virus isolates in China during 2005–2012 reveals a new length in hemagglutinin–neuraminidase. Infect Genet Evol. 2014;21:359-366.

- 24. Benson DA, Clark K, Karsch-Mizrachi I, Lipman Dj, Ostell J, Sayers EW. GenBank. Nucleic Acids Res. 2014;42(D1):D32-37.
- 25. Wan H, Wu Y, Liu X, Zhang R. Sequence analysis of the fusion (F) protein genes of four Newcastle disease virus strains causing clinical disease in geese. Wei sheng wu xue bao= Acta microbiologica Sinica. 2002; 42(2):208-213.
- 26. Wu S, Wang W, Yao C, Wang X, Hu S, Cao J, et al. Genetic diversity of Newcastle disease viruses isolated from domestic poultry species in Eastern China during 2005–2008. Arch Virol. 2011;156(2): 253-261.
- 27. Cho SH, Kwon HJ, Kim TE, Kim JH, Yoo HS, Kim SJ. Variation of a Newcastle disease virus hemagglutinin-neuraminidase linear epitope. J Clin Microbiol. 2008:46(4):1541-1544.
- 28.Suarez D, et al. Newcastle Diseases, Other Avian Paramyxoviruses, and Avian Metapneumovirus Infections. Iowa. 2013, John Wiley & Sons, Inc.
- 29. Hines NL, Killian ML, Pedersen JC, Reising MM, Mosos NA, Mathieu-Benson C, et al. An rRT-PCR assay to detect the matrix gene of a broad range of avian paramyxovirus serotype-1 strains. Avian Dis. 2012; 56(2):387-395.
- 30. Dortmans J, Venema-Kemper S, Peeters BPH, Koch G. Field vaccinated chickens with low antibody titres show equally insufficient protection against matching and non-matching genotypes of virulent Newcastle disease virus. Vet Microbiol. 2014;172(1-2):100-107.
- 31. Chen XQ,.Li ZB, Hu GX, Gu SZ, Zhang S, Ying Y, Gao FS. Isolation, Identification, and Sequencing of a Goose-Derived Newcastle Disease Virus and Determination of Its Pathogenicity. Avian Dis. 2015;59(2):235-243.
- 32. Susta L, Miller PJ, Afonso Cl, Brown CC. Clinicopathological characterization in poultry of three strains of Newcastle disease virus isolated from recent outbreaks. Vet Pathol. 2011;48(2):349-360
- 33. TANG, Xf, et al. Cloning and sequencing of fusion protein gene of Newcastle disease virus isolates in Guangxi [J]. Chin J Vet Sci Technol. 2005;5:001.
- 34. Umali DV, Ito H, Suzuki T, Shirota K, Katoh H, Ito T. Molecular epidemiology of Newcastle disease virus isolates from vaccinated commercial poultry farms in non-epidemic areas of Japan. Virol J. 2013:10(1):330.
- 35. Xiao S, Paldurai A, Nayak B, Samuel A, Bharoto EE, Prajitno TY, et al. Complete genome sequences of Newcastle disease virus strains

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- circulating in chicken populations of Indonesia. J Virol. 2012;86(10):5969-5970.
- 36. Dimitrov, K.M., et al., Temporal, geographic, and host distribution of avian paramyxovirus 1 (Newcastle disease virus). Infection, genetics and evolution, 2016. 39: p. 22-34.
- 37. Xue C, Cong Y, Yin R, Sun Y, Ding C, Yu S, et al. Genetic diversity of the genotype VII Newcastle disease virus: identification of a novel VIIj sub-genotype. Virus Genes. 2017;53(1): 63-70
- 38. Susta L, Jones MEB, Cattoli G, Cardenas-Garcia S, Miller PJ, Brown CC, et al., Pathologic characterization of genotypes XIV and XVII Newcastle disease viruses and efficacy of classical vaccination on specific pathogen-free birds. Vet Pathol. 2015;52(1):120-131.
- 39. Boroomand Z, Jafari RA, Mayahi M. Molecular characterization and phylogenetic study of the fusion genes of Newcastle disease virus from the recent outbreaks in Ahvaz, Iran. Virus Dis. 2016; 27(1):102-105.

- 40. Kelleher C, Halvorson DA, Newman J, Senne DA. Isolation of avian paramyxoviruses from sentinel ducks and turkeys in Minnesota. Avian Dis. 1985;29(2):400-407.
- 41. Spalatin J, Hanson R. Epizootiology of Newcastle disease in waterfowl. Avian Dis. 1975;19(3): 573-582.
- 42. Fathi E, Nourbakhsh SA, Jafarian M. Phylogenetic analysis and characterization of Newcastle disease virus by molecular methods in Chaharmahal-e- Bakhtiari province. Iran Vet J. 2008; 5: 50–55.
- 43. Kianizadeh M, Aini I, Omar AR, Yusoff K, Sahrabadi M, Kargar R. Sequence and phylogenetic analysis of the fusion protein cleavage site of Newcastle disease virus field isolates from Iran. Acta Virol. 2002;46(4):247-251.
- 44. Conan A, Goutard FL, Sorn S, Vong S. Biosecurity measures for backyard poultry in developing countries: a systematic review. BMC Vet Res. 2012;8(1):240.