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Molecular identification of reovirus in broiler type flocks in Golestan province, Iran

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Summary

Background: Avian reovirus (ARV) has a global distribution in nature and most clinical signs are found in broiler type chickens. **Aims:** This study was conducted to detect and identify reovirus infections from vaccinated breeder chickens and their progenies. **Methods:** A total of 20 tissue and blood samples were collected from vaccinated broiler breeders and their progenies with gastrointestinal or performance problems during peak production. Antibody titers were measured by indirect enzyme-linked immunosorbent assay (ELISA) tests. RNA extraction from tissue samples was performed and cDNA was prepared and directly used in the polymerase chain reaction (PCR). Nucleotide sequences were bilaterally determined using internal primers. The analysis of the nucleotide sequences and their related amino acids was performed by the specialized Molecular Evolutionary Genetics Analysis software (6th version). **Results:** The virus variant was detected in two vaccinated broiler breeders and five broiler flocks. The vaccine strains in breeder flocks included S1133, SS412, 1733, 2408 belonging to genotype 1 from the reovirus phylogenetic tree. Sequence 7 from the isolated reovirus based on the σC revealed that they were different from the reovirus vaccine, and that 6 isolates belonged to genotype 1 of the phylogenetic tree while 1 isolate belonged to branch 4 of the phylogenetic tree. **Conclusion:** The study showed that the new reovirus strain isolated from vaccinated birds differs from common strains used in the vaccines. It is therefore essential to prevent the effects of the field reovirus on the performance of industrial poultry, by updating and inventing new commercial vaccines, live and killed, against the reovirus.

Key words: Broiler, Broiler breeder, Phylogenetic tree, Reovirus, σC protein

Introduction

Avian reovirus (ARV) has a global release in nature and creates a wide range of diseases in different bird species like poultry, pheasants, turkeys, ducks, geese, pigeons, quails, hunting birds, and parrots (Jones and Swane, 2013; Lu, 2015). Avian reoviruses belong to Orthoreovirus genus of the Reoviridae family with 10 segments of double-stranded RNA (dsRNA) without any covers, all of which being classified according to their electrophoretic stimulation into three large (LI-L3), medium (M1-M3) or small (S1-S4) groups (Mcnulty *et al*., 2008; Jones, 2009; Jones and Swane, 2013). The virus size ranges from 4/0 to 5/2 kbp (Tang and Huaguang, 2015) and the segmented genome includes eight structural proteins (λA, λB, λC, μA, μB, σA, σB, σC) and four non-structural proteins (μNS, P10, P17, σNS) (Tang and Huaguang, 2015). The virus has 6 branches in the phylogenetic tree (Huang *et al*., 2015). Most clinical signs are observed in broiler poultry and breeder chickens (Lu, 2015). Reovirus infections in domestic birds have numerous noticeable destructive economic effects. Clinical signs in young chickens include tenosynovitis, malabsorption syndrome, runting-stunting syndrome (RSS), digestive problems, immune system suppression, and secondary infections caused by a bacterium or a virus (Jones and Swane, 2013; Lu, 2015). Simultaneous infection with other pathogens such as mycoplasma sinwellia leads to severe immune deficiency, faintness, weight loss, egg production reduction, and more particularly, the creation of slaughterhouse waste (Senties-Cue *et al*., 2005; Reck, 2013). The findings indicate that the σC protein encoded by genomic S1 is a cell binding agent and the main determinant of the antigenicity of ARVs (Hoseini *et al*., 2015; Lu, 2015). Genomic S1 segment in the current ARVs strain has been well identified and evaluated in poultry viruses (Spackman *et al*., 2005; Mcnulty *et al*., 2008). Avian reoviruses are usually based on molecular functions especially the nucleotide and the amino acid sequence determination of the σ protein (Jones and Swane, 2013; Hoseini *et al*., 2015). Recent studies based on the phylogenetic analysis of the σC immunogenic protein indicate many varieties that are different from the current cluster of ARVs (Kant *et al*., 2003). New isolates seem to be distinct from conventional vaccine strains (Kant *et al*., 2003; Hoseini *et al*., 2015). Recent studies in Australia showed that there is a potential for genetic reassortment when the cell is infected with more than one reovirus, hence the emergence of new variants. Reoviruses are found everywhere and a large number of the isolates (over 80%) are non-pathogens (Rosenberger

et al., 1989). Horizontal transmission of the virus is one of the main ways of infecting birds (Robertson *et al*., 1989; Mcnulty *et al*., 2008; Jones and Swane, 2013). Horizontal transmissions are often carried out through faecal contamination and can also be transmitted through the respiratory tract. Reoviruses can also be transmitted vertically (Mcnulty *et al*., 2008; Jones and Swane, 2013). Vaccination of breeder flocks is essential to transfer breeder antibodies to the progenies and protect the birds from infection (Kant *et al*., 2003). However, breeder vaccination will be effective in protecting the progeny when there is a homologue between the strains of the vaccine and the virus circulating in the fields. There have been recent reports of reovirus infections despite the vaccination of breeder flocks and their progeny (Jones, 2009; Troxler, 2013; Hoseini *et al*., 2015; Mayahi *et al*., 2015). The present study was conducted to identify possible reoviruses in vaccinated breeder chicken flocks and their progenies.

Materials and Methods

Sampling

Initially, a history of target broiler breeder flocks from the Golestan province was gathered including information about location, capacity, age, flock performance, reovirus infection signs and vaccination programs against reovirus. This information was then documented in a work sheet (Hoseini *et al*., 2015). During peak production season, 5 to 10 cm sections of duodenum, jejunum, ileum, and rectum of 10 birds of each of the 20 reovirus-vaccinated broiler breeder flocks with digestive disorders or performance problems were collected, and placed in a container consisting of saline solution. These samples were then slowly mixed and numbered as one sample. After a 30 min centrifuge cycle, the supernatant was separated, transferred to a test tube and immediately sent to the laboratory on dry ice. In flocks with lameness symptoms, samples was taken from the tibiotarsal joint and gastrocnemius and finger retraction muscle tendons, all pooled and numbered as one sample (Table 1). In addition, blood samples were collected from the wing vein of the 20 birds of each breeder broiler flock. Twenty broiler farms that raised chicks from the same broiler breeder flocks were investigated at the age of about three weeks, for signs of gastrointestinal, enteritis problems, growth retardation or limb problems. Blood samples from the wing vein and

Table 1: Breeder flock features and vaccination programs

Number	Breeder, hybrid flock codes	Site of flocks	Vaccination program	Age of sampling (week)	
				Breeder flocks	Progeny
1	1-Ross 308	Golestan	A live vaccine (merial) at 12 days and two killed vaccines (ceva) at weeks 12 and 20	35	2
2	3-Hubbardf15	Golestan	A live vaccine (MSD) at 11 days and a killed vaccine (MSD) at week 20	37	3
3	5-Ross 308	Golestan	A live vaccine (MSD) at 11 days and a killed vaccine (MSD) at week 20	33	4
4	6-Ross 308	Golestan	A live vaccine (MSD) at 12 days and a killed vaccine (ceva) at week 20	38	2
5	9-Arbor acres	Golestan	A live vaccine (merial) at 35 days and a killed vaccine (MSD) at week 20	40	2
6	11-Arbor acres	Golestan	A live vaccine (MSD) at 30 days and a killed vaccine (MSD) at week 20	33	2
7	13-Ross 308	Golestan	Two live vaccines (merial) at week 1 and 10 and two killed vaccine (ceva) at weeks 7 and 18	32	3
8	15-Ross 308	Golestan	Two live vaccines (merial) at 7 and 27 days and a killed vaccine (MSD) at week 18	35	3
9	17-Hubbardf15	Golestan	Two live vaccines (merial) at10 and 35 days and a killed vaccine (ceva) at week 20	28	3
10	19-Ross 308	Golestan	A live vaccine (merial) at 12 days and two killed vaccines (MSD&ceva) at weeks 12 and 20	35	3
11	21-Ross 308	Mazandaran	A live vaccine (MSD) at 12 days and a killed vaccine (MSD) at week 20	36	2
12	23-Ross 308	Golestan	Two live vaccines (merial) at 32 days and week 21 and a killed vaccine (ceva) at week 20	29	3
13	25-Ross 308	Golestan	A live vaccine (merial) at 12 days and a killed vaccine (MSD) at week 20	36	2
14	26-Arbor acres	Golestan	Two live vaccines (merial) at week 4 and 8 and a killed vaccine (ceva) at week12	34	3
15	27-Ross 308	Golestan	A live vaccine (MSD) at 41 days and a killed vaccine (ceva) at week 20	30	2
16	29-Hubbardf15	Golestan	Two live vaccines (merial) at 19 days and week 10 and a killed vaccine (Biomune) at week 17	33	3
17	32-Ross 308	Mazandaran	A live vaccine (MSD) at 12 days and two killed vaccines (MSD&ceva) at weeks 12 and 20	40	2
18	33-Ross 308	Mazandaran	A live vaccine (merial) at 12 days and two killed vaccines (ceva) at weeks 7 and 18	36	2
19	34-Ross 308	Mazandaran	A live vaccine (merial) at 12 days and two killed vaccines (ceva) at weeks 7 and 18	35	2
20	39-Hubbardf15	Golestan	Two live vaccines (MSD) at 9 and 70 days and two killed vaccines (MSD) at week 5 and 20	30	3

10 intestine samples (pieces of duodenum, jejunum, ileum, and rectum) were collected along with samples from the tibiotarsal joint, gastrocnemius and finger retraction muscle tendons of 20 birds (Hoseini *et al*., 2015).

Experimental method

Serology

The anti-reovirus antibody titer in the blood serum of broiler chickens and their progeny was measured using Biocheck Company's enzyme-linked immunosorbent assay (ELISA) kit.

Autopsy

Birds with clinical signs were euthanized and autopsy was carried out to take tissue samples from joints, tendons, and the gastrointestinal tract (Troxler, 2013).

RT-PCR

Identification of the reovirus

RNA extraction: Homogeneous tissue samples were centrifuged at 4°C and 3000 rpm for 10 min. The supernatant solution of the intestinal contents was transferred to a microtube for RNA extraction. The RNA extraction from samples was performed using the RNX-plus solution (CinnaGen, Tehran, Iran) according to the manufacturer's protocol. Isolated RNAs were either used immediately for the reverse transcription-polymerase chain reaction (RT-PCR) or stored at -80°C.

Synthesis of cDNA (RT reaction)

After extracting RNA for cDNA synthesis, the Random Hexamer Primer and Canadian Revert aid first stand cDNA Synthesis kit were used. These primers are attached to the RNA nonspecifically and as a result, the entire RNA was converted to cDNA (Troxler, 2013).

The cDNA was prepared and used in the PCR reaction immediately. In order to run the PCR reaction, two pairs of primers were used as Nested-PCR. These primers are specific to the *S1* gene of reovirus and allow the detection of reovirus with high sensitivity from clinical specimens (Hoseini *et al*., 2015). The primer's sequence was as follows: S1C 5´ ATT GAA TTC TCT CTG TTA TCT AAC CTTG3´738 bp, S1D 5´AAG GAA TTC GTT GAG AAC AGA AGT AGG3´738 bp, S1E 5´TCT GAA TTC ATC CGC AGC GAA GAG AGG TG3´324 bp and S1F 5´AGT GAA TTC AGT ATC GCC GTG CGC AG3´ 342 bp (Tang and Huaguang, 2015). In all cases, positive and negative controls were used simultaneously.

The master mix used contained 2 µL PCR buffer 10X, 1 µL $MgCl_2$ (50 mM/µL), 0.25 µL dNTPs (10 mM/µL), 0.25 µL of each 10 mM/µL primer, 6 µL cDNA, 10 µL distilled water, and the final addition of 0.25 µL Taq DNA polymerase (5 IU/µL). The thermal programs were similar to those presented in Table 2. Finally, the PCR products were separated in 2% agarose gel containing safe stain (CinnaGen, Tehran, Iran), using an ultraviolet trans-illuminator.

Table 2: Reverse transcription-polymerase chain reaction thermal program of reovirus

Phase	Temperature (°C)	Time
Early denaturation	94	3 min
Denaturation	94	10 s
Annealing	53	15 s
Elongation	72	20 s
Final elongation	72	15 min

Determination of nucleotide sequences and phylogenetic analysis

In order to determine the sequence, PCR products of the identified positive samples were purified using a Roche commercial kit. The determination of nucleotide sequences using internal primers was bilaterally conducted by BioNeer Co., South Korea. Analysis of the nucleotide sequences and their related amino acids was conducted using the specialized Molecular Evolutionary Genetics Analysis Software (6th version).

Nucleotide sequences of the *S1* gene of these isolates were compared with each other and with the number of different reoviruses in the GeneBank. Multiple alignment was then performed using the CLUSTAL W method for all nucleotide sequences. The nucleotide sequences were then evaluated based on the codes equivalent to the corresponding amino acids. The amino acid sequences were then investigated along with those of the nucleotides. Phylogenetic analysis based on the nucleotide sequence and the amino acid was performed by the neighbor joining (NJ) method using the Tamura-NEI model. The validity and accuracy of the phylogenetic tree were then evaluated using the bootstrapping method and 1000 replications. All analyses were performed with the Pro Molecular Evolutionary Genetics Analysis software (6th version).

Results

Despite being vaccinated, some clinical symptoms like lameness and gastrointestinal problems were observed in the broiler breeder flocks. Moreover, through a molecular study and PCR test, in two cases of broiler breeder flocks (codes 13 and 29), positive reovirus variants were reported. In 25% of the broiler flocks (codes 4, 7, 14, 30, and 40) originating from the broiler breeder flocks vaccinated against reovirus, the PCR test was positive for the virus variant (Table 3, Fig. 1). In reovirus-positive flocks, clinical symptoms like lameness and lack of growth were observed and in the autopsy, swelling and rupture of gastrocnemius tendons were found. The sequence of the isolates of code samples (4, 14, 29, 30, and 40) was determined and classified in the phylogenetic tree (Figs. 2 and 3). Samples 7 and 1 were related to the gastrointestinal tract and tendon, respectively. Table 4 shows the degree of similarity of the isolates of this study with the vaccine strain.

In the serologic test, the highest antibodies titer were measured in the breeder flocks which received live and killed vaccines twice. In the progeny, the maternal

Table 3: ELISA test and RT-PCR results

Number	Breeder, hybrid flock codes	Tissue sample	Clinical features	PCR result	Mean Ab titre	CV (%)	Broiler flock codes	Tissue sample	PCR result	MAS/VA	Mean Ab titre	CV (%)
1	1-Ross 308	Tendon & gut	MAS/VA	-/-	8321	38	10	gut	-	MAS	2496	46
2	3-Hubbardf15	Tendon & gut	MAS/VA	-/-	7739	27	2	Tendon & gut	-/-	MAS/VA	3224	51
3	5-Ross 308	Tendon & gut	MAS/VA	-/-	7500	45	4	Tendon & gut	-/+	MAS/VA	6016	32
4	6-Ross 308	Tendon & gut	MAS/VA	-/-	10700	23	20	gut	-	MAS	2996	41
5	9-Arbor acres	Tendon & gut	MAS/VA	-/-	8300	34	7	gut	+	MAS	16709	110
6	11-Arbor acres	Tendon & gut	MAS/VA	-/-	22352	32	12	Tendon & gut	-/-	MAS/VA	3751	36
7	13-Ross 308	gut	MAS/VA	+	9707	29	14	Tendon & gut	-/+	MAS/VA	2890	63
8	15-Ross 308	Tendon	MAS/VA	-	8420	32	16	gut	-	MAS	2216	91
9	17-Hubbardf15	Tendon & gut	MAS/VA	-/-	9638	48	18	gut	-	MAS	2641	30
10	19-Ross 308	Tendon & gut	MAS/VA	-/-	11643	33	22	Tendon & gut	-/-	MAS/VA	7034	47
11	21-Ross 308	Tendon & gut	MAS/VA	-/-	14845	47	8	gut	-	MAS	2924	31
12	23-Ross 308	Tendon & gut	MAS/VA	-/-	11906	35	24	Tendon & gut	-/-	MAS/VA	5988	74
13	25-Ross 308	Tendon	VA	-	7120	40	38	gut	-	MAS	270	91
14	26-Arbor acres	Tendon & gut	MAS/VA	-/-	10954	38	28	gut	-	MAS	1971	44
15	27-Ross 308	gut	MAS	-	13255	33	31	Tendon & gut	-/-	MAS/VA	3438	56
16	29-Hubbard f15	Tendon & gut	MAS/VA	-/+	6854	35	30	gut	+	MAS	4220	50
17	32-Ross 308	Tendon & gut	MAS/VA	-/-	10086	44	35	gut	-	MAS	1561	59
18	33-Ross 308	Tendon & gut	MAS/VA	-/-	9611	38	36	gut	-	MAS	2607	60
19	34-Ross 308	Tendon & gut	MAS/VA	-/-	3065	45	37	gut	-	MAS	2240	56
20	39-Hubbard f15	Tendon & gut	MAS/VA	-/-	6481	44	40	Tendon & gut	+/+	MAS/VA	512	128

The sample related to the gastrointestinal tract and malabsorption (MAS: Mal absorption syndrome), the sample related to joint problems (VA: Viral arthritis), positive sample (+), negative sample (-), first or second positive or negative sample (+/-), Coefficient of variation: CV, and Ab: Antibody

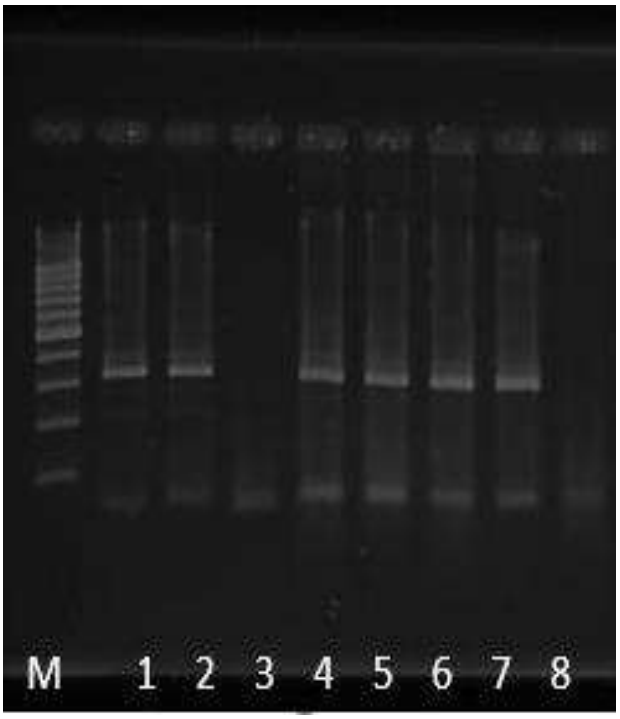

Fig. 1: The 324 base pair RT-PCR products. M: DNA marker (100 bp), Lines 1-2 and 4-6: Positive samples, Line 3: Negative sample, No. 7: Positive control, and Line 8: Negative controls

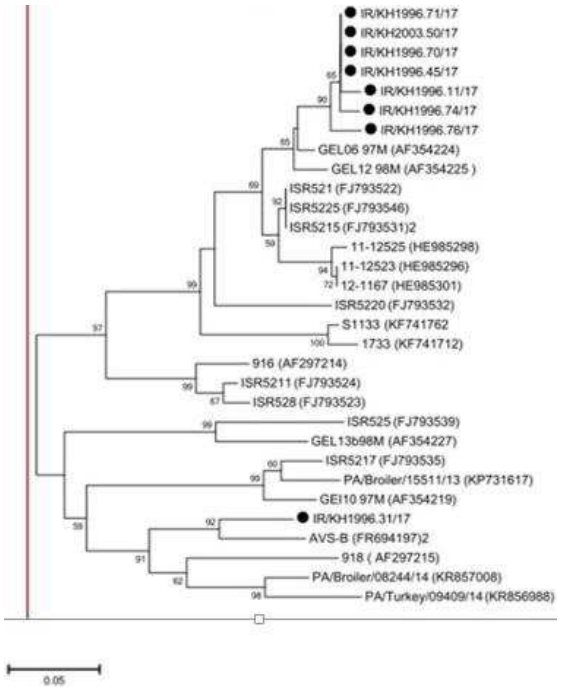

Fig. 2: The phylogenetic tree based on the sequence of amino acids in Iranian poultry along with isolates of the poultry virus whose sequences existed in the GeneBank. The isolates related to the present study are marked with a bold circle

Table 4: Similarity of this study's isolates with vaccine strains based on amino-acid sequences

Number	Strain	Accession number	% Identity with vaccine strain S1133	Phylogeny phylum	Farm codes
1	IR/kH1996.71/17	MG922798	81	1	40
2	IR/KH2003.50/17	MG922799	81	1	40
3	IR/KH1996.70/17	MG922800	81	1	29
4	IR/KH1996.45/17	MG922801	81	1	29
5	IR/KH1996.11/17	MG922802	81	1	4
6	IR/KH1996.74/17	MG922803	79.5	1	29
7	IRK/H1996.76/17	MG922804	81	1	30
8	IR/H1996.31/17	MG922805	57.5	4	14

IR is related to the isolated country, code 1996 belongs to gastrointestinal samples, code 2003 belongs to tendon samples, numbers are related to the samples, and 17 is related to the year of isolation

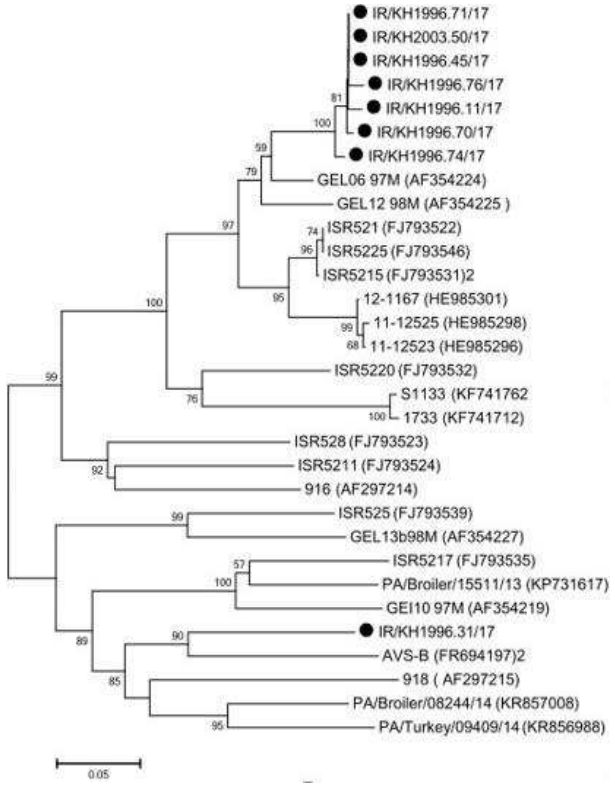

Fig. 3: The phylogenetic tree was based on the sequence of amino acids in Iranian poultry along with isolates of the poultry virus whose sequences existed in the GeneBank. Branch spacing represents the degree of sequence difference. The isolates related to the present study are marked by a bold circle

antibody derived from the breeder flock was detectable. In the infected flocks, however, the level of antibody in the ELISA test increased (Table 3).

Discussion

Evaluation of lameness and growth retardation in broiler flocks showed a high prevalence of reovirus in industrial poultry farms (Mayahi *et al*., 2015). In the past, good protection was provided by vaccination in the breeder flocks due to the similarity of the vaccine virus and the circulating virus. In the broiler flocks, on the other hand, this protection was due to breeder antibody transmission. Recently, however, genetic changes in the virus have reduced vaccine effectiveness. The *σC* gene is the main cause of cellular connection and the induction of neutralizing antibodies and is considered to be the variable segment of the virus. The most important way to control the activity of the virus in poultry farms is to vaccinate the breeder flocks with an effective vaccine to protect the vertical transmission of the virus as well as the progeny through the breeder's antibody against the circulating virus. The sequencing of the *σC* gene of S1 segment of reovirus helps differentiate the reovirus vaccine virus from the circulating reovirus virus. The present study was hence conducted to identify possible reoviruses in the vaccinated breeder flocks and their progenies. Bokaie *et al*. (2008) investigated the seroprevalence of reovirus infections in broiler chicken flocks of Tehran on 582 serum broiler chickens originating from non-vaccinated breeders using the ELISA test. Of the total samples, 572 serum samples were positive with a serum prevalence of 98.3% (Spackman *et al*., 2005). Hedayati and Shojadoust (2012) examined the reovirus of tenosinovitis in birds from Iranian breeder flocks using RT-PCR and restriction fragment length polymorphism (RFLP) methods. The analysis of the segments obtained from the enzymatic digestion of PCR products in all positive samples indicated that the enzymatic digestion patterns of the samples were consistent with the standard digestive pattern of each S1133 vaccine. Mayahi *et al*. (2015) reported the occurrence of reovirus in broiler chicks originating from vaccinated broiler breeders in Golestan province. According to their clinical study, signs of lameness were detected from the second week in a number of rooster chicks of broiler farms, and the presence of reovirus in the gastrointestinal tract and gastrocnemius tendons of the infected chicks was confirmed using RT-PCR. In the present study, the investigation of the phylogenetic tree based on the σC segment showed that most isolates belonged to branch one, and one isolate belonged to branch 4 of the tree. Vaccine strains such as S1133 and 1733 belonged to branch one of the phylogenetic tree, but the isolates studied in this research were different from the vaccine strains. Most closely related to the isolates were German GEL06 97M and GEL12 98M isolates which were identified in 1997 and 1998 with malabsorption symptoms from branch one of the phylogenetic tree. A single case of one isolate from broiler flocks and growth retardation syndrome belonged to branch 4 and showed the most similarity with the isolate of the American AVS B in branch four of the phylogenetic tree. In the study

conducted by Hoseini *et al*. (2015), cases of tenosynovitis and lameness in broiler flocks were observed despite the vaccination of breeder flocks. In all cases, hock joint inflammation, flexor digital tendon, swelling of the foot and subsequently poor performance of the flocks were observed. In the serological study, all affected flocks were found to have high levels of antibodies in the ELISA test. S1 segment sequences that codify the *σC* gene were examined. After being separated from the first broiler flock in the Ardehal area in central Iran, this isolate was named Ardehal variant. Investigations showed that the Ardehal variant belonged to branch one of the phylogenetic tree but differed from the strains of the vaccine which also belonged to branch one of the phylogenetic tree. Broiler breeder flocks in Iran are protected against reovirus by live and killed reovirus vaccine. Thus, chicks of these breeders are also expected to be protected against reoviruses. However, recent cases of prevalence of the reovirus in broiler flocks indicate that vaccination of breeder flocks with current vaccines is not enough to provide immunity to chicks. Tang *et al*. (2015) reported severe clinical disease and economic loss in Pennsylvanian turkey breeder flocks since 2011. The study of molecular identification of the 114 *cu* gene of field isolates from 2011 to 2014 indicated that the isolated reovirus included only 21.93% of the 114 farm isolates of a genotypic branch (genotypic branch1) such as the vaccine strain (S1133, 1733, 2408). The remaining 78.7% of the strains belonged to a different genotypic branch of the vaccine (Branch 2, 3, 4, 5, and 6) and indicated the emergence of new strains of reovirus. In this report, the emerging genotype six was reported for the first time (Lu, 2015). Troxler (2013) reported a severe economic loss in French broiler breeder farms due to reovirus infection despite routine vaccinations in broiler farms. Clinical signs included lameness, growth retardation, and lack of uniformity in flocks. Observations of autopsy and bacteriological and serologic tests confirmed the presence of tenosynovitis caused by reovirus infections. Moreover, sequencing the *cσ* gene and the phylogenetic evaluation of the RT-PCR product determined the new genotype of the reovirus. The virus was not neutralized by monovalent serums of the vaccinated chickens in the neutralization test. It was concluded that these reovirus isolates were serologically and genetically distinct from conventional reoviruses used in commercial vaccines and could not, therefore, provide protection and prevention against disease. Kort *et al*. (2013) identified, classified, and determined the genotype of the Tunisian strains of bird reovirus using the RT-PCR test, which was performed on both *σC* and *σB* genes, and subsequent RFLP tests to better identify Tunisian isolates. The replicated segment in the RT-PCR test showed 15 Tunisian strains with lengths of 738 and 540 bp for *σC* and *σB*. Using Acil and Msel enzymes in the RFLP test showed that all isolates could be clearly distinct from the vaccine strain. Tang and Huaguang (2015) determined genotypes of the reovirus isolates in Pennsylvania. The reovirus strain (Reo/PA/Broiler/05682 /12) was isolated from broiler breeders suffering from tenosynovitis and severe joint infections, all reovirus genomes being sequenced by RT-PCR and rapid amplification of cDNA ends (RACE) methods. The size of the complete isolated reovirus was 23494 bp and contained C+G 50%. A phylogenetic study of the nucleotide sequence of 10 genome segments in Pennsylvania showed moderate to highly noticeable differences in the nucleotide sequence of the strain used in the reovirus S1133 vaccine and 138 isolates. This genomic data indicated that the new Pennsylvania field strain is a reovirus that produces tenosynovitis and differs from the common strain used in the vaccine.

In the present study of broiler breeder flocks, despite their good performance, some clinical signs such as growth retardation, lameness, and gastrointestinal problems were observed. Vaccinating the broiler breeder flock helped prevent clinical symptoms; however, as shown in codes 13 and 29, both breeder and broiler flocks (codes 14 and 30) showed severe cases of growth retardation. In the molecular investigation of other breeder flocks, the virus was not detected despite clinical signs. In some broiler breeder flocks reovirus was not detected but the virus was detected in their progenies. Despite that the virus was not detected in some broiler breeder flocks, their progenies (codes 4, 7, and 40) showed clinical signs of growth retardation, gastrointestinal problems, and lameness. Environmental contamination seems to play a major role in developing infections in growing broiler flocks and due to the heterologous nature of the some reovirus and vaccine strains, the breeder's antibody does not have the ability to protect and prevent infection.

Comparison of the nucleotide sequence σC of 8 virus isolates with the standard virus suggests the emergence of a variant in the circulating virus that does not show enough vaccine immunity against the reovirus. Genetic rearrangement is a noticeable characteristic of the reovirus (Troxler, 2013), and in the *σC* gene, segment S1 appears more often than other gene segments. In addition to the horizontal transmission of the reovirus that is very common among the flocks, vertical transfer is observed in codes 13, 14 and 29, 30. Regarding the breeder flock (code 13) with the isolate belonging to branch one of reovirus and the broiler flock (code 14) with the isolate belonging to branch four of the reovirus, the possibility of vertical transmission of the virus is rejected. However, regarding the breeder flock (code 29) and the broiler flock (code 30), while both isolates belonged to the same branch, there is a high possibility of the vertical transmission of the reovirus. On the other hand, in cases such as code 7, in spite of the detection of severe lameness in the flock, a segment of the bird's intestine was removed and examined, indicating the traceability of the reovirus in the tissues as well as the body's capability to clear the virus. Moreover, it was shown that the gastrointestinal tract was the main reovirus replication location. This genomic data suggests that the new field strain of reovirus in Iran has been responsible for the development of tenosynovitis and growth retardation problems.

The study showed that the new reovirus strain isolated from vaccinated birds was different from common strains used in the vaccines and that it is essential to prevent the effects of the field reovirus on the performance of industrial poultry by updating and making new commercial vaccines, live and killed, against the reovirus. It is necessary, therefore, to design further experiments to show this.

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Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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