Explor Anim Med Res.
Vol. 13, Issue 2, 2023
DOI: 10.52635/eamr/13.2.277-282

ISSN 2277-470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Published under the CC BY-NC 4.0 license

Short Communication

FALSE LAYER SYNDROME IN A COMMERCIAL LAYER FLOCK ASSOCIATED WITH INFECTIOUS BRONCHITIS VIRUS VARIANT

Hamid Shoushtari[1], Najmeh Motamed[2*]

Received 03 December 2022, revised 19 August 2023

ABSTRACT: False Layer Syndrome is a permanent condition that prevents hens from laying eggs as a consequence of early infectious bronchitis (IB) virus infection. Cystic oviduct associated with the reduced peak of egg production in apparently healthy layer flock was diagnosed. Considering the flock history different viral and bacteriological tests were done. Any evidence of bacterial infection was not found. IB virus (IBV) was detected in caecal tonsil samples, suggesting early IB virus infection, an isolated virus phylogenetically belonging to the Variant 2 genotype. However, it is the first report of a Variant 2 genotype causing cystic oviducts in Iran, complementary studies are needed to investigate the pathogenicity of these viruses for oviduct and their ability to develop cystic oviducts.

Keywords: Layer flock, Infectious Bronchitis, Cystic oviduct, Infection.

The poultry industry suffers from the effects of various viral diseases of the reared birds, and continuous monitoring and vaccination are the weapons used to combat the situation [1, 2, 3]. Many poultry viruses cause a direct effect on some organs and indirect delayed effects in some other systems. From the first report of infectious bronchitis disease in the 1930s by Schalk and Hwan as a respiratory disease, there have been several reports of IB disease outbreaks showing not only respiratory but also other organ involvement such as kidney and reproductive systems [4]. In general, the pathogenicity of the virus in the reproductive system usually leads to decreased egg production and eggshell quality. Occasionally, permanent lesions in the reproductive leads to false layer syndrome. Sevoian and Levine, in 1957 [5] reported sexually mature hens ovulated usually but were unable to lay eggs, suffering from large oviductal cysts and free of any obvious clinical signs. Some birds show waddling or stand upright, but many have normal behavior. The ovaries may appear normal with evidence of recent ovulation and release of yolk in the abdomen, which may lead to secondary egg peritonitis. Normal development of the ovary and cystic oviduct are hypothesized as causes of false layer syndrome [6]. Some Massachusetts (Mass) type, Australia T and QX virus strains were shown to develop false layer syndrome [6,7] and more recently, DMV/1639 strain from Canada since 2011 [8], not only in layers but in some non-layer chickens [9]. It is suggested that the earlier birds are infected with the virus, the more severe damage can be observed in the reproductive system. On the other hand, higher viral loads, especially following imperfect or deficient maternal antibodies in young chickens, are predisposing factors for it. Immuno-suppression and coexisting pathogens should not be ignored [6].

The study

History and sampling

Ten (10) hens from a commercial layer flock were presented at Razi´s poultry disease research and diagnostic clinic, showing dilated abdomen. The flock was in an active phase of production and had apparent healthy conditions. At necropsy, the abdomen was dilated, and very thin to be torn easily (Fig. 1), and

[1]*Department of Poultry Diseases Research and Diagnostics,* [2]*Department of Poultry Vaccine Research and Production, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization, Karaj, Iran.*
**Corresponding author. e-mail: drmotamed95@gmail.com*

serum-like fluid-filled oviducts were observed (Fig. 2). Ovaries had active follicles and, in some birds, egg yolk resulted in egg peritonitis. No macroscopic lesion was found in other internal organs Trachea, liver, spleen, oviduct, kidney, and caecal tonsils were collected.

The trachea, liver, spleen, oviduct, kidney, and caecal tonsils were collected, and divided into two parts for molecular assays and bacterial cultures. The first part of the samples were placed in brain heart infusion broth, incubated at 37 °C for 24 h, and then cultured aerobically in blood agar and MacConkey agar for potential *E. coli* infection. The second part was processed to prepare 20% tissue suspensions. After low-speed centrifugation for 10 minutes, 200µl of the supernatant was inoculated to 9-11 days specific pathogen-free embryonated chicken eggs and incubated at 37ºC for 5-7 days. After 3-5 days, embryonated egg passages dwarfing, and curling of embryos in comparison with normal embryos was observed (Fig. 3A and Fig. 3B).

Molecular investigations

DNA extraction was done by boiling method and allantoic fluids were investigated for *Mycoplasma gallisepticum* and, or *Mycoplasma synoviae* genome by reported primers (Table 1) [10]. duplex nested PCR was set up in two rounds, the first reaction was run by the external primers (pMGAFo/pMGARo; MS1.2Fo/ MS1.2Ro) and thermal cycles began from the denaturation step at 95 °C for 5 min then 35 cycles including 95°C for 45 sec (denaturation), 53ºC for 30 sec (annealing), and 72 °C for 30 sec (elongation) and finished with a final extension step at 72°C for 5 min. Then, the amplified product was subjected to the second round with the inner set of primers (pMGAF1i/pMGAR1i; MS1.2F1i/MS1.2R2i) under the conditions described above except that the annealing temperature was 60°C.

Viral RNA extraction was done on allantoic fluids using a High pure Viral RNA kit (Roche; Germany) according to the manufacturer's instructions. A BioFact™ 2X RT-PCR Pre-Mix kit used for cDNA synthesis. Nested PCR was done by Eppendorf 5333 Mastercycler Thermalcycler instrument, using two primer pairs (Table 1) [11]. The thermal condition for the two partial S1 gene amplifications was: 94°C/2 min, followed by a 35 cycle of 94°C/ 20 sec; 50°C/30 sec; 72°C /30 sec, and finally 72°C /7 min elongation. The PCR product of the first step of nested PCR was used as a template for the second step (nested) of the PCR reaction with the same thermal cycle mentioned above. Final PCR products were electrophoresed on a 1% agarose gel, and positive samples were used for cloning and further processing (Fig. 4). To the genetic and phylogenetic analysis of detected infectious bronchitis amplified DNA products were purified using a High Pure PCR Purification kit (Roche, Germany), sent for sequencing in both directions using above forward and reverse primers (Faza Pajooh, Tehran, Iran). Obtained chromatograms and sequences were blasted with those on the NCBI, phylogenetic tree (Fig. 5) was constructed after the alignment of sequences using the MEGA.X version.11 software using the suggested method of Valastro *et al.* [12].

Results and discussion

All tissue samples were negative for *E. coli*, *M. gallisepticum*, and *M. synoviea*, only cecal tonsils were positive for infectious bronchitis virus. The virus was phylogenetically related to the IS-1494-like (Variant 2) genotype based on S1 gene sequencing [11]. Early exposure of day-old chickens to IB viruses when vaccinations are inappropriately done or by an IBV strain that is antigenically different from and has no cross-protection with field virus, leads to severe IBV infection and damage to the oviduct. So the virus cannot be detected in cystic oviducts several weeks after infection [8, 13]. Ovaries are affected and hyperemic just in the acute phase of IBV infection, and after a few weeks, they will recover [9]. Some bacteria and mycoplasma spp. infections in chickens cause pathological changes in the oviduct, but they are usually associated with fibrin-necrotic or caseous exudates in the abdomen, air sacs, or other systemic involvements such as pericarditis or perihepatitis, emaciation, enlarged liver or spleen [19]. QX-like genotypes, which were detected in the middle of the 1990s showed more tendency or tropism to the genital system mainly, when the infection occurred early in chicken life, less than three weeks [6, 14, 15]. The severity of lesions in a reproductive system depends on the virus strain, lay period, and host-specific factors. IB virus tropism for epithelial cells especially ciliated epithelial surfaces such as the respiratory and reproductive system leads to excess goblet cell secretion and fluid accumulation in cystic oviduct. Goblet cells are responsible for egg albumin secretion in healthy hens, which are altered in IB infection with a serum-like fluid secretion [16]. So far, only a few Mass types, Australia T, and most recently Delmarva (DMV/1639) strains were reported to be the primary cause of developing cystic oviduct and FLS [15, 17].

Table 1. Oligonucleotide sequences used in this study.

Primers	Primers 5´-3´	PCR product size/Reference	Target region	Reference
pMGAFo pMGARo	GTG AAG AAA AAA AAC ATA TTA AAG TTTCTA AGA TGG ATT TGA AAC ATT AGTCTA GTT AAT ACT AGT	1900	pMGA1.2	
pMGAF1i pMGAR1i	GAT CAA GTG AAA CTA TTG AAC ATT GTT CTT TGG AAC CAT CAT	500		[10]
MS1.2Fo MS1.2Ro	AAA CTA CAA AAC TTT GTA ATG GCT TTA CAA GTA CGG TGT TTA ATC AAT	1200	MS2/12	
MS1.2F1i MS1.2R2i	ATT ACC AAG CAG ATG GTT ACG ACG T AGT TAT AGT AAC TCC GTT TGT TCC A	450		[10]
SX1-SX2	CACCTAGAGGTTTGCTA/T GCAT TCCACCTCTATAAACACCC/T TT	490 bp	S1 gene	
SX3-SX4	TAATACTGG C/TAATTTTTCAGA AATACAGATTGCTTACAACCAC	390 bp		[11]

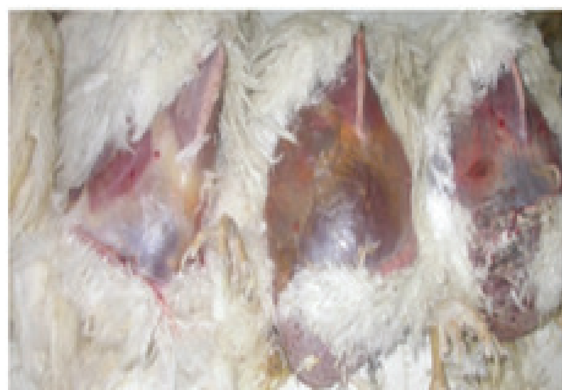

Fig. 1. Dilated abdomen of dead birds.

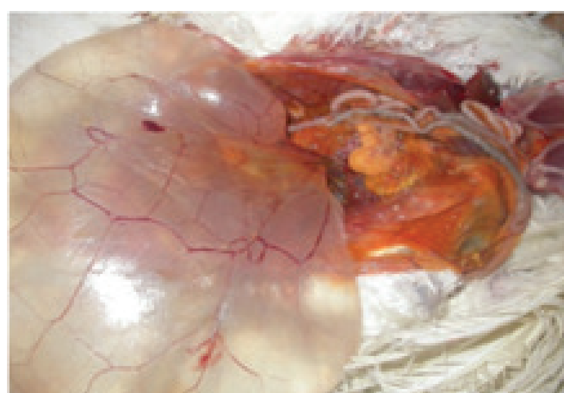

Fig. 2. Serum-like fluid filled oviduct.

Fig. 3. Dawrafing, retarded growth and curling of IBV infected emberyos in comparision with normal-uninfected emberyos [(A) Infected embryos, (B) Normal Embryos].

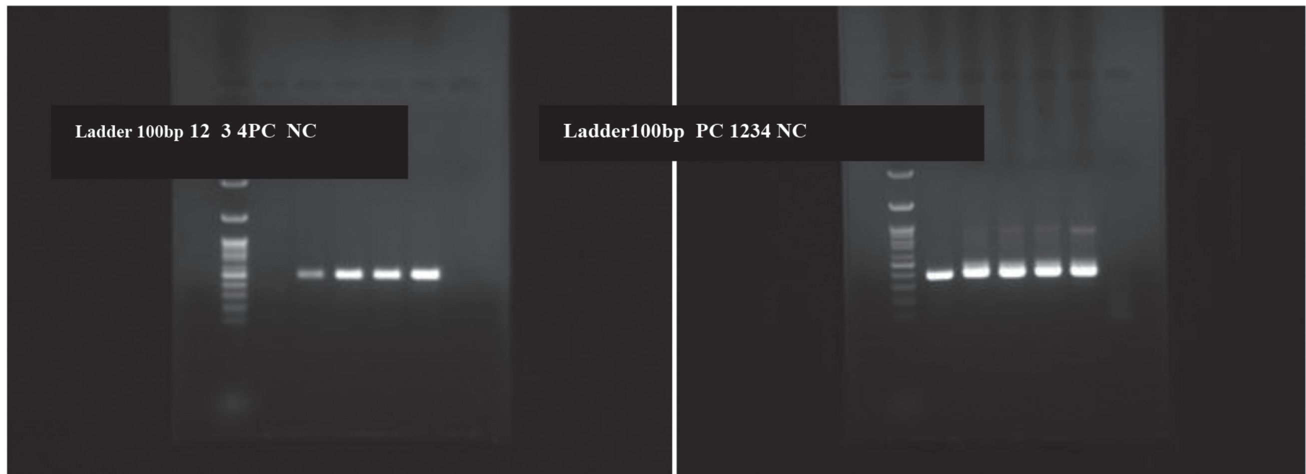

Fig. 4. Results of Nested PCR targeting IBV partial S1 gene-left picture is Sx1-Sx2 and the right picture is Nested PCR. [(100bp ladder; samples are specified as numbers 1,2,3 and 4; Positive control (PC) and Negative control (NC)].

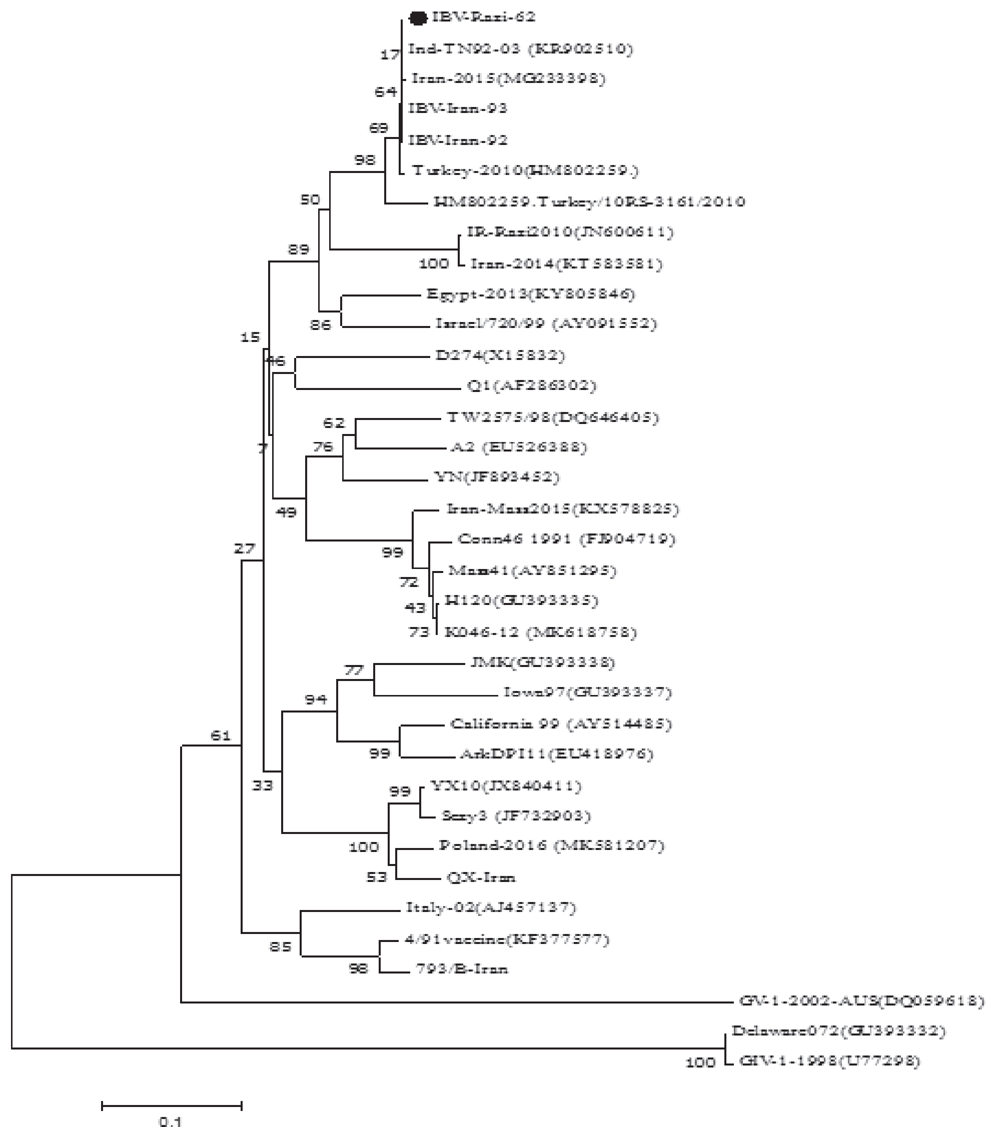

Fig. 5. Phylogenetic analysis of S1 gene of IBV isolate with other sequences from the NCBI. [Isolate of this study is indicated by black circles. The phylogenetic tree was constructed by the Neighbor-joining statistical method and Maximum Composite Likelihood model with Bootstrap 1,000 using MEGA 7 software].

Variant 2 (IS-1494-like) viruses were described in 2010 as a nephron-pathogenic strain in Iran, but an association of the genotype with cystic oviduct has been reported recently in a layer flock in Turkey [11, 18], regarding the high mutation and recombination capacity of the IBV. However, IBV is conventionally classified by the S1 gene sequence, but other viruses´ genes may affect its pathogenicity and tissue tropism; thereupon, we may have the same strain, but different pathogenicity or tissue tropism [6], *e.g.*, DMV/1639 was first time reported as a nephron-pathogenic strain for broilers in Delmarva USA but today it is one of major causes of false layer syndrome in Canadian poultry flocks [17].

Conclusion

This study is the first case study reporting a Variant 2 genotype virus detection associated with false layer syndrome in commercial poultry flocks in Iran. Because IBV is continuously evolving genetically and pathogenically, it is not so unusual to see new distinct properties or features related to a specific strain far from the original virus, such as causing cystic oviduct complications. Additionally, improper vaccination, vaccination with a heterologous genotype with less protective and immunosuppressive conditions, should be considered as stimulating factors for the evolution of the virus by mutation and recombination, leading to raising new viruses with different tissue tropism and pathogenicity even without genotype changing.

ACKNOWLEDGEMENT

The authors would like to acknowledge the kind and special support of our colleagues in the poultry disease diagnostics department of Razi Institute, Iran.

REFERENCES

1. Mousa M, Mohammed F, El-Deeb A, Amer F, Ahmed K. Pathological monitoring of visceral lesions induced by velogenic Newcastle disease in experimentally infected broiler chickens at different ages with special reference to pulmonary inflammatory cytokines expression. Explor Anim Med Res. 2021;11(1), DOI: 10.52635/eamr/11.1.67-79.

2. Parthiban SKJ, Ramesh A, Vidhya M, Rajalakshmi S, Rajasekaran R, Thangavelu A. Pathotyping of Newcastle disease virus by mean death time and Real-time PCR assay: an empirical comparison. Explor Anim Med Res. 2022; 12(1), DOI: 10.52635/eamr/12.1.69-73.

3. Mahapatra CS, Dhar P (2021) Characterization of IVRI-CSF-BS cell culture adapted vaccine strain for Newcastle disease virus exaltation (END) and correlation of virus titre by END method with FAT. Explor Anim Med Res 11(1), DOI: 10.52635/eamr/11.1.24-29.

4. Najimudeen MS, Barboza-Solis C, Ali A, Buharideen SM, Isham MI *et al.* Pathogenesis and host responses in lungs and kidneys following Canadian 4/91 infectious bronchitis virus (IBV) infection in chickens. Virology. 2022; 566: 75-88, DOI: 10.1016/j.virol.2021.11.013.

5. Muneer MA, Halvorson DA, Sivanandan V, Newman JA, Coon CN. Effects of infectious bronchitisvirus (Arkansas strain) on laying chickens. Avian Dis. 1986; 30(4): 644-647.

6. de Wit JJ, Nieuwenhuisen-van Wilgen J, Hoogkamer A, van de Sande H, Zuidam GJ, Fabri THF. Induction of cystic oviducts and protection against early challenge with infectious bronchitis virus serotype D388 (genotype QX) by maternally derived antibodies and by early vaccination. Avian Pathol. 2011; 40(5): 463-471, DOI: 10.1080/03079457.2011.599060.

7. de Wit JJ, Cook JKA, van der Heijden HMJF. Infectious bronchitis virus variants: a review of the history, current situation and control measures. Avian Pathol. 2011; 40(3): 223-235, DOI:10.1080/03079457.2011.566260.

8. Hassan MSHA, Buharideen SM, Goldsmith D, Coffin CS, Cork SC *et al.* Pathogenicity of the Canadian delmarva (DMV/1639) infectious bronchitisvirus (IBV) on female reproductive tract of chickens. Viruses 2021; 13(12): 2488. DOI: 10.3390/v13122488.

9. Chhabra R, Chantrey J, Ganapathy K. Immune responses to virulent and vaccine strains of infectious bronchitis viruses in chickens. Viral Immunology. 2015; 28(9): 478-488, DOI: https://doi.org/10.1089/vim.2015.0027.

10. Mardassi BBA, Mohamed RB, Gueriri I, Boughattas S, Mlik B. Duplex PCR to differentiate between *Mycoplasma synoviae* and *Mycoplasma gallisepticum* on the basis of conserved species-specific sequences of their hemagglutinin genes. 2005; 43(2): 948-958, DOI: 10.1128/JCM.43.2.948-950.2005.

11. Motamed N, Bashashati M. Comparative genetic features of S1 gene from six massachusetts and variant avian 2 infectious bronchitis viruses isolated in Iran. J Archives Razi Institute. 2022; 77(5): 1611-1619, DOI: 10.22092/ARI.2022.356836.1924.

12. Valastro V, Holmes EC, Britton P, Fusaro A, Jackwood MW *et al.* S1 gene-based phylogeny of infectious bronchitis virus: An attempt to harmonize virus classification. Infect Genet. 2016; DOI: 10.1016/j.meegid.2016.02.015.

13. Benyeda Z, Szeredi L, Mató T, Süveges T, Balka G *et al.* Comparative histopathology and immunohistochemistry

of QX-like, Massachusetts and 793/B serotypes of infectious bronchitis virus infection in chickens. J Comp Pathol. 2010; 143(4): 276-283, DOI: 10.1016/j.jcpa.2010.04.007.

14. De Wit JJ, Swart WAJM, Fabri THF. Efficacy of infectious bronchitis virus vaccinations in the field: association between the α-IBV IgM response, protection and vaccine application parameters. Avian Pathology. 2010; 39(2):123-131, DOI: 10.1080/03079451003604639.

15. Zhong Q, Hu Y-x, Jin J-h, Zhao Y, Zhao J, Zhang G-z. Pathogenicity of virulent infectious bronchitis virus isolate YN on hen ovary and oviduct. Vet Microbiol. 2016; 193: 100-105, DOI: 10.1016/j.vetmic.2016.08.017.

16. Hassan MSH, Najimudeen SM, Ali A, Altakrouni D, Goldsmith D *et al.* Immunopathogenesis of the Canadian Delmarva (DMV/1639) infectious bronchitis virus (IBV): Impact on the reproductive tract in layers. Microbial Pathogene. 2022; 166: 105513.

17. Mueller SA, Franca M, Jackwood M, Jordan B. Infection with IBV DMV/1639 at a young age leads to increased incidence of cystic oviduct formation associated with false layer syndrome. 2022; 14(5): 852 - 858, DOI: https://doi.org/10.3390/v14050852.

18. Ongor H, Timurkaan N, Coven F, Karabulut B, Eroksuz H *et al.* Detection of Israel variant 2 (IS/1494/06) genotype of infectious bronchitis virus in a layer chicken flock. Veteriner Fakültesi Dergisi. 2021; 68(2): 167-172.

19. Srinivasan P, Balasubramaniam GA, Murthy TR, Balachandran P. Pathomorphological studies of polyserositis in commercial caged layer chicken. Asian Pac J Trop Med. 2014; 7(1): S313-S320, DOI: 10.1016/S1995-7645(14)60252-2.

Cite this article as: Shoushtari H, Motamed N. False layer syndrome in a commercial layer flock associated with infectious bronchitis virus variant. Explor Anim Med Res. 2023; 13(2), DOI: 10.52635/eamr/13.2.277-282.