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A review of the Infectious Bursal Disease (IBD) in Iran

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A B S T R A C T

Infectious bursal disease (IBD) is one of the most important diseases in the poultry industry worldwide. It is a viral disease caused by a birnavirus, which weakens the immune system. The disease was observed for the first time in 1962 in the Gumboro area of Delaware, USA. The first report of this disease in Iran dates back to 1994. Since then, various investigations have been conducted in Iran on the isolation and identification of IBDV isolates, cognitive immunological studies and vaccine testing and design, disease pathogenesis and pathology, and recently in the field of genetic engineering. The main procedure for IBD control is vaccination in productive and commercial herds. Therefore, studies related to vaccination and immunogenicity play an essential role in designing preventive measures against this disease. In this manuscript, a comprehensive review of the studies conducted in Iran on IBD has been discussed.

Keywords: *Birnavirus, Infectious bursal disease, Iran, poultry, vaccination*

1 History of the disease

As cited by Eterradossi and Saif (1), Infectious bursal disease or Gumboro or avian nephrosis was first reported from the Gumboro area in Delaware, USA, in the sixty's and then spread to the most parts of the United States. These researchers also mentioned that IBD was spread to Europe, the Middle East, South and West Africa, India and Australia between 1962 and 1974 (1). Infectious bursal disease it is now considered an epidemic in many parts of the world (2). The first identified "type" strains of IBDV were identified in 1960 and 1964 in vaccinated broiler flocks in the United States, mainly by two epidemiologic episodes (2). The second epidemiological event was the emergence of IBDV in 1962 in the Netherlands, Belgium and France; since then, the virulence of IBD viruses has been increased, and mortality rate due to IBD has reached up to 90% (2). Investigations on the immunosuppressive nature of IBDV has been reviewed by van den Berg et al. (3). Discovery of antigenic diversity among IBDVs and identification of serotype 2 occurred in 1980 by McFerran and coworkers (4), and the identification of major antigenic variants also was reported in 1984 by Saif (5). Very virulent strains of infectious bursal disease (vvIBDV), which were much more severe than classical IBDV strains and caused 90% mortality, were first identified in the Netherlands in 1986 (3).

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Almost until 1987, IBDV viruses were of low virulence, causing less than 2% specific mortality, and were satisfactorily controlled by vaccination. But then, vaccination failures were described in different parts of the world. In the United States, new IBDV strains were shown to be affected by antigenic drift against which classical IBDV vaccines did not satisfactorily protect. At the same time in Europe, the first cases of vvIBDV were described. Surprisingly, some of the first acute outbreaks in broilers occurred at the end of the rearing period, in farms where all sanitary and preventive measures had been implemented (6). After its spread to the UK in 1988, vvIBDV was also identified in Japan and Belgium (3). Also, vvIBDV infections were observed in Africa, Asia and in South America (7).

2 Identification and characterization of IBD viruses in Iran

Infectious bursal disease was reported for the first time in Iran in 1994 (8). Also, the report of the first isolation of the very virulent virus of infectious bursal disease was published in 1996 (9). In 2004, a new isolate of the Gamboro disease virus was isolated, identified and named, IR499 (10). In an experimental study, strain IR499 caused 85% and 22% mortality in SPF chickens and normal broilers, respectively. Also, using the RFLP method, digestion of the 552 bp PCR product with HhaI and SacI restriction enzymes showed that there are no sites for these enzymes in the VP2 region of the IR499 isolate. While digestion of the same PCR product with restriction enzymes SspI and StuI showed a single site in isolate IR499 for each enzyme (10). In another study, molecular identification of the infectious bursal virus was performed based on the replication and gene sequence of segment A of the virus (11). The phylogeny results of this study showed that the virus isolated from Iran was very close to the very virulent strain isolated from other parts of Asia. These results reinforced the possibility of a common origin between the virus isolated from Iran and other parts of the world (11). In 2008, Bahmanejad and coworkers also reported isolating infectious bursal virus from layer flocks in Iran (12). These researchers used various methods to identify the isolated virus, including different serological methods such as AGP, immunoperoxidase staining, electron microscopy, and inoculation into embryonated eggs. Using RT-PCR and RFLP and phylogenetic analysis, the presence of very virulent strains was confirmed and the similarity of 96.3 to 99.8% of these strains with other vvIBDV strains in other parts of the world was shown (12). Razmyar and Peighambari (13) conducted research and collected bursal samples from 49 broiler and layer pullet flocks suspected of IBD infection from different parts of Iran during 2005-2006. RT-PCR was used to amplify 743 bp-fragment of VP2 gene among IBDV field isolates (13). Two restriction enzymes, BspMI and SacI, were used for further characterization of each amplified product. It was found that 37 (75.5%) out of 49 samples were positive for IBDV and digestion with two used restriction enzymes showed patterns compatible with vvIBDV and classical IBDV strains in 34 (91.9%) and 3 (8.1%) IBDV-positive samples, respectively (13). The procedure followed in this study was demonstrated to be useful for rapid differentiation between vvIBDV and classical IBDV isolates (13). The same researchers later characterized nine Iranian IBDV isolates from their previous study by sequencing the 743-bp amplified fragment of the VP2 gene, comparing the obtained data with published sequences of IBDV strains from Iran and around the world and analyzing their phylogenetic relationships altogether (14). The findings revealed that three isolates had a close relation to classical attenuated IBDVs and the other six isolates had sequences common in European and Asian strains of vvIBDVs. Amino acid sequences of three Iranian vvIBDVs were 100% identical and resembled vvIBDV strains from European (UK661), Asian (HK46, GZ96), and Iranian origins (IR01, SDH1). Some unique amino acid substitutions after major hydrophilic peak A, including 231S-L, 231S-P, and 233N-K were also observed among Iranian vvIBDVs of this study (14).

Infectious bursal virus also infects turkeys but does not cause disease. Razmyar and Peighambari (15) identified and confirmed the presence of IBDV in a turkey flock by amplifying a 743-bp gene fragment of the VP2. The results of this investigation showed that the IBD virus isolates obtained from Bursa Fabricius of turkeys were genetically very similar to vvIBDV strains isolated from chicken flocks and had a common origin with chicken isolates (15).

Genetic analysis and identification of the IBD virus are not limited to VP2 gene. Researchers (16) performed a different genetic analysis on the 715 bp fragment of the VP1 coding gene. this research studied strains whose virulence pathotype had been previously determined by molecular analysis of the VP2 gene (16). The results showed that in 76% of cases, the VP1 and VP2 gene analyses matched each other, also, due to the mismatch in other 24% of cases, the hypothesis of the presence of reassortant viruses was proposed (16). In 2014, the same researchers conducted another study on the VP1 gene of three strains of Iranian

IBDVs (17). They amplified a 715 bp fragment of the VP1 gene of IBDV strains by using reverse transcriptase-polymerase chain reaction (RT-PCR), sequenced, compared the obtained data with the published sequences of IBDV strains from around the world and analyzed the phylogenetic relationships between those IBDV strains (17). In phylogenetic analyses, all three Iranian strains clustered together with vvIBDVs. One Iranian strain, JRMP30IR, was more closely related to two European strains (HOL and UK661) and two southeast Asian strains (OKYM and ZJ2000) (17). However, the other two Iranian strains, JRMP07IR and JRMP14IR, were closer to two Turkish strains (OA/G1 and OE/G2) and a Malaysian strain (UPM94) (17).

During 2014-2015, another group of researchers collected and prepared 12 pooled bursal tissue samples, showing macroscopic lesions typical of IBD, todetect of IBDV (18). In this study, the 474 bp gene fragment from the hypervariable region of the VP2 protein coding gene was used for analysis and sequencing (18). Ten isolates had the characteristic amino acid (AA) positions of vvIBDV viruses, and the other two isolates were identified as attenuated vaccine strains (18). Very virulent IBDV isolates showed a point mutation at AA position 254 when compared to other Iranian isolates (18). A 253Q AA position mutation, which is not found in the D78 strain, was also observed in two attenuated vaccine strains. This mutation led to the virulence of the vaccine strains studied in this research (18). In 2017, Shoushtari reported the isolation of IBDVs from broiler flocks in Tehran province (19). In this study, Bursa of Fabricius samples were taken from 25 broiler flocks at the age of 10 to 12 days before IBD vaccination (19). The IBDV was identified in two by using RT-PCR (19). In Gilan province of Iran, sampling from 40 flocks suspected to IBD revealed four positive flocks using RT-PCR and amplifying of a 643-bp fragment located in the hypervariable region of the VP2 gene (20). For further confirmation, all four positive samples were also analyzed by Nested PCR, which identified vvIBDV isolates using a specific primer leading to a 552-bp product (20). Using RFLP and digestion with SacI and BspMI enzymes, the presence of vvIBDV was re-confirmed in the four flocks based on the RE patterns that shown (20).

A group of Iranian researchers used real-time RT-PCR and high-resolution melt (HRM) curve analysis, which is a relatively fast method with high precision, to differentiate IBDVs isolated from Iranian poultry flocks (21). In this study, high melting resolution at temperatures ranging from 81 to 92°C was performed (21). The results showed that the viruses were classified from A to D. Three vaccine strains of D78, Gumbokal and Bursa CE were placed in group A; IBD L and Bursine 2 belonged to groups B and C, respectively, and all field viruses were classified in group D. High resolution melting (HRM) curve analysis after normalization also showed all viruses of this study were placed in 4 HRM genotypic group (21). These researchers concluded that the real-time RT-PCR HRM technique was cost-effective and reliable among the currently used methods and could be used for the differentiation of IBDV isolates (21).

In 2018, Najafi and coworkers collected bursal samples from pullet flocks suspected of IBD and with mortalities up to 40%, performed RT-PCR based on the highly variable region of the VP2 gene, followed by sequencing, and showed a high similarity between vvIBDV isolated from Iranian pullet flocks and recent vvIBDVs originating from Iran (UT-PCR-Keivanfar-2019) and some countries from Middle East such as Kuwait, Iraq and Turkey (22).

Infectious bursal disease virus consists of a two-segmented double-stranded RNA genome, which can easily undergo genomic recombination or reassortment during mixed infections. In 2020, Ghorbani et al. characterized a previously identified Iranian IBDV strain (JRMP29IR) in specific-pathogen-free (SPF) chickens, evaluated the presence of a mixed and/or reassortant virus population in this strain and examined the frequency of genomic recombination and reassortment in publicly available IBDV genomes through bioinformatics (23). The SPF chickens were challenged with the JRMP29IR strain via oral and intraocular routes (23). Bursal tissues were used for histopathological examination, and RT-PCR was followed by Sanger sequencing. Putative recombinations and reassortments were evaluated using the Recombination Detection Program 5 (23). Through genomic sequencing of the viruses from the bursas of infected chickens, the JRMP29IR strain was found to contain viruses from the classic, variant and very virulent IBDV genotypes (23). Through bioinformatics, numerous putative recombination and reassortment events that naturally occured throughout the IBDV genome were identified (23). Parental JRMP29IR appears to be derived from a flock undergoing a mixed IBDV infection (13, 14). The high frequency of recombination and reassortment among IBDVs suggested that these events were evolutionarily beneficial for the virus (23).

3 Experimental studies

In 2005, to evaluate the severity of the infectious bursal disease virus, an experiment was designed based on the relationship between the intensity of apoptosis changes and the severity of the virus (24). Two groups of 21-day-old SPF Leghorn chicks were selected. Three subgroups (n=30) of group 1 were inoculated via the ocular/nasal route with the IR499 strain (vvIBDV), D78 intermediate vaccine strain and saline serum (control), respectively (24). And two subgroups (n=10) of group 2 were inoculated with IR499 and saline serum (control) via the oral route. After inoculation, tissues samples from the spleen and bursa of Fabricius were taken and processed for histopathological examination with H&E staining and tunnel method. The results showed apoptotic changes in spleen and bursal B cells in all treated subgroups compared to the control subgroup (24). The statistical analyses indicated a significant correlation between the severity of the IBD virus strain and the amount of apoptosis that occurred in B cells (24).

Using immunohistochemistry, Siavosh Haghighi et al. (25) investigated the pathogenicity process and virulence characteristics of IBDV in the early stages of infection in SPF chickens. For this purpose, 15 four-week-old chickens were inoculated with IR499 strain (vvIBDV) with a dose of 103 EID50/100 µl through the ocular/nasal route (25). Also, five chickens received an equal volume of PBS through the route as a control group. Then, blood and tissue samples from the bursa of Fabricius, cecal tonsils, liver, spleen, thymus, and thigh muscle were taken at 3, 6, 12, 24 and 48 hours post inoculation (p.i.). Viral antigens were as first observed 3 h p.i. in lymphoid cells of cecum tonsils (favorable organ for initial virus replication) and liver Kupffer cells (25). Positive signals for the presence of virus in the bursa were observed 6 h p.i., indicating the occurrence of primary viremia. After secondary viremia, the virus was first seen in the spleen and thymus 12 h p.i. These results showed that, in the early stages of IR499 virus infection, this strain acts as a very virulent strain of IBDV with a rapid and generalized course (25).

Isolation of IBD virus in turkeys has also been shown in various studies. In 2017, Hashemzadeh et al. investigated the effects of IBDV in turkeys (26). Following experimental IBDV infection induced in turkeys, its effects on the response to the H9N2 influenza virus were investigated (26). The results showed that despite not affecting mortality, IBDV infection can affect the severity of the damage caused to the bird as well as the duration of the conflict (27).

4 Vaccine related studies

The Pathogenicity and immunogenicity of four commercial IBD vaccines were studied in an experimental study by Hedayati et al. (27). In this work, cloned D78®, Bursine-2®, Bursimune® and Cevac Gambo-L® vaccines were evaluated in 100 SPF chickens. No clinical signs and mortality were observed in any groups. The findings of this investigation found cloned D78® and Cevac Gambo-L® vaccines to be more pathogenic by causing more severe bursal lesions but to be stronger immunogenic as measured by ELISA titers (27).

Immune complex vaccines are not affected by maternal antibodies. These vaccines are injected in ovo in hatcheries or to day-old chicks. In 2006, Sadrzadeh et al investigated the immunosuppressive effects of an immune complex IBDV vaccine administered in ovo or to day-old chicks (28). The IBD vaccinated groups were vaccinated against Newcastle Disease (ND) with an entrotropic apathogenic (at day 1) or B1 (day 7) and LaSota (day 18) strains. Birds in all groups were challenged with a virulent HERTZ33 ND virus strain at 31 days of age. It was found that in ovo or day-old vaccination of chicks against IBD with an IBD-immune complex vaccine did not have any significant immunosuppressive effects on broilers (28).

Developing of new vaccines using newly discovered strains is one of the procedures to develop new vaccines. In 2013, Ebrahimi et al isolated and identified a new IBDV strain named IBD07IR for vaccine development (29). The IBD07IR strain was identified as one of the vvIBDV strains using different serological tests and restriction fragment length polymorphism (RFLP) (29). Then, this strain was attenuated through passaging in SPF chicks at four-day intervals. After preparing the vaccine, the clinical evaluation was performed on 60 SPF chickens in two groups via ocular and drinking water routes. The results showed the vaccine efficacy and the induction of sufficient immunity (29).

The best way to use the immune complex vaccine is in ovo injection. This method faces financial and technological limitations due to the need for advanced equipment. Therefore, alternative methods should be used to use immune complex vaccines. In 2014, Sadrzadeh et al (30) investigated the difference between two methods of in ovo and subcutaneous (SC) injections in day-old chicks in terms of average weight gain at the end of the production period, antibody response, the bursal weight/body weight ratio and histopathological lesions found on the bursal tissue (30). The ELISA was used to evaluate the antibody response at 17 and

25 days of age, and no significant difference was observed between the two mentioned methods. At 32 days of age and after, in both vaccinated groups, a significant decrease was found in bursal weight compared to the total body weight (30). The results of this study confirmed the use of the immune complex vaccines by both methods of in ovo and SC injections without any significant difference (30).

In 2016, Mayahi et al. investigated the clinical efficacy of two commercial intermediate vaccines against IBD manufactured by Razi Vaccine and Serum Research Institute (RVSI) of Iran and Lohmann Company (Germany) (31). In this study, the exact time of vaccination in chickens was determined using Deventer's formula. Both vaccines were administered via drinking water at the age of 17 and 23 days in broilers. In this study, factors such as average feed consumption, feed conversion rate and average weight gain at the ages of 16, 23 and 42 days were measured (31). The results showed that both vaccines had a significant negative impact on the average weight gain at 23 days of age and at the end of the period. Still, they did not have a significant impact on the average feed consumption and feed conversion rate (31). In the continuation of the same author's research work, the effect of these two vaccines in antibody response against Newcastle disease (ND) was evaluated (32). In this study, B1 strain and killed AI+ND vaccines were administered via ocular and SC routes, respectively, at days 9 of age. IBD vaccination occurred at 16 and 23 days of age, similar to the previous study. The results showed that vaccination with any of the Iranian and foreign IBD vaccines did not have any immunosuppressive effect on live and killed AI+ND vaccines (32). Ebrahimi et al. (33) compared the immunogenicity of four commercial IBD intermediate vaccines in Iranian broiler flocks in areas with vvIBDV infection history (33). The four IBD intermediate vaccine brands named as Dn, Vc, Ch, and Razi. The findings showed that serum antibody titers were not affected by the vaccine brands at 28, 35, and 42 days of age ($P>0.05$). Other production parameters did not differ significantly in various vaccinated groups ($P>0.05$). In general, the potential of the IBD Razi vaccine was comparable to the other investigated foreign IBD vaccine (33).

Live vaccines are usually used to vaccinate chickens against IBD. In addition to the type of vaccine, the route of immunization also, is important in the induction of an immune response. Sadrzadeh et al. (34) investigated the impact of the routes of live vaccine administration against IBD on the induction of antibody response in broiler chickens. A single dose vaccination of an intermediate IBD vaccine strain was administered at 21 days of age through five routes, including subcutaneous (SC), intramuscular (IM), drinking water, eye drops, and course spray (34). Antibody response was shown in all routes by commercial IDEXX ELISA kit. These researchers concluded that a single dose SC injection of an intermediate IBD vaccine was capable of mounting higher antibody response and improving bursal health and performance of chickens as compared with birds immunized via drinking water (34).

Killed or inactivated vaccines are one of the vaccine types used to combat IBD. These vaccines are mainly used before the start of production in breeder flocks in order to induce passive immunity and its transfer to progeny. In 2020, Ebrahimi and coworkers used an IBDV strain to design an inactivated vaccine by using formalin, beta-propiolactone (BPL) and binary ethylenimine (BEI) to inactivate the virus (35). All three antigen preparations were adjuvanted separately with ISA-70 and then injected SC in to groups of three-week-old SPF chickens. The results showed that the lowest concentrations that could fully inactivate the infectivity of the IBD virus were 2.5 mM for BEI, 0.15% for BPL and 0.1% for formalin (35). Inactivated preparations of 2.5 mM BEI and 0.15% BPL showed no apparent adverse effect on IBDV infectivity and showed a reliable inactivation unlike antigens inactivated with 0.1% formalin that demonstrated an antigenicity decrease after one year (35). Serum antibody titers were raised against IBDV in all treated groups as detected by ELISA. BEL-inactivated antigen generally showed much better antigenicity stability than other preparations (35).

The development of VP2-based DNA vaccines with biological adjuvants has recently attracted attention due to their effectiveness in provoking antibody and cellular immune responses. In 2023, Soleimani et al. used a bioinformatics approach to design a bioadjuvant candidate vaccine targeting IBDV using viral VP2 fusion and chicken IL-2 antigenic epitope (36). The physicochemical properties, molecular dynamic simulations, and antigenic site determination of the final 3D structure of the VP2-L-chiIL-2105-129 were characterized (36). The findings resulted in the development of non-allergenic candidate vaccine with the potential for antigenic surface display potential and adjuvant activity. However, the potential of the proposed vaccine in the induction of immune response in avian hosts should be investigated (36).

5 Genetic engineering studies

Genetic engineering has been used to express the genes of various proteins in various of pathogens. In 2006, Shahbazzadeh et al. expressed the VP2 gene of IBDV vaccine strain D78 in Pichia pastoris yeast (37). The recombinant DNA plasmid containing VP2 gene was transferred into the chromosome of this yeast and the expression of this protein was confirmed by SDS-PAGE and western blotting tests. Also, for accurate detection of this protein, it was bound with anti-IBDV chicken polyclonal antibodies and confirmed by western blot. The results of this study showed that this yeast was effective in expressing the VP2 protein of the D78 strain virus (37).

The VP2 protein, a major immunogen, is crucial for protecting chickens against IBDV. Considering the importance and development of new technology for the production of recombinant proteins such as vaccines, antibodies and pharmaceuticals, the production of recombinant VP2 protein has attracted special attention among researchers. Pourseyedi et al. (2009) used Agrobacterium to produce this protein in tobacco, alfalfa and lettuce plants. For this purpose, the VP2 gene, which contains 1356 base pairs and is located under the CaMV35S promoter, was transferred to the tissue of fresh leaves of these plants (38). Transient protein expression in transgenic leaves was measured using ELISA and protein staining. Using the agroinfiltration method and transfer of the VP2 expression structure, the highest expression level of this protein was obtained in alfalfa and tobacco transgenic leaves, and the lowest expression level was obtained in lettuce transgenic leaves. other researchers can use the findings of this study to make recombinant vaccines (38).

In another study in 2010 by Ghafari et al., the coding region of the VP2 gene of the IBDV D78 vaccine strain was cloned into a eukaryotic gene expression vector, pSec Tag2A (39). This gene which is controlled by human cytomegalovirus (hCMV) imm (29)ediate early enhancer and promoter was positioned downstream of Ig κ chain leader sequence. The pSec Tag2A-VP2 construct was transfected in COS-7 cell line and VP2 expression and secretion were evaluated by dot blot and antigen capture ELISA (39). A neutralizing monoclonal antibody (A61) against VP2 was used in the immunological assay. The positive reaction with the antibody suggested that the construct was useful with respect to the expression and secretion of a native VP2. The results of this study, like the previous study, can be used to produce recombinant vaccines (39).

6 Conclusion

With the expansion of the poultry industry in Iran, the importance of infectious diseases in various types of poultry production has also expanded. Annually, many direct and indirect costs are incurred to reduce the economic losses of these diseases. Despite these costs and the widespread use of vaccination programs, the economic losses caused by IBD are still significant after several decades. Therefore, dealing with this disease requires permanent and unstoppable extensive monitoring. In this review, almost all studies conducted regarding IBD in Iran were reviewed from the early years when IBD was diagnosed for the first time in Iran until the present time that Iranian researchers have investigated various aspects of IBD. More studies, especially in the field of examining subclinical cases that are usually ignored, can shed more light on this disease.

Conflict of Interest

The authors declared no conflicts of interest.

Author Contributions

AS drafted the manuscript, and SMP critically reviewed and revised it. Both authors have read and approved the final manuscript and agreed to the published version of the manuscript.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

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