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# Molecular and *in vivo* Characterization of an Iranian Infectious Bursal Disease Virus Containing a Mixed Virus Population

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## Abstract

**BACKGROUND:** Infectious bursal disease (IBD) is a highly contagious immunosuppressive disease of young chickens caused by infectious bursal disease virus (IBDV). IBDV consists of a two-segmented double-stranded RNA genome which can easily undergo genomic recombination or reassortment during mixed infections.

**OBJECTIVES:** The objectives of this study were to characterize a previously identified Iranian IBDV strain (JRMP29IR) in specific-pathogen-free (SPF) chickens, evaluate the presence of a mixed and/or reassortant virus population in this strain, and examine the frequency of genomic recombination and reassortment in publicly available IBDV genomes through bioinformatics.

**METHODS:** The SPF chickens were challenged with the JRMP29IR strain via oral and intraocular routes. Bursal tissues were extracted for histopathological evaluation and reverse transcription-polymerase chain reaction (RT-PCR) followed by Sanger sequencing. Putative recombinations and reassortments were evaluated using the Recombination Detection Program 5.

**RESULTS:** 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. Through bioinformatics, numerous putative recombination and reassortment events were identified that naturally occured throughout the IBDV genome.

**CONCLUSIONS:** Parental JRMP29IR appears to be derived from a flock undergoing a mixed IBDV infection. High frequency of recombination and reassortment among IBDVs suggests that these events are evolutionarily beneficial for the virus.

**KEYWORDS:** Infectious bursal disease virus, Mixed infection, Molecular characterization, Reassortment, Recombination

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## Introduction

Infectious bursal disease (IBD) is a highly contagious immunosuppressive disease of young chickens with active lymphoid tissues (Hemida *et al.*, 2019; Eterradossi and Saif,

2020). IBD was first recognized as avian nephrosis in 1962. Almost after a decade, the causative agent of IBD was proposed as infectious bursal disease virus (IBDV). Owing to its lymphotropic nature, IBDV causes huge economic loss to the poultry industry both through high levels of mortality and severe immunosuppression which often leads to secondary infections and vaccination failure in the infected flocks (Hemida *et al.*, 2019; Eterradossi and Saif, 2020).

IBDV consists of a two-segmented double-stranded RNA genome (segments A and B), which encodes the five viral proteins designated as VP1-VP5 (Hemida *et al.*, 2019; Eterradossi and Saif, 2020). Although IBDVs are divided into two main serotypes (serotypes 1 and 2), so far, all pathogenic IBDVs have been characterized as serotype 1 (Eterradossi and Saif, 2020). Within the IBDV serotype 1, antigenic drifts have caused a significant change in virus antigenicity mainly through amino acid changes in the mid-third of viral protein 2 (VP2), also known as the VP2 hypervariable domain (Eterradossi and Saif, 2020). While the antigenic changes in VP2 hypervariable domain appear to play a major role in generation of the viral antigenic variants, larger antigenic shifts through genetic recombination and/or reassortment (mixing of the entire or parts of the genes between two different viruses) are likely to occur in the case of mixed infections (Hemida *et al.*, 2019; Touzani *et al.*, 2019; Eterradossi and Saif, 2020).

Genetic reassortments can play a vital role in the evolution of viruses with segmented genomes by creating viruses with novel genetic and antigenic makeups (McDonald *et al.*, 2016; Jackwood *et al.*, 2018). With further advancement of rapid molecular sequencing techniques, a putative reassortant IBDV strain from Europe was described for the first time in 1996 (Brown and Skinner, 1996). Since then, several studies have described additional reassortment events in IBDVs from across the world (Yamaguchi *et al.*, 1997; Kong *et al.*, 2004; Le Nouen *et al.*, 2006; Wei *et al.*, 2006; Chen *et al.*, 2012; Kasanga *et al.*, 2013; Pikuła *et al.*, 2018). Majority of the genetic reassortments in the previous studies occurred between the very virulent and attenuated or classic IBDV strains. Therefore, vaccination with live-attenuated IBDV strains is likely to be involved in these reassortment events. However, the exact mechanisms governing the generation of reassortant IBDVs and their clinical attributes are yet to be elucidated.

In a series of previous studies, a number of IBDV viruses were identified from the infected poultry farms in Iran during 2005-2006 (Razmyar and Peighambari, 2008; Razmyar and Peighambari, 2009; Ghaniei *et al.*, 2011). Our first study conducted by Razmyar and Peighambari (2008 and 2009) using combination of reverse transcriptase-polymerase chain reaction/restriction fragment length polymorphisms (RT-PCR/RFLP) and nucleotide sequence analyses of the VP2 hypervariable region, revealed that the majority of Iranian IBDVs (34 out of 37) belong to the very virulent IBDV genotypes (encoded by segment A) (Razmyar and Peighambari, 2008; Razmyar and Peighambari, 2009). Only 3 out of 37 studied viruses from Iran were identified as classic or standard IBDV genotypes based on their VP2 gene (Razmyar and Peighambari, 2008). Next, Ghaniei performed a second RT-PCR/RFLP analysis on the VP1 (encoded by segment B) of the previously identified viruses using a primer set specific to the very virulent IBDV strains (Ghaniei *et al.*, 2011). Interestingly, one of the studied viruses, JRMP29IR, which was originally identified as a classic strain based on its VP2 gene could be identified as a very virulent strain based on its VP1 gene. Of note that, JRMP29IR was collected from an IBDV-vaccinated farm with high mortality (~15%) and clinical signs similar to infection with very virulent IBDV strains (e.g., extensive hemorrhage in cloacal bursa) (Razmyar and

Peighambari, 2008). Collectively, these results suggested that JRMP29IR may contain a mixed or reassortant IBDV population.

Mixed infections may result in the emergence of recombinant or reassortant viruses with novel antigenic and/or pathogenic makeups (Patel *et al.*, 2016; Jackwood *et al.*, 2018; Kim *et al.*, 2018). For instance, viruses with novel antigenic makeups can effectively break through the host immunity developed against the previous vaccinations (Jackwood, 2017; Jackwood *et al.*, 2018). A better understanding of possible recombination and reassortment events within the virus genome and their effects on the virus phenotype can help on designing novel vaccines and therapeutics. The aim of this study was to further characterize the JRPM29IR strain in SPF chickens and evaluate the presence of a mixed and/or reassortant IBDV population in this strain. Further, we evaluated the frequency of genomic recombination and reassortment in publicly available IBDV genomes through bioinformatics, providing a strong evidence for the presence of numerous naturally occurring recombinant and reassortant IBDV strains.

## Materials and Methods

### Viruses

The JRMP29IR strain was collected from a broiler flock with 15% mortality, previously vaccinated with a live-attenuated IBDV vaccine, located in Tabriz, Iran in 2006, as previously described (Razmyar and Peighambari, 2008; Razmyar and Peighambari, 2009). Aliquots of the JRMP29IR infected bursal tissues (100 mg) were obtained from the Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. Bursal aliquots were homogenized using the sterile scalpel blades, mixed with 5 mL sterile PBS, and filtered through sterile 0.2 µm filters to remove cell debris and bacterial contaminants. Chicken bursal passage (CBP) subclones of JRMP29IR were collected from the bursa of individual infected chickens in this experiment.

### Chicken Experiment and Ethics Statement

Three-week-old white leghorn SPF chickens were obtained from the Razi Vaccine and Serum Research Institute (Karaj, Alborz, Iran). The birds were divided into two subgroups of 5 chickens and kept inside large cages (700 $cm^2$) for the experiment duration. IBDV challenge was performed at 3 weeks of age in SPF chickens via oral and intraocular routes (1:1 ratio, v/v) with 600 µL of the JR-MP29IR bursal tissue homogenates per bird. The health status of chickens was monitored twice daily throughout the experiment. Chic-kens were humanely euthanized when they displayed severe symptoms such as ruffled feathers, reluctance to move, and respiratory distress. Blood and tissue collection from the euthanized or dead birds were performed immediately after euthanasia or daily checks.

### Histopathology

Histopathology and bursal lesion scoring were performed as previously described (Jackwood *et al.*, 2011). Briefly, bursal tissues were fixed in 10% neutral buffered formalin, sectioned at 4-5 µm and stained using hematoxylin and eosin (H&E). Bursal tissues were scored under a light microscope based on the extent of lymphocyte necrosis, follicular depletion and atrophy. The lesion scores from 0 to 4 indicate relative degree of severity with the score of '0' indicating no lesions, and scores of 1 to 4 indicating lesions in <25%, 25 to 50%, 50 to 75% and >75% of the bursal follicles, respectively (Jackwood *et al.*, 2011).

### RNA Extraction and RT-PCR

Bursal tissues were collected from the experimentally infected birds at 3-4 days post-challenge. The collected tissues were cut in half and pressed onto Whatman FTA cards (GE Healthcare Life Sciences, Pittsburgh, PA). Viral

RNA was extracted from the FTA cards as previously described in detail (Michel and Jackwood, 2017). Briefly, FTA card punches were vortexed with 300 µL of TE buffer pH 8.0 with 1 mg/mL of proteinase K (Invitrogen, Carlsbad, CA) and 0.5% SDS (Sigma-Aldrich, St. Louis, MO) and incubated at 56°C for 60 min. The samples were lysed by adding 300 µL of RNA Lysis Buffer (Zymo Research, Irvine, CA) followed by incubation at room temperature for 9 min. Total RNA was extracted using the Quick-RNA™ MiniPrep Kit (Zymo Research) according to the manufacturer's instructions, precipitated with 3 M sodium acetate buffer and ethanol, and resuspended in 25 µL of 90% dimethyl sulfoxide (Sigma-Aldrich) (Michel and Jack-wood, 2017). The RT-PCR was performed to amplify a 579-bp fragment of the VP2 hypervariable region using the Qiagen One-Step RT-PCR Reagents Kit (Qiagen, Valencia, CA) by 743-F (5′-GCC CAG AGT CTA CAC CAT -3′) and 1331-R (5′-ATG GCT CCT GGG TCA AAT CG-3′) primers, as previously described (Michel and Jackwood, 2017). The RT-PCR was performed at 48°C for 30 min and 95°C for 10 min, followed by 35 cycles of 95°C for 30 s, 57°C for 90 s and 72°C for 90 s, with a final extension at 72°C for 5 min (Michel and Jackwood, 2017).

**Nucleotide and Amino Acid Sequence Analysis**

The RT-PCR products were extracted from the agarose gel using Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI) and Sanger sequenced at the Molecular and Cellular Imaging Center, The Ohio State University (Wooster, OH). BioEdit (Version 7.2.5) was used to assemble and translate the nucleotide sequences into putative protein sequences. Nucleotide and amino acid ali-gnments were performed with the NCBI GenBank reference sequence UK661 (GB#: NC-004178) using the ClustalW alignment option in MEGA-X (Version 10.0.5) (Kumar *et al.*, 2018). Phylogenetic analysis was carried out for 182 amino acids of the VP2 hypervariable region using the Maximum Likelihood method and JTT matrix-based model with 1000 bootstrap replicates.

**Recombination Analysis**

A total of 113 nucleotides of complete IBDV genomes were downloaded from the NCBI's Virus Database as of July 2020. To analyze the putative intra-segment recomb-ination events, segments A (n=58) and B (n=49) of each complete genome were separately aligned using MUSCLE alignment option in MEGA-X (Version 10.0.5) (Kumar *et al.*, 2018). To explore the potential presence of inter-segment recombination events (i.e., genomic reassortments), genomic sequences of segments A and B from each strain was pooled (n=51) and MUSCLE aligned before being used for the downstream analysis. Putative recombination events were identified using Recombination Detection Program 5 (RDP5; Version 5.5), which uses multiple methods to minimize the false recombination discovery (Martin *et al.*, 2015). All genomes were analyzed using 3Seq, Chimaera, SiScan, MaxChi, Bootscan, Geneconv, and RDP detection methods (cut-off value $P \leq 0.001$) and only the recombination events predicted by at least six out of the seven methods were considered positive (Hoxie and Dennehy, 2020).

**Statistical Analysis**

Statistical analysis and data visualization were performed using GraphPad Prism version 8 (GraphPad Software, San Diego, CA). The statistical analyses were performed through one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A P-value less than 0.05 was considered significantly different.

## Results

**Pathogenicity of JRMP29IR in SPF Chickens**

We determined and confirmed the pathogenicity of the JRMP29IR strain in 3-week-old

SPF chickens. At 2 days post-challenge, all infected chickens became depressed and showed the typical signs of IBD including ruffled feathers, pasty vents, diarrhea and a reluctance to move. Mortality started from the second day after challenge and reached to 60% at 4 days post-challenge (Figure 1). At necropsy, the bursas from all birds were found enlarged and edematous, and either contained yellowish exudates or varying degrees of hemorrhages, resembling an intermediate clinical profile for JRMP29IR between the variant, classic, or very virulent IBDV viruses. In addition, ecchymotic hemorrhages were observed on the mucosal surface at the proventriculus and gizzard junction, on the heart fats, and thigh muscles. Microscopic lesions including follicular lymphoid depletion and atrophy with severe lymphoid necrosis were observed in the bursal tissues of all infected chickens (Figure 2). The extent of microscopic lesions in bursas also suggested an intermediate pathogenicity index for JRMP29IR between the variant, classic and very virulent IBDV strains.

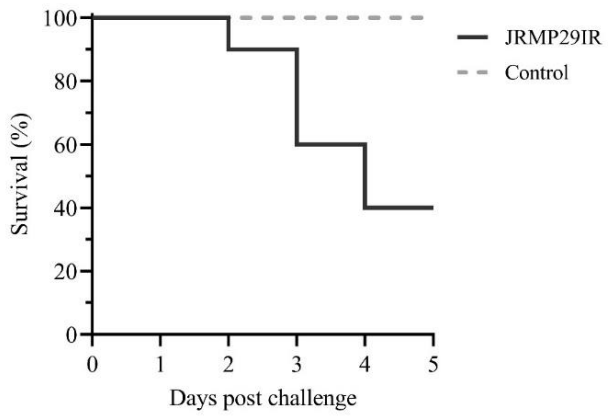


**Figure 1.** Mortality of chickens inoculated with the JRMP29IR strain. Three-week-old SPF chickens were challenged with the JRMP29IR-infected bursal homogenates via the oral and intraocular routes, as described in the materials and methods. Percent survival of chickens was calculated with 10 birds per group and the counting number of birds that died each day for 5 days post challenge.

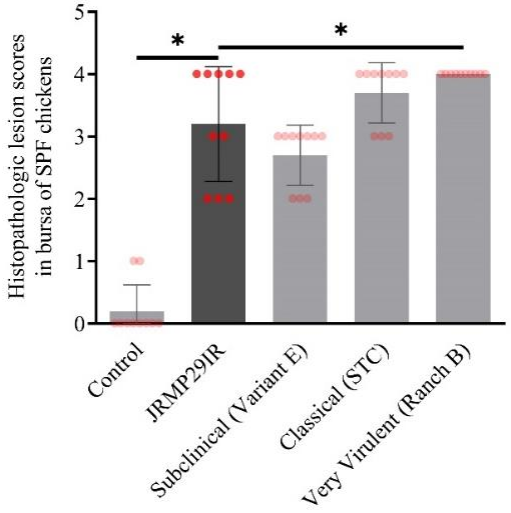


**Figure 2.** Severity of histopathologic lesion in the bursa of chickens inoculated with the JRMP29IR strain. Three-week-old SPF chickens were challenged with the JRMP29IR-infected bursal homogenates via the oral and intraocular routes, as described in the materials and methods. The severity of the microscopic lesions in the bursa of infected chickens was graded based on the extent of the lymphocyte necrosis, follicular depletion and atrophy, as described in the materials and methods. Histopathologic scores for the very virulent (Ranch B), classical (STC), and subclinical variant (Variant E) viruses were obtained from a previously published study by Jackwood *et al.* (2011) using the same lesion scoring criteria. Dots represent the scores for individual samples. Bars indicate mean ± standard deviation for groups. *, $P \leq 0.05$ (one-way analysis of variance with Tukey's test).

**Molecular Characterization of JRMP29IR from the Bursa of SPF Chickens**

The RT-PCR amplified VP2 hypervariable region from five individual chickens was sequenced. The evolutionary analysis by Maximum Likelihood method revealed that the five sequenced samples belong to different lineages of viruses apart from the original JRMP29IR strain (Figure 3). Three out of 5 sequenced samples (CBP2-4) were closely related to the typical European very virulent strain UK661. Other two sequenced samples were more closely related to the classical and variant IBDV viruses (CBP1 and CBP5, respectively). The amino acid sequences across the four major and minor hydrophilic peaks within the VP2 hypervariable region represented the profiles identical to very virulent strains for the CBP2-CBP4 (Figure 4). However, minor deviations within these antigenic sites could be observed for CBP1 (253N) and 5 (315S) compared to their closest sequence relatives (1-UK and Variant OH, respectively). Collectively, our results revealed that the original JRMP29IR strain is likely to contain a mixture of viruses from three different IBDV pathotypes (classical, variant, and very virulent) possibly due to a concurrent mix infection in the original flock.

**Strong Evidence for Recombination and Reassortment in Multiple IBDV Strains**

We first analyzed the complete genomic sequences of the segments A (n=58) and B (n=49) separately. Through these analyses, we identified a total number of 6 (8 strains) and 8 (15 strains) putative recombination events within the segments A and B of the available IBDV sequences, respectively (Table 1). Next, we attempted to analyze the putative reassortment events in the available complete genome including both segments A and B of the IBDV strains (n=44). Interestingly, 25 out of the 51 available complete IBDV genomes appeared to contain reassortments between their genomic segments which may result in formation of new virus lineages (Figure 5). All the seven previously described reassortant IBDVs with publicly available full-length genome were successfully detected as recombinant using our approach coupled with the most stringent criteria on RDP5 (Figure 5; marked with red asterisks).

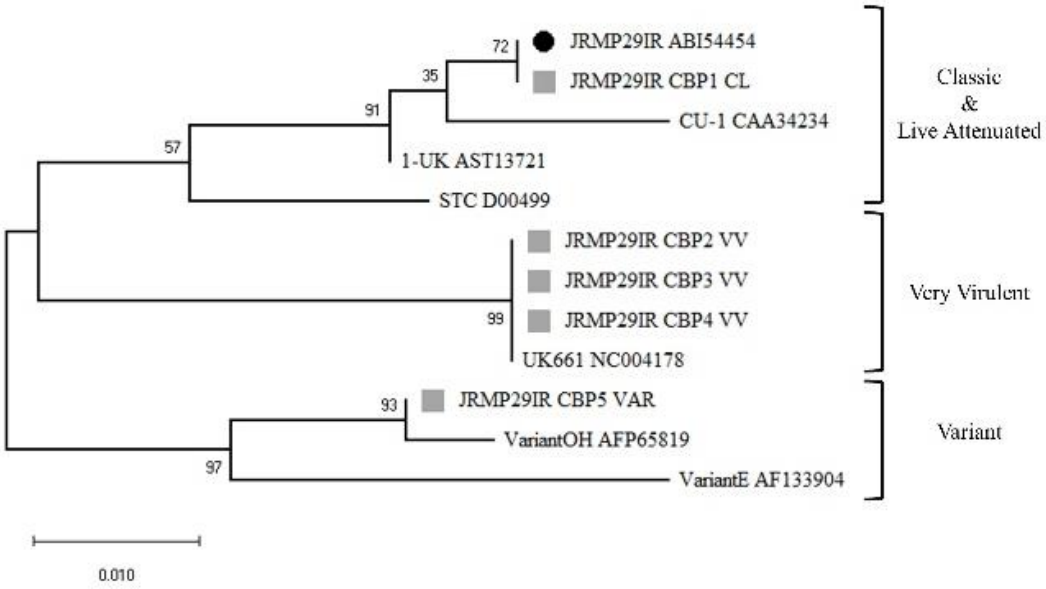


**Figure 3.** Phylogenetic analysis of VP2 hyper variable regions of the JRMP29IR and chicken bursal passaged (CBP). The evolutionary history was inferred by using the Maximum Likelihood method and JTT matrix-based model with a total of 182 amino acid positions and 1000 bootstrap replicates in MEGA X. GenBank accession numbers are followed by the common name of each virus. CL, VV, and VAR after CBP# are short for classical, very virulent, and variant.

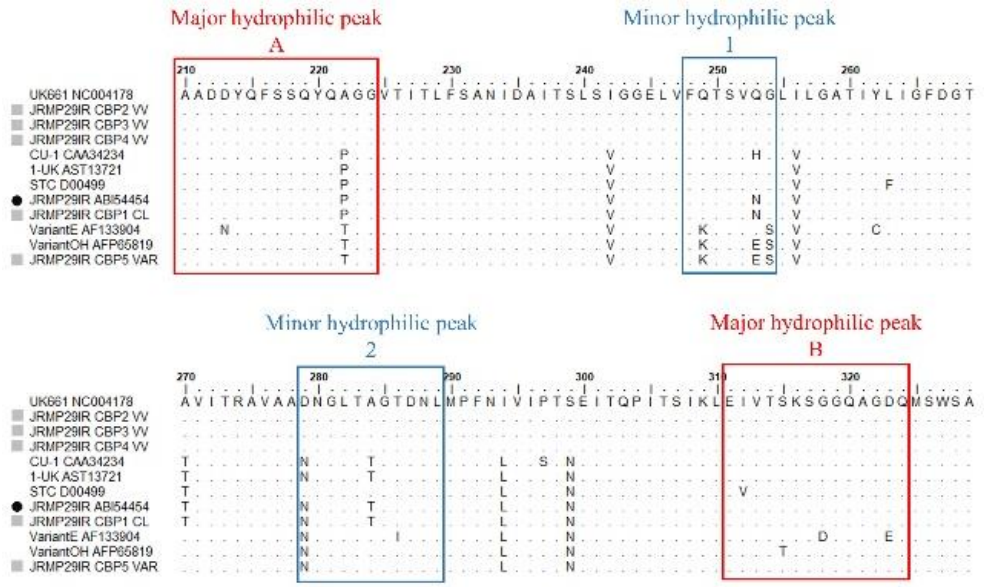


**Figure 4.** Alignment of predicted amino acid sequence of the VP2 hyper variable domain of IBDV strains. Dots indicate positions with amino acids identical to the reference UK661 strain. Major hydrophilic peaks are boxed with red lines, and minor hydrophilic peaks are boxed with blue lines.

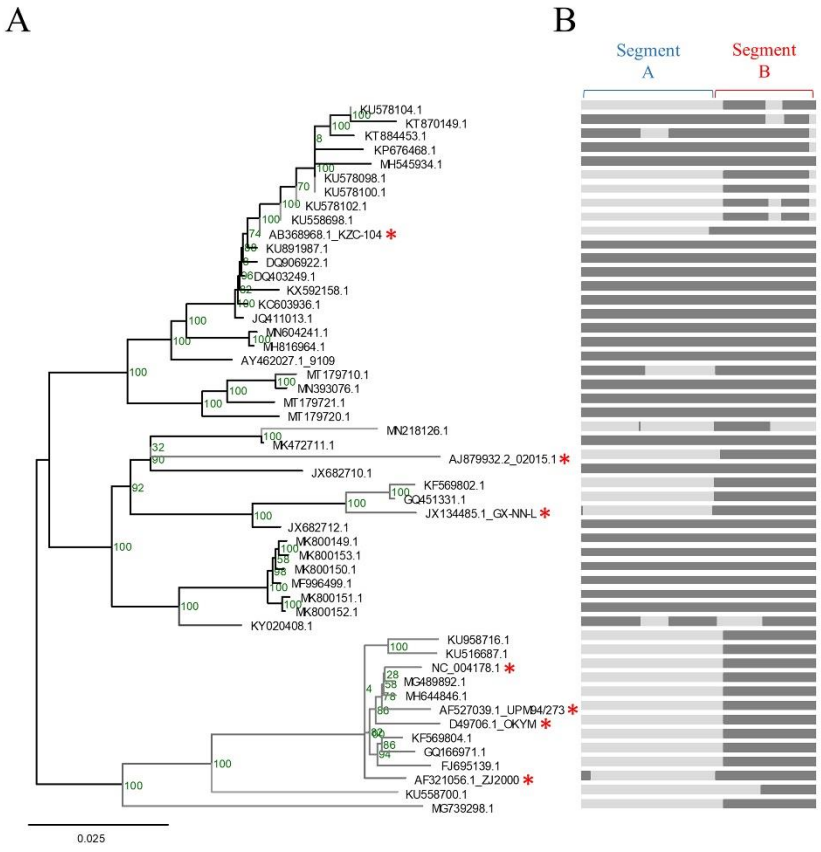


**Figure 5.** An overview of putative recombination and reassortment events occurred throughout the IBDV genome. A total number of 51 complete IBDV genomes including both segments A and B were MUSCLE aligned in MEGA-X and used to identify the putative recombination events in RDP5. (A) FastNJ Tree for the non-recombinant regions of the putative recombinant strains. Red asterisks represent the previously described reassortant strains. (B) Visual representation of putative recombination events. Dark grey areas represent the nucleotides corresponding to the major recombination parents used for generation of the FastNJ Tree.

**Table 1.** Recombination events observed in multiple independent IBDV strains from GenBank.

| Recombined segment | Event number | Breakpoints | | Number of independent isolates | Accession numbers |
|---|---|---|---|---|---|
| | | Start | End | | |
| A[a] | 1 | 52 | 1498 | 1 | MN218126.1 |
| | 2 | 464 | 1481 | 1 | KY020408.1 |
| | 3 | 1442 | 2812 | 2 | KU578098.1 |
| | | | | | KU578100.1 |
| | 4 | 1443* | 2126 | 2 | ^KT884452.1 |
| | | | | | KY020408.1 |
| | 5 | 1564 | 3216 | 1 | MT179710.1 |
| | 6 | 2292 | 2992 | 1 | KU578102.1 |
| B[b] | 1 | 48 | 682 | 1 | KY020409.1 |
| | 2 | 683* | 1158 | 1 | KY020409.1 |
| | 3 | 1110* | 1846 | 1 | KU558700.1 |
| | 4 | 1162 | 1758 | 1 | KU558700.1 |
| | 5 | 1247 | 1686 | 1 | KT870149.1 |
| | 6 | 1331 | 1644 | 3 | KU578103.1 |
| | | | | | KU578105.1 |
| | | | | | KU558698.1 |
| | 7 | 1362 | 2794 | 1 | MN218127.1 |
| | 8 | 2312 | 2804 | 6 | KU578099.1 |
| | | | | | KU578101.1 |
| | | | | | KU578103.1 |
| | | | | | KU578105.1 |
| | | | | | KU558698.1 |
| | | | | | KU558700.1 |

a and b= The total number of 58 and 49 complete genome sequences were used in this analysis for segments A and B, respectively.
*= The actual breakpoint position is undetermined (it was most likely either overprinted by a subsequent recombination event or off the edges of the analyzed sequence fragments).
^= The recombinant sequence may have been misidentified (one of the identified parents might be the recombinant)

## Discussion

Genomic recombination and reassortment are among major evolutionary processes through which viruses maintain a healthy level of diversity and adaptation. These processes allow the virus to evade the population bottlenecks (e.g., immune pressure) by maintaining a higher level of sequence polymorphism. Both genomic recombination and reassortment happen when a host is co- or mixed-infected with more than one virus strain.

Therefore, it is important to rule out the possibility of mixed infections before considering a virus as a recombinant or reassortant strain. In this study, we evaluated the possibility of mixed infection in the JRMP29IR strain which was previously flagged as a potential reassortant virus with the segments A and B from classics and very virulent viruses, respectively. We found that the JRMP29IR strain contained viruses from three different IBDV pathotypes (classic, variant and very virulent) suggesting a mixed infection at the time of sampling. We then explored the frequency of recombination and reassortment events in all available IBDV genomes using bioinformatics. Our results suggested that both recombination and reassertment events are common among IBDV strains and are likely to play an important role in the evolutionary dynamics of IBDVs in nature.

Three out of five viruses from the bursa of the experimentally infected SPF chickens in this study were characterized as very virulent IBDVs. This is while the original parental JRMP29IR strain was shown to be closely related to a cell culture-adapted classical virus strain (CU-1) and was presumably derived from a live-attenuated vaccine strain. This is not surprising because very virulent strains of IBDV have been shown to replicate at a higher rate in the infected animals causing the virus to rapidly outcompete the other co-infecting virus populations (Van Den Berg, 2000). Mixed infection situations can also provide a fertile ground for generation of novel recombined or reassortant genotypes which may be more fit than their predecessors. Hence, it is not surprising to isolate novel reassortant viruses from the JRMP29IR strain after subsequent passages and/or purifying selections in animals or cell cultures.

The JRMP29IR strain appears to have an intermediate pathotype between classic/variant and very virulent IBDV strains as observed based on the mortality, clinical signs, and histopathology. This is in contrast with the findings that most identified viruses from the bursas of infected chickens belonged to the very virulent genotype. This discrepancy seems to be related to the ability of co-infecting viral populations to affect the function of the parental virus through a phenomenon known as competitive inhibition (Whitaker-Dowling and Youngner, 1987). Although the dynamics of co-infecting virus populations in the infected chickens cannot be easily determined, our genotyping on the parental and chicken bursal passaged viruses suggest that the population composition in JRMP29IR has shifted toward the very virulent IBDV strains in the chicken bursas. A naturally occurring reassortant IBDV combined of segment A from a very virulent serotype 1 and segment B from a non-pathogenic serotype 2 viruses was shown to be less pathogenic in SPF chickens compared to the typical very virulent IBDV strains (Jackwood *et al.*, 2011). Naturally occurring reassortant IBDVs with either segment A or B from attenuated strains from serotype 1 were also shown to replicate at a slower pace in cell cultures or be less pathogenic for chickens compared to their very virulent parental viruses (Le Nouen *et al.*, 2006; Wei *et al.*, 2006). However, the exact mechanisms governing the reduced pathogenicity of very virulent IBDV strains in the host co-infected with a classic or variant IBDVs is yet to be determined.

Both recombination and reassortment events seem to frequently occur in IBDVs. Recombination may be the result of lineage-specific rate variation and/or convergent evolution but can also be related to sequencing and alignment errors, mixed infection and contamination, and/or improper bioinformatics analysis (Hoxie and Dennehy, 2020). Therefore, multiple steps should be taken to reduce the chance of incorrect recombination detections. One of the main steps is to ensure the samples do not contain multiple virus genotypes due to potential mixed

infections. In the case of mixed infection, samples must be carefully plaque purified prior to sequencing. Although such precautions cannot be done for the sequences obtained from the online repositories, careful bioinformatics analyses can greatly minimize possible errors (Hoxie and Dennehy, 2020). Herein, we set a stringent criterion for the recombination detection and only reported the events consistently detected by at least 6 out of the seven detection methods in RDP5. Further, multiple recombination events were repeatedly detected in more than one independent strain, making it less likely that the event is spurious or a consequence of improper sequence analysis or experimental techniques (Table 1). We used a modified alignment containing both genomic segments from each strain to explore the presence of reassortment events in IBDVs. We found numerous sequences with similar recombination breakpoints coinciding with the inter-segment linker regions in our alignment dataset, suggesting reassortments between the two genomic segments. In addition, our analysis was able to correctly identify all the seven previously reported reassortant IBDV strains from 1996 to 2013 (Brown and Skinner, 1996; Yamaguchi *et al.*, 1997; Kong *et al.*, 2004; Le Nouen *et al.*, 2006; Wei *et al.*, 2006; Chen *et al.*, 2012; Kasanga *et al.*, 2013). Altogether, this result provides a strong evidence for high frequency of genomic reassortments in circulating IBDV strains.

## Conclusion

In conclusion, the parental JRMP29IR strain contains multiple IBDV genotypes and appears to be derived from a flock undergoing a mixed IBDV infection. Subsequent passages of the JRMP29IR can facilitate the generation of true recombinant or reassortant viruses which can be effectively purified through virus plaque purification. Recombination and reassortment events are very common among naturally occurring IBDVs, suggesting that they are evolutionarily beneficial for the virus. Future studies should focus on generation of the laboratory reassortant IBDVs which can be useful for engineering the future live-attenuated IBDV vaccines.

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## Conflict of Interest

The authors declare that there are no conflicts of interest.

## References

Brown, M.D., Skinner, M.A. (1996). Coding sequences of both genome segments of a European 'very virulent' infectious bursal disease virus. *Virus Res, 40*, 1-15. [DOI:10.1016/0168-1702(95)01253-2]

Chen, F., Liu, J., Yan, Z., Liu, D., Ji, J., Qin, J., ... Xie, Q. (2012). Complete genome sequence analysis of a natural reassortant infectious bursal disease virus in China. *J Virol, 86*(21), 11942-11943 [DOI:10.1128/JVI.02043-12] [PMID] [PMCID]

Eterradossi, N., Saif, Y.M. (2020) *Infectious Bursal Disease. In: Diseases of Poultry*.

D.E. Swayne (Ed.), (14th ed.) New Jersey: John Wiley and Sons, Inc.. 257-283. [DOI:10.1002/9781119371199.ch7]

Ghaniei, A., Peighambari, S., Razmyar, J. (2011). Identification of very virulent infectious bursal disease viruses by RT-PCR of VP1gene and speculation about the possible presence of reassortant viruses. *J Vet Res*, *66*, 153-159.

Hemida, M. G., Al-Mubarak, A. I., Abdelaziz, A. M., & Ibrahim, A. M. (2019). Infectious Bursal Disease Virus (IBDV). In *Recent Adv Anim Virol* (pp. 237-252). Springer, Singapore. [DOI:10.1007/978-981-13-9073-9_13]

Hoxie, I., Dennehy, J.J. (2020). Intragenic recombination influences rotavirus diversity and evolution. *Virus Evol*, *6*, vez059. [DOI:10.1093/ve/vez059] [PMID] [PMCID]

Jackwood, D.J. (2017). Advances in vaccine research against economically important viral diseases of food animals: infectious bursal disease virus. *Vet Microbiol*, *206*, 121-125. [DOI:10.1016/j.vetmic.2016.11.022] [PMID]

Jackwood, D.J., Schat, K.A., Michel, L.O., de Wit, S. (2018). A proposed nomenclature for infectious bursal disease virus isolates. *Avian Pathol*, *47*, 576-584. [DOI:10.1080/03079457.2018.1506092] [PMID]

Jackwood, D.J., Sommer-Wagner, S.E., Crossley, B.M., Stoute, S.T., Woolcock, P.R., Charlton, B.R. (2011). Identification and pathogenicity of a natural reassortant between a very virulent serotype 1 infectious bursal disease virus (IBDV) and a serotype 2 IBDV. *Virology*, *420*, 98-105. [DOI:10.1016/j.virol.2011.08.023] [PMID]

Kasanga, C., Yamaguchi, T., Munang'andu, H., Ohya, K., Fukushi, H. (2013). Genomic sequence of an infectious bursal disease virus isolate from Zambia: classical attenuated segment B reassortment in nature with existing very virulent segment A. *Arch Virol*, *158*, 685-689. [DOI:10.1007/s00705-012-1531-4] [PMID]

Kim, H., Webster, R.G., Webby, R.J. (2018). Influenza virus: dealing with a drifting and shifting pathogen. *Viral Immunol*, *31*, 174-183. [DOI:10.1089/vim.2017.0141] [PMID]

Kong, L.L., Omar, A., Hair-Bejo, M., Aini, I., Seow, H.-F. (2004). Sequence analysis of both genome segments of two very virulent infectious bursal disease virus field isolates with distinct pathogenicity. *Arch Virol*, *149*, 425-434. [DOI:10.1007/s00705-003-0206-6] [PMID]

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol*, *35*, 1547-1549. [DOI:10.1093/molbev/msy096] [PMID] [PMCID]

Le Nouen, C., Rivallan, G., Toquin, D., Darlu, P., Morin, Y., Beven, V., de Boisseson, C., Cazaban, C., Comte, S., Gardin, Y. (2006). Very virulent infectious bursal disease virus: reduced pathogenicity in a rare natural segment-B-reassorted isolate. *J Gen Virol*, *87*, 209-216. [DOI:10.1099/vir.0.81184-0] [PMID]

Martin, D.P., Murrell, B., Golden, M., Khoosal, A., Muhire, B. (2015). RDP4: Detection and analysis of recombination patterns in virus genomes. *Virus Evol*, *26*;1(1):vev003. [DOI:10.1093/ve/vev003] [PMID] [PMCID]

McDonald, S.M., Nelson, M.I., Turner, P.E., Patton, J.T. (2016). Reassortment in segmented RNA viruses: mechanisms and outcomes. *Nat Rev Microbiol*, *14*, 448. [DOI:10.1038/nrmicro.2016.46] [PMID] [PMCID]

Michel, L.O., Jackwood, D.J. (2017). Classification of infectious bursal disease virus into genogroups. *Arch Virol, 162*, 3661-3670. [DOI:10.1007/s00705-017-3500-4] [PMID] [PMCID]

Patel, A.K., Pandey, V.C., Pal, J.K. (2016). Evidence of genetic drift and reassortment in infectious bursal disease virus and emergence of outbreaks in poultry farms in India. *Virus Dis, 27*, 161-169. [DOI:10.1007/s13337-016-0306-z] [PMID] [PMCID]

Pikuła, A., Lisowska, A., Jasik, A., Śmietanka, K. (2018). Identification and assessment of virulence of a natural reassortant of infectious bursal disease virus. *Vet Res, 49*, 89. [DOI:10.1186/s13567-018-0586-y] [PMID] [PMCID]

Razmyar, J., Peighambari, S. (2008). Molecular characterization of Iranian infectious bursal disease viruses. *Avian Dis, 52*, 665-669 [DOI:10.1637/8280-031008-Reg.1] [PMID]

Razmyar, J., Peighambari, S.M. (2009). Rapid differentiation between very virulent and classical infectious bursal disease viruses isolated in Iran by RT-PCR/REA. *Iran J Vet Med, 2*, 111-117.

Touzani, C.D., Fellahi, S., Gaboun, F., Fihri, O.F., Baschieri, S., Mentag, R., El Houadfi, M. (2019). Molecular characterization and phylogenetic analysis of very virulent infectious bursal disease virus circulating in Morocco during 2016-2017. *Arch Virol, 164*, 381-390. [DOI:10.1007/s00705-018-4076-3] [PMID]

Van Den Berg, T.P. (2000). Acute infectious bursal disease in poultry: a review. *Avian Pathol*, 29, 175-194. [DOI:10.1080/03079450050045431] [PMID]

Wei, Y., Li, J., Zheng, J., Xu, H., Li, L., Yu, L. (2006). Genetic reassortment of infectious bursal disease virus in nature. *Biochem Biophys Res Commun*, 350, 277-287. [DOI:10.1016/j.bbrc.2006.09.040] [PMID]

Whitaker-Dowling, P., Youngner, J.S. (1987). Viral interference-dominance of mutant viruses over wild-type virus in mixed infections. *Microbiol Rev, 51*, 179. [DOI:10.1128/MR.51.2.179-191.1987]

Yamaguchi, T., Ogawa, M., Miyoshi, M., Inoshima, Y., Fukushi, H., Hirai, K. (1997). Sequence and phylogenetic analyses of highly virulent infectious bursal disease virus. *Arch Virol, 142*, 1441-1458. [DOI:10.1007/s007050050171] [PMID]

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# تعیین هویت ملکولی و بالینی یک سویه ایرانی ویروس بیماری بورس عفونی حاوی جمعیت مخلوط ویروسی

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**زمینه مطالعه:** بیماری بورس عفونی پرندگان، یک بیماری بسیار واگیردار و سرکوب‌کنندۀ سیستم ایمنی در مرغ‌های جوان است که به‌وسیلۀ ویروس بیماری بورس عفونی ایجاد می‌شود. ویروس بیماری بورس عفونی حاوی ژنوم دوقطعه‌ای از نوع RNA دو رشته‌ای است. پدیدۀ نوترکیبی و یا بازآرایی ژنتیکی در خلال عفونت همزمان با دو یا چند ژنوتیپ از این ویروس محتمل می‌شود.

**هدف:** اهداف این مطالعه، شامل تعیین هویت یک سویه از قبل شناسایی‌شده ویروس بیماری بورس عفونی (JRMP29IR) در مرغ‌های عاری از پاتوژن مشخص (SPF)، ارزیابی حضور جمعیت‌های مختلف ویروسی و یا واجد بازآرایی ژنتیکی در این سویه، و آزمایش فراوانی نوترکیبی یا بازآرایی های ژنومی در ویروس بیماری بورس عفونی به‌وسیلۀ بررسی توالی ژنومی موجود در بانک جهانی ژن از طریق روش‌های بیوانفورماتیک بوده است.

**روش کار:** مرغ‌های SPF از طریق خوراکی و داخل چشمی با سویۀ JRMP29IR چالش داده شدند. بافت بورس پرندگان بیمار برای ارزیابی‌های هیستوپاتولوژیکی و تعیین توالی ژنوم ویروسی پس از انجام واکنش زنجیره‌ای پلیمراز رونویسی معکوس استخراج گردید. نوترکیبی و بازآرایی‌های ژنتیکی محتمل توسط نرم‌افزار Recombination Detection Program 5 ارزیابی شدند.

**نتایج:** نتایج تعیین توالی ژنومی ویروس‌های جداشده از بورس‌های عفونی مرغ‌ها، حاکی از آن بود که سویۀ JRMP29IR حاوی ویروس‌هایی از ژنوتیپ‌های کلاسیک، واریانت و بسیار حاد بیماری بورس عفونی است. از طریق بیوانفورماتیک، تعداد قابل توجهی از وقایع محتمل نوترکیبی و بازآرایی، که به نظر می‌رسد به‌طور طبیعی در ژنوم ویروس بیماری بورس عفونی اتفاق افتاده‌اند، شناسایی گردید.

**نتیجه‌گیری نهایی:** به نظر می‌رسد که سویه JRMP29IR از گله‌ای در خلال عفونت همزمان با چندین ژنوتیپ ویروس بیماری بورس عفونی جدا شده است. با توجه به فراوانی بالای نوترکیبی و بازآرایی در بین ویروس‌های بیماری بورس عفونی به نظر می‌رسد که این وقایع نقش پر اهمیتی در تکامل این ویروس‌ها در گذر زمان ایفا می‌کنند.

**واژه‌های کلیدی:** بازآرایی، تعیین هویت ملکولی، نوترکیبی، ویروس بیماری بورس عفونی

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