Bulgarian Journal of Veterinary Medicine, 2017, **20**, Suppl. 1, 234–239
ISSN 1311-1477 (print); ISSN 1313-3543 (online)

DETECTION AND FREQUENCY OF INFECTIOUS BURSAL DISEASE VIRUS (IBDV) VP2 GENE VARIANTS IN CHICKENS IN TURKEY

N. TURAN[1], A. GUREL[2], A. YILMAZ[1], U. Y. CIZMECIGIL[1], O. E. BAMAC[2], O. AYDIN[1], E. BAYRAKTAR[3], B. ÇAKAN[3] & H. YILMAZ[1]

[1]Department of Virology, Veterinary Faculty, Istanbul University, Istanbul, Turkey; [2]Department of Pathology, Veterinary Faculty, Istanbul University, Istanbul, Turkey; [3]CEVA Hayvan Sagligi, Turkey

Summary

Turan, N. A. Gurel, A. Yilmaz, U. Y. Cizmecigil, O. E. Bamac, O. Aydin, E. Bayraktar, B. Çakan & H. Yilmaz, 2017. Detection and frequency of infectious bursal disease virus (IBDV) VP2 gene variants in chickens in Turkey. *Bulg. J. Vet. Med.*, **20**, Suppl. 1, 234–239.

Infectious bursal disease (IBD-Gumboro) is an economically important viral disease in chickens. The disease is caused by infectious bursal disease virus (IBDV) which initiates immunosuppression and immunosuppressed chickens can not have a good immune response to vaccinations against other viral infections such as Newcastle disease or infectious bronchitis. Also, the risk of secondary infections increase. In IBD, the degree and duration of immunosupression varies depending on the genotype and virulence of virus detected. This study was aimed to investigate IBDV genotypes in chickens and the lesions occurred in bursa Fabricius during infections. For this purpose, bursa Fabricius from 80 broiler flocks located in different regions in Turkey were collected. In these samples, presence of IBDV and viral load were investigated by SYBR-Green real time RT-PCR. Of the samples with high CT value, the variable region of the VP2 gene was amplified, sequenced and phylogenetic analyses were performed to generate phylogenetic tree. Results of SYBR-Green real time RT-PCR showed that IBDV-RNA was detected in 60 (75%) samples and CT values were found to be between 19 and 37. Phylogenetic analyses revealed very virulent genotypes were present in 6 broiler flocks. In addition, classical and vaccine strains were identified. On histopathology, lesions indicating varying degrees of immunosuppression were observed in infected bursa of Fabricus. In conclusion, IBDV continues to be a health proplem in our country in chickens. Especially subclinical immunosuppressions were noticed and therefore this point needs attention. Therefore, it will be useful to reevaluate the current preventive and control measurements for IBD.

Key words: chicken, infectious bursal disease, phylogenetic analysis

INTRODUCTION

Infectious bursal disease (IBD-Gumboro) is an acute and highly contagious disease that occurs in many countries around the world, including our country, and causes immunosuppression, especially at an early age. This disease, caused by the infectious bursal disease virus (IBDV) classified in the *Birnaviridae* family, causes lymphocy-

tolysis and immunosuppression as rapidly replicating in developing B-lymphocytes in bursa of Fabricius (Jackwood , 2016). The virus has different strains as classical, variant and very virulent. The major A segment of the viral genome encodes VP2, VP3, VP4 and VP5 viral proteins, while the smaller B segment encodes VP1 viral protein (Mahgoub, 2012). Antigenic variations are more likely to occur in the VP2 gene of the virus and therefore this gene is used in genotyping studies (Jackwood , 2016).

The disease has acute and subclinical forms, and the emergence of these forms generally depends on the virulence and subtypes of the virus (Van den Berg *et al*., 2004). Very virulent IBDV has a much higher mortality rate than conventional IBDV. Very virulent IBDV has a mortality of 100% in SPF chickens, 60% in laying hens and 30% in broilers (Van den Berg *et al*., 2004). Very virulent IBDV has caused economic losses in poultry industry, which has caused major outbreaks in our country and around the world. In order to distinguish between classic IBDV and very virulent IBDV, we should investigate the VP2 gene, which contains variable region and is highly susceptible to mutation. Sequence analysis of vaccine and field strains and reporting the VP2 gene variants to the GenBank and establishing the current vaccination practices by ensuring colaboration with the poultry sector is of great importance in terms of poultry industry and economy of our country (Jackwood , 2016).

In this study, in 2016, detection and frequency of IBDV virus were investigated in bursa of Fabricius samples collected from the Marmara, Western Black Sea, Mediterranean and Central Anatolian regions of Turkey. Squence analyses of the VP2 gene and phylogenetic analysis were peformed to determine the genotype distribution of IBDV viruses in these regions. Also lesions of bursa Fabricius were examined.

MATERIAL AND METHODS

Samples, RNA extraction and reverse transcription

In this study, bursa of Fabricius from 80 broiler chickens in Marmara, Western Black Sea, Mediterranean and Central Anatolia regions were investigated by SYBR-Green real time RT-PCR for the presence of IBDV. RNeasy Mini Kit (Qiagen, Cat. No. 74106) was used for viral RNA extraction from tissue samples. The Improm-II kit (Improm-II reverse transcriptase-Promega, Cat. No. A3803) was used to obtain cDNA from RNA. Reverse transcription was performed in two steps as described by others (Yilmaz *et al*., 2011).

Real-Time RT-PCR, VP2 gene sequencing and phylogenetic analyses

SYBR-Green real-time RT-PCR was used for rapid detection of the IBDV and to determine viral loads in the samples by using the the primers and PCR conditions as recommended by others (Tomas *et al*., 2012). Samples having low CT value (high viral load) in real time PCR were selected and VP2 gene was partially sequenced (Medsantek) as described by others (Toroghi *et al.*, 2001). Phylogenetic analyses were performed by using MEGA-6 program and a phylogenetic tree was generated by aligning the VP2 gene of IBDV reported to GenBank from other countries.

Necropsy and histopathology

For this, a total of 28 chickens could be examined. Bursa of Fabricius was exam-

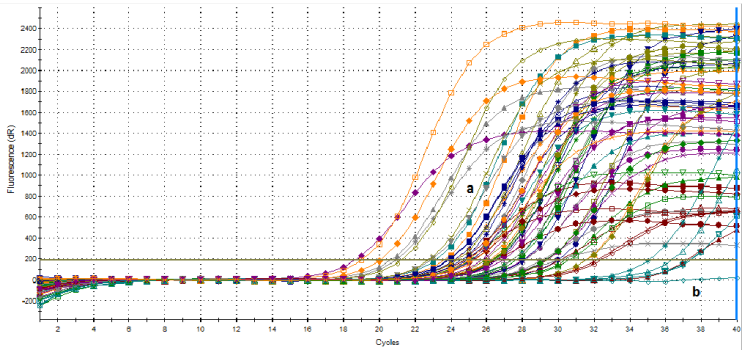

Fig. 1: IBDV positive detected samples by SYBR-Green real-time RT-PCR.
a: positive control; b: negative control; Other curves show IBDV positive samples

ined for the presence of edema, necrosis, discoloration as well as decrease or increase in size. Histopathological analyses were performed by conventional methods.

RESULTS

SYBR-Green real time RT-PCR and phylogenetic analyses

A 95 bp product was obtained in agarose gel electrophoresis after the SYBR-Green real-time RT-PCR. IBDV positivity was detected in 60 (75%) of 80 farms with SYBR-Green Real-Time RT-PCR. CT values of the positive samples and the positive control ranged from 19 to 37 (Fig. 1).

A 522 bp product was obtained in agarose gel electrophoresis after RT-PCR for sequencing (Fig. 2). In this way, partial sequence analysis of the VP2 variable region was performed in 36 samples. According to the VP2 gene partial sequence analysis and phylogenetic analysis (Fig. 3). IBDV sequences detected in 6 broiler farms in different regions were found to be similar to the very virulent IBDV genotypes, vv-IBDV-FS10, vv-IBDV-FS12, AL13 vvIBDV, Jordan vvIBDV, V90/TW95, West Bengal/HBL-07-15-b reported to the GenBank. Classical and vaccine strains were also detected.

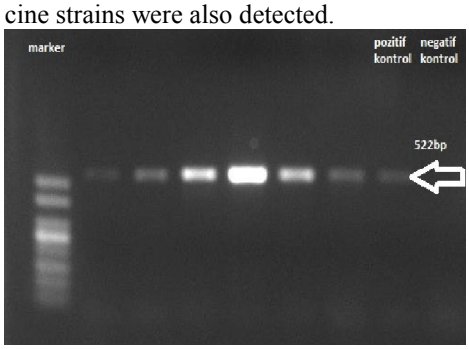

Fig. 2. Bands seen on agarose gel electrophoresis performed for RT-PCR for IBDV sequencing.

Necropsy and histopathological findings

Some of the bursa of Fabricus from IBDV positive chickens were athrophied and found smaller than their normal size. Microscopic examinations have revelaed that some of the IBDV positive bursa of Fabricus had large numbers of vacuolisations in the lamina epithelium of the plica, odema in interfollicular area and enlargement of RES cells. More than one follicle; with expansion and necrosis in the medullar areas, some follicles showed marked endothelial-type macrophage accumulation, widespread vacuolisation, and atrophy in a large number of follicles (Fig. 4) were also seen.

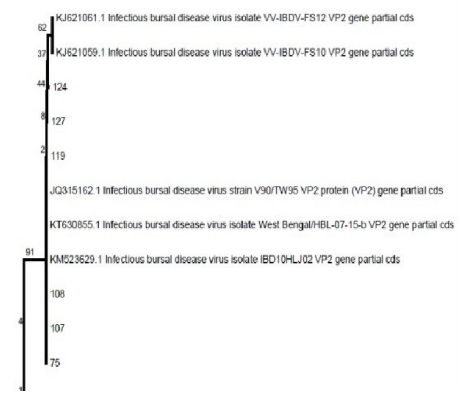

Fig. 3. vvIBDV strains (75, 106, 107, 119, 124, 127) detected in this study and similarity to other vvIBDV VP2 genes reported globally to GenBank.

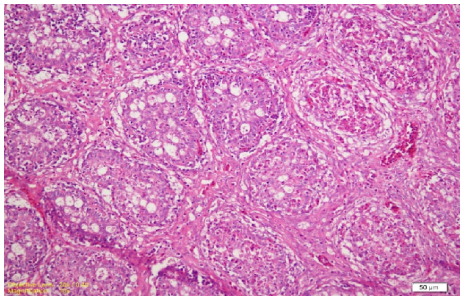

Fig. 4. Histopathological changes of bursa of Fabricius in RT-PCR-IBDV positive chickens. Vacuolisation, necrosis, RES cell infiltrations in medullar areas in follicles in the bursa of Fabricius, expansion in interfollicular areas, RES cell and fibrous tissue cell growth and atrophy in the follicles. H.E. Bar ; 50 µm.

DISCUSSION

Since in 1962 in US, IBDV has become a major economic problem for the poultry industry worldwide (Jackwood , 2016). At the beginning of the 1990s, very virulent IBDV strains emerged in Europe, spreading rapidly to Russia and then to all of Asia (Domanska *et a*l 2004, Van Den Berg, 2000). Only North America, New Zealand and Australia are considered to be free from very virulent IBDV (Jackwood & Sommer, 2005). The presence of maternal immunity and endemic variant strains has been shown to cause very virulent IBDV to spread so rapidly in other countries that it is limited to only a few cases in the USA (Jackwood , 2011).

It has been found in Turkey that in beginning of the 1990s and following years the outbreaks were caused by strains of very virulent IBDV (Ture *et a*l., 1998, Çeribaşı *et a*l 2007). In 2014, in a study conducted in Iraq, the phylogenetic analysis of vvIBDV strains found similarity to vvIBDV strains previously reported in Turkey and Iran and presence of the virus could be due to a limited number of live animal transits from these countries (Amin & Jackwood, 2014).

Sequencing and phylogenetic analysis of the VP2 gene is recommended to isolate the very virulent IBV strain from other strains. However, studies on the VP1 gene can also be performed (Hon *et al*., 2006, Le nouen *et al* 2006). In this study, VP2 gene variations of IBDV was investigated. According to the partial VP2 gene sequencing and phylogenetic analyses, very virulent IBDV was detected in 6 broiler farms in different regions and were found to be similar to very virulent IBDV genotypes, vv-IBDV-FS10, vv-IBDV-FS12, AL13 vvIBDV, Jordan vvIBDV, V90/TW95, West Bengal/HBL-07-15-b reported to the GenBank.

In conclusion, the results of this study show that vvIBDV starins are circulating in chickens in our country. Also, the time of vaccination and vaccines used needs to be reevaluated since virulence of existing genotypes may lead to disorders and immunosuppression in bursa of Fabricius. Even if only a single amino acid of the VP2 protein replaces, the virulence might change (Jackwood *et al*., 2008). Therefore, it would be useful to perform phy-

logenetic studies related to the very virulent IBDV strain in order to avoid economic losses in the poultry sector. For this, epidemiological surveys should be done to find out which genotypes are circulating in the farms to prevent and control IBDV infections in the farms.

REFERENCES

Amin, O. G. M. & D. J. Jackwood, 2014. Identification and molecular analysis of infectious bursal disease in broiler farms in Iraq. *Tropical Animal Health and Production*, **46**, 1297–1301.

Çeribaşı, A. O., H. Bulut, İ. Gülaçtı, Y. Eröksüz & Y. Bolat, 2007. Presence of a very virulent genotype of infectious bursal disease virus in vaccinated layer hens in Turkey. *Turkish Journal of Veterinary and Animal Science*, **31**, 105–111.

Domanska, K., T. Mato, G. Rivallan, K. Smietanka, Z. Minta, C. deBoisseson, D. Toquin, B. Lomniczi, V. Palya & N. Enterradossi, 2004. Antigenic and genetic diversity of early European isolates of infectious bursal disease virus prior to the emergence of the very virulent viruses: early European epidemiology of infectious bursal disease revisited? *Archives of Virology*, **142**, 465–480.

Hon, C. C., T. Y. Lam, A. Drummond, A. Rambaut, Y. F. Lee, C. W. Yip, F. Zeng, P. Y. Lam, P. T. Ng & F. C. Leung, 2006. Phylogenic analysis reveals a correlation between the expansion of very virulent infectious bursal disease virus and reassrotment of its genome segment B. *Journal of Virology*, **80**, 8503–8509.

Jackwood, D. J. & S. E. Sommer, 2005. Molecular studies on suspect very virulent infectious bursal disease virus genomic RNA samples. *Avian Diseases,* **49**, 246–251.

Jackwood. D. J., B. Sreedevi, L. J. LeFever & S. E. Sommer-Wagner, 2008. Studies on naturally occuring infectious bursal disease viruses suggest that a single amino acid substitution at position 253 in VP2 increases pathogenicity. *Virology*, **377**, 110–116.

Jackwood, D. J., 2011. Viral competition and maternal immunity influence the clinical disease caused by very virulent infectious bursal disease virus. *Avian Diseases*, **55**, 398–406.

Jackwood, D. J., 2016. Advances in vaccine research against economically important vial disease off food animals: Infectious Bursal Disease Virus. *Veterinary Microbiology,* **206**, 121–125.

Le Nouen C., G. Rivallan, D. Toquin, P. Darlu, Y. Morin, V. Beven, C. de Boisseson, C. Cazabaan, S. Comte, Y. Gardin & N. Eterradossi, 2006. Very virulent infectious bursal disease virus: Reduced pathogenicity in a rare natural segment-B-reassorted isolate. *Journal of General Virology*, **87**, 535–539.

Mahgoub, H. A., 2012. An overview of infectious bursal disease. *Archives of Virology,* **157**, 2047–2057.

Tomas, G., M. Hernandez, A. Maradino, Y. Panzera, L. Maya, D. Hernandez, A. Pereda, A. Banda, P. Villegas, S. Aguirre & R. Perez, 2012. Development and validation of a TaqMan-MGB real-time RT-PCR assay for simultaneous detection andd characterization of infectious bursal disease virus. *Journal of Virological Methods*, **185**, 101–107.

Toroghi, R., M. Kataria, K. C. Verma, R. S. Kataria & A. K. Tiwari, 2001. Amino acid changes in the variable region of VP2 in three infectious bursal disease viruses with different virulence, originating from a common ancestor. *Avian Pathology*, **30**, 667–673.

Türe, O., Y. M. Saif & D. J. Jackwood, 1998. Restriction fragment length polymorphism analysis of highly virulent strains of infectious bursal disease viruses from Holland, Turkey and Taiwan. *Avian Diseasesm* **42**, 470–479.

Van Den Berg, T. P., 2000. Acute infectious bursal disease in poultry: A review. *Avian Pathology,* **29**, 175–194.

Van den Berg, T. P., D. Morales, N. Eterradossi, G. Rivallan, D. Toquin, R. Raun, K. Zierenberg, M. F. Zhang, Y. P. Zhu, C. Q. Wang, H. J. Zheng, X. Wang, G. C. Chen, B. L. Lim & H. Müller, 2004. Assessment of genetic, antigenic and pathotypic criteria for the characterization of IBDV strains. *Avian Pathology*, **33**, 470–476.

Yilmaz, H., N. Turan, E. Altan, K. Bostan, A. Yilmaz, C. R. Helps & K. O. Cho, 2011. First report on the phylogeny of bovine norovirus in Turkey. *Archives of Virology,* **156**, 143–147.