VETERINARSKI ARHIV 82 (1), 115-124, 2012

Characterization of infectious bursal disease virus field strains in Jordan using molecular techniques - a short communication

Dergham A. Roussn[1]*, Walid Al-Saleh[2], Ghassan Y. Khawaldeh[1], and Waheed S. Totanji[1]

[1]*Provimi, Technical Department, Amman Jordan*

[2]*CEVA Sante Animale, Poultry Range Technical Manager, Middle East Area, Jordan*

ROUSSN, D. A., W. AL-SALEH, G. Y. KHAWALDEH, W. S. TOTANJI: Characterization of infectious bursal disease virus field strains in Jordan using molecular techniques. Vet. arhiv 82, 115-124, 2012.

ABSTRACT

The reverse transcriptase-polymerase chain reaction/restriction fragment length polymorphism (RT-PCR/RFLP) and RT-PCR specific primer to detect very virulent (vv) strains of infectious bursal disease virus (IBDV) were used for identification and characterization of Jordanian field isolates of IBDV that caused severe outbreaks. In this study, 80 bursa of fabricius samples were used from 20 commercial broiler chicken flocks in Jordan with clinical symptoms of IBDV. The RT-PCR/RFLP was conducted on a 743-bp fragment of the VP2 gene with the restriction enzymes *Bst*NI and *Mbo*I. The results indicate the existence of IBDV field strains in Jordan. In addition, 60% of IBDV Jordanian isolates had unique RFLP patterns different from those previously published elsewhere. However, 20% of local IBDV strains were positive on using a specific primer for vvIBDV and had a unique RFLP pattern that differed from the Jordanian IBDV isolate and those previously published elsewhere.

Key words: broiler, Jordan, infectious bursal disease, restriction fragment length polymorphism

Introduction

Infectious bursal disease virus (IBDV) is a member of the genus *Avibirnavirus* within the family *Birnaviridae* (PRINGLE, 1998). Due to the high mortality rates in acute infections and severe immuno-suppression by subclinical infections, IBDV is of major economic importance to the poultry industry (KIBENGE et al., 1998). Two serotypes of IBDV can be differentiated by the virus neutralization test. Serotype 1 contains the pathogenic strains, whereas serotype 2 strains cause neither disease nor protection against serotype 1 strains in chickens (ISMAIL and SAIF, 1991; ISMAIL et al., 1998; MEFERRAN et

*Corresponding author:

Dr. Dergham Ahmad Roussn Roussan, DVM, MAM, Technical Department, Provimi - Jordan, P.O. Box: 499, Amman 11118, Jordan, Phone: +962 64 711 476, Fax: +962 64 711 478, E-mail: droussan@provimi.com.

ISSN 0372-5480
Printed in Croatia

al., 1980). Pathogenic serotype 1 IBDV field strains can be grouped into classical virulent (cv) strains, antigenic variant strains and very virulent (vv) strains (BROWN et al., 1994; KIBENGE et al., 1998; SNYDER, 1990; VAKHARIA et al., 1994; ZIERENBERG et al., 2000; ZIERENBERG et al., 2001).

The use of molecular techniques to detect and identify IBDV strains has increased in recent years. Scientists have used reverse transcriptase-polymerase chain reaction (RT-PCR) to amplify sections of the IBDV genome (JACKWOOD and JACKWOOD, 1994; JACKWOOD and JACKWOOD, 1997; JACKWOOD and NIELSEN, 1997; JACKWOOD and SOMMER, 1997; JACKWOOD and SOMMER, 1998; LIU et al., 1994; WU et al., 1992). Two enzymes (*Bst*NI and *Mbo*I) have been previously used for characterization of infectious bursal disease (IBD). The *Bst*NI and *Mbo*I enzymes were used to generate restriction fragment length polymorphism (RFLP) patterns that distinguished viruses into molecular groups (JACKWOOD and SOMMER, 1997; JACKWOOD and SOMMER, 1998; JACKWOOD and SOMMER, 1999; ZIERENBERG et al., 2001). The amplified sections of the viral genome are then examined with restriction enzymes that cut the amplified DNA, resulting in a number of fragments that can be separated and visualized by electrophoresis. The VP2 gene is commonly studied because it encodes for the major protective epitopes, contains determinants for pathogenicity, and is highly variable among strains (ABDEL-ALIM et al., 2003; BAYLISS et al., 1990; OPPLING et al., 1991; VAKHARIA et al., 1994).

In Jordan, where the poultry industry is of great importance in terms of its social and economic impact, a preventive program is crucial to avoid virus spread and disease appearance. The infectious bursal disease virus continuous to be a serious problem in Jordan (DERGHAM et al., 2007; Jordanian Ministry of Agriculture, Annual Report 2010) as it does in other poultry producing countries all over the world. Until now, diagnosis in Jordan has based mainly on clinical signs, gross lesions, histopathology, isolation, and conventional identification tests (immunofluorescence assay, ELISA, agar gel immunodiffusion assay etc). These techniques and tests are all laborious, time consuming, and in some cases are expensive and require specialized laboratory personal. Detection of IBDV in clinical samples by RT-PCR is superior to the conventional techniques mentioned above, in that it has greater sensitivity and specificity (JACKWOOD and SOMMER, 1997; JACKWOOD and SOMMER, 1998).

Although IBDV is frequently isolated and causes significant losses to the poultry industry, no information is currently available about the type of IBDV strains present in Jordan. The objectives of this study were: i) to characterize IBDV field strains in commercial broiler flocks in Jordan using RT-PCR/RFLP, ii) to detect vvIBDV using a specific primer, and iii) to evaluate comparatively the Jordanian RT-PCR/RFLP patterns with those previously identified and published elsewhere.

Materials and methods

Samples. A total of 80 of bursa of fabricius samples (edematous and hemorrhagic) were collected from 20 commercial broiler flocks (4 samples from each flock) in Jordan, during the period from November 2007 to November 2010 and they were sent to the Provimi Jordan laboratory . The age of the chickens varied from 19 to 26 days. All flocks were contacted through the appropriate veterinarians and all owners agreed to participate in this study. All flocks were vaccinated against IBD, but there were different vaccination approaches (one dose intermediate or two dose intermediate vaccines). All flocks in this study had a history of poor growth performance and secondary infections. Mortality varied between 25%-35% in all flocks, except in 4 flocks where mortality was 75-80%. An intermediate vaccine of IBDV, CEVAC® GUMBO L (CEVA Sante Animale, Libourne, France) currently used in Jordan, was taken from the manufactures for the analysis of the vaccine strain.

Extraction of RNA and RT-PCR. Total RNA was extracted directly from bursa of fabricius samples and vial vaccine with Trizol® Reagent (Life Technology, Carlsbd, CA), according to the manufacturer's instruction. Briefly, 4 bursa fabricius were pooled and homogenized in 10% (w/v) TNE (Tris-HCL, EDTA, NaCl) buffer. Trizol® was added to supernatants from homogenized tissue samples and incubated for 5 min at 25 ºC. The mixtures were then extracted with chloroform (200 µL per mL of Trizol® Reagent) and centrifuged at 10,000 Xg for 15 min at 4 ºC. The aqueous phases were precipitated with isopropyl alcohol and the pellets washed with 75% ethanol, dried, and suspended in RNase-free water (Promega Corp., Madison, WI). Lyophilized vaccine viruses were reconstituted by adding 2 mL phosphate-buffered saline prior to RNA extraction. Universal primers (Table 1) were used in this study to determine the presence of IBDV. One-step RT-PCR was performed by using an Access RT-PCR System kit (Promega Corp) according to the manufacturer's instructions. Briefly, a 50 µL reaction volume per sample was prepared by adding 10 µL of avian myeloblastosis virus/ reverse transcriptase/ *Thermus filiformis* DNA polymerase 5X reaction buffer, 1 µL of deoxy nucleotide 50-triphosphate mixture (10 mM each deoxy nucleotide 50-triphosphate), 1 µL (50 pmol/µL) of each downstream and upstream primer (Alpha DNA, Montreal, Quebec, Canada), 2 µL of 25 *mM* MgSO4, 1 µL of avian myeloblastosis virus reverse transcriptase (5 u/µL),1 µL of *T. filiformis* DNA polymerase (5 u/µL), 28 µL of nuclease-free water, and 5 µL of RNA template. Reverse transcription PCR was carried out in a DNA Engine thermal cycler (BioRad Laboratories Ltd., Mississauga, Ontario, Canada) for 1 reverse transcription cycle of 60 min at 45 °C, followed by 94 °C for 5 min, then 40 PCR cycles of 94 °C for 45 s, 59 °C for 45 s, and 72 °C for 90 s, with a final extension cycle at 72 °C for 10 min. Reverse transcription PCR produces a 736-bp fragment. A negative control (nuclease-free water) (Promega Corp) was also used in each run.

RT-PCR/RFLP. The RT-PCR products were digested with the restriction enzymes *Bst*NI and *Mbo*I (New England Biolabs Inc., Beverly, MA). The *Bst*NI and *Mbo*I enzymes were used to generate RFLP patterns that distinguished viruses into molecular groups. The reactions were carried out following the manufacture's instructions.

Agarose gel electrophoresis. The RT-PCR and RFLP products were electrophoresed on a 2% agarose gel in Tris-acetate-EDTA buffer (40 mM of Tris and 2 mM of EDTA, with a pH value of 8.0) containing ethidium bromide (Promega Corp.) for 50 min at 100 V and visualized under ultraviolet light (AlphaImager; Alphainnotech, San Leandro, CA).

Detection of vv of IBDV. Specific primers (Table 1) were used to investigate the presence of the vvIBDV strain. Extraction of RNA and the RT-PCR program were performed as described above, except for the annealing temperature (50 ºC). Visualization of bands was done as above.

Results

Infectious bursal disease virus RNAs were detected in all bursa of fabricius samples obtained from 20 commercial broiler flocks and in IBDV vaccine (CEVAC® GUMBO L) as evidenced by amplification of the 743-bp region of VP2 gene by RT-PCR (Fig. 1). There were 3 different RFLP patterns (A-C) (Fig. 2) (Table 2) after digestion of local IBDV strains with *Bst*NI and *Mbo*I enzymes.

Four (20%) strains showed the identical RLFP pattern A, 12 (60%) strains showed the identical RFLP pattern B, and 4 (20%) strains showed the identical RFLP pattern C. On other hand, a different RFLP pattern was obtained after digestion of the IBDV vaccine CEVAC® GUMBO L (Fig. 2) (Table 2). The vvIBDV strains were detected in 4 (20%) flocks (Fig. 3) and all of these isolates had the same RFLP pattern (C) and the flocks suffered high mortality (75-80%).

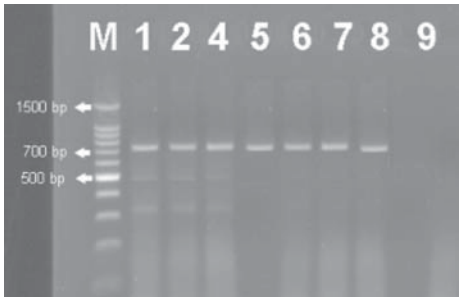

Fig. 1. Agarose gel electorophpresis (2% agarose gel) of 743-bp fragment of the VP2 gene. M = 100-1500 bp DNA ladder marker (Promega Corp., Madison, WI); Lanes 1 to 8 = Local IBDV strains (positive; band at 743 bp). Lane 8 = IBDV vaccine (CEVAC® GUMBO L vaccine (CEVA Sante Animale, Libourne, France) (positive; band at 743 bp). Lane 9 = Negative control.

Table 1. Polymerase chain reaction primers used for IBDV and vvIBDV

Type of primer	Primer's name	Sequences	Expected RT-PCR product size (bp)	References
Universal	Forward Reverse	5'- GCCCAGAGTCTACACCAT -3' 5'- CCCGGATTATGTCTTTGA -3'	743	JACKWOOD NIELSEN, 1997
Specific*	Forward Reverse	5'- CCGAGGCCACAGATAACCTTAAA -3' 5'- CCTCTAAACGGGTTGAAC -3'	715	ASHRAF et al., 2007

*This primer was used to detect vvIBDV strain.

Table 2. Reverse transcriptase-polymerase chain reaction/restriction fragment length polymorphism (RT-PCR/RFLP) assay results for vaccine and field strains. Values are the length in base pairs of restriction fragments. Marked boxes designate the presence of a restriction fragment after digestion with restriction enzymes[1].

RFLP patterns	*Bst*NI							*Mbo*I			
	528	424	209	172	154	139	119	403	362	269	229
A (20%)[2]		√		√			√		√		√
B (60%)[2]		√	√	√	√		√	√		√	
C (20%)[2]	√	√		√			√		√		√
CEVAC® GUMBO L[3]			√	√	√		√		√		√

[1]All flocks (n = 20) in this study had a history of clinical symptoms of IBDV, [2]Values in parentheses represent the percentage of RFLP molecular patterns. [3]Vaccine strain.

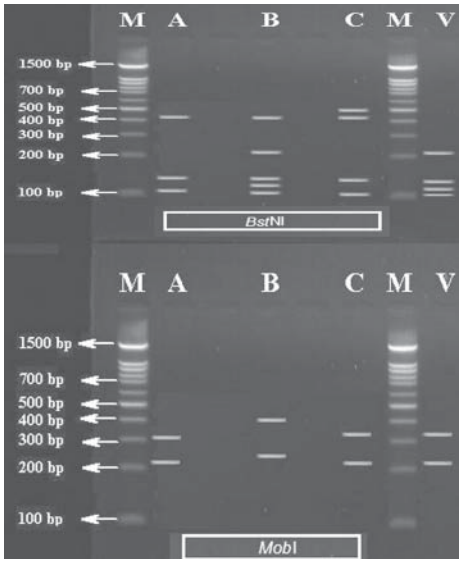

Fig. 2. *Bst*NI and *Mob*I restrictions of RT-PCR amplicons of IBDV RNAs obtained from flocks reared commercially in Jordan. M = 100-1500 bp DNA ladder marker (Promega Corp., Madison, WI); A = RLFP molecular pattern for 4 IBDV field samples digested by *Bst*NI and *Mob*I enzymes; B = RLFP molecular pattern for 11 IBDV field samples digested by *Bst*NI and *Mob*I enzymes. C = RLFP molecular pattern for 5 IBDV field samples digested by *Bst*NI and *Mob*I enzymes. V = RLFP pattern of vaccine virus (CEVAC® GUMBO L vaccine (CEVA Sante Animale, Libourne, France) digested by *Bst*NI and *Mob*I enzymes.

Fig. 3. Agarose gel electorophpresis (2% agarose gel) of RT-PCR with specific primers for vvIBDV strains. M = 100-1500 bp DNA ladder marker (Promega Corp., Madison, WI); Lanes 1-4 = vvIBDV field strains (positive; band at 715 bp). Lanes 5-8 = IBD field strains (positive with universal primers). Lane 9 = IBDV vaccine (CEVAC® GUMBO L vaccine (CEVA Sante Animale, Libourne, France) (positive; band at 715 bp). Lane 10 = Negative control.

Discussion

Classification of IBDV strains by molecular techniques, such as RT-PCR and RFLP, has been frequently reported in the last decade and has been demonstrated to be a good tool to identify and classify viral isolates. JACKWOOD and SOMMER (1998) classified IBDV strains into five molecular groups by RT-PCR of a 743-bp fragment of VP2 gene, followed by RFLP of this fragment with the restriction enzymes *Bst*NI and *Mbo*I, and they found a high degree of heterogenicity among field strains of IBDV. In the present study, 20 IBDV field strains were characterized and classified by RT-PCR and RFLP of a 743-bp fragment of VP2 gene and RFLP of this fragment with the enzymes *Bst*NI, and *Mbo*I.

The results of this study showed the high genetic heterogenicity that exists among the IBDV field strains detected in Jordan. When RT-PCR products of all local strains of IBDVs were digested with *Bst*NI and *Mbo*I enzymes, 3 different RFLP profiles were obtained (A-C) (Fig. 1) (Table 2). The RFLP profile A (20%) corresponded to the RFLP patterns of previously identified (ABDEL-ALIM and SAIF, 2001; JACKWOOD and SOMMER, 1998; TURE et al., 1998; SAREYYUPOGLU and AKAN, 2006). The RFLP profile B (60%) was found to be the most common and unique molecular pattern detected in Jordan which did not resemble any IBDV described earlier (ABDEL-ALIM et al., 2003; ABDEL-ALIM and SAIF, 2001; JACKWOOD and SOMMER, 1997; JACKWOOD and SOMMER, 1998; LIN et al., 1993; TURE at al., 1998, SAREYYUPOGLU and AKAN, 2006). The RFLP profile C (20%) was also a unique molecular pattern, which did not resemble any of the IBDV RFLP in Jordan and those previously published elsewhere (ABDEL-ALIM et al., 2003; ABDEL-ALIM and SAIF, 2001; JACKWOOD and SOMMER, 1997; JACKWOOD and SOMMER, 1998; LIN et al., 1993; TURE at al., 1998; SAREYYUPOGLU and AKAN, 2006). On other hand, the RFLP pattern obtained after digestion of the IBDV vaccine (CEVAC® GUMBO L) by the *Bst*NI and *Mbo*I enzymes, was different from those of local strains.

A specific *Ssp*I site has previously been identified in all very virulent IBDV (vvIBDV) strains (LIN et al., 1993; ZIERENBERG et al., 2000; ZIERENBERG et al., 2001). Consequently, this *Ssp*I site has been used as a genetic marker to predict a very virulent phenotype that must be confirmed by *in vivo* studies. However, not all vvIBDV have this marker and some non-vvIBDV strains have been found to contain the *Ssp*I marker (ETERRADOSSI et al., 1999; LIM et al., 1999; SAPATS and IGNJATOVIC, 2002). For these reasons we used specific primer (ASHRAF et al., 2007) to detect vvIBDV. In the present study 4 (20%) of the local IBDV isolates were positive (Fig. 3) when we used a specific primer to detect vvIBDV. It is interesting that all our vvIBDV isolates had the same RFLP pattern (pattern C) that differs from the patterns detected in Jordan or that have been previously published. Hence, the detection of vvIBDV strains in Jordan provides an explanation for

the occurrence of severe outbreaks of IBDVs in chicken flocks in Jordan that have been previously vaccinated with IBDV vaccines.

In conclusion, this is the most comprehensive study of molecular typing of IBDV strains detected in Jordan, and also the first report showing new molecular patterns different from previously published RT-PCR/RFLP patterns.

Acknowledgements

This study was funded by Provimi Jordan. Our deepest gratitude extends to all members of Provimi Jordan.

References

ABDEL-ALIM, G. A., M. H. H. AWAD, Y. M. SAIF (2003): Characterization of Egyptian field strains of infectious bursal disease virus. Avian Dis. 47, 1452-1457.

ABDEL-ALIM, G. A., Y. M. SAIF (2001): Immunogenicity and antigenicity of very virulent strains of infectious bursal disease viruses. Avian Dis. 45, 92-101.

ASHRAF, S., Y. TANG, Y. M SAIF (2007): Development of differential RT-PCR assays and molecular characterization of the complete VP1 gene of five strains of very virulent infectious bursal disease virus. Avian Dis. 51, 934-941.

BAYLISS, C. D., U. SPIES, K. SHAW, R. W. PETERS, A. PAPAGEORGIOU, H. MULLER, M. E. BOURSNELL (1990): A comparison of the sequences of segment A of four infectious bursal disease virus strains and identification of a variable region in VP2. J. Gen. Virol. 71, 1303-1312.

BROWN, M. D., P. GREEN P, M. A. SKINNER (1994): VP2 sequences of recent "very virulent" isolates of infectious bursal disease virus are closely related to each other but are distinct from those of "classical" strains. J. Gen. Virol. 75, 675-680.

DERGHAM, A. R., R. HADDAD, G. KHAWALDEH, I. SHAHEEN (2007): Newcastle disease in vaccinated commercial broiler chicken flocks experiencing subclincal infectious bursal disease in Jordan. J. Poult. Sci. 44, 446-452.

ETERRADOSSI, N., C. ARNAULD, F. TEKAIA, D. TOQUIN, H. LE COQ, G. RIVALLAN, M. GUITTET, J. DOMENECH, T. P. VAN DEN BERG, M. A. SKINNER (1999): Antigenic and genetic relationships between European very virulent infectious bursal disease viruses and a early West African isolate. Avian Pathol. 28, 36-46.

ISMAIL, N., Y. M. SAIF (1991): Immunogenicity of infectious bursal disease viruses in chickens. Avian Dis. 35, 460-469.

ISMAIL, N., Y. M. SAIF, P. D. MOORHEAD (1998): Lack of pathogenicity of five serotype 2 infectious bursal disease viruses in chickens. Avian Dis. 32, 757-759.

JACKWOOD, D. J., R. J. JACKWOOD (1994): Infectious bursal disease viruses: molecular differentiation of an-tigenic subtypes among serotype 1 viruses. Avian Dis. 38, 531-537.

JACKWOOD, D. J., R. J. JACKWOOD (1997): Molecular identification of infectious bursal disease virus strains. Avian Dis. 41, 97-104.

JACKWOOD, D. J., C. K. NIELSEN (1997): Detection of infectious bursal disease viruses in commercially reared chickens using the reverse transcriptase/polymerase chain reaction-restriction endonuclease assay. Avian Dis. 41, 137-143.

JACKWOOD, D. J., S. E. SOMMER (1997): Restriction fragment length polymorphisms in the VP2 gene of infectious bursal disease viruses. Avian Dis. 41, 627-637.

JACKWOOD, D. J., S. E. SOMMER (1998): Genetic heterogeneity in the VP2 gene of infectious bursal disease viruses detected in commercially reared chickens. Avian Dis. 42, 321-339.

JACKWOOD, D. J., S. E. SOMMER (1999): Restriction fragment length polymorphism in the VP2 gene of infectious bursal disease viruses from outside the United States. Avian Dis. 43, 310-314.

KIBENGE, F. S. B., A. S. DHILLON, R. G. RUSSELL (1998): Biochemistry and immunology of infectious bursal disease virus. J. Gen. Virol. 69, 1757-1775.

LIM, B. L., Y. CAO, T. YU, C. W. MO (1999): Adaptation of very virulent infectious bursal disease virus to chicken embryonic fibroblasts by site-directed mutagenesis of residues 279 and 284 of viral coat protein VP2. Virol. 73, 2854-2862.

LIN, Z., A. KATO, Y. OTAKI, T. NAKAMURA, E. SASMAZ, S. UEDA (1993): Sequences comparisons of a highly virulent of infectious bursal disease virus prevalent in Japan. Avian Dis. 37, 315-323.

LIU, H. J., J. J. GIAMBRONE, T. DORMITORIO (1994): Detection of genetic variations in serotype I isolates of infectious bursal disease virus using polymerase chain reaction and restriction and nuclease analysis. J. Virol. Methods 48, 281-291.

MEFERRAN, J. B., M. S. MCNULTY, E. R. MCKILLOP, T. J. CONNER, R. M. MCCRACKEN, D. S. COLLINS, G. M. ALLAN (1980): Isolation and serological studies with infectious bursal disease viruses from fowl, turkey and duck: demonstration of a second serotype. Avian Pathol. 9, 395-404.

OPPLING, V., H. MULLER, H. BECHT (1991): Heterogenecity of the antigenic site responsible for the induction of neutralizing antibodies in infectious bursal disease virus. Arch. Virol. 119, 211-223.

PRINGLE, C. R. (1998): The universal system of virus taxonomy of the International Committee on Virus Taxonomy (ICTV), including new proposals ratified since publication of the Sixth ICTV Report in 1995. Arch Virol. 143, 203-210.

TURE, O., Y. M. SAIF, D. J. JACKWOOD (1998): Restriction fragment length polymorphism analysis of highly virulent strains of infectious bursal disease virus from Holland, Turkey, and Taiwan. Avian Dis. 42, 470-479.

SAPATS, S. I., J. IGNJATOVIC (2002): Restriction fragment length polymorphism analysis of the VP2 gene of Australian strains of infectious bursal disease virus. Avian Pathol. 31, 599-566.

SAREYYUPOGLU, B., M. AKAN (2006): Restrection fragment length polymorphisim typing of infectious bursal disease virus field strains in Turkey. Avian Dis. 50, 545-549.

SNYDER, D. B. (1990): Changes in field status of infectious bursal disease virus. Avian Pathol. 19, 419-423.

VAKHARIA, V. N., J. HE, B. AHAMED, D. B. SNYDER (1994): Molecular basis of antigenic variation in infectious bursal disease virus. Virus Res. 31, 265-273.

WU, C. C., T. L. LIN, H. G. ZHANG, V. S. DAVI, J. A. BOYLE (1992): Molecular detection of infectious bursal disease virus by polymerase chain reaction. Avian Dis. 36, 221-226.

ZIERENBERG, K., H. NIEPER, T. P. VAN DEN BERG, C. D. EZEOKOLI, M. VOSS, H. MULLER (2000): The VP2 variable region of African and German isolates of infectious bursal disease virus: comparison with very virulent, "classical" virulent, and attenuated tissue culture-adapted strains. Arch. Virol. 145, 113-125.

ZIERENBERG, K., R. RAUE, H. MÜLLER (2001): Rapid identification of "very virulent" strains of infectious bursal disease virus by reverse transcription-polymerase chain reaction combined with restriction enzyme analysis. Avian Pathol. 30, 55-62.

Received: 2 December 2010
Accepted: 14 July 2011

ROUSSN, D. A., W. AL-SALEH, G. Y. KHAWALDEH, W. S. TOTANJI: Karakterizacija terenskih izolata virusa zarazne bolesti Fabricijeve burze u Jordanu uporabom molekularnih metoda - kratko priopćenje. Vet. arhiv 82, 115-124, 2012.

SAŽETAK

Lančana reakcija polimerazom uz prethodnu reverznu transkripciju/polimorfizam dužine restrikcijskih fragmenata (RT-PCR/RFLP) i RT-PCR specifične početnice za jako virulentne sojeve virusa zarazne bolesti Fabricijeve burze (ZBFB) bile su rabljene za identifikaciju i karakterizaciju jordanskih terenskih izolata toga virusa koji su uzrokovali teški oblik bolesti. Za istraživanje je bilo rabljeno 80 uzoraka tkiva Fabricijeve burze uzetih iz 20 komercijalnih jata tovnih pilića u Jordanu u kojih su ustanovljeni klinički znakovi ZBFB-a. RT-PCR/RFLP proveden je na odsječku gena VP2 od 743-bp s restrikcijskim enzimima *Bst*NI i *Mbo*I. Rezultati upućuju na postojanje terenskih sojeva virusa ZBFB-a od kojih je 60% izolata imalo jedinstveni RFLP obrazac različit od onoga već objavljenog drugdje. Ipak, 20% lokalnih sojeva virusa bilo je pozitivno uporabom specifične početnice za jako virulentni virus i imalo je jedinstveni obrazac RFLP različit u jordanskih izolata u odnosu na izolate objavljene drugdje.

Ključne riječi: tovni pilići, Jordan, zarazna bolest Fabricijeve burze, polimorfizam dužine restrikcijskih fragmenata