VETERINARSKI ARHIV 83 (1), 57-67, 2013

Genetic characterization of turkey astroviruses identified in Croatian poultry flocks

Marina Biđin[1]*, Darko Majnarić[2], Zdenko Biđin[1], and Ivana Lojkić[3]

[1]*Department of Poultry Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[2]*Veterinary Department Križevci, Croatian Veterinary Institute, Križevci, Croatia*

[3]*Department of Virology, Croatian Veterinary Institute, Zagreb, Croatia*

BIĐIN, M., D. MAJNARIĆ, Z. BIĐIN, I. LOJKIĆ: Genetic characterization of turkey astroviruses identified in Croatian poultry flocks. Vet. arhiv 83, 57-67, 2013.

ABSTRACT

The identification and genetic characterization is described of turkey astroviruses (TAstV) detected by polymerase chain reaction (PCR) in Croatian turkey and chicken flocks. Multiple organ samples were collected from 16 turkey and 7 chicken flocks and tested by specific primers for the presence of turkey astrovirus-1 (TAstV-1) and turkey astrovirus-2 (TAstV-2). TAstV-1 was detected in 6 turkey flocks, and TAstV-2 in 4 turkey and 2 chicken flocks, which represents the first finding of turkey-origin astrovirus in chicken hosts. Amplified polymerase gene fragments were sequenced and analysed. Phylogenetic analysis was based on the alignments of 82 amino acids deduced from 250 nucleotide (nt) ORF 1b sequences from a total of 30 TAstV isolates. Molecular and phylogenetic analysis of astrovirus sequences revealed the existence of one TAstV-2 and two TAstV-1 genotypes detected in commercial poultry flocks in Croatia. Within each cluster of astroviruses, the polymerase gene proved to be highly conserved.

Key word: turkey astrovirus-1, turkey astrovirus-2, genetic characterization, phylogenetic analysis

---

Introduction

Turkey astroviruses (TAstV) are members of the genus *Avastrovirus*, from the *Astroviridae* family. They are non-enveloped, single-stranded and positive sense RNA viruses, approximately 27 to 30 nm in size (COOK and MYINT, 1995; KOCI et al., 2000). The astrovirus genome consists of three open reading frames (ORF): ORF 1a, ORF 1b and ORF 2. ORF 1a and ORF 1b code for non-structural proteins, while ORF 2 encodes a structural, capsid precursor protein (CUBITT, 1996). ORF 1b is considered the most

*Corresponding author:

Marina Biđin, PhD, Department of Poultry Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb, 10000 Zagreb, Heinzelova 55, Croatia, Phone: +385 1 2390 281; Fax: +385 1 2390 280; E-mail: mbidin@vef.hr

ISSN 0372-5480
Printed in Croatia

conserved region among avian and mammalian astroviruses, and codes for RNA-dependant RNA polymerase (RdRp) (MONROE et al., 1993; KOCI and SCHULTZ-CHERRY, 2002). It overlaps with ORF 1a in approximately 70 nucleotides (nt), and is introduced to the open reading frame by a stem loop (MARCZINKE et al., 1994). Unlike ORF 1b, ORF 2 appears to be a hypervariable region of the astrovirus genome, responsible for classification in different serotypes (SMYTH et al., 2009; PANTIN-JACKWOOD et al., 2006; 2011).

Turkey astrovirus was first detected in 1980 by electron microscopy (EM) of faecal samples from turkeys suffering from diarrhoea (McNULTY et al., 1980). Later, based on the genome characterization and serological investigation, the existence of two genetically and immunologically distinct types of TAstV (TAstV-1; TAstV-2) was confirmed (KOCI et al., 2000; TANG et al. 2005a; 2005b). TAstV-1 and TAstV-2 isolates, when compared, shared only 35% nucleotide similarity in the capsid gene (KOCI et al. 2000; BEHLING-KELLY et al., 2002). Both TAstV types, but primarily TAstV-2, are associated with poultry enteritis and mortality syndrome (PEMS), causing constant economic problems in commercial turkey production (PANTIN-JACKWOOD et al., 2006; 2008). PEMS is a multi-causal disease of young turkeys, characterized by diarrhoea, maldigestion, weight loss and poor growth performance. Apart from astroviruses, which are considered the most frequently detected enteric viruses in turkeys, rotaviruses, reoviruses, coronaviruses and parvoviruses are viral agents also associated with the complex aetiology of this syndrome (BARNES and GUY, 1996; PANTIN-JACKWOOD et al., 2007; SPACKMAN et al., 2010) .

Infection with TAstV is a relatively common finding in commercially reared turkeys in countries with developed turkey production. In Croatia, TAstV-1 and TAstV-2 have been recently detected by polymerase chain reaction (PCR) in young turkeys affected by enteric disease (BIĐIN et al., 2009; LOJKIĆ et al., 2010). In order to reveal the genetic specificities of TAstV, we based our research on an molecular and phylogenetic analyses of the obtained TAstV polymerase gene sequence data. Here we report an investigation of TAstV found to circulate in commercial turkey and chicken flocks in Croatia.

Material and methods

Poultry flocks. Sixteen turkey and seven chicken flocks from commercial farms in Croatia were included in this investigation. Both turkeys and chickens were found dead at the age of 2 to 40 days. Ten to 20 carcasses from each flock were collected and dissected. The intestines and lymphoid organs (timus, spleen and bursa of Fabricius) were sampled for the purpose of molecular diagnostic testing.

Sample preparation and viral nucleic acid extraction. All organ samples collected from poultry were pooled, so each sample contained approximately five organs per flock. Finally, there was a total of three pooled samples of each type of organ (intestines, timus,

spleen and bursa of Fabricius) per each turkey and chicken flock. Viral RNA was extracted from samples using a High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Manheim, Germany) according to the manufacturer's instructions. The final product was purified viral RNA in a volume of 70 μL.

Reverse transcription (RT) and polymerase chain reaction (PCR). Reverse transcription was conducted using 5 μL RNA in a 50-μL reaction volume containing 20 U RNaseH-M MLV reverse transcriptase (SuperScript™ III reverse transcriptase; Invitrogen, Carlsbad, California, USA), 5 pmol random hexamer primer, 0.5 mM dNTPs, 10 mM dithiothreitol, 50 mM Tris-HCl, 75 mM KCl and 3 mM $MgCl_2$. The reaction was carried out in the GenAmp PCR System 2400 (Applied Biosystems, Foster City, California, USA) and the mixture was incubated at 50 °C for 45 min followed by 72 °C for 10 min. The cDNA obtained was then used for amplification in PCR assays.

The primers used for detection of TAstV-1 (forward primer: 5'-GAT GGT GGG TAG CCT ATT GTG TTC -3' and reverse primer: 5'-AGC TYA TGM GGT TCT TTC TTC TYG-3') and TAstV-2 (forward primer: 5'-TGG ACC GAC CCR RTT ITY ACC A-3' and reverse primer: 5'-GGC CCG ACY TCA GGM AGT TGT-3') were designed to amplify 250 base pairs (bp) fragment of TAstV-1 and 911 bp fragment of TAstV-2 polymerase gene (DAY et al., 2007). The PCR reaction (total volume 50 μL) contained 5 μL cDNA, 25 μL GoTaq® Green Master Mix (Promega, Madison, USA) and 0.20 μL of forward and reverse primer. Thermal cycling parameters were adjusted for each virus, as described by DAY et al. (2007). The PCR reaction products were analysed by 2% agarose gel electrophoresis and stained with ethidium bromide.

The PCR products that showed the expected amplicon length were considered positive and purified by ExoSAP-IT® (Affymetrix UK Ltd., USB® Products, High Wycombe, UK). Sequencing was performed in both directions by Macrogen Inc. (Seoul, Korea).

Molecular and phylogenetic analysis. The sequence data were initially aligned using the BLAST search program. Molecular analysis and multiple sequence alignment were performed using the Clustal W program implemented in the Mega 5 software package (TAMURA et al., 2011) and BioEdit (HALL, 1999). For the phylogenetic analysis, all TAstV-1 and TAstV-2 partial polymerase gene sequences used in this study were aligned together to 250 nt corresponding to 4132-4381 nt of the TAstV-1 referent isolate Y15936. Eighty-two amino acids were deduced from 18 astrovirus nucleotide sequences, and analysed. Phylogenetic analysis was performed by the Neighbour-joining method, based on the *p-distance*, using Mega 5 software.

Gen Bank accession numbers. Accession numbers of the sequences submitted through this study are listed in Table 1. TAstV-1 sequences used in this study were from turkeys (Y159362, DQ324822, DQ324823, DQ324825, DQ324826, HQ317706, HQ317715) and ducks (JF815370, JF815371). The accession numbers of the TAstV-2 sequences

compared from turkeys were EU143844, EU143845, EU143850 and AF206663, and from ducks JQ692620, JQ692621 and JQ69262.

Results

Virus detection. Out of all the tested flocks, PCR assays showed astrovirus presence in the organ samples from eleven turkey and two chicken flocks (Table 1). TAstV-1 was found positive in six, and TAstV-2 in four turkey flocks. The ORF 1b fragment of TAstV-2 was also amplified in samples belonging to two chicken flocks. Neither of the tested chicken flocks were found to be infected with TAstV-1.

Table 1. Poultry flocks positive to TAstV, organs in which the virus was detected, isolate names and Gen Bank accession numbers.

Flock	Age/ days	Virus	Positive organs	Isolate	Accession number
1-turkeys	15	TAstV-1	Intestine	TAstV-1/CRO-53	JX083354
2-turkeys	18	TAstV-1	Intestine	TAstV-1/CRO-21	JX083355
3-turkeys	10	TAstV-1	Intestine	TAstV-1/CRO-61	JX083356
4-turkeys	20	TAstV-1	Spleen, intestine*	TAstV-1/CRO-510	JX083357
5-turkeys	21	TAstV-1	Intestine	TAstV-1/CRO-286	JX083358
6-turkeys	12	TAstV-1	Intestine	TAstV-1/CRO-528	JX083359
7-turkeys	6	TAstV-2	Timus, spleen, intestine*	TAstV-2/CRO-169	JX083366
8-turkeys	15	TAstV-2	Timus	TAstV-2/CRO-201	JX083367
9-turkeys	2	TAstV-2	Spleen	TAstV-2/CRO-552	JX083368
10-turkeys	40	TAstV-2	Timus, spleen, intestine, bursa Fabricii*	TAstV-2/CRO-05	JX083974
11-chicken	7	TAstV-2	Intestine	TAstV-2/CRO-06	JX083370
12-chicken	21	TAstV-2	Intestine	TAstV-2/CRO-739	JX083371

* Organ from which the virus was sequenced.

Sequence characterization. The samples that showed the expected amplicon length were sequenced, and the nucleotide sequence data obtained (Table 1) were used for further molecular and phylogentic analyses. Croatian TAstV-1 sequences (250 nt in length) derived from turkeys showed a higher similarity to each other on an amino acid (96.3-100%) than on a nucleotide level (93.2-98.8%). Compared with TAstV-1 from ducks and turkeys, they shared high nucleotide (90.8-99.2%) and amino acid (92.7-100%) identities. Generally, Croatian TAstV-1 sequences were more similar in the polymerase gene fragments compared to TAstV-1 from ducks than to turkey-origin TAstV-1 sequences. Croatian TAstV-1 sequences were characterized with seven unique

nucleotide changes that resulted in substitution of three amino acids. Corresponding to the nucleotide positions of the referent TAstV-1 isolate Y15936, the substitutions were as follow: at 4346 nt (serine instead of alanine in TAstV-1/CRO-53), at 4349 nt (histidine instead of leucine also in TAstV-1/CRO-53) and 4373 nt position (glutamine instead of leucine in TAstV-1/CRO-528).

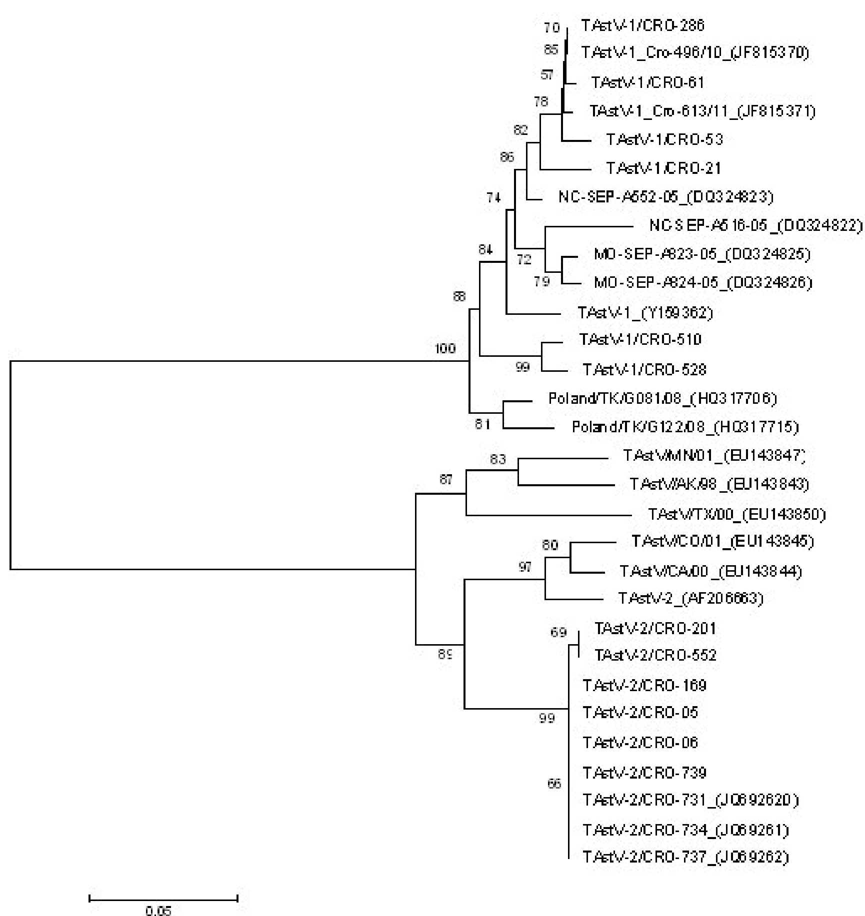

Fig. 1. Neighbour-joining tree based on alignments of 82 amino acids deduced from 250 nt fragment of polymerase gene of 15 TAstV-1 and 15 TAstV-2 sequences. The evolutionary distances were computed using *p-distance*, with 1000 bootstrap replicates.

The polymerase gene fragment of six TAstV-2 positive samples was sequenced in the length of 883 nt. Croatian TAstV-2 sequences from turkeys and chickens showed genetic parity. They were identical in amino acid composition, whilst they differed on a nucleotide level in only 0.4% of cases. They shared the same range of nucleotide and amino acid percentage identities with duck TAstV-2 isolates, also originating from Croatia. The similarities with foreign turkey TAstV-2 sequences were greater on the amino acid (92.7-98.7%) than on the nucleotide level (87.2-92.4%). The sequence TAstV-2/CRO-739 detected in chickens was characterized by three unique nucleotide changes at the 4041, 4045-6 nt position of the TAstV-2 referent strain TAstV/CO/01 (EU143845). This resulted in substitution of three amino acids: alanine-histidine-asparagine instead of glutamic acid-glutamine-aspartic acid. Although it had no effect on amino acid composition, the specificity of all Croatian turkey and chicken TAstV-2 sequences was adenine (at the 4024 nt position of the referent strain), while other compared sequences had guanine located at this position.

Aligned together, all TAstV-1 and TAstV-2 sequences shared 59.2-62% nucleotide and 59.2-65% amino acid similarities in the compared 250 nt ORF 1b fragment.

Phylogenetic analysis. A phylogenetic Neighbour-joining tree was constructed using *p-distance*, based on the alignments of 82 amino acids deduced from 15 TAstV-1 and 15 TAstV-2 nucleotide sequences (Fig. 1). Phylogenetic analysis was consistent with molecular characterization of astrovirus sequences. A Neighbour-joining tree formed two distinct clusters; one consisted of TAstV-1 and the other consisted of TAstV-2 sequences. Within the cluster of TAstV-1 sequences, Croatian TAstV-1 isolates from turkeys (except TAstV-1/CRO-510 and TAstV-1/CRO-528) were branched with TAstV-1 from ducks, but showing a close evolutionary relationship with other TAstV-1 sequences. TAstV-1/CRO-510 and TAstV-1/CRO-528 were more phylogenetically related to TAstV-1 sequences originating from Poland and with the referent sequence (Y15936) from the U.S. Within the TAstV-2 cluster, all Croatian TAstV-2 isolates from turkeys, chickens and ducks were grouped separately from foreign TAstV-2 sequences, forming a ‘’Croatian subgroup’’.

Discussion

The present research was designed to investigate the occurrence and genetic diversity of turkey astroviruses circulating in commercial turkey and chicken flocks in Croatia. As avian astroviruses are known to be associated with enteric syndromes (PEMS and RSS) affecting commercially reared poultry, their molecular specificities and phylogenetic relationship have recently been of the great interest. The majority of studies regarding avian astroviruses have been done in Northern Ireland and the U.S. (McNULTY et al., 1990; McNEILLY et al., 1994; PANTIN-JACKWOOD et al., 2006; 2007; 2008; 2011; SPACKMAN et al., 2010; TODD et al. 2009; 2011), while in Croatia there have been only two limited

surveys dealing with astroviruses found in chickens and turkeys (BIĐIN et al., 2009; LOJKIĆ et al., 2010). To investigate the presence of turkey astroviruses, for the purpose of this study we tested two types of avian hosts: turkeys and chickens. Although there are no published data about turkey-origin astroviruses infecting chickens, we found it to be epizootiologically important to investigate the possibility of astrovirus transmission between avian species. Recently, an astrovirus similar to TAstV-2 was detected in guinea fowl (CATOLLI et al., 2007), chicken-origin astroviruses were identified in pigeons (ZHAO et al., 2011), ducks and geese (BIĐIN et al., 2011; 2012), while turkey-origin astroviruses have also been detected in ducks (BIĐIN et al., 2012).

From the 16 turkey and 7 chicken flocks included in this survey, TAstV-1 was found positive only in turkeys (6 flocks), while TAstV-2 was detected both in turkeys (4 flocks) and chickens (2 flocks). We suspect that TAstV-2 infection occurred in chickens due to insufficient biosecurity and disinfection of poultry houses. To clarify TAstV-2 transmission to another avian host, further research is required to determine possible changes in virus-host interaction. Of all the types of organ tested (intestines and lymphoid organs), astroviruses were the most frequently detected in intestinal samples (Table 1). There were only three flocks in which the virus was detected in multiple organ samples. The finding of TAstV-1 or TAstV-2 in lymphoid organs (flock 4, 7, 8, 9 and 10) indicates the early stage of astrovirus infection, in which the virus replicates in lymphoid tissues (SCHULTZ-CHERRY et al., 2000). Considering that the age of TAstV-positive poultry varied from 2-40 days, it is obviously that astrovirus infection spreads by vertical and horizontal routes. Moreover, because TAstV-2 was found in multiple lymphoid organs and intestines, it is likely that turkeys aged 40 days (flock 10) were infected with TAstV-2 later in the production cycle, rather than before or shortly after hatching. Conversely, we may suspect that the flocks in which the virus was only detected in intestinal samples were found dead later in the course of astrovirus infection. Although it would not be wise to attribute the death of turkeys and chickens to astroviruses, the TAstV-1 or TAstV-2 infection surely acted as a contributing factor in immunosuppression and poor performance.

To characterize the TAstV-1 and TAstV-2 detected in Croatian poultry flocks, the polymerase gene from each astrovirus positive sample was amplified, sequenced and analysed. The molecular characterization of TAstV-1 sequences identified in turkey flocks showed that they were highly similar in nucleotide and amino acid composition to TAstV-1 isolates from ducks originating from Croatia, indicating that there were no significant differences in genotypes of TAstV-1 found in various avian hosts. These four sequences from turkeys and two from ducks were branched together (Fig. 1), showing the greatest phylogenetic relationship with the sequence NC-SEP-A552-05 (DQ324823), originating from the U.S. turkey flock. Unlike them, the sequences TAstV-1/CRO-510 and TAstV-1/CRO-510 were phylogenetically closer to European TAstV-1 isolates. Although

there is a small evolutionary distance between amino acid sequences calculated by the Neighbour-joining tree, the analysis performed suggests that there were two TAstV-1 genotypes detected to be circulating in Croatian poultry flocks.

Nucleotide and amino acid sequence characterization of the amplified polymerase gene fragment of TAstV-2 detected in Croatia revealed that one genotype was infecting turkeys, chickens and ducks. Based on the molecular analysis and the Neighbour-joining tree constructed, Croatian TAstV-2 sequences derived from various avian species belonged to the same genetic group, so there were no host-specific TAstV-2 genotypes (Fig. 1).

The alignment and comparison of 250 nt and 82 deduced amino acid of TAstV-1 and TAstV-2 revealed that polymerase gene sequence similarities were consistent with the results obtained in other studies (PANTIN-JACKWOOD et al., 2006; 2011). Within each cluster of turkey astroviruses, the polymerase gene was highly conserved, without significant variability in nucleotide or amino acid sequences. Although it shared a high level of identity with other Croatian TAstV-2 isolates, by having three unique amino acid substitutions, the sequence TAstV-2/CRO-739 was the most diverse TAstV-2 sequence.

Conclusion

The molecular and phylogenetic analysis performed allowed us to infer several conclusions regarding turkey astroviruses identified in Croatian poultry flocks. The finding of TAstV-2 in chickens represents the first detection of turkey-origin astrovirus in chicken hosts, indicating that astroviruses are not host-specific. Molecular and phylogenetic analysis of astrovirus sequences revealed the existence of one TAstV-2 and two TAstV-1 genotypes detected in commercial poultry in Croatia. Analysis of the partial ORF 1b sequences confirmed that polymerase gene is highly conserved within astroviruses.

Acknowledgements

This research was supported by grant No. 053-0531863-1856 from the Ministry of Science, Education and Sports, Republic of Croatia.

References

BARNES, J., J. C. GUY (1996): Spiking mortality of turkeys (SMT) and related disorders - an update. Turkeys 44, 11-14.

BARNES, H. J., J. S. GUY, J.-P. VAILLANCOURT (2000): Poult enteritis complex. Rev. sci. tech. Off. int. Epiz. 19, 565 -588.

BEHLING-KELLY, E., S. SCHULTZ-CHERRY, M. KOCI, L. KELLEY, D. LARSEN, C. BROWN (2002): Localization of astrovirus infected turkeys as determined by *in situ* hibridization. Vet. Pathol. 39, 595 -598.

BIĐIN, Z., I. LOJKIĆ, M. BIĐIN, M. TIŠLJAR, M. MIKEC, D. MAJNARIĆ, Ž. STANIŠIĆ (2009): Dosadašnji rezultati istraživanja enteritisa i uginuća purića u Hrvatskoj. VIII. Simpozij Peradarski dani (25.-28.03.2009.). Zbornik Peradarski dani 2009., 114.-118.

BIĐIN, M., I. LOJKIĆ, Z. BIĐIN, M. TIŠLJAR, D. MAJNARIĆ, M. MIKEC (2011): Detection and characterization of avian nephritis virus in ducklings. Avian Pathol. 40, 173 -177.

BIĐIN, M., I. LOJKIĆ, M. TIŠLJAR, Z. BIĐIN, D. MAJNARIĆ (2012): Astroviruses associated with stunting and pre-hatching mortality in duck and goose embryos. Avian Pathol. 41, 91-97.

CATOLLI, G., C. De BATTISTI, A. TOFFAN, A. SALVIATO, A. LAVAZZA, M. CERIOLI, I. CAPUA (2007): Co-circulation of distinct genetic lineages of astroviruses in turkeys and guinea fowl. Arc. Virol. 152, 595-602.

COOK, N., S. MYINT (1995): Astroviruses. J. Med. Microbiol. 42, 1-2.

CUBITT, W. D. (1996): Historical background and classification of caliciviruses and astroviruses. Arch. Virol., Suppl. 12, 225-235.

DAY, J. M., ERICA SPACKMAN, MARY PANTIN-JACKWOOD (2007): A multiplex RT-PCR test for the differential identification of turkey astrovirus type 1, turkey astrovirus type 2, chicken astrovirus, avian nephritis virus and avian reovirus. Avian Dis. 51, 681-684.

HALL, T. A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41, 95-98.

KOCI, M. D., S. SCHULTZ-CHERRY (2002): Avian astroviruses. Avian Pathol. 31, 213-227.

KOCI, M. D., B. S. SEAL, S. SCHULTZ-CHERRY (2000): Molecular characterization of an avian astrovirus. J. Virol. 74, 6173-6177.

LOJKIĆ, I., M. BIĐIN, Z. BIĐIN, M. MIKEC (2010): Viral antigens associated with poult enteritis in Croatian commercial turkey flocks. Acta Vet. Brno 79, 125-131.

MARCZINKE, B., A. J. BLOYS, T. D. BROWN, M. M. WILLCOCKS, M. J. CARTER, I. BRIERLEY (1994): The human astrovirus RNA-dependent RNA polymerase coding region is expressed by ribosomal frameshifting. J. Virol. 68, 5588-5595.

McNULTY, M. S., W. L. CURRAN, J. B. McFERRAN (1980): Detection of astroviruses in turkey faeces by direct electron microscopy. Vet. Rec. 106, 561.

McNEILLY, F., T. J. CONNOR, V. M. CALVERT, J. A. SMYTH, W. L. CURRAN, A. J. MORLEY, D. THOMPSON, S. SINGH, J. B. McFERRAN, B. M. ADAIR, M. S. McNULTY (1994): Studies on a new enterovirus-like virus from chickens. Avian Pathol. 23, 313-327.

MONROE, S. S., B. JIANG, S. E. STINE, M. KOOPMANS, R. I. GLASS (1993): Subgenomic RNA sequence of human astrovirus supports classification of *Astroviridae* as a new family of RNA viruses. J. Virol. 67, 3611-3614.

PANTIN-JACKWOOD, M. J., E. SPACKMAN, P. R. WOOLCOCK (2006): Molecular characterization and typing of chicken and turkey astrovirus circulating in the United States: Implication for diagnostics. Avian Dis. 50, 397-404.

PANTIN-JACKWOOD, M. J., E. SPACKMAN, J. M. DAY, D. RIVES (2007): Periodic monitoring of commercial turkeys for enteric viruses indicates continous presence of astrovirus and rotavirus on the farms. Avian Dis. 51, 674-680.

PANTIN-JACKWOOD, M. J., J. M. DAY, M. W. JACKWOOD, E. SPACKMAN (2008): Enteric viruses detected by molecular methods in commercial turkey flocks in the United States between 2005-2006. Avian Dis. 52, 235-244.

PANTIN-JACKWOOD, M. J., K. O. STROTHER, E. MUNDT, L. ZSAK, J. M. DAY, E. SPACKMAN (2011): Molecular characterization of avian astroviruses. Arch. Virol. 156, 235-244.

SCHULTZ-CHERRY, S., D. R. KAPCZYNSKI, V. M. SIMMONS, M. D. KOCI, C. BROWN, H. J. BARNES (2000): Identifying agent(s) associated with poult enteric mortality syndrome: Importance of the thymus. Avian Dis. 44, 256 -265.

SMYTH, J. A., T. J. CONNOR, F. M. McBEILLY, D. A. MOFFET, V. M. CALVERT, M. S. McNULTY (2007): Studies on the pathogenicity of entero-virus-like viruses in chickens. Avian Pathol. 36, 119 -126.

SMYTH, V. J., H. L. JEWHURST, B. M. ADAIR, D. TODD (2009): Detection of chicken astrovirus by reverse transcriptase-polymerase chain reaction. Avian Pathol. 38, 293-299.

SPACKMAN, E., J. M. DAY, M. J. PANTIN-JACKWOOD (2010): Astrovirus, reovirus, and rotavirus concomitent infection causes decreased weight gain in broad-breasted white poults. Avian Dis. 54, 16-21.

TAMURA, K., D. PETERSON, N. PETERSON, G. STECHER, M. NEI, S. KUMAR (2011): Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol. Biol. Evol. 28, 2731-2739.

TANG, Y., M. M. ISMAIL, Y. M. SAIF (2005a): Development of antigen-capture enzyme-linked immunosorbent assay and RT-PCR for detection of turkey astroviruses. Avian Dis. 49, 182-188.

TANG, Y., M. V. MURGIA, Y. M. Saif (2005b): Molecular characterization of the capsid gen of two serotypes of turkey astrovirus. Avian Dis. 49, 514-519.

TODD, D., V. J. SMYTH, N. W. BALL, B. M. DONNELLY, M. WYLIE, N. J. KNOWLES, B. M. ADAIR (2009): Identification of chicken enterovirus-like viruses, duck hepatitis virus type 2 and duck hepatitis virus type 3 as astrovirus. Avian Pathol. 38, 21-29.

TODD, D., J. TRUDGETT, V. J. SMYTH, B. DONNELLY, N. McBRIDE, M. D. WELSH (2011): Capsid protein sequence diversity of avian nephritis virus. Avian Pathol. 40, 249-259, 2011.

ZHAO, W., A. L. ZHU, C. L. YUAN, Y. YU, C. X. ZHU, D. L. LAN, Z. B. YANG, L. CUI, X. G. HUA (2011): Detection of astrovirus infections in pigeons (*Columbia livia*) during an outbreak of diarrhoea. Avian Pathol. 40, 361-365.

Received: 16 June 2012
Accepted: 5 December 2012

BIĐIN, M., D. MAJNARIĆ, Z. BIĐIN, I. LOJKIĆ: Genska karakterizacija puranskih astrovirusa dokazanih u hrvatskim jatima peradi. Vet. arhiv 83, 57-67, 2013.

SAŽETAK

Opisana je identifikacija i genska karakterizacija puranskih astrovirusa (TAstV) dokazanih lančanom reakcijom polimerazom (PCR) u hrvatskim jatima purića i pilića. Organi uzorkovani od 16 jata purića i 7 jata pilića pretraženi su specifičnim početnicama na prisutnost puranskoga astrovirusa-1 (TAstV-1) i puranskoga astrovirusa-2 (TAstV-2). TAstV-1 dokazan je u 6 jata purića, a TAstV-2 u 4 jata purića i 2 jata pilića, što predstavlja prvi nalaz astrovirusa puranskog podrijetla u novog domaćina-kokoši. Umnoženi odsječci polimeraze bili su sekvencirani i analizirani. Filogenetska analiza temeljena je na sravnjivanju 82 aminokiseline izvedene iz 250 nukleotida (nt) sekvencije ORF 1b od ukupno 18 izolata puranskog astrovirusa. Molekulska i filogenetska analiza otkrile su postojanje jednog genotipa TAstV-2 i dva genotipa TAstV-1 dokazanih u komercijalnim jatima peradi u Hrvatskoj. Polimeraza je unutar obje skupine astrovirusa pokazala visoki stupanj postojanosti.

Ključne riječi: puranski astrovirus-1, puranski astrovirus-2, genska karakterizacija, filogenetska analiza