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RESEARCH ARTICLE

Rotavirus Diarrhea Outbreaks in Arabian Thoroughbred Foals in A Stud Farm, Turkey

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Summary

In this study, the aetiological agent of diarrhea in Arabian thoroughbred foals housed in a stud farms and it's molecular characterization were reported. Out of sampled seven foals with diarrhoea, five were detected positive by RT-PCR depending on the amplification of the VP6 gene of rotavirus. Then the aetiological agent was characterized genetically by sequence analysis of the genome segments encoding VP6, VP7, VP4 and NSP4 of rotavirus. Findings revealed that the Turkish equine rotavirus circulating within this stud farm belongs to G3 and P[12] genotype with E2 NSP4 and I6VP6. This is the first study to report the G and P genotypes of equine group A rotaviruses in Turkey.

Keywords: *Rotavirus, Equine, Genotyping, Diarrhoea, Turkey*

Türkiye'de Bir Arap Atı İşletmesindeki Taylarda Rotavirus İshali Salgını

Özet

Bu çalışmada, bir at yetiştiriciliği işletmesinde bulunan safkan arap taylarındaki ishal olgusunun etiyolojik ajanının tespiti ve moleküler karakterizasyonu bildirildi. İshal semptomlu yedi taydan sağlanan materyallerin beşinde RT-PCR tekniği ile rotavirus VP6 geni yönünden pozitiflik saptandı. Daha sonra enfeksiyona neden olan virusun VP6, VP7, VP4 ve NSP4 gen bölgelerini kodlayan gen bölgelerinin dizi analizi yapıldı. Elde edilen verilere dayanılarak söz konusu at yetiştiriciliği işletmesinde enfeksiyona neden olan ERV saha suşunun E2 NSP4 ve I6VP6 ile ilişkili G3P[12] genotipinde olduğu belirlendi. Bu çalışma, Türkiye'de atlardaki grup A rotavirusların G ve P genotiplerinin bildirildiği ilk çalışmadır.

Anahtar sözcükler: *Rotavirus, At, Genotip, İshal, Türkiye*

INTRODUCTION

Group A rotaviruses (GARV) are one of the most important causative agents of severe diarrhea resulting in dehydration in human infants and the offspring of many animal species including cattle, equine, goat, etc. [1-3] and have severe economic impact on stud farming [4,5]. The rotavirus genome consists of 11 segments of double-stranded RNA (dsRNA), which encode 12 viral proteins in which 6 structural (VP1-VP4, VP6 and VP7) and 6 non-structural proteins (NSP1-NSP6). The structural protein, VP6, bears group spesific antigenic determinants. As genotyping analysis of VP6 gene, 16 different VP6 gene were recognized [6]. The nonstructural transmembrane glycoprotein NSP4 from group A rotaviruses, the viral enterotoxin, have been genetically classified into 14 genotypes, E1 and E14, recently [6]. The virus has two outer capsid proteins, VP7 and VP4, which are independently associated with the serotype specificities for G serotype (for glycoprotein) and the P serotype (for protease-sensitive protein), respectively [1]. In human and animal rotaviruses 27 G genotypes and 35 P genotypes have been identified so far [6].

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Equine GARVs have been identified in foals with diarrhea in a lot of countries as Ireland [7], Australia [8,9], India [10], Greece [5], Germany [11], Italy [12], Argentina [13] and Japon [14-16]. Reports reveals that the majority of rotavirus strain from foals are either G3P[12] or G14P[12] [5,7,9,11-14,16,17]. The other strains detected in foals belonging to genotype G5, G8, G10, G13 and P[18], P[3], P[11], P[1], P[7] types [4,13,17-21]. But there is no report on the G and P genotypes of equine rotavirus in Turkey.

In this study, it is aimed i-) to investigate of rotavirus as causative agent in Arab thoroughbred foals with diarrhea, houses in a stud farm and ii-) to report of the molecular characterization of the VP6, VP4, VP7 and NSP4 genes of a rotavirus detected in these foals.

MATERIAL and METHODS

Field Sample

In the present study, faecal samples from 9 to 24 days old foals (n=7) with clinical symptoms as mild diarrhea, loss of appetite and intestinal cramping, bred on a stud farm consisting of approximately 350 mares with different age, were used for the detection of rotavirus. Outbreaks of diarrhea during the foaling period in 2010-2011 were detected and foals tested in this study were sampled in February 2011. Foals were lactated naturally from their mother. As a knowledge given the veterinarians working this stud farm, diarrhea cases in foals had also been seen in the foaling period in the years before. In this farm, to prevent to the some viral, bacterial and parasite infections, regular administration of drugs and vaccination programs have been subjected to the farmed mares, except rotavirus vaccine.

RNA Extraction, RT-PCR and Genotyping of ERV

The extraction of rotavirus genomic RNA was performed using a QIAamp Viral RNA Mini Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions. All faeces samples were analyzed for rotavirus presence by VP6 RT-PCR with the primers [22]. Then, all positive samples for the amplicons (379 bp) for VP6 genes were characterized rotavirus G and P types by seminested PCR. For amplification of full length VP7 (1062 bp) gene [23] with Beg9-End9 primers and VP4 (876 bp) specific regions [24] with Con2-Con3 primers were used. For G typing, primers for G3, G13, G14 types [14] and End9 [23] were used [14]. For P typing, the second round PCR was performed with primer Con3 [24] and primer specific for genotypes P[12] and P[18] [15]. Additionally, the expected size amplicons were produced for NSP4 gene region from all samples positive for rotavirus with primers and protocols elsewhere [25]. Either full-length or partial sequences of the VP4, VP6, VP7 and NSP4 genome segments of the Turkish equine rotavirus strain, RVA/horse-wt/TUR/Eskisehir/2011, were determined after RT-PCR amplifications with specific primer sets. The list of the primer sets used in this study and their detailed specifications including expected product sizes, etc. were given in *Table 1*. The results from sequencing of amplicons compared with cognate sequences of reference viruses available in the databases. Sequence editing and multiple alignments were performed with Bioedit software package version 2.1 [26]. Phylogenetic analysis was carried out with the software package MEGA version 5.0 [27], using the Neighbor-Joining model with Kimura 2-parameter correction and bootstrap analysis (1.000 replicates).

RESULTS

Out of 7 faces samples, 5 were found positive for rotavirus by RT-PCR based on the detection the expected sizes (379 bp) of amplicon for VP6 gene of rotavirus. The RT-PCRs used for the detection and molecular characterization of equine rotaviruses detected (n=5) revealed expected sizes of DNA products (876 bp for VP4, 1062 bp for VP7 and 743 bp for NSP4) in five faces samples in concordance with reference viruses. As results of PCRs for G and P

Table 1. *Primers used for RT-PCR and sequence analysis of VP4, VP6, VP7 and NSP4 gene region of rotavirus*
Tablo 1. *Rotavirusun VP4, VP6, VP7 ve NSP4 gen bölgelerinin RT-PCR ve dizin analizinde kullanılan primerleri*

Procedure	Primer Sequence (5'→3')	Specifity	Location	Product Size (in bp)	Reference
VP4 gene amplification	TGGCTTCGCTCATTTATAGACA ATTTCGGACCATTTATAACC	P-F(con3) P-R(con2)	11-32 887-868	876	[24]
P genotype	CCATTATAAACCCATAGCTG ATGCACCATCTAATGTTTGC	P12 P18	545-526 463-444	535 452	[15]
VP7 gene amplification	GGTCACATCATACAATTCTAATCTAAG GGCTTTAAAAGAGAGAATTTCCGTCTGG	G-R(end9) G-F(beg9)	1062-1036 1-28	1062	[23]
G genotype	CAATCGAAGAGATTGCGACAG GGAGTAAATCACAAAATAAATCTC GACGAAGCATTGCAATTA	G3 G13 G14	683-703 742-765 481-498	374 321 582	[14]
VP6 gene amplification	GACGGVGCRACTACATGGT GTCCAATTCATNCCTGGTGG	VP6-F VP6-R	747-766 1126-1106	379	[22]
NSP4 gene amplification	GGCTTTWAAAAGTTCTGTTCCGAGAGAG TAAGACCRTTCCYTCCATTAAC	151 152	1-28 743-722	743	[25]

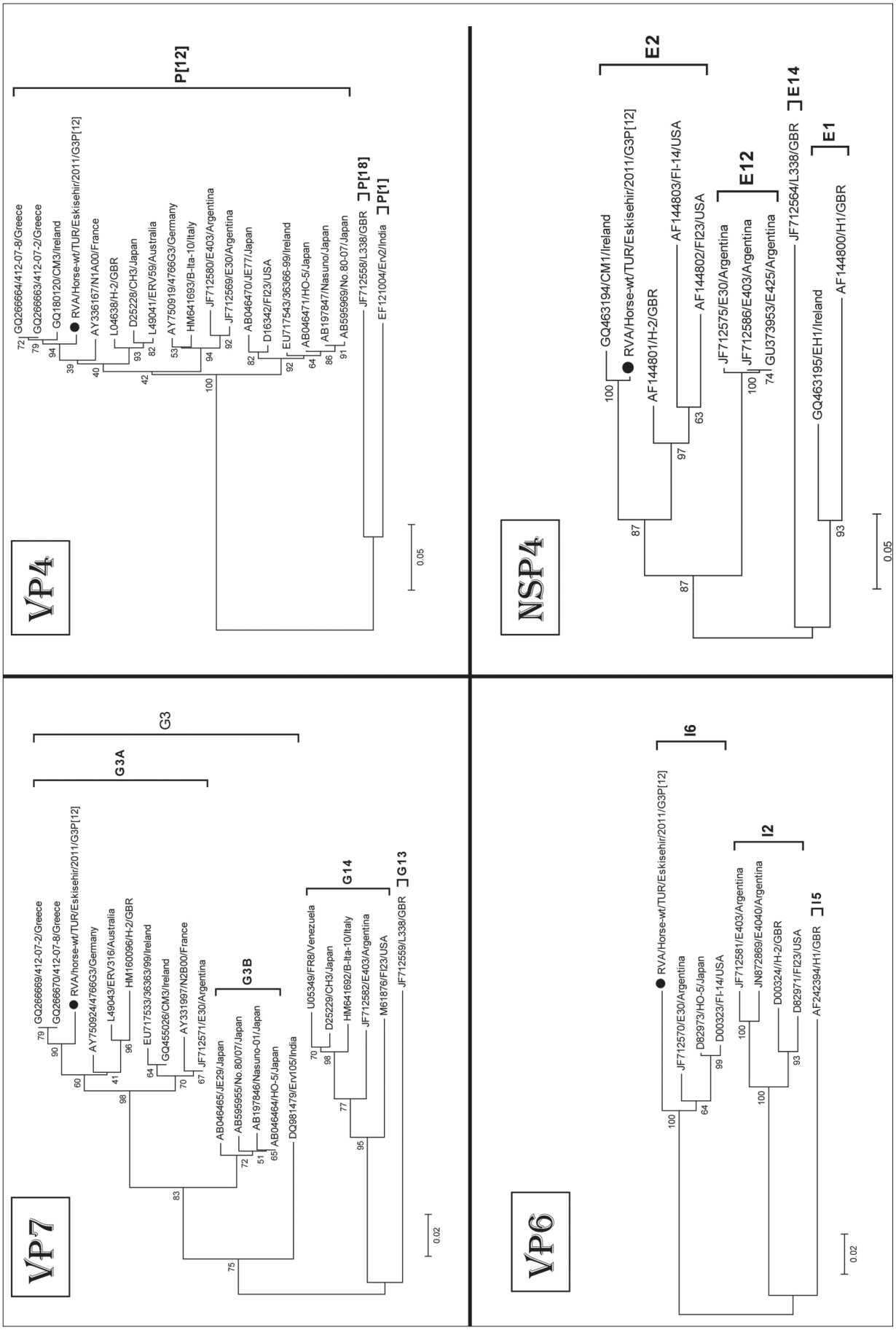

Fig 1. Phylogenetic analysis (Neighbor Joining method) of the VP4, VP6, VP7 and NSP4 genes of RVA/horse-wt/TUR/Eskisehir/2011 and some rotavirus strains deposed in GenBank.

Şekil 1. Gen Bankasından alınan birkaç rotavirus suşu ile RVA/horse-wt/TUR/Eskisehir/2011 adlı suşun VP4, VP6, VP7 ve NSP4 gen bölgeleri düzeyinde, Neighbor Joining metoduna göre yapılmış filogenetik analizi

typing, the binary combination of rotavirus isolates were characterized as G3P[12].

According to the results of phylogenetic analysis using the Neighbor Joining method, Turkish equine rotavirus, RVA/horse-wt/TUR/Eskisehir/2011, have been characterized as G3P[12] with E2 NSP4 and I6 VP6 genotypes *(Fig. 1)*. The accession numbers of the sequences are: JQ687223, JQ687220, JQ687221 and JQ687222 for NSP4, VP4, VP6, VP7 of a Turkish equine rotavirus, RVA/horse-wt/TUR/Eskisehir/2011/G3P[12], analyzed in this study, respectively.

DISCUSSION

The Arabian horses are worldwide, including the United State and Canada, The United Kingdom, continental Europe, some South American countries and Middle East, and also they have a importance for the equestrian activity. In Turkey, races for two equine breed (Arabian and English horses) has been organized for a long time in a seven different places/city according to the climatic conditions/seasons. Then, the breeding of horses has economical importance for the private breeders and also state stud farms responsible to protect the genes and pedigree of Arabian thoroughbred horses in Turkey. To date, there is no report about rotavirus or other viruses causes diarrhea in foals housed in stud farm sampled in this study, and also other state or private stud farms, althought owners reported the diarrhea cases in newborn foals when we asked them to be presence these events.

Equine rotavirus belong to genotype G3 was predominantly detected throughout the world. This genotype followed the G14 strains isolated in different countries (UK, Japon, Australia, Venezuela and US, etc. [9,14,17,28] while the other G genotypes (G5, G8, G10, G13) from foals were detected sporadically [4,13,17-19]. While the P genotypes of rotavirus from human and other some animal species as bovine and porcine especially had been classified into 35 P types, equine rotaviruses are predominantly in P [12] genotype. At this date, at least six different P types (P[12], P[3], P[11], P[1], P[18] and P[7]) of equine rotaviruses has been reported [4,13,17,18,29]. The known binary combinations in group A equine rotaviruses are especially G14P[12] [14] and G3P[12] [14], followed P[1] with combination G10 and G8, P[7] with combination G13 [17,20].

In this paper, Turkish equine rotavirus were clustered in phylogenetic analyses based on VP4 and VP7 gene regions of viral genom. The sequence analyses of rotavirus defined in this study (RVA/horse-wt/TUR/Eskisehir/2011/G3P[12]) shown that the mentioned virus belongs to G3A and P[12] genotypes *(Fig. 1)* as most of the rotaviruses from horses deposited in GenBank, detected in an European countries.

The vaccination programs had been used to the prevention of the rotavirus infection in human and bovine worldwide. Similarly, some inactivated vaccines for equine rotavirus had been developed and licenced USA and some European contries (the vaccine including H2 strain) and Japon (the vaccine including H0-5 strain), except Turkey [13,29,30]. However it is remained that the insufficient of the vaccine have been documented on several occations if foals are not supported by keeping the stable clean and by outsiding of their mares on the pastures. Additionally, it is reported that the differences of the genotypes of field strains can be causes the vaccine breakdown. Thus, it is reported that the vaccine (RotaCli Equina®) including the prototype ERV H2 (G3P[12]), simian rotavirus (SRV - SA11 G3P[2]), and bovine rotavirus (BRV- NCDV-Lincoln G6P[1]) strains had been used in Argentina since 1996 and also ERV diarrhea incidence reduced in the high level depend on the this vaccine application to the pregnant mares [13]. In the same paper, the researchers reported that the rates of the G14 rotavirus detection and the evaluating of the incidence of rotavirus cases at the last years studied and that the ERV vaccine should be updated according to the detected G and/or P type rotaviruses in countries, as previously described for human and bovine rotavirus vaccines.

It is known that risks for horses to acquire rotavirus are high because of the increased movement of horses from one farm to another for different reason or their presence in the race area . The stud farm sampled in current study is one of the three state stud farms which are the producer (for Arabian horses) of thoroughbred foals in Turkey. These stud farms have an exchange procedure of horses for reproductive reasons especially. To date, there is no investigation based on the molecular characterization of VP4 and VP7 gene regions of GARVs from horses while the knowledge were reported about GARVs from other species in Turkey [31,32]. It's possible reasons are the inadequate knowledge on the rotavirus infection in foal diarrhea cases, disregarding of the mild diarrhea. In the future studies, we will investigate the presence of the rotavirus infection in foals with different age, race, management conditions and its molecular epidemiology for the deeper understanding of the epidemiology of rotavirus infections in horses, in Turkey.

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