Eurasian Journal
of Veterinary Sciences

www.eurasianjvetsci.org www.ejvs.selcuk.edu.tr

RESEARCH ARTICLE

Differential diagnosis of *Fasciola hepatica* from different animal hosts by PCR

Osman Selcuk Aldemir[1], Mederle Narcisa[2]

[1]Department of Parasitology, Faculty of Veterinary Medicine, Adnan Menderes University, Isıklı, Aydin, Turkey,
[2]Universitatea de StiinNe Agricole si Medicina Veterinara a Banatului Timisoara, Facultatea de Medicina Veterinara, Timisoara, Romania
Received: 10.02.2014, Accepted: 26.02.2014
*oselcuk9@hotmail.com

Özet

Aldemir OS, Narcisa M. Farklı konaklardan elde edilen *Fasciola hepatica*'nın PCR ile ayırıcı teşhisi.

Amaç: Bu çalışmanın amacı koyun ve keçiden elde edilen *Fasciola hepatica*'nın PCR ile ayırıcı teşhisinin yapılmasıdır.

Gereç ve Yöntem: Örnek materyal (*F. hepatica*) koyun ve keçilerden elde edildi. Elde edilen parazitler birkaç kez pH:7.4 fosfat bufer solusyonunda yıkandıktan sonra, genomik DNA ekstraksiyonu amacıyla fenol ekstraksiyon/etanol presipitasyon metodu uygulandı. PCR koşullarının optimizasyonunda her bir *F. hepatica*'nın DNA'sını amplifiye edebilmek için rastgele 5 adet primer seçildi.

Bulgular: Hem koyun, hemde keçilerden elde edilen *F. hepatica*'ların RAPD-PCR tekniği ile amplifikasyonu sonucu farklı büyüklük ve sayıda bandlar elde edildi. Elde edilen bu bandların büyüklükleri ve sayıları sayesinde *F. hepatica*'ya ait genetik varyasyonlar identifiye edildi. Koyun ve keçi orjinli parazitlere (*F. hepatica*) ait bu bandlar tür spesifiktir.

Öneri: Bu çalışma ile koyun ve keçilerde bulunan *F. hepatica*'nın ayırıcı teşhisinde RAPD-PCR tekniğinin kullanılmasının daha uygun olacağı saptanmıştır.

Anahtar kelimeler: Ayırıcı teşhis, *Fasciola hepatica*, RAPD-PCR, koyun, keçi.

Abstract

Aldemir OS, Narcisa M. Differential diagnosis of *Fasciola hepatica* from different animal hosts by PCR.

Eurasian J Vet Sci, 2014, 30, 2, 85-88
DOI:10.15312/EurasianJVetSci.201425924

Aim: The objective of this study was evaluated differentiational diagnosis of *Fasciola hepatica* from sheep and goat by PCR.

Materials and Methods: *F. hepatica* were obtained from sheep and goat. These parasites were washed several times with pH:7.4 phosphate-buffered saline and phenol extraction/ethanol precipitation method was employed in genomic DNA extraction. In order to optimization of the PCR conditions, five primers were selected to amplify the DNA of each *F. hepatica*.

Results: Both sheep and goat were amplified products in different numbers and sizes by RAPD-PCR technique. The results show that different primers gave different bands (fragments) and allowed to identify genetic variations of *F. hepatica*. These bands were species-specific to *F. hepatica* from goat and sheep.

Conclusions: The RAPD-PCR method can be useful for the differentiational diagnosis of *F. hepatica* of sheep and goat.

Keywords: Differentiational diagnosis, *Fasciola hepatica*, RAPD-PCR, sheep, goat.

Introduction

Fasciolosis, caused by digenean trematodes of the genus Fasciola (Platyhelminthes: Trematoda: Digenea), is one of the most important plant-borne helminth infections of human and livestock in many parts of the world (Soulsby 1986, Bruijnesteijn et al 2009, Van Lieshout and Verweij 2010, Ronki et al 2010).

Fasciola hepatica occurs in the bile ducts of sheep, goat, ox and other ruminants, pig, hare, rabbit, beaver, coypu, elephant, horse, such as man and the horse, the fluke may be found in the lungs, under skin or other situations (Soulsby 1986). Transmission of *F. hepatica* is dependent on prisence of its lymnaeid snail intermetiate hosts (Soulsby 1986, Aldemir 2006).

The snail is commonly seen in poorly drained land, drainage ditches, areas of seepages of spring or broken drains. Following ingestion of metacercaria by the hosts, the juvenile worms burrow through the host gut walls and migrate to the liver, where they cause extensive damage before moving into the bile ducts walls and develop into mature forms that live in the microenvironment of the bile ducts. *F. hepatica* causes important economic losses due mainly to liver condemnation and reduced production of meat and milk (Sineo et al 1993, Aldemir 2006, Ronki et al 2010).

Nowadays, in diagnosis and identification of parasitic diseases are successfully used molecular techniques such as PCR and its variants. Also, molecular techniques are used for the development of specific antigens for serological tests and for studying the immune response in the patients (Kramer and Schnieder 1998, Heckeroth and Tenter 1999, Mostafa et al 2003).

Understanding genetic structure and status of genetic variation of the *Fasciola hepatica* populations has important implications for epidemiology and effective control of fasciolosis (Sineo et al 1993). In our previous study (Aldemir 2006) were assessed to characterize molecularly and to differentiate between cattle and sheep originated *Fasciola hepatica* using a RAPD-PCR assay.

In this study, we aimed to evaluate differentiational dioagnosis of *F. hepatica* from sheep and goat by RAPD-PCR.

Materials and Methods

Sample collection

Materials were obtained from sheep and goat at abattoir. The obtained F.hepatica were washed several times with pH 7.4 phosphate-buffered saline and then incubated in the same buffer at 37°C for 3 h to eliminate any residual host matter.

Genomic DNA extraction

The Genomic DNA extraction was performed according to the protocol described previously study (Vargas et al 2003, Guclu et al 2004, Aldemir 2006, Ronki et al 2010). The method was slightly modified as follows; the parasites were homogenized in a lysis solution (8% Triton 100X, 0.25 M Sucrosa, 50 mm EDTA, pH:7.4). The homogenates were centrifuged at 10.000 g for 10 min at 4°C. Genomic DNA was extracted by SDS-proteinase K digestion, followed by phenol chloroform extraction. The recovered DNA was dissolved in 10 mM Tris-HCl, 1 mM EDTA pH:7.6 (Tris-EDTA buffer) and contaminating RNA was removed by incubation with RNAse for 1 h at 37°C, followed by a second phenol chloroform extraction and ethanol precipitation. The suspensions were stored at -20°C until required.

Primers and optimization

Primers in optimization OSA 09, OSA 10, OSA 11, OSA 12 and OSA 13 were randomly selected to amplify the DNA of *F. hepatica* from sheep and goat, as reported by Vargas et al (2003), Guclu and Aldemir (2004), Aldemir (2006), Ronki et al (2010). The DNA concentrations were determined spectrophotometrically (260 nm(A260)) for the amplification to obtain complete and reproducible band patterns for the genomic DNA (5 ng) of each parasite used as a template DNA. The reaction was carried out in a final volume of 25 mL containing 10X(500 mM KCI, 200 mM Tris- HCl pH:8.4), 2.0 mM MgCI2, 400 µM dNTPs (Promega, USA). Each reaction tube contained 2 units of Taq polymerase. PCR was performed in a Hybaid Omni-Gene thermocycler (Hybaid, UK). DNA was amplified in 45 cycles with a denaturing step at 95°C for 5 seconds, primer annealing at 32°C for 60 seconds, and an extension step at 72°C for 7 seconds. Incubation at 72°C for 5 seconds for primer extension has been suggested by previous studies (Vargas et al 2003). However, a longer extension period produced shadow formation. On the basis of several attempts over different time periods, it is concluded that 10 seconds at 72°C is optimum for primer extension. Agarose gel (1.4%) was stained with ethidium bromide prior to visualization and photography. It was used as indicating of molecular weight to ladder 100 pb. in cremnts.

Results

The RAPD-PCR technique is used to amplify short regions of an organism's genome without prior sequence information has great potential in identifying genetic markers, tagging genes and chromosomes and performing population studies. In this study, different DNA fragments were obtained from parasites of sheep and goat according to the primers used (Table 1). The results in Table 1 show that different primers gave different bands (fragments). These bands are species-specific to *F. hepatica* from goat and sheep.

The sizes of DNA fragments from goat and sheep originated *F. hepatica* respectively amplified by the OSA 09 primer were approximately 450 bp and 150-450 bp but obtained line from sheep 450 bp was poorly amplified (Figure 1). Using the OSA 10 primer, only DNA fragments (150 bp) from sheep parasite have been evidenced, whereas no amplification has been achieved with goat worm DNA (Figure 2). In amplification by the OSA 11 primer 1 DNA fragments of approximately 450 bp were obtained only from goat and no band from sheep worm was visualized (Figure 3). The sizes of DNA fragments of *F. hepatica* from sheep by the OSA 12 primer, only DNA fragments (450 bp) have been evidenced for sheep, whereas

Table 1. Number and size of amplified genomic DNA fragments from sheep and goat using different primers.

	Goat Worm		Sheep Worm	
Primer	Number	Size(bp)	Number	Size(bp)
OSA-09 5'- TCG TCG CATT -3'	1	450	2	150-450
OSA-10 5'- AGC AGC AGGC -3'	0	-	1	150
OSA-11 5'- GGG TAA CGCC -3'	1	450	0	-
OSA-12 5'-GGA TAG GGT -3'	0	-	1	450
OSA-13 5'- GTA GGT GTC -3'	1	450	0	-

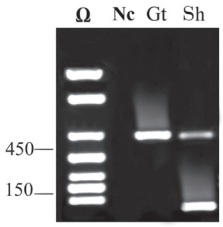

Figure 1

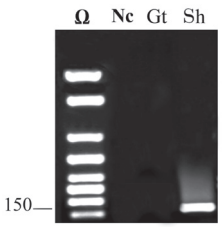

Figure 2

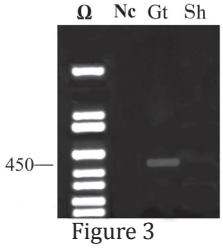

Figure 3

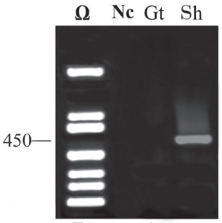

Figure 4

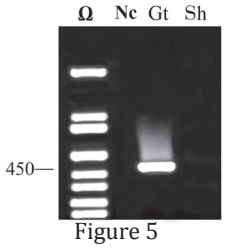

Figure 5

'Ω: Marker, Nc: Negative control, Gt: Goat Worm, Sh: Sheep Worm.

no amplification has been achieved with goat worm DNA (Figure 4). Using finally, after amplification by the OSA 13 primer 1 DNA fragments of approximately 450 bp were obtained and this band is the most clear band in study, no band from sheep worm was visualized (Figure 5).

Discussion

Molecular techniques are utilized for the diagnosis of parasitic diseases and identification of parasites, for the development of specific antigens for serological tests and studying immune response in the patients. Molecular techniques have become widely accepted in the world. They provide more specific method than conventionally employed in epidemiological studies (Aldemir ve Dik 2003, Guclu et al 2004, Aldemir 2006, Christen and Henrik 2012).

Studies on genetic variability within and between populations of Fasciola have important implications for epidemiology, control and diagnosis of fasciolosis. Different DNA-based molecular techniques have been applied to these flukes. In studies about morphological characteristics can be difficult to identification for soft-bodied animals such as digenean trematodes. Molecular biology is constantly evolving. Especially the amplification of specific DNA regions via the polymerase chain reaction (PCR) and improved direct dideoxy sequencing techniques, may allow closely related species to be distinguished by comparing their DNA (Mostafa et al 2003, Aldemir 2006, Caron et al 2010, Khan et al 2012, Le et al 2012).

Random Amplified Polymorphic DNA-PCR tech¬nique has a great potential in identifying genetic markers, tagging genes and chromosomes, and performing population studies, and gives species-specific DNA fragments which may be used as diagnostic probes among organisms and consequently would allows determination of their genetic relatedness (Williams et al 1990, Guclu et al 2004, Aldemir 2006).

Used primers in the study gave different band in size according to the host, that these fragments were specific for goat and sheep. A 150-450 bp length bands were obtained from the sheep whereas a 450 bp length DNA fragment was identified from the goat by OSA 09 primer. These results were in agreement with previous reports (Shubkin et al 1992, Vargas et al 2003, Aldemir 2006, Ronki et al 2010) and the band of *F. hepatica* from goat was firstly detected in this study.

Using the OSA 10 primer, only DNA fragments (150 bp) from sheep parasite have been evidenced, whereas no amplification has been achieved with goat worm DNA. This result was agreement with our previous study (Aldemir 2006). In amplification by the OSA 11 primer 1 DNA fragments of approximately 450 bp were obtained only from goat and no band from sheep worm was visualized. The result was firstly obtained with the primer. By the OSA 12 and OSA 13 primers in the study gave same bands in previous study and these results were in agreement with previous reports (Shubkin et al 1992, Vargas et al 2003, Aldemir 2006, Ronki et al 2010).

Conclusions

In explaining of the genetic relation *F. hepatica* obtained from different hosts can be useful RAPD-PCR technique. Both sheep and goat were amplified products in different numbers and sizes by RAPD-PCR techniques. Therefore this technique has the potential to differentiate these two worm species. These findings may also form a base for future studies aimed at determining genetically the phylogenic evolution of worm species.

References

Aldemir OS, 2006. Differentiation of cattle and sheep originated *Fasciola hepatica* by RAPD-PCR. Rev Med Vet, 157, 2, 165-167.

Aldemir, OS, Dik B, 2003. Koyunlardaki Sarcocystis Türlerinin RAPD-PCR ile Teşhisi. T Parazitol Derg, 27, 255-259.

Bruijnesteijn LE, Wallinga JA, Ruijs GJ, Bruins MJ, Verweij JJ, 2009. Parasitological diagnosis combining an internally controlled real-time PCR assay for the detection of four protozoa in stool samples with a testing algorithm for microscopy. Clin Microbiol Infect, 15, 869-874.

Caron Y, Righi S, Lempereur L, Saegerman C, Losson B, 2010. An optimized DNA extraction and multiplex PCR for the detection of Fasciola sp. in lymnaeid snails. Vet Parasitol, 178, 1-2, 93-99.

Christen RS, Henrik VN, 2012. Comparison of microscopy and PCR for detection of intestinal parasites in Danish patients supports an incentive for molecular screening platforms. J Clin Microbiol, 50, 2, 540-541.

Güçlü F, Aldemir OS, Güler L, 2004. Differential identification of cattle Sarcocystis spp. by Random Amplified Polymorphic DNA–Polymerase Chain Reaction (RAPD-PCR). Rev Med Vet, 155, 440-444.

Heckeroth AR, Tenter AM, 1999. Development and validation of species-specific nested PCR for diagnosis of acute Sarcocystosis in sheep. Int J Parasitol, 29, 1331-1349.

Khan I, Khan AM, Ayaz S, Khan S, Anees M, Khan SA, 2012. Molecular detection of *Fasciola hepatica* in water sources of District Nowshehra Khyber Pakhtunkhwa Pakistan. Int J Advance Res and Techno, 1, 7, 2278-7763

Kramer F, Schnieder T, 1998. Sequence heterogeneity in a repetitive DNA element of Fasciola. Int J Parasitol, 28, 1923-1929.

Le TH, Nguyen KT, Nguyen NT, Doan HT, Le XT, Hoang CT, De NV, 2012. Development and evaluation of a single-step duplex PCR for simultaneous detection of *Fasciola hepatica* and Fasciola gigantica (family Fasciolidae, class Trematoda, phylum). J Clin Microbiol, 50, 8, 2720-2726.

Mostafa OM, Taha HA, Ramadan G, 2003. Diagnosis of Fasciola gigantica in snail using the polymerase chain reaction (PCR) assay. J Egyptian Soc Parasitol, 33, 733-742.

Ronki MB, Mirhendi H, Behnia M, Fasihi Harandi M, Jalalizand N, 2010. Molecular characterization of *Fasciola hepatica* isolates by RAPD-PCR and Ribosomal ITS1 sequencing. Iran Red Crescent Me, 12, 27-32.

Shubkin C, White M, Abrahamsem M, 1992. A Nucleic Acid-Based test for detection of *Fasciola hepatica*. J Parasitol, 78, 817-821.

Sineo L, Martini R, Borghi G and Failli M, 1993. Analysis of genetic markers by Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR). Bol Chim Farm, 132, 201-202.

Soulsby EJL, 1986. The English Language Book Society and Bailliere Tindal, 7th edition, Printed in Great Britain by Williams Clauses Limited, London, UK, pp: 40-44.

Van Lieshout L, Verweij JJ, 2010. Newer diagnostic approaches to intestinal protozoa. Curr Opin Infect Dis, 23, 488-493.

Vargas D, Vega M, Gloria C, 2003. Aproximacion a una caracterizacion molecular *Fasciola hepatica* por la tecnica RAPDs-PCR. Parasitol, 58, 11-16.

Williams JGK, Kubelik AR, Jivak KS, Rafalksi JA, Tingey SV, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res, 18, 6531-6535.