Analysis of microsatellite sequence in major histocompatibility complex region in horses

ELISKA HORECKA [1, 2], CENEK HORECKY[1, 2], PETR HORIN[3, 4], ALES KNOLL[1, 2]
[1]Department of Animal Morfology, Physiology and Genetics
[2]CEITEC MENDELU
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[3]Department of genetics
[4]CEITEC VFU
Veterinary and Pharmaceutical University Brno
Palackeho trida 1/3, 612 48 Brno
CZECH REPUBLIC

eliska.horecka@mendelu.cz

Abstract: Major histocompatibility complex (MHC) is one of the immunologically important mammalian genome regions. Genes in MHC encode proteins, which are involved in innate and adaptive immune response. Genetic changes in this region can affect immune reactions in animals. But complex documentation of genetic variation in MHC is still missing in most domestic animals, including horses. In horses MHC is located on chromosome 20, region 27 403 526 – 33 865 081 bp. The aim of this study was utilisation of described microsatellite markers to analyse the genetic diversity of selected populations of horses. And then to determine number of alleles and compare results with the original authors. Even and odd alleles were identified for microsatellites ABGe17416 a 305-93, thus further sequencing will be performed to reassert in/del in PCR product.

Key-Words: MHC, horse, microsatellite, pathogen, alleles

Introduction

The major histocompatibility complex (MHC) is a fundamental part of the vertebrate immune system, and the high variability in many MHC genes is thought to play an essential role in recognition of parasites [2]. The function of MHC molecules is to bind peptide fragments derived from pathogens and display them on the cell surface for recognition by the appropriate T cells. The consequences are almost always deleterious to the pathogen—virus-infected cells are killed, macrophages are activated to kill bacteria living in their intracellular vesicles, and B cells are activated to produce antibodies that eliminate or neutralize extracellular pathogens. Thus, there is strong selective pressure in favor of any pathogen that has mutated in such a way that it escapes presentation by an MHC molecule [3].

The MHC contains some of the most polymorphic genes of the vertebrate genome, but alleles of some of these genes are ancient and predate speciation of related taxa [4]. Because trans-species polymorphisms confound analysis of MHC history and haplotype structure by single nucleotide polymorphisms (SNPs), many investigators have turned to microsatellite repeats and microsatellite–SNP combinations as a source of more rapidly diversifying gene markers to provide a better understanding of MHC evolution and function [8]. Brinkmeyer-Langford *et al*. [1] states for 69 primer pairs tested, 37 (53%) amplicons were polymorphic and 30 (41%) were monomorphic among the IHRFP horses (International Horse Reference Family Panel). Among the polymorphic microsatellites 31 were newly described markers and six previously described markers [6, 7, 9, 10, 11].

The aim of this study was utilisation of described microsatellite markers to analyse the genetic diversity of selected horses populations.

Material and Methods

Characterization of study population

In this study 325 individuals from two regions with different infection pressure were included.

Populations from pathogen rich regions were: Camarque, Romanian horse, Campolino, Marajo, Galiceno and Mangalarga Marchador. And populations of Gotland, Yakut and Icelandic horse were set as pathogen free region.

Testing of different polymerases

In this study different types of polymerase were tested : AmpliTaq Gold DNA polymerase (Thermo Fisher Scientific Inc., Waltham, USA), PPP Master Mix (Top-Bio, Vestec, Czech Republic), CombiTaq (Top-Bio, Vestec, Czech Republic), HotStarTaqTM MasterMix (Qiagen, Hilden, Germany). Polymerases were used in multiplex with primers taken from Tseng et al. [11].

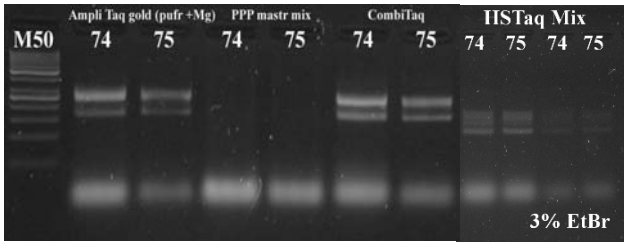
Microsatellite genotyping

PCR amplification was performed in two multiplex reactions, each in the total volume of 6 µl. The first multiplex included primers: 25 pmoles UMN-JH34-2, UM011, COR114 and 40 pmoles COR112, COR113 (primer sequences according to Tseng et al. [11]). The second multiplex included primers 20 pmoles 305-93, ABGe9019, HMS082, ABGe17416 and TKY3324 (primer sequences according to Brinkmeyer-Langford et al. [1]). Multiplex contained 1.6 U CombiTaq polymerase and 1x complete buffer for CombiTaq (Top-Bio, Vestec, Czech Republic), 100 µM dNTP (Thermo Fisher Scientific Inc., Waltham, USA) and PCR water (Top-Bio, Vestec, Czech Republic) adjusted to 6µl. Primers were modified on 5' end by fluorescent labels 6-FAM, PET, NED and VIC so that samples could be separated and analysed in one mix. Temperature profile of PCR reaction 1 was 95/3min; (95/30; 58/30; 72/60) 30x; 72/60min; 7/∞. Temperature profile of PCR reaction 2 was: 95/3min; (95/30; 58/30; 72/30) 30x; 72/60min; 7/∞. Designed panel of the microsatellites was analysed using genetic analyser ABI PRISM® 3500 (Applied Biosystems, Foster City, CA, USA) and sized with LIZ500 dye size standard (Applied Biosystems, Foster City, CA, USA). Results were analysed using the GeneMapper v4.1 software (Applied Biosystems, Foster City, CA, USA).

Results and Discussion

In this study different types of polymerase were tested. Results are displayed in Figure 1. The best results were achieved by using CombiTaq and AmpliTaq Gold DNA polymerases. For further analysis was used only CombiTaq polymerase because the price was only one-third compared to AmpliTaq Gold DNA.

Fig. 1 Using different types of polymerase



Specific microsatellites were selected to cover all three classes of MHC region. From class I located in region 27 403 526 – 30 620 000 bp, two microsatellites were selected: 305-93 (MS_6) and UMN-JH34-2. From class III, which covers region 31 321 086 – 31 572 317 bp, two microsatellites were selected: ABGe9019 (MS_2) and HMS082 (MS_3). From the largest part of MHC class II, which occurs in location 32 621 480 – 33 849 668 bp, six microsatellites were used: ABGe17416 (MS_4), TKY3324 (MS_5), COR112, COR113, UM011 and COR114. Number of alleles were determined for individual microsatellite (Tab. 1).

Table1 Information about microsatellite markers used in this study

Markers	Class MHC	Range of amplikon	Number of alells	Repetition
305-93	I	331-347	16	CA
UMN-JH34-4	I	186-218	17	CA
ABGe9019	III	288-330	13	CA
HMS082	III	189-219	13	TG
ABGe17416	II	276-317	24	CA
TKY3324	II	244-266	12	CA
COR112	II	228-258	13	TG
COR113	II	251-271	10	TG
UM011	II	160-180	11	CA
COR114	II	219-245	12	TG

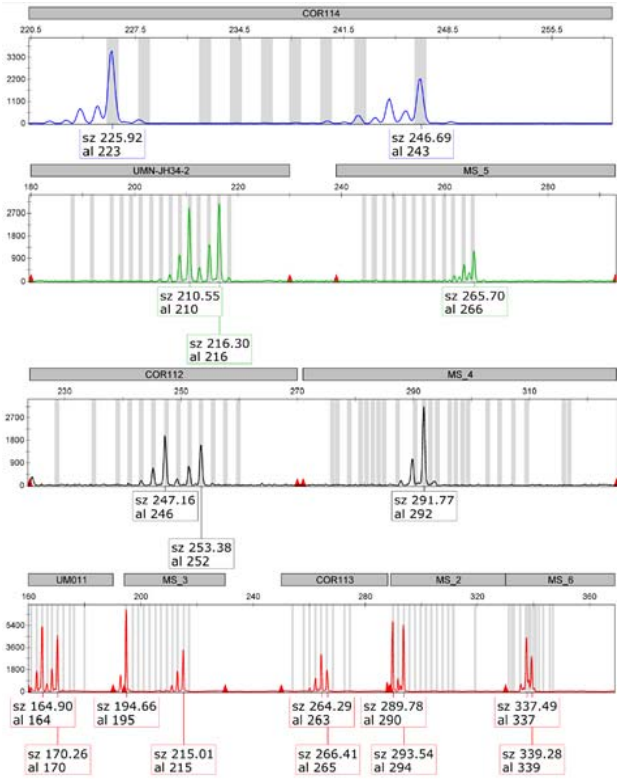
Comparison of our results and alleles by Brinkmeyer-Langford et al. [1], Tseng et al. [11] are listed in Table 2.

Table2 Comparison of the Brinkmeyer-Langford et al. (2012) and our results

Markers	Number of alells by Brinkmeyer-Langford et al./ Tseng *et al*	Number of alells discovered in this study
305-93	7	16
UMN-JH34-4	0/12	17
ABGe9019	11	13
HMS082	10	13
ABGe17416	13	24
TKY3324	13	12
COR112	11/13	13
COR113	10/8	10
UM011	11/13	11
COR114	9/10	12

Tseng et al. [11] describes even and odd alleles in microsatellite UM011, but we confirmed only even alleles. For microsatellite ABGe17416 and 305-93 even and odd alleles occurred, therefore, the numbers of alleles were significantly different than found by Brinkmeyer-Langford et al. (2012). This finding will be specified by sequencing the PCR product to reassert in / del, or odd and even alleles. In the picture 2 is shown an example of genetic analyzer ABI PRISM® 3500 result.

Fig. 2 Examples of microsatellite markers and their size



Conclusion

This study extends the original panel of five microsatellites (Klumplerová et al., 2013) to another five microsatellites for better and uniform coverage of whole MHC region. This panel of ten microsatellites together with SNPs determined in MHC region and with the panel of neutral microsatellites will be used for more accurate analysis of genetic diversity and evolution of MHC in horses.

Acknowledgement

This study was supported by CEITEC - Central European Institute of Technology with help of research infrastructure financed by project CZ.1.05/1.1.00/02.0068 from the European regional development fund.

References:

[1] Brinkmeyer-Langford CL, et al., Microsatellite variation in the equine MHC, *Animal Genetic,* 44, 2012, pp. 267-275

[2] Hedrick PW, et al., Major histocompatibility complex variation in the endangered Przewalski's horse, *Genetics*, 152, 1999, pp. 1701–1710.

[3] Janeway CA Jr, et al., Immunobiology: The Immune System in Health and Disease. 5th edition, New York: Garland Science; 2001, ISBN 780815336426

[4] Klein J, Origin of major histocompatibility complex polymorphism: the trans-species hypothesi, *Human Immunology,* 1987, 19, pp. 155–62.

[5] Klumplerová M, et al., Major histocompatibility complex and other allergy-relatedcandidate genes associated with insect bite hypersensitivityin Icelandic horses, *Molecular Biology Reports*, 40, 2013, pp. 3333–3340.

[6] Meyer AH, et al., Sixteen new polymorphic equine microsatellites. *Animal Genetics,* 28, 1997, pp. 69–70

[7] Milenkovic D, et al., Characterization and localization of 17 microsatellites derived from BACs in the horse. *Animal Genetics*, 36, 2005, pp. 164–6.

[8] Mountain JL, et al., SNPSTRs: empirically derived, rapidly typed, autosomal haplotypes for inference of population history and mutational processes. *Genome Research*, 2002, 12, pp. 1766–72.

[9] Roberts MC, Murtaugh J., Valberg S. J., Mickelson J. R., Alexander L. J., Twenty-one new equine dinucleotide repeat microsatellites. *Animal Genetics*, 31, 2000, pp. 141.

[10] Tozaki T, et al., Characterization of equine microsatellites and microsatellite-linked repetitive elements (eMLREs) by efficient cloning and genotyping methods. *DNA Research*, 8, 2001, pp. 33–45.

[11] Tseng CT, et al., Identification of equine major histocompatibility complex haplotypes using polymorphic microsatellites. *Animal Genetics*,2010, 41, pp. 150–153.