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ELA-DRA polymorphisms are not associated with Equine Arteritis Virus infection in horses from Argentina

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ABSTRACT

Polymorphisms at Major Histocompatibility Complex (MHC) genes have been associated with resistance/susceptibility to infectious diseases in domestic animals. The aim of this investigation was to evaluate whether polymorphisms of the *DRA* gene the *Equine Lymphocyte Antigen* is associated with susceptibility to Equine Arteritis Virus (EAV) infection in horses in Argentina. The equine *DRA* gene was screened for polymorphisms using Pyrosequencing® Technology which allowed the detection of three *ELA-DRA* exon 2 alleles. Neither allele frequencies nor genotypic differentiation exhibited any statistically significant (*P*-values = 0.788 and 0.745) differences between the EAV-infected and no-infected horses. Fisher's exact test and OR calculations did not show any significant association. As a consequence, no association could be established between the serological condition and *ELA-DRA*.

Several studies have been conducted to define the role of the Major Histocompatibility Complex (MHC) polymorphisms concerning susceptibility/resistance to infectious diseases in horses (Brostrom et al., 1988; Horin et al., 2004; Chung et al., 2004). However, the extent to which the *Equine Lymphocyte Antigen* (*ELA*) in controlling the Equine Viral Arteritis (EVA), a highly infectious disease among equids, remains unclear. In 2001, the EAV virus was first isolated in South America from an imported stallion (Echeverría et al., 2003). The EAV prevalence of the disease has been relatively low (0.5%) until the 2010 epidemic in Argentina (Metz et al., 2008).

In functional MHC class II genes, exon 2 encodes amino acids associated with the peptide-binding sites (PBS) in the first domain of the molecule (Brown et al., 1993). Previously, *DRA* gene has shown to be highly conserved in most mammalian species (Ellis and Ballingall, 1999), by this it has not been well studied. Sequence polymorphisms of *DRA* gene have been observed in water buffalo (Sena et al., 2003), rhesus macaques (de Groot et al., 2004), and cattle (Zhou et al., 2007). In *Equidae* however extensive polymorphism has been reported in *Equidae* species (Albright-Fraser et al., 1996; Brown et al., 2005; Díaz et al., 2008; Janova et al., 2009; Kamath and Getz, 2011), where 17 alleles have been detected, with four of them being exclusive to domestic horse breeds.

To determine if an association between these *DRA* alleles and EAV infection exists, we analyzed the *ELA-DRA* exon 2 polymorphisms in horses that were exposed to EAV during the epidemic of 2002 in Argentina. Serum samples from 133 Silla Argentino horses (N_{EAV} infected = 84, N_{EAV} non-infected = 49) representing two farms in Buenos Aires and Córdoba Provinces were used in this analysis. During the 2002 epidemic, serum samples had been sent to the Virology laboratory for routine EAV testing using the virus neutralization method (Timoney and McCollum, 1993). Most of these samples had numeric code identification without any reference to pedigree, age or sex records for any of the horses. Genomic DNA was isolated from frozen serum with DNAzol reagent (Invitrogen, CA, USA) and quantified using the NanoVue equipment (GE Healthcare, USA).

ELA-DRA exon 2 Pyrosequencing® was performed using procedure and conditions described previously (Díaz et al., 2008) using a PSQTM96 System instrument and Pyrosequencing software (Biotage AB). Allelic and genotypic frequencies, unbiased expected heterozygosity (h_e), observed heterozygosity (h_o) and Hardy–Weinberg equilibrium (HWE) were estimated using the Probability Test Estimation of exact *p*-values of the Markov chain method (dememorization = 10,000, batches = 20, iterations per batch = 5000). Genetic differences between the EAV-infected and no-infected groups were evaluated using the GENEPOP 4.0 software program (Rousset, 2008) and the *G* exact test and Woolf–Haldane odds ratio (OR) (Bland and Altman, 2000).

Three out of four of the reported horse DRA alleles (*ELA-Eqca-DRA*0101*, *ELA-Eqca-DRA*0201* and *Eqca-DRA*0301*) were detected in both the EAV-infected and EAV-non infected horses (Table 1).

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Table 1
ELA-DRA allele frequencies and association results (OR, odds ratio; CI, confidence interval) in EAV-infected and EAV-no infected horses.

Allele	EAV-no infected ($N = 49$)	EAV-infected ($N = 84$)	OR	95% CI	Fisher's exact test (p)
	F_{IS} ([a])				
DRA∗0101	0.233 (0.476)	−0.044 (0.520)	0.84	0.509–1.380	–
DRA∗0201	−0.010 (0.292)	−0.407 (0.255)*	1.20	0.685–2.111	0.093
DRA∗0301	−0.162 (0.232)	−0.163 (0.225)	1.04	0.576–1.893	0.119

[a] Allelic frequencies between brackets. F_{IS} was computed as in Weir and Cockerham (1984).
* $p \leqslant 0.05$ was considered statistically significant for those with the allele versus those without the allele.

Table 2
ELA-DRA genic and genotypic differentiation for each population pair (exact *G* test).

Locus/allele	EAV-no infected ($N = 49$)	EAV-infected ($N = 84$)
h_e	0.62	0.64
h_o	0.59	0.76
HWE (F_{IS} – p value)	0.124	0.0002*
Genic differentiation	0.777[a]	
Genotypic differentiation	0.745[a]	

Number of individuals (N) between brackets.
[a] p-Values of the genic and genotypic differentiation for each population pair (exact *G* test).
* $p < 0.05$ was considerede statistically significant.

The association analysis did not show significant values for *ELA-DRA* alleles (p values, 0.093 and 0.119). HWE was observed only among the EAV-infected horses ($p = 0.0002$), while h_e as well as h_o estimates were not significantly different between both the infected and non-infected horses (Table 2). Comparisons between groups by the genetic and genotypic differentiation tests also did not exhibit any significant differences (p-values, 0.788 and 0.745).

Through experimental infections, Castillo-Olivares et al., (2003) have suggested that EAV restricts the genetic mechanism of cytolytic activity. Since the T cell immune response is dependent on antigen processing and presentation, MHC polymorphism, and T cell recognition, class II molecules should contribute restriction determinants for antigen-specific T cell recognition of processed epitopes. Many factors could be the underlying causes for the lack of association observed here between *ELA-DRA* polymorphisms and EAV infection. Firstly, only six amino acid changes, four of them within the PBS regions, occurred in the four horse alleles. Two SNPs that were present in the three alleles that were detected produce non-synonymous changes in PBS 47 (R/H) and 49 (A/T). These differences among *DRA* alleles would not be sufficient to affect the antigen-binding pocket structure, and therefore, their affinity to antigenic peptides that may cause a modification in the immune response. Selection models applied to the *DRA* exon 2 sequences have revealed signs of limited selection, with amino acid site 49 being more strongly selected and site 47 being more weakly selected (Janova et al., 2009).

Furthermore, class I polymorphisms could also be involved in the response to a virus infections, but class II products may also interact in later and specific stages of the immune response.

Our investigation focused on determining the relevance of specific ELA candidate gene polymorphisms on EAV resistance/susceptibility, and we have defined the resistance as the presence of virus, by this reason we cannot discard the role of *DRA* locus in the progression of the disease. Since our strategy did not include genome-wide analysis of gene polymorphisms, it is not unexpected that variability at other genes other than those within the MHC could also be affecting the EAV immune response contributing to disease susceptibility. Population heterogeneity was not evidenced in the lack of association of *DRA* and the EAV serological condition. This kind of population structure should allow easier identification of weaker associations between genetic variables and resistance/susceptibility. Likewise, it might be inferred that while an association is not significant in our population of Silla Argentino horses, this may not be true for a more mixed population.

Conflicts of interest

The authors declare no conflict of interests.

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References

Albright-Fraser, D.G., Reid, R., Gerber, V., Bailey, E., 1996. Polymorphism of *DRA* among equids. Immunogenetics 43, 315–317.
Bland, J.M., Altman, D.G., 2000. Statistics notes: the odds ratio. British Medical Journal 320, 1468.
Brostrom, H., Fahlbrink, E., Dubath, M.L., Lazary, S., 1988. Association between equine leucocyte antigens (*ELA*) and equine sarcoid tumors in the population of Swedish half-breds and some of their families. Veterinary Immunology and Immunopathology 19, 215–223.
Brown, J.H., Jardetzky, T.S., Gorga, J.C., Stern, L.J., Urban, R.G., Strominger, J.L., y Wiley, D.C., 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature 364, 33–39.
Brown, J.J., Thomson, W., Clegg, P., Eyre, S., Kennedy, L.J., Matthews, J., Carter, S., Ollier, W.E.R., 2005. Polymorphisms of the equine major histocompatibility complex class II DRA locus. Tissue Antigens 64, 173–179.
Castillo-Olivares, J., Tearle, J.P., Montesso, F., Westcott, D., Kydd, J.H., Davis-Poynter, N.J., Hannant, D., 2003. Detection of equine arteritis virus (EAV)-specific cytotoxic CD8+ T lymphocyte precursors from EAV-infected ponies. Journal of General Virology 84, 2745–2753.
Chung, C., Mealey, R.H., McGuire, T.C., 2004. CTL from EIAV carrier horses with diverse MHC class I alleles recognize epitope clusters in Gag matrix and capsid proteins. Virology 327, 144–154.
Díaz, S., Echeverría, M.G., It, V., Posik, D.M., Rogberg-Muñoz, A., Pena, N.L., Peral-García, P., Giovambattista, G., 2008. Development of an *ELA-DRA* gene typing method based on Pyrosequencing technology. Tissue Antigens 72, 464–468.
de Groot, N., Doxiadis, G.G., de Groot, N.G., Otting, N., Heijmans, C., Rouweler, A.J.M., Bontrop, R.E., 2004. Genetic makeup of the DR Region in Rhesus Macaques: gene content, transcripts, and pseudogenes. Journal of Immunology 172, 6152–6157.
Echeverría, M.G., Pecoraro, M.R., Galosi, C.M., Etcheverrigaray, M.E., Nosetto, E.O., 2003. The first isolation of equine arteritis virus in Argentina. Revue scientifique et technique (International Office of Epizootics) 22, 1029–1033.
Ellis, S.A., Ballingall, K.T., 1999. Cattle MHC: evolution in action? Immunological Reviews 167, 159–168.
Horin, P., Smola, J., Matiasovic, J., Vyskocil, M., Lukeszova, L., Tomanova, K., Kralık, P., Glasnak, V., Schroffelova, D., Knoll, A., Sedlinska, M., Krenkova, L., Jahn, P., 2004. Polymorphisms in equine immune response genes and their associations with infections. Mammalian Genome 15, 843–850.
Janova, E., Matiasovic, J., Vahala, J., Vodicka, R., Van Dyk, E., Horin, P., 2009. Polymorphism and selection in the major histocompatibility complex DRA and DQA genes in the family *Equidae*. Immunogenetics 61, 513–527.
Kamath, P.L., Getz, W.M., 2011. Adaptive molecular evolution of the Major Histocompatibility Complex genes, DRA and DQA, in the genus *Equus*. BioMedCentral Evolutionary Biology 11, 128.
Metz, G.E., Serena, M.S., Martin Ocampos, G., Panei, C.J., Fernandez, V.L., Echeverria, M.G., 2008. Equine arteritis virus: a new isolate from the presumable first carrier stallion in Argentina and its genetic relationships among the four reported unique Argentinean strains. Archives of Virology 153, 2111–2115.

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Rousset, F., 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Molecular Ecology Resources 8, 103–106.
Sena, L., Schneider, M.P.C., Brenig, B., Honeycutt, R.L., Womack, J.E., Skow, L.C., 2003. Polymorphisms in MHC-DRA and -DRB alleles of water buffalo (*Bubalus bubalis*) reveal different features from cattle DR alleles. Animal Genetics 34, 1–10.
Timoney, P.J., McCollum, W.H., 1993. Equine viral arteritis. The veterinary clinics of North America. Equine Practice 9, 295–309.
Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. Evolution 38, 1358–1370.
Zhou, H., Hickford, J.G.H., Fang, Q., Byun, S.O., 2007. Identification of allelic variation at the bovine DRA locus by polymerase chain reaction-single strand conformational polymorphism. Journal of Dairy Science 90, 1943–1946.

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