Short communication

Low diversity in the major histocompatibility complex class II *DRA* exon 2 in dromedaries from northern Oman

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Abstract

Dromedaries in Oman provide essential resources for emerging and rapidly increasing demands in (peri-) urban milk and meat markets. The supply of products from healthy animals is important as dromedaries can be reservoirs not only for economically challenging, but also human transmissible (zoonotic) diseases. To learn more about the immune response genes in dromedaries we investiaged the genetic diversity in an important region of the major histocompatibility complex (MHC) class II. We targeted the *DRA* exon 2, which is highly variable and antigen-binding site encoding, in 60 dromedaries from Oman that were infected or non-infected with trypanosomes. We successfully amplified 18 sequences, which all belonged to a single haplotype *Cadr-DRA*02* (Genbank: KT936411) including one individual (J16), which was found heterozygous with a second haplotype *Cadr-DRA*03* (Genbank:KT936410). The haplotypes identified in this study from Oman were identical to sequences of dromedaries originating from Jordan and Australia. In general, all *Cadr-DRA* haplotypes are shared between the three Camelini species (dromedary, Bactrian camel, wild two-humped camel). These findings indicate a low diversity of *DRA* exon 2 gene in the dromedaries examined in Oman.

Keywords: Immune response genes, MHC, *Camelus dromedarius*

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Introduction

The major histocompatibility complex (MHC) has a crucial role in the immune response of the host and its interactions with pathogens. It has a unique genetic structure compared to the rest of the genome characterized by high levels of linkage disequilibrium (LD) and homologies (Matzaraki et al., 2017) and three major sub-regions of the MHC from the telomeric to the centromeric end; the classical class I, class III, and classical class II (Shiina et al., 2009). In camels, the MHC was identified on the longer arm of chromosome 20 with the MHC class II located closer to the centromere and the MHC class I more distant (Plasil et al., 2016). MHC class I and II genes encode antigen-presenting

molecules for dual recognition of antigenic peptides on the cell surface (Janeway et al., 2001). MHC class II polymorphisms are mainly concentrated in the alpha and beta chain genes exons 2, which are highly variable and encode the antigen-binding site in the extracellular domains (Alcaide et al., 2014; Xi et al., 2014). However, only few variation has been found in MHC class II genes of camels (Plasil et al., 2016). Dromedaries can be reservoirs of economically important as well as human transmittable (zoonotic) diseases, like the Middle East Respiratory Syndrom corona virus (MERS-CoV; Lado et al., 2021a) or the Crimean-Congo hemorrhagic fewer virus (CCHFV; Lado et al., 2021b). Especially trypanosoma infection has caused production and economic losses in dromedaries from Oman with three species of this blood parasites detected, *T. evansi*, *T. congolensis* and *T. brucei*. It has been shown that there is an association between low levels of genetic diversity, increased susceptibility of a pathogen and high pathogen loads (Meyer-Lucht & Sommer, 2009; Sommer, 2005). Therefore it is important to study the diversity of MHC and its sub-regions in different species (Lighten et al., 2014). The objective of this study was to screen MHC class II *DRA* exon 2 diversity in dromedaries from Oman infected and non-infected with trypanosomes, and to identify potential associations between haplotypes and trypanosome infection.

Materials and methods

A total of 142 dromedaries from different ages and both sexes were collected during a large survey about trypanosome infection in dromedaries in five governorates in the north of The Sultanate of Oman (Al-Buraimi, Al-Dakhiliyah, Al-Batinah, Al-Sharqiyah, and Al-Dhahirah; Fig. 1) approved by the Ministry of Agriculture and Fisheries and Water Resource, Sultanate of Oman. From each animal, ten ml of EDTA blood and serum were collected to be screened for trypanosome infection using Card Agglutination test (CATT) for *Trypanosome evansi* as described in Al-Kharusi et al. (2023) and molecular analysis with polymerase chain reaction (PCR; Al-Kharusi et al. (2022). In this immune genetic study, we included 60 dromedary samples from different districts in Oman (Fig. 1) that could successfully and reliably be grouped into trypanosome positive and negative animals based on recently published CATT (Al-Kharusi et al., 2023) and PCR results (Al-Kharusi et al., 2022).

DNA was purified using the E.Z.N.A. SQ blood DNA kit (Omega, USA) following the manufacturer's instructions. The extracted DNA was quantified using NanoDrop spectrophotometer 2000c (Thermo Fisher Scientific, Waltham, USA) at a wave length of 260/280 nm and stored at -20 ^{o}C until usage. The DNA concentration ranged between 5-18 ng/µl. The *DRA* exon 2 (923 bp) gene fragments were amplified using camel specific DNA primer pairs (DRA-ex2-F CCCTGGAATTCGGGTTTAAG/ DRA-ex2-R GGCTGAAAAAGCAGTTGAGC) with an annealing temperature of 57°C (Plasil et al., 2016). The PCR contained 15 µl of master mix (Thermo Fisher Scientific), 5 µl of nuclease free water, 200 pMol of forward and reverse primer, 3 µl of DNA template. The PCR reaction was started in 25 µl for 95°C at 10 min. The 35 cycles initiated with denaturation for 30 sec at 95°C, following by annealing for 45 sec at 57°C and extension for 50 sec at72°C. The final extension was carried out for 7 min at 72°C. The PCR product was checked on an 1.5% agarose gel.

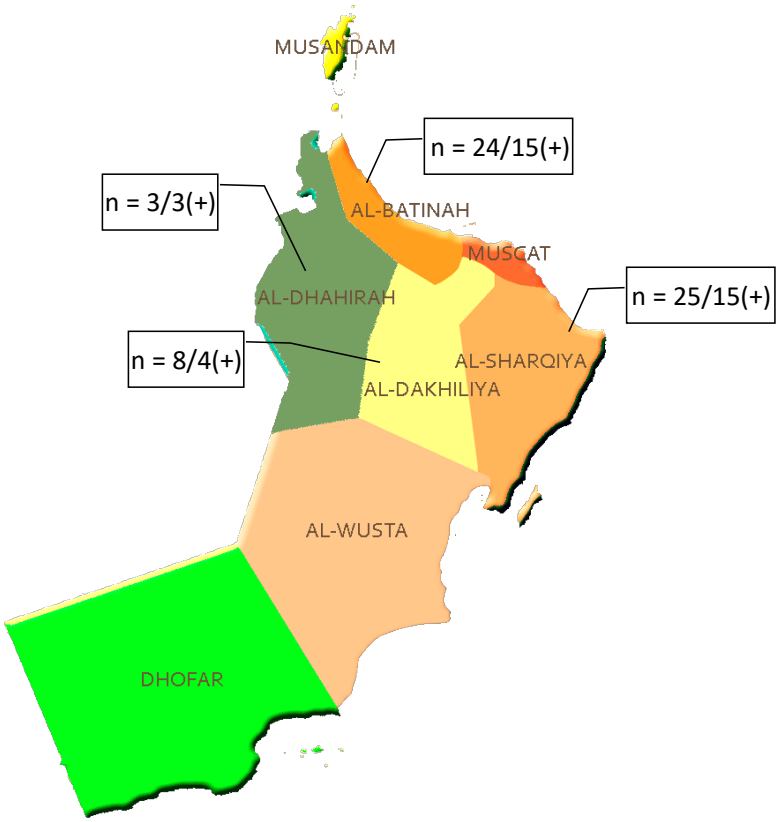

Figure 1. Districts of Oman with the number of dromedaries included in this study. The number of trypanosome positive individuals (+) according to Al-Kharusi et al. (2022 and 2023) is provided after the diagonal slash.

Succesfully amplified PCR products were purified with FastAP (Thermo Fisher Scientific) and the sequencing reaction was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific). Sanger sequencing of the *DRA* exon 2 gene in both directions was performed on an ABI 3130 DNA Analyzer. Sanger sequences were visually checked and aligned to the *DRA* exon 2 [GenBank: AGVR01020883.1|:30918–31163] reference sequences using CodonCode Aligner v.3.7.1.2 (Codon Code Corporation, USA). We identified the Kimura-2-parameters best-fit evolutionary model following the Akaike Information Criterion with correction for small sample size to build a neighbor-joining phylgenetic tree in MEGAX (Kumar et al., 2018), including two additional dromedary *DRA* exon 2 sequences (haplotypes) from Jordan (GenBank: KT936411) and Australia (GenBank: KT936412)

Results and discussion

We successfully amplified and sequenced 18 samples of DRA exon 2 sequences out of 60 samples from trypanosome infected and non-infected camels from Oman. The low amplification rate of the fragment in the dromedaries despite multiple iterations was disappointing and might be attributed to a low DNA quality and long (924 bp) *DRA* exon 2 fragment. All of the 18 sequences belonged to one haplotype *Cadr-DRA*02* (Genbank: KT936411) and were found homozygous in the dromedaries except one individual (J16), which was heterozygous for a second haplotype *Cadr-DRA*03* (Genbank: KT936410) (Figure 2).

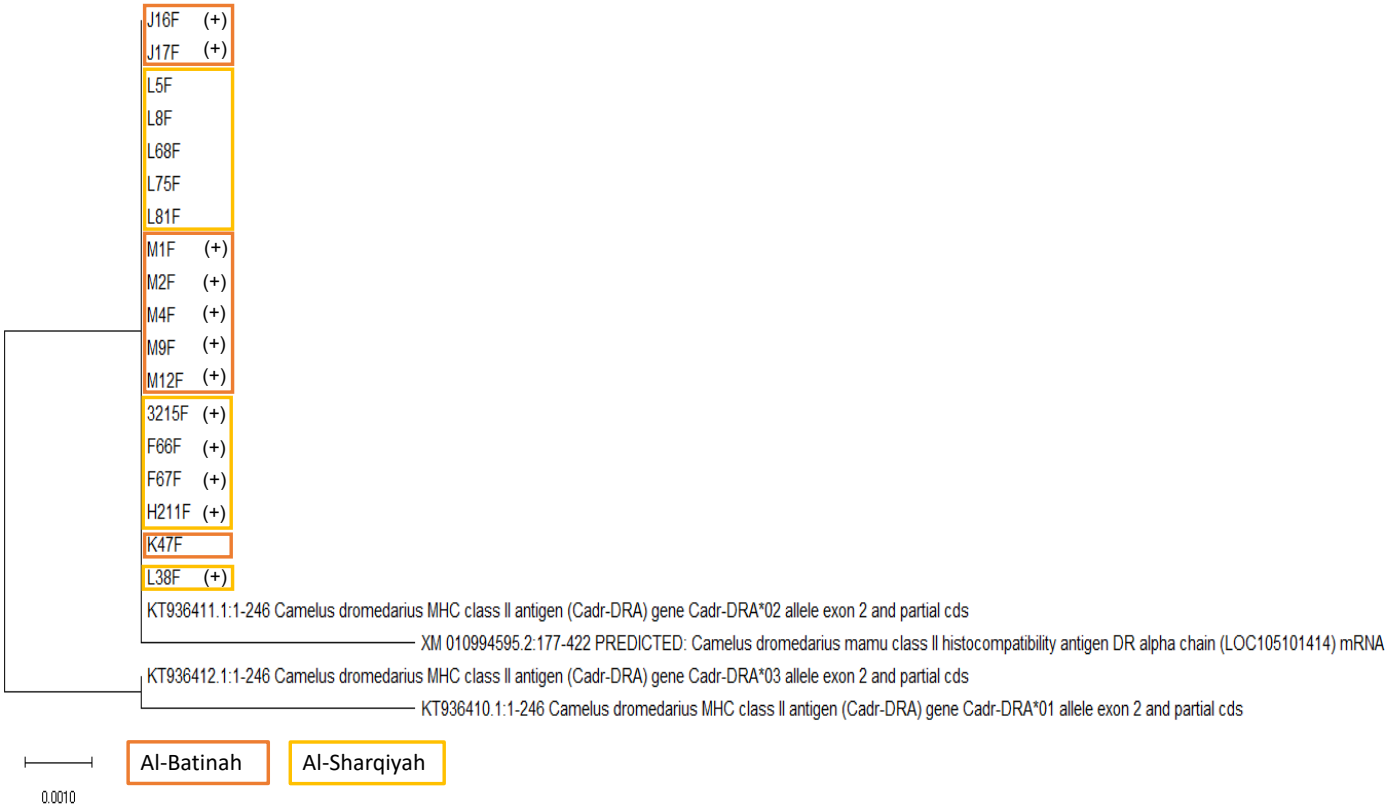

Figure 2. Neighbor-joining tree of *DRA* exon 2 in dromedaries from Northern Oman. The 18 successfully sequenced dromedaries shared one haplotype *Cadr-DRA*02* (Genbank: KT936411). Samples are colour-coded based on their origin from Al-Batainah (orange) and Al-Sharqiyah (yellow). The female (F) sex of the individuals as well as the positive (+) trypanosome status (Al-Kharusi et al., 2022 and 2023) are indicated after the sample ID.

Overall, lower genomic heterozygosity in dromedaries compared to domestic and wild Bactrian camels was observed (Fitak et al., 2020). The *DRA* exon 2 haplotypes amplified in this study were identical to the sequences of dromedaries originating from Jordan and Australia as well as to Bactrian camel (*Camelus bactrianus*) and wild two-humped camel (*Camelus ferus*) (Plasil et al., 2016). In general, the *Cadr-DRA* haplotypes are shared between the three Camelini species, dromedary, Bactrian camel, and wild camel. Their allele distribution is across species and not only found in specific geographical regions of populations. Moreover, the MHC of dromedary camels has a similar structure like the MHC of Bactrian camel, wild two-humped camel, llamas and bovids (Plasil et al., 2016). In camels, MHC class II genes had a higher mean nucleotide diversity compared to all other gene groups. But there were no differences in diversity between innate and adaptive immune response gene groups in dromedaries (Lado et al., 2020). Pathogen-mediated selection is the major force in maintaining the high diversity at MHC loci (Bernatchez & Landry, 2003). In the adaptive immune system, the MHC plays an important role and has been used to estimate levels of adaptive genetic variation (Elbers et al., 2017). However, due to the low diversity observed over all investigated dromedary samples, we were not

able to identify a potential relationship between a specific *DRA* exon 2 haplotype and trypanosome infection in Omani dromedaries. We suggest additional genotyping to perform genome-wide association studies, e.g., using the newly developed 180K SNP camelid Affymetrix array (Burger et al., in preparation), including 11K SNPs located in known immune response genes in trypanosome infected and non infected samples. Another interesting approach would be performing targeted sequencing of the MHC I and II using PacBio or nanopore long-read sequencing to capture the entire haplotypes of these important immungenomic region.

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