Analysis of genetic variation in the SLC11A1 gene in Holstein Friesian and Brown Swiss cattle, its influence on the expression of NRAMP1 and potential association with resistance to bovine tuberculosis.

Rachel Garty[1], Angela Holder[1], Bernardo Villarreal-Ramos[2], Charlotte Elder[1], Dirk Werling[1]

[1] Royal Veterinary College, Department of Pathobiology and Population Sciences

[2] APHA, Weybridge

Abstract

Bovine tuberculosis (bTB) is a chronic insidious disease caused by *Mycobacterium bovis* which infects both domestic livestock and wildlife. The increasing prevalence of bTB across the UK has impacted animal movement, public health and the agricultural industry's economy. Previously, efforts have focused on expanding veterinary diagnostic capability, generating vaccines and controlling maintenance hosts. However, given the extent of the disease within the UK wildlife population and the unlikely probability of eradication in the near future, alternative control strategies need to be investigated.

The identification of bTB resistant bovine genotypes could enable the introduction of breeding strategies to minimise the risk of infection by propagating more genetically resistant individuals. The SLC11A1 gene, a member of the solute carrier family 11, encodes for the natural resistance-associated macrophage protein 1 (NRAMP1), a specific membrane transporter involved with iron metabolism believed to be vital for controlling resistance to infection by intracellular pathogens. Single nucleotide polymorphisms (SNPs) in the coding region of the SLC11A1 gene and a microsatellite in the 3' UTR of this gene have previously been implicated as possible genetic targets associated with resistance to intracellular infections such as *M. bovis*, *Brucella abortus* and *M. avium paratuberculosis*. This study aimed to delineate the different genotypes for SLC11A1 in two breeds of UK dairy cattle, Holstein Friesian and Brown Swiss, identified as having differing susceptibilities to bTB. The possible functional effect of this genotypic variation will then be assessed using an ELISA to measure the concentration of NRAMP1 protein in macrophages generated from CD14+ cells cultured with GM-CSF or M-CSF for 6 days. CD14+ cells were cultured from 12 Brown Swiss and 12 Holstein Friesian cattle and the concentration of NRAMP1 protein was measured. Analysis of the SLC11A1 gene in cDNA samples generated from peripheral blood mononuclear cells by PCR amplification and Sanger sequencing identified 3 SNPs (c.650C>T, c.961G>A, c.1066C>G).

All 3 SNPs were found to be non-synonymous, leading to changes in the amino acid sequence, but two of them (c.650C>T, c.961G>A) were present only at very low frequencies (<10%). The third SNP (c.1066C>G) was found to occur at a higher frequency in Brown Swiss compared to Holstein Friesians, but this difference was not statistically significant. Additionally, a microsatellite in the 3'UTR (c.1647+61) containing either 10, 11 or 12 GTs was found to be present at similar frequencies in both Holstein Friesian and Brown Swiss. The NRAMP1 ELISA identified differences in the amount of NRAMP1 protein present when comparing macrophages generated from either Brown Swiss or Holstein Friesian cattle, cattle that were homozygous or heterozygous for the c.1066C>G SNP, and cattle which had different genotypes for the microsatellite. However, none of these differences were found to be statistically significant. Due to small sample size the study may benefit from increasing the

sample population to elucidate the differences in the amount of NRAMP1 protein expressed by the two different breeds.