An interdisciplinary approach to investigate the *English spotting* locus and its association with megacolon in the domestic rabbit: a new putative model of enteric neuronal dysfunction

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ABSTRACT

Studies on coat colour genetics in the domestic rabbit, carried out at the beginning of the last century, led to the identification of the *English spotting* locus, characterized by an incomplete dominant mutant allele (*En*). Homozygous non mutant *en*/*en* rabbits have solid coloured phenotype. Heterozygous *En*/*en* rabbits possess far larger patches of coloured fur compared to the homozygous *En*/*En* animals. The latter ones may be almost completely white. The *En*/*en* genotype is selected for show purposes and a few breeds have a recognized standard, that according to these early studies, should be the result of this allele combination. The *En*/*En* genotype seems to be subvital probably due its association with an underlying megacolon syndrome. Here we studied this locus starting from the confirmation of its mode of inheritance and effects on coat colour. To follow the segregation of the *English spotting* alleles, a F1 population was created crossing Checkered Giant rabbits. Chi square test indicated no deviation from the classical Mendelian ratio of 25% (almost completely white animals), 50% (normal spotted animals), 25% (solid coloured animals). However, the extent and position of the patches in the animals classified as "almost completely white" or "normal spotted" varied, probably due to the action of modifier genes. In addition, segments of cecum and colon of two *En*/*En* and two *en*/*en* rabbits have been harvested. Tissue specimens have been fixed in Zamboni's solution, embedded in paraffin, cut with a microtome and stained with cresyl violet or prepared as whole mounts and processed for indirect immunofluorescence. These techniques unravelled an apparently normal enteric neural network on both putative megacolon tissues and controls. On the contrary, some other colon specimens of *En*/*En* rabbits that were processed for transmission electron microscopy showed important anomalies of enteric neurons and nerve endings. Sequencing of a few candidate genes identified several polymorphisms. In conclusion, these preliminary results suggest a genetically determined enteric neuropathy responsible for megacolon in the investigated rabbit model.