BOVINE NEURONAL CEROILD LIPOFUSINOSIS IN AUSTRALIAN DEVON CATTLE

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SUMMARY

Neuronal ceroid lipofusinoses (NCL) are a group of lethal inherited progressive neurodegenerative disorders and more than 20 different genes have been associated with these diseases. In Australian Devon cattle there is strong evidence that NCL is caused by a single nucleotide insertion in the *CLN5* gene (c.662dupG). The aim of the present study was to estimate the frequency of the disease-causing allele in the Australian Devon cattle population. Samples from 300 randomly selected animals were requested, 190 samples were received and genotyped using a previously described DNA test. All animals were homozygous normal and the allele frequency of NCL in Australian Devon cattle was therefore estimated to be zero.

INTRODUCTION

Neuronal ceroid lipofusinoses (NCL) are a group of inherited lethal progressive neurodegenerative disorders in humans and many other animal species (Mole *et al.* 2011). Affected individuals show a progressive loss of visual, motor and mental function, often suffer from seizures and die prematurely. Characteristic findings are the accumulation of auto-fluorescent storage bodies comprised of either subunit C of mitochondrial ATP synthase or saponins in lysosomes, especially in neurones (Mole *et al.* 2011). NCL are mostly recessively inherited and at least 21 different genes have been associated with these diseases in humans and various other animal species (Bond *et al.* 2013; Mole 2013). Currently no cure is available but clinical trials for gene therapy, stem cell therapy and various pharmacological approaches are in progress (Mole *et al.* 2011, Mole 2013).

Both naturally occurring and artificially induced mutations can cause NCL in various animal species (Bond *et al.* 2013). Naturally occurring bovine NCL has been reported in Beefmaster (Read and Bridges 1969), Devon (Harper *et al.* 1988) and in a single Holstein-Friesian bull (Hafner *et al*. 2005). So far, a disease causing mutation has only been proposed for the NCL variant in the Devon breed and a direct DNA test was developed identifying a nucleotide duplication in exon 4 (c.662dupG) of the bovine *CLN5* gene on bovine chromosome 12 (Houweling *et al.* 2006). *CLN5* codes for a protein with unknown function (Kollmann *et al.* 2013). The previous study by Houweling *et al.* (2006) was only focused on a single herd within New South Wales (NSW), Australia, and it was therefore not possible to make a statement about the allele frequency or the prevalence of the disease in the Australian Devon population.

As Devon cattle are raised nationwide we describe here large scale sampling and genotyping in order to estimate the allele frequency of NCL in the Australian Devon breed. The results of this study are aimed to assist the Devon breed organisation to make informed decisions about the impact of NCL in Devons.

MATERIALS AND METHODS

Random sampling. The Devon Cattle Breeders' Society of Australia approved access to their electronic herd book and a data extract was obtained from ABRI, University of New England. The extract compromised of 65,535 animals born between 1922-2011 and listed more than 100 owner

identifiers in Australia and New Zealand. A random sampling approach was applied to the subset of 4880 animals born after 2005. The sampling size was calculated using an estimated relative targeting allele frequency for the disease. Considering an estimated allele frequency in the original herd (p = 0.03) as the worst case for NCL in the Devon population, sample size (n) was determined using the following formula, $se(p) = \sqrt{p(1-p)/(2n)}$ where p is the allele frequency, se(p) is the standard error of the estimate. A relative error se(p)/p less than 0.3 was chosen as a minimum requirement suggesting that a minimum of 200 animals are needed to be genotyped to be reasonably confident that a true allele frequency greater than zero can be detected (Table 1). In consideration of an incomplete response rate 300 samples were requested.

Table 1. Effect of varying sample sizes (n) for different allele frequencies p. The results are shown as the relative error se(p)/p. Relative errors less than 20% and 30% of the true allele frequency are shown in italics and bold, respectively.

Frequency	Sample size (n)								
P	100	150	200	250	300	350	400	450	500
0.005	1.00	0.81	0.71	0.63	0.58	0.53	0.50	0.47	0.45
0.01	0.70	0.57	0.50	0.44	0.41	0.38	0.35	0.33	0.31
0.015	0.57	0.47	0.41	0.36	0.33	0.31	*0.29*	*0.27*	*0.26*
0.02	0.49	0.40	0.35	0.31	*0.29*	*0.26*	*0.25*	*0.23*	*0.22*
0.025	0.44	0.36	0.31	*0.28*	*0.25*	*0.24*	*0.22*	*0.21*	**0.20**
0.03	0.40	0.33	*0.28*	*0.25*	*0.23*	*0.21*	**0.20**	**0.19**	**0.18**
0.035	0.37	*0.30*	*0.26*	*0.23*	*0.21*	**0.20**	**0.19**	**0.18**	**0.17**
0.04	0.35	*0.28*	*0.24*	*0.22*	**0.20**	**0.19**	**0.17**	**0.16**	**0.15**
0.045	0.33	*0.27*	*0.23*	*0.21*	**0.19**	**0.17**	**0.16**	**0.15**	**0.15**
0.05	*0.31*	*0.25*	*0.22*	**0.19**	**0.18**	**0.16**	**0.15**	**0.15**	**0.14**

Letters including labelled sampling bags, a consent sheet, an animal ethics information statement, an NCL information sheet, a hair sampling guideline, a support letter from the Devon Cattle Breeders' Society of Australia and a stamped return envelope were sent to the 41 owners, who were asked to provide tail hair samples for requested animals (or equivalent replacement animals) and additionally semen straws of any available artificial insemination (AI) bulls. The study was approved by the University of Sydney animal ethics committee (N00/8-2011/3/5581).

DNA test. For tail hair DNA extractions, 3-4 hair roots per animal were boiled for 15 minutes with 50 μl of 200 mM NaOH. After brief centrifugation, the solution was neutralized with 50 μl of 200 mM HCl and 100 mM Tris-Cl pH 8.5. The mixture was vortexed and centrifuged 2 minutes at 13,000 rpm and diluted 1:10 with Milli-Q water. DNA was extracted from AI semen straws using the method described by Heyen *et al.* (1997). The DNA test was conducted as described by Houweling *et al.* (2006) and involved PCR and visualisation of products on 8% polyacrylamide gels using a LI-COR 4200 sequencer. The normal allele is expected to produce a product of 62 bp whereas the disease allele yields a product of 63 bp. DNA samples of homozygous affected and carrier animals were available and used as positive controls (Houweling *et al.* 2006)

RESULTS

Out of 300 hair samples requested from 41 farmers, only 54.3% (163 animals) of the hair samples were received from 36.6% (15 owners) of the owners. These included replaced samples (72.3%, n=118) selected by owners due to inaccessibility to the originally targeted animals and 13

voluntarily donated samples. In addition, 27 semen samples were donated. All hair and semen samples were processed for DNA extraction, PCR amplification and gel electrophoresis. All 190 samples were successfully genotyped and tested as homozygous normal.

DISCUSSION

NCL has been previously identified in a single herd of Devon cattle in NSW (Harper *et al.* 1988). Advanced pedigree analysis could not identify a common founder (Tammen *et al.* 2002) but molecular characterisation identified that these cattle are a model for the Finnish variant late infantile disease in humans and a direct DNA test was developed to eradicate the disease allele within the initial herd (Houweling *et al.* 2006). The present study suggests that the allele frequency of bovine NCL in registered Devon cattle in Australia was zero or very close to zero and thus a management program is currently not indicated.

However, there are several limitations in the present study. In relation to the sampling size ideally a relative error of 10% should have been considered. However, this would have required sampling of a large proportion of the whole population. The statistic used does assume that all samples are unrelated, which is unlikely to be the case. Furthermore, the samples received were less than requested (a total of 190) and the majority of these were not randomly sampled. This could have been related to the relatively short return time line of 1 month. Herdbook data might not have been updated in regards of 'death' or 'sales', which resulted in a high number of replacement animals. Sampling for this study was totally depending on the willingness of the owners to participate and a support letter from the breed organisation might be the reason for relative high response rates. Access to additional semen samples would have been of interest as they could have provided a more historic view and would have allowed testing of animals with a broader impact on the population.

A follow up study using advanced pedigree analysis such as the GeneProb software (Kerr and Kinghorn 1996) where DNA test results of a subset of animals (including carrier and affected animals identified by Houweling *et al.* (2006)) and pedigree information of all animals in the population are used to predict genotypes for all animals is recommended. This can be used to identify the founder animal and to verify that the risk for the current population is extremely low. Such a study might also provide us with some insight in why the mutation that was only ever found in Australian Devon cattle in a single herd in NSW was not found in this population screen. Considering that the owner of the initial affected animals acted very responsible and immediately notified the breed society and other breeders, the possibility of a *de novo* mutation in this herd that was then contained within the herd and eradicated (initially by culling affected animals and obligate carriers and after DNA testing of the whole herd by culling remaining carriers) might be a possible explanation.

Any suspected clinical case of NCL disease should be reported and investigated.

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