
K.G. Reichmann¹, R.D. Drinkwater², D.J.S. Hetzel², R.W. Hielscher³, P.J. Healy⁴

¹Department of Primary Industries, Animal Research Institute, Yeerongpilly, Q 4105, Australia.
²CSIRO, Division Tropical Animal Production, University of Queensland, Brisbane, Q 4072, Australia.
³Department of Primary Industries, Bruce Highway, Rockhampton, Q 4700, Australia.
⁴Elizabeth McArthur Agricultural Institute, PMB 8, Camden, NSW 2570, Australia.

INTRODUCTION

Generalised glycogenosis (glycogen storage disease type II; Pompe’s disease) is an autosomal recessive disease that has been recorded in humans (Pompe, 1932) and various animal species including Beef Shorthorn (Jolly et al., 1977) and Brahman (O’Sullivan et al., 1981) cattle in Australia. This disease is caused by a deficiency of the enzyme acid a glucosidase (AAG) which results in an excessive accumulation of glycogen within lysosomes in many tissues.

In man, glycogenosis is expressed as several different clinical syndromes of varying severity. These syndromes are associated with variation in residual AAG activity which, in turn, reflects molecular heterogeneity within the AAG gene (McKusick, 1990). In contrast, the majority of Brahman cases reported in Australia presented with a similar clinical disease (Reichmann et al., 1993). This is consistent with the hypothesis that the disease has arisen through dissemination of a mutation via a founder effect in a breed derived from a narrow genetic base.

FEATURES OF THE DISEASE

Typically, affected calves fail to thrive and by 6 months of age display evidence of muscular weakness and a degree of incoordination. After weaning the severity of the clinical disease increases rapidly. Affected animals have sunken eyes, adopt a wide-based stance and have a concave arch of the neck reflecting muscular weakness. Incoordination and ataxia impair locomotion. Death usually occurs between 6 and 9 months of age. Some calves often die by misadventure. However, with careful nursing affected calves may survive until 12-13 months of age. Only a small proportion of actual cases are reported. This is a consequence of the non-specific nature of the clinical signs shown by cases, the extensive grazing conditions this breed is subject to and the random nature of presentation of sick, single animals.

Necropsy fails to show any consistent macroscopic abnormalities. In contrast, PAS-positive cytoplasmic vacuolation of skeletal muscle and neurones is a consistent histological finding. Similar vacuolation can also be found in Purkinje fibres, myocardial cells, smooth muscle of the gastrointestinal tract, hepatocytes, distal tubular cells of the kidney and lymphocytes. Increased number and frequency of PAS-positive vacuoles in circulating lymphocytes is a useful aid to the diagnosis of glycogenosis. Similarly, progressive elevation of AST and CK activities in blood and excretion of high molecular weight oligosaccharides in urine are characteristic of the disease and can be indicators of glycogenosis in suspect animals (Reichmann et al., 1993).

Usually, diagnosis of glycogenosis is confirmed by demonstration of a severe deficiency of AAG in blood lymphocytes (typically < 2% of normal). Similar low activities are found in most
tissues (liver, kidney, brain, cardiac muscle) whilst a greater residual level (10% of normal) is found in skeletal muscle (Reichmann et al., 1989). Wisselaar et al. (1993), however, reported that there was a complete lack of AAG synthesis and mRNA production in this tissue. Glycogen levels are elevated by at least 2 to 3 fold in most tissues.

A small number of atypical cases, some involving blindness, have been reported in calves aged less than 6 weeks but in most cases the diseases were considered coincident with glycogenosis (Reichmann et al., 1993). Subsequent cases from a breeding program using obligate heterozygotes (Reichmann unpublished) have included a stillborn calf and one that died at 1 week of age showing nervous signs and ataxia.

**TESTING PROGRAMS**

(a) Enzyme Based Test:- A heterozygote detection program was initiated in response to a desire of members of the Australian Brahman Breeders’ Association (ABBA) to reduce the prevalence of the disease in commercial herds. The activity of AAG relative to that of hexosaminidase and ß-galactosidase in blood mononuclear cells (Healy, 1982) was used to identify heterozygotes in bull-breeding herds. As expected with the gene dosage phenomenon, the enzyme activity in heterozygotes was approximately half that of normal animals. In each submission a histogram of the relative enzyme activities was plotted to help differentiate populations of normal and heterozygous animals. Within herds, analytical results for each individual were considered in relation to pedigree information to arrive at a diagnosis of probable glycogenosis genotype.

The efficiency of the enzyme test program was limited by a number of factors. As with many enzymeopathies there was overlap in the populations of normal and heterozygous animals. An overlap in relative AAG activity between genotypes of at least 7% was estimated from observations of animals of known genotypes (Healy et al., 1987) and predicted by statistical modelling (Macbeth et al., 1992). Overlapping populations and/or misdiagnosis of genotype could be a consequence of other genes affecting AAG activity with a heritability of 0.44 being reported for this enzyme activity (McPhee and Reichmann, 1990). This could be manifested in varying levels of AAG in subclasses of lymphocytes (Healy et al., 1987) whose proportions could be influenced by physiological state, secondary disease and environmental factors. Some of these factors could be minimised by testing on a herd basis, however this necessitated sampling a larger number of animals.

The presence of a naturally occurring inhibitor of AAG further complicated the testing program. This inhibitor, Castanospermine, from the seeds of *Castanospermum australe* was found to be a potent inhibitor of AAG from all tissues (Reichmann et al., 1987). A simple plasma test, based on the differential inhibition of acid and neutral AAG, was subsequently developed to identify samples from animals consuming these seeds.

Misclassification of genotype was observed in the small number of known twins tested. Twins are haemopoietic chimeras as a result of having conjoint placentae in utero. Therefore their circulatory blood cells will include populations derived from stem cells from their cotwin whose genotype may be different.

(b) DNA Based Test:- In an effort to improve the efficiency of heterozygote detection a DNA test was sought. A Msp1 RFLP was found to be closely associated with the disease (Hetzel et al., 1988; Drinkwater and Hetzel, 1992). Using polymerase chain reaction (PCR) the RFLP
has been adapted to a routine test that provides a very high level of probability for detection of Brahman animals heterozygous for glycogenosis. The ability to utilise a variety of samples, but usually heparinised blood, as a source of target DNA provides significant advantages over the enzyme based assay in a large-scale disease control program. Furthermore, the PCR test has the advantages of avoiding the complications caused by C. australe consumption, the sample requirements are less stringent and there is no requirement for herd sampling. The prime advantage, however, is the improved accuracy of the test. Initial accuracy estimates indicate that the specificity and sensitivity of the PCR test is 98% and 100%, respectively, whilst those for the enzyme test are both approximately 90%. The PCR test has correctly identified all obligate heterozygotes (n=57) and clinically diagnosed affected animals (n=30) indicating the existence of the single mutation in, at least, this sample of animals.

RESULTS OF TESTING PROGRAMS

Approximately 30,000 and 1600 animals, predominantly from studs, were tested in the enzyme based testing program and the DNA test program, respectively. The incidence of heterozygotes was estimated by the enzyme testing program to be approximately 13%. DNA testing on properties not previously tested by the enzyme based method revealed a heterozygote prevalence of 12% (n=250). Similar frequencies were predicted by modelling analysis of populations of animals based on their enzyme activities and also by genetic investigations which determined the contribution to the current gene pool of ancestor animals found to have been the likely source of the defective gene in Australian herds. Despite the limitations of the enzyme testing program it has successfully reduced the number of heterozygotes in breeding herds.

Assuming random mating, the incidence of glycogenosis resulting from a heterozygote prevalence of 13% would be 0.42%. While the economic costs of this disease may not appear to be highly significant when considering the overall population, it has an important impact on some properties where the frequency of heterozygotes have been found to exceed 30%. Jolly and Townsley (1980) found a screening program for mannosidosis in Angus cattle was economically viable when the heterozygote prevalence was 6%. Within Brahman herds, cost benefit analysis could be used to determine the suitability of introducing various control programs.

The greatest potential for disseminating genetic defects with subsequent economic loss occurs through artificial breeding programs. The ABBA's policy has made it mandatory that bulls and cows intended for AI and ET programs, respectively, are tested. The consequences are exemplified by a case where, prior to the testing program being initiated, 5153 doses of semen were collected from a bull and subsequently used before it was shown to be an obligate heterozygote. The ABBA has continued with its policy that the testing program for its members be a voluntary one, where natural mating is involved. However, the majority of studs making dominant contributions to the breed hierarchy, have proceeded with testing so that the advantages of breeding from normal animals will disseminate throughout the breed.

The use of accurately kept pedigree records should supplement any genetic testing program for stud animals. These can be helpful in establishing the likely probability of an animals' genotype. A comprehensive database containing information on tested animals and obligate and inferred heterozygotes from current and previous generations, can be used to determine the probability of the genotype of the majority of stud animals.
IMPLICATIONS

It has been established that glycogenosis is a cause of significant mortalities in Australian Brahman herds. The principal source of Brahman cattle over the last 60 years has been the US. Evidence from two genetic investigations showed that some of the cattle from early importations are likely to have been the primary source of the mutation (Reichmann unpublished). One of these analyses compared the frequency of appearance of ancestors in groups of different genotype whilst the other identified the first appearance of a common ancestor in the sire and dam pedigrees of affected calves. Heterozygotes have been detected amongst recent importations from the US, some being parents of affected calves (ie obligate heterozygotes), whilst others were diagnosed as heterozygotes on the basis of both enzyme and DNA tests. More recently, DNA testing on samples collected from bulls in the US suggest a heterozygote prevalence of 11% (n = 54). Consequently, we endorse the current policy of the Australian Quarantine Inspection Service and the ABBA that a homozygous normal genotype is mandatory for importation of Brahman germplasm to Australia. To support this policy, greater certainty in diagnosis of genotype in animals of both Australian and non-Australian origin, is required. It is important, therefore, that the mutation responsible for glycogenosis in Brahmans is defined to improve the specificity of the heterozygote detection test. Furthermore, the existence of multiple mutations, as occurs in the human condition, needs to be closely monitored.

REFERENCES