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Risk factors for congenital chondrodystrophy of unknown origin in beef cattle herds in south eastern Australia

Peter J. White^{*}, Peter A Windsor, Navneet K. Dhand and Jenny-Ann L.M.L. Toribio

Faculty of Veterinary Science, The University of Sydney, 425 Werombi Road,
Camden,
NSW 2570, Australia

*Corresponding author. Tel.: +61 2 9351 1610; E-mail address: p.white@usyd.edu.au

Abstract

A case control study was conducted on 46 farms in south eastern Australia with a recent history of congenital chondrodystrophy of unknown origin (CCUO) between 2002 and 2007. For each farm data was collected using face-to-face interviews concerning the management of case and control mobs during the gestation period in which affected calves were born. Data concerning the paddocks in which gestating cattle were maintained was also collected for analysis. Three separate multivariable models were constructed using generalised linear mixed models (GLMM). The first model was based on the dichotomous outcome of mob status (affected/not affected) and included explanatory variables for management and environment factors. The second model used a novel approach, taking into account the number of cases in affected mobs in order to utilise available data. The outcome events/ trials was used where the numerator was equal to the number of affected calves in a mob, and the denominator was equal to the total number of calves in the mob. The third model used the dichotomous outcome paddock status and included environmental and soil variables for paddocks involved with case and control mobs. Confounding for dam age and year affected was included in the mob outcome models, and random effects for paddock and farm were incorporated into the models.

The birth of CCUO calves was associated with dams grazing native pastures on hilly terrain during gestation. Low levels of pasture were also associated with the outcome. The two models used for the mob outcome were similar in many respects. The

events/trial model included the use of supplemental feed and an interaction term. This study demonstrates an association between a maternal nutritional disturbance and the occurrence of CCUO.

Keywords

Risk factors, congenital chondrodystrophy, case control study, epidemiology, Australia

1. Introduction

A congenital chondrodystrophy of unknown origin (CCUO) has been reported with increasing incidence in free range beef calves in south eastern Australia since 2002 affecting over 1000 calves on more than 135 farms (Cave *et al.*, 2008). Cases of CCUO are characterised by disproportionate dwarfism due to lesions affecting the epiphyseal growth plates of long bones (McLaren *et al.*, 2007). Signs include shortened and rotated limbs, superior brachygnathia and spinal deformities (Cave *et al.*, 2008; McLaren *et al.*, 2007). Deformities of the trachea and nasal turbinates may lead to perinatal death and most affected calves are not viable in the long term (McLaren *et al.*, 2007).

In south-east Australia, the incidence of CCUO appears to vary between affected farms with apparently similar characteristics. On affected farms, there is evidence to support differences in CCUO presence at the mob level. A previous attempt to investigate risk factors for CCUO using limited available data on an outbreak in 1991 suggested an association with low pasture availability and supplemental feeding (White *et al.*, 2009). The possibility that CCUO occurrence is associated with nutritional factors is also confirmed from studies into similar cases of CCOU in other countries (Gunn *et al.*, 1997; Mee, 2001; Ribble *et al.*, 1989; Staley *et al.*, 1994; Valero *et al.*, 1990). The implication of a nutritional disturbance involving trace mineral availability warrants inclusion in any study where possible (Hidioglou, 1980).

Pastures or supplemental feeds low in nutrients may be a source of primary nutritional deficiency. Due to the retrospective nature of this case-control study however, analysis of feedstuffs and pastures was not possible for the period of interest. The collection of soil samples, while influenced by fertilizer history and climate, was undertaken to describe any soil deficiencies or excesses that may affect the nutritional quality of plants and lead to a maternal nutritional disturbance (Whitehead, 2000). In addition to soil and nutritional data, the collection of all farm management and environment data permitted the evaluation of other potential risk factors. Farm management data was collected including general farm size, enterprise, CCUO history, mob structure, dam age at calving, animal treatments, condition score, breed, calving period and grazing history. Environmental data concerning paddock topography, level of tree cover and species, pasture type and species, water source and

fertilizer and pesticide history were also collected. These factors were included in the study to determine if the occurrence of CCUO was associated with a particular management factor or the environment of gestating cows.

Currently, there are no practical methods for control of CCUO for southeast Australian beef farmers. Methods of controlling CCUO in other countries rely on nutritional advice and the avoidance of poor quality hay-only or silage-only diets during gestation. The purpose of this study was to identify risk factors that farmers might be able to manipulate to reduce the occurrence of CCUO affected calves.

2. Materials and Methods

2.1 Study design and sampling methodology

The target population for this case-control study was all beef herds in south-eastern Australia affected by CCUO for the years 2002 to 2007 inclusive. The study population was herds that met the following criteria:

- beef herds reported by either a private veterinarian or government veterinarian as experiencing the birth of calves fitting the description of CCUO, including signs of disproportionate dwarfism, since 2002; and
- located in the regions of south-eastern New South Wales (NSW) or north eastern Victoria.

The signs of disproportionate dwarfism required for inclusion in the study were shortening of one or more limbs, superior brachygnathia and spinal deformities including torticollis, scoliosis or kyphosis.

The University of Sydney Human Ethics Committee (approval number 02-2008/10640) approved procedures involving contact with and the collection of information from farmers. Names and addresses of farm owners or managers with reported affected herds were provided by private and government veterinarians. Contact with farmers was initiated by mail and a follow up telephone call was made two weeks later to confirm herd eligibility based on reports of at least one calf from the herd showing signs of CCUO between 2002 and 2007 and farmer willingness to participate in the study. A small payment was offered to farmers to compensate for the time required to conduct an interview in an attempt to improve the participation rate. When a farmer was not prepared to participate in an interview the herd was excluded from the study. A face to face interview was then conducted with each consenting farm owner or manager.

During the interview, information about mobs of pregnant cattle present on the farm during years of CCUO occurrence was used to identify case mobs and control mobs. A case mob was a mob reported by the farmer in which at least one CCUO calf was born in a calving season. A control mob was a mob reported by the farmer to be free of CCUO calves in the same calving year as the case mob/s. For farmers that experienced cases in more than one year, details were collected for case mobs and control mobs for each affected year. Similarly, information was gathered about the

paddocks on the farm grazed by pregnant cow mobs during years of CCUO occurrence and used to identify case and control paddocks. A case paddock was defined as a paddock in which a case mob had grazed for at least the last two trimesters of pregnancy. A control paddock was defined as a paddock grazed by a control mob for at least the last two trimesters of pregnancy.

Using Win Episcopy 2.0 (<http://www.clive.ed.ac.uk>) it was determined that a sample size of at least 56 case mobs and 113 control mobs was required to provide 95% confidence of detecting a significant difference of odds ratio of three with 80% power, assuming a 10% exposure of controls.

2.2 Farmer Interview

A questionnaire was used to collect information from farmers for each enrolled herd about potential risk factors associated with the occurrence of CCUO calves. The questionnaire, designed following standard guidelines (Dillman, 2007), had four sections titled farm history and management, case mob management and environment, control mob management and environment, and paddock history. It consisted of 24 open and 34 closed questions and was modified for ease of use after trialing on three herds in NSW during March 2008. As these changes to the questionnaire did not alter the quality or quantity of information collected, data collected for these three herds were included in the study. A complete copy of the questionnaire (in English) is available from the corresponding author on request.

2.3 Soil Collection

Soil samples were collected for testing from paddocks identified as case paddocks and control paddocks except when recent soil test results from an accredited soils laboratory were available and obtained from the farmer. For each paddock 25 to 30 samples of the top 10cm of soil were collected by walking the paddock in a zigzag pattern, avoiding atypical areas such as fence lines, cattle and sheep camps, dung piles, fire sites and wetlands, in order to obtain a representative pooled sample. Samples were labeled, sealed in plastic bags and held at 4°C before submission to an accredited commercial laboratory (Incitec Pivot Lab Services, Werribee, Victoria) for analysis. Soil samples were analysed using standard methods accredited by the National Association of Testing Authorities. Measurements of the chemical characteristics of each sample were carried out according to standardised procedures using calibrated equipment. Calculated parameters were created using standard industry formulae (Anonymous, 2009). The location and altitude of each paddock was determined using a global positioning system meter (Garmin GPS 205).

2.4 Data Management

Microsoft Access (Microsoft 2003) was used to create a relational database to manage the questionnaire and laboratory data. Data concerning fertilizer history, supplemental feeding, breed and age were categorized and then all data entry completed and

checked against the hard records for errors. For statistical analysis this data was imported into SAS statistical software (release 9.2, SAS Institute Inc., Cary, NC, USA).

2.5 Statistical Analysis

The general features of study herds were described using standard descriptive statistics and their locations, based on GPS data, were mapped using ESRI® ArcGIS™ software (ArcGIS Professional 8.0, MapInfo Corporation, USA). Deformities reported in affected calves were documented and percentage of case mobs with various categories of deformity calculated. The proportion of affected calves born in each case mob was calculated and summarized across case mobs using median, percentiles and range.

Two separate datasets were constructed for analysis. The mob dataset contained data on herd management and environment variables for 168 mobs. The paddock dataset contained data on soil and paddock environment variables for 125 paddocks. Separate analyses were conducted on each dataset.

2.5.1 Outcome Variables

Two outcomes were used in analysis of the mob dataset. First, the outcome variable mob status was coded as 1 for case mobs and 0 for control mobs. Second, a novel approach using the outcome variable mob events-trials consisted of the number of affected calves (events) in a mob and the total number of calves born in a mob (trials) with the number of events for each control mob being zero. This model was used to reflect the severity of CCUO in case mobs and to complement the model based on mob status. In the paddock dataset, the outcome variable paddock status was coded 1 for case paddocks and 0 for control paddocks.

2.5.2 Explanatory variables

The explanatory variables in the mob dataset included 23 categorical and two continuous farm management and environment variables. In the paddock dataset, the explanatory variables included 23 continuous soil variables and nine categorical environment variables.

2.5.3 Descriptive Analysis

Descriptive analyses were performed on all explanatory variables. Frequency distributions, bar charts, and bar charts classified by the outcome were created for categorical variables. Mean, standard deviation and range were determined for continuous variables. In addition, histograms and box plots for continuous variables with outcome were produced. All descriptive analyses were performed using SAS statistical software and UniLogistic Macro (Dhand, 2009).

2.5.4 Univariable analysis

For each dataset, the unconditional association between each explanatory variable and each respective outcome of interest was investigated on an individual basis using the likelihood-ratio chi-square test implemented in UniLogistic Macro for SAS (Dhand, 2009). Variables with an unconditional association with the outcome at a significance of $P < 0.25$ were selected for further investigation. First, these variables (all selected variables had some inherent order or were created by categorization of continuous data) were tested in pairs for collinearity using Spearman rank correlation and highly correlated variables (coefficient $> |0.70|$) were then evaluated using either chi-square test or Fisher exact test (for categorical data without and with a number of expected cell counts < 5 , respectively). For significant associations ($P < 0.05$), one of the collinear pair of variables was selected for inclusion in further analyses based on an opinion of biological plausibility. Second, the selected variables were assessed for missing values and those with $> 10\%$ missing values were excluded from further analyses. Third, variables with less than 10 responses per category were also excluded. The remaining explanatory variables were selected for inclusion in the multivariable model.

2.5.5 Multivariable analysis

Separate generalised linear mixed models using logit transformation were constructed for each of the mob outcomes using the SAS GLIMMIX procedure and a manual stepwise approach (Dohoo *et al.*, 2003). A forward entry was made to the base model containing random effects and a variable retained when significant ($P < 0.1$). After a variable addition, a backward check was then conducted for each variable in the model and variables retained if significant ($P < 0.1$). First order interaction terms were added to the final model and retained when significant at $P < 0.05$. The variables damage, year affected and mob size were then added to the model one at a time to check for substantial changes ($> 10\%$) in the estimates of variables in the model. If confounding was present, or if the variable was statistically significant ($P < 0.05$), it was retained. Random-effect variables for farm and paddock were included in each model to account for clustering of CCUO occurrence within farm and paddock. Standardised residuals were plotted using the SAS GRAPHICS application and outliers checked for data entry errors. The intra-cluster correlation (ICC) coefficient was estimated using the latent variable approach described by Browne *et al.* (2005) to determine the proportion of total variance accounted for by random effects. Similarly, generalised linear mixed models were constructed using the SAS GLIMMIX procedure with the dichotomous outcome paddock status. The general procedure for model building was similar to that described for the two mob outcomes. A random effect variable for farm was included in the base model. Continuous variables significant in the final model were visually checked for linearity by categorizing the variable using quartile ranges and plotting the log odds for each

category against the midpoint of each category. Standardised residuals and ICC were checked using similar techniques as for mob status models.

3. Results

Of the 90 farm owners or managers reported with affected herds and invited to participate in this study, contact could not be made with 14 (15.6%) farmers, 19 (21.1%) declined to participate citing a lack of time, memory or interest, and nine (10.0%) had sold their farm or were no longer in cattle production and declined. The remaining 48 (53.3%) farmers were interviewed between March 2008 and February 2009, with a further two (2.2%) farms subsequently excluded from the study as affected cattle were purchased just prior to calving and did not graze on the farm during gestation. Each interview was conducted over a period of three to five hours including time for soil collection. From these interviews information was collected for 66 case mobs with a total of 6417 dams and 102 control mobs with a total of 6498 dams. Soil samples were collected for 50 case paddocks and 75 control paddocks.

3.1 General Farm Characteristics

The geographic distribution of farms is shown in Figure 1 and extended from Young, NSW in the north to Tooborac, Victoria in the south. Fourteen farms were located in Victoria and 32 in NSW. The farms ranged in size from 70ha to 7000 ha (mean 1214 ha) with an elevation ranging from 170m to 1100m (mean 405m) above sea level (measured at the homestead).

A total of 50 case paddocks were identified from the study. These paddocks ranged from 16ha to 250 ha (mean 92, median 100, SD 80) in size and were located on steep land in 37 (74.0%) cases and contained areas of native pastures in 28 (56.0%) paddocks and mixed pastures in 13 (26.0%) paddocks. Soils on these paddocks were all derived from granite (farmer reported) and usually contained large granite rocks protruding from the soil. Water in these paddocks was supplied from dams in 38 (76%) paddocks, from creeks in 10 (20%) paddocks and from a reticulated system in two (4%) paddocks.

Seventy-five control paddocks ranging in size from 6ha to 350 ha (mean 68, median 65, SD 59) were identified, 62 (82.6%) of which were flat to undulating. Fifty-four (72%) paddocks contained improved pasture and 13 (17%) contained mixed pastures. Soils were derived from granite in all paddocks (farmer reported). Water was supplied from creeks or rivers in 21 (28%), dams in 48 (64%) and reticulated water systems in six (8%) of the control paddocks.

Beef production was the major enterprise on all farms, with 31 farms also producing sheep, four of which included cropping as part of their activity. Herd sizes ranged from 10 to 800 head of breeding cows, with a mean herd size of 329 breeding cows. The number of cattle mobs per farm ranged from one to 30 with a mean of 5.6. On five farms, farmers reported observing similar deformities in their lambs, and in one case, American Bison (*Bison bison*) were present in a case mob of cattle and produced

deformed calves along with the cattle. The mean stocking rate was 0.9 head per hectare (range 0.1 to 3.8) for case mobs and 1.0 head per hectare (range 0.8 to 3.4) for control mobs. and the average mob size was 97 head (range 115 to 520) for case mobs and 59 head (range 35 to 200) for control mobs. Movement of stock during gestation was practiced on four farms, however in severe drought the practice of leaving paddock gates open and running cattle as one mob was practiced on three farms. Calving in beef herds in the region under investigation coincided with the seasons of autumn or spring. Interviews with farmers revealed a trend in recent years away from autumn calving to spring calving to better utilize available pastures. All affected calves were born during the spring calving season on all farms. On five farms where autumn calving was still practiced, no affected calves were born in these autumn calving mobs, but cases were reported for spring calving mobs. Farmers reported a total of 799 affected calves born in the 66 case mobs from 2002 to 2007 as shown in table 1.

Dystocia was reported in 23 (34.8%) case mobs and none of the control mobs. Related reproductive problems reported for case mobs were retained fetal membranes in 3 (4.5%) mobs, and low conception rates and abortion in 4 (6.0%) mobs. Aborted calves were reported as deformed and near full term and all dams with retained membranes had produced deformed calves. The high level of dystocia in case mobs compared to control mobs was related to limb deformities in affected calves, in particular a varus (inward bowing) deformation of both front limbs making unassisted birth impossible in many cases.

Calves surviving birth were reported to grow in stature in all affected mobs, though signs of dwarfism remained into adulthood. Five farmers reported an increased incidence of bloat in affected calves, with one farmer losing 20 calves at eight-months-old to bloat in a one-week period. Three farmers reported that badly affected calves developed a form of spastic paresis in the hind limbs. The onset was slow and progressive, commencing at three to four months of age. Either one or both limbs were involved and the limbs become rigid, resulted in swinging of one leg while the other remained on the ground. Walking appeared difficult, and affected animals sat in a dog sitting posture with hind limbs protruding between the front limbs. These animals also exhibited kyphosis, shortened front limbs and facial deformities to varying degrees.

3.2 Generalised linear mixed models for mob status and mob events-trials

Contingency tables for categorical mob management and environment explanatory variables investigated are shown in table 2 for the mob status outcome and in table 3 for the mob events-trials outcome.

Of the 25 farm management and environment explanatory variables, three variables were eliminated due to low numbers per category. Of the remainder, 15 variables were unconditionally associated with the outcome mob status at $P < 0.25$. High

correlation detected between feed type and supplemental feed provided led to exclusion of supplemental feed provided.

After deletion of one highly correlated variable (supplemental feed provided), 21 of the explanatory variables were unconditionally associated with the outcome variable mob events-trials at $P < 0.25$. The final models for mob status and for mob events-trials are presented in table 4 and table 5 respectively.

For the mob status final model there was no suggestion of over dispersion and standardised conditional residuals were normally distributed with no unusual influence of any observation. The ICC for the random effect paddock was calculated as 0.46 and that for farm as 0.11. This indicates that the random effect paddock accounts for a significant portion of the total variance in the final model.

There was no suggestion of over dispersion in the final model for mob events-trials (Generalised chi-square/d.f = 1.09) however standardised conditional residuals were right skewed and there were a number of influential observations. These observations were examined and no data entry errors were found. The ICC for the farm random effect was 0.16 and for the paddock random effect 0.59 indicating again that a significant portion of the total variance was attributed to paddocks.

3.3 Generalised linear mixed model for paddock status

A description of the soil variables is shown in table 6 and a contingency table for environment variables in table 7. Eight of the 23 soil explanatory variables were excluded from analysis due to missing data. Highly correlated variables were detected and resulted in the exclusion of three other variables. Six continuous soil variables and five environment variables were unconditionally associated with the outcome variable paddock status at $P < 0.25$.

The final model for paddock status is shown in table 8. There were no significant interaction terms in the model. The association of soil potassium was linear and the standardised conditional residuals were normally distributed with no influential observations. The ICC for the random effect farm was 0.28 in this model.

4. Discussion

The level of participation in this study was reflective of the attitude of many farmers during a difficult period where continued drought has produced hardship. For many farmers the problems associated with CCUO were negligible compared to other difficulties they experienced such as lack of income and the availability of feed for stock. Farmers who experienced low numbers of CCUO calves (one or two) seemed most likely to refuse to participate as they considered CCUO as a comparatively minor problem. In contrast, farmers who had experienced the birth of high numbers of CCUO calves in their herds were most likely to participate. The use of a payment to farmers for participation in the study provided enough incentive for some farmers to change their minds and take part and increased the overall participation rate. The possibility that selection bias has occurred in this study requires consideration during

interpretation of the results. The sample selected for this study is not a representative sample of all affected farms in south-eastern Australia and should be extrapolated to other farms with caution. However the sample size of 66 case mobs was sufficient to satisfy the requirements for 80% power and 95% confidence based on the minimum requirement of 56 case mobs.

4.1 Control of confounding

In order to minimise confounding bias, all potential confounders were tested and added into multivariable models where appropriate. Dam age at calving and year affected were forced into both mob status and mob events-trials models. Farmers anecdotally reported grazing heifer groups more commonly on hilly paddocks with native pastures in an attempt to keep off weight and improve fitness for calving. This may account for the higher incidence in heifer groups than mixed groups of cattle. Drought periods were common throughout south-eastern Australia for the entire period of interest in this study (2002-2007) and the association between drought and CCUO occurrence requires further investigation.

4.2 Farm management and environment factors

The models produced for the outcomes mob status and mob events-trials are similar in a number of respects and both show significant associations of variables with the outcome. In both models, the risks associated with hilly terrain, grazing native pastures, grazing inadequate pastures and the age grouping of cows are positive and significant. The wider confidence intervals calculated in the mob status model are indicative of the lower sample size used when compared to the mob events-trials model. While the use of the events-trials outcome is unusual in case control studies, the model developed in this study complements the model based on the dichotomous mob status outcome.

The results of this analysis lend support to the role of maternal nutrition in the aetiology of CCUO. Nutritional factors including pasture type and low availability of pasture (farmer reported) were strongly associated with the birth of affected calves in both the mob status and mob events-trials models. The low availability of pasture was more likely a direct result of rainfall deficiency rather than poor management or over-grazing.

Low levels of available pasture may affect maternal nutrition in several ways. Inadequate pasture levels result in basic malnutrition, with inadequate levels of intake of all nutrients including energy, protein and minerals. Basic nutritional needs of gestating dams cannot be met under these conditions unless provided with a suitable supplemental feed. The ingestion of large quantities of soil may also result from grazing short pastures, with cattle reported to consume between 400 and 1400 grams per day (Reid and Horvath, 1980). Geophagia has been demonstrated to increase the level of trace minerals ingested by animals, but also to decrease the availability of trace minerals *in vivo* (Reid and Horvath, 1980). A nutritional disturbance in pregnant dams resulting from geophagia may contribute to CCUO.

The grazing of native and mixed pasture, compared to improved pasture, were significantly associated with the outcome in both mob status and mob events-trials models. The quantity of nutrients found in different pasture types is variable and dependant upon a number of factors including soil factors (mineral levels, moisture content), temperature, the amount of incident sunlight, the stage of plant growth, fertiliser history and species (Reid and Horvath, 1980). It is possible that climatic conditions during the gestation period of dams resulted in native and mixed pastures deficient in essential nutrients. Due to the retrospective nature of this study, it was not possible to analyse pastures at the time of risk during the gestation of dams.

The association of supplemental feed type with the outcome mob events-trials and its interaction with inadequate levels of pasture is difficult to interpret without access to nutritional data concerning the feeds used and without data concerning the amounts and frequency of supplemental feed used. The interaction terms suggest that a combination of inadequate pasture and supplement feed use provided more risk than either factor alone. This may be due to farmers using poor quality supplemental feed for cattle grazing low levels of poor quality pasture. During drought periods, many hay and silage supplements are of low nutritional value and may not contribute significantly to the nutrition of the animal. In reports of CCUO in other countries, an association between CCUO and grass-silage only feeding has been demonstrated (Hidioglou *et al.*, 1992; Proulx and Ribble, 1992; Ribble *et al.*, 1989). Addition of grain or hay to the diet was found to reduce the occurrence of CCUO and even prevent the birth of affected calves. It is worth noting that no farmers in the current study reported the use of grain supplements in years affected by CCUO. The highest risk involving inadequate pasture and pellet feeding (table 5) cannot be accurately interpreted without considering the amount, frequency or quality of the supplement used. In addition, it should be recognised that not all cattle in a mob receive the same amount of supplements. In Australia feed is commonly placed in feeding troughs or in lines on the ground. Some cattle may be bullied and receive only a small component of any feed provided. It may be that these cows are more likely to produce CCUO calves, but this cannot be determined.

The fact that all affected calves were born in the spring calving period suggests that a seasonal component may contribute to the birth of CCUO calves. The incidence and severity of drought periods may explain the difference in incidence between years affected. The fact that autumn calving herds were not affected on properties experiencing CCUO calves in spring suggests that drought at a certain stage of gestation may be a significant factor in CCUO production. The role of drought is worthy of further investigation as climate change may result in an increased incidence of drought and possibly an increase in incidence of CCUO (Hennessey and Mpelasoka, 2007).

The association of hilly to steep paddocks with the outcome in both mob models is in agreement with other reports (Cave *et al.*, 2008). These paddocks are more likely to contain native pastures due to the difficulty in farming the land, and often receive less fertilizer. Runoff on these paddocks may be more severe during rainfall, resulting in drier soils. These paddocks may also contain increased tree cover compared to other

paddocks, resulting in less sunlight on pastures. It is unknown as to whether these factors combined may result in changes in mineral availability in pastures leading to a nutritional deficiency.

While not significant in the final model, other factors unconditionally associated with the outcome at a significant level include a higher level of tree cover, the existence of forest adjacent to paddocks, the presence of earthen dams as a water source and the grazing of trees. Hilly areas are more likely to border natural areas of forest such as national parks or state forests and to have an increased level of tree cover. Grazing of trees is more likely to occur due to the increased level of tree cover and the poor quality of pasture. Earthen dams appear as the most likely source of water in these paddocks due to the high runoff on steeper ground.

4.3 Paddock Factors

The results of the paddock status model reinforce the previous findings by demonstrating a strong association between pasture type and hilly terrain with paddock status. The univariable associations of soil variables with the outcome did not suggest any significant difference between case and control paddocks for the majority of variables of interest. Soil zinc levels were below 3mg/kg on 113 (90.4%) paddocks and soil manganese levels were below the minimum level of 30mg/kg on 54 (43.2%) paddocks. The role of increased soil potassium in the production of CCUO does not appear to be biologically plausible (Whitehead, 2000). There are no known interactions between potassium and trace minerals or other nutrients that could implicate potassium in the aetiology of this condition known at this time (Whitehead, 2000).

4.4 Study strengths and limitations

A major limitation of this study stems from the confirmation of diagnosis of CCUO on farms included in this study. The exclusion of eight farms was precautionary, as reports from these farms did not describe any dwarfism in deformed calves, and only one or two calves were born on each farm. These exclusions may reduce the power of the current study if in fact these farms were experiencing CCUO, however, it is possible that these farms experienced another condition not associated with CCUO, such as Pestivirus and their exclusion is necessary to avoid misclassification bias. Restrictions on location of farms (south-eastern Australia) and the time period for birth of deformed calves (2002-2007) were used to increase the likelihood that farms included in the study were suffering from the same condition.

In addition to problems with CCUO confirmation, farm records on selected farms were found to be minimal and of poor quality. Most farmers recorded events such as joining, calving period, weaning period, treatments and sales, but failed to keep detailed records on stock movement, fertiliser, herbicide and pesticide treatments and specific details on cattle including parity, calf mortality and supplemental feeding. Questions in the study relying on farmer recall may have been subject to recall bias. The use of personal interview with a single interviewer reduced this bias through

carefully asked questions and the elimination of answers that appeared to be guesses or that were unclear. Personal interview also helped to reduce misclassification bias by avoiding the misinterpretation of questions.

5. Conclusion

The results of this study indicate that a maternal nutritional deficiency cannot be ruled out as contributing to CCUO in south-eastern Australia. A trace mineral deficiency resulting from a soil deficit is not likely based on these results. However, due to the complex interaction between soil, plants and animals, a trace mineral deficiency is still possible due to low uptake of minerals by plants or poor absorption. Low uptake of nutrients by plants may be influenced by soil factors, rainfall, temperature, fertilizer history, stage of growth and species. Absorption of nutrients may be influenced by a number of factors including antagonism, acid-base balance, genetic factors and the age and health of the animal (Reid and Horvath, 1980). The environmental factors associated with the outcome are also associated with pasture type and pasture availability. Hilly terrain is more likely to contain native pastures due to difficulty in farming the land with machinery. Runoff from hills results in drier ground and less pasture growth during dry periods (Whitehead, 2000). The role of drought has been implicated in previous reports and may result in altered plant uptake of minerals and a reduction in pasture availability. Further investigations are required to determine any association of drought periods during the gestation of dams with the occurrence of CCUO.

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Figure 1 Distribution of the 46 farms with affected calves from 2002-2007 in south-eastern Australia that participated in the case-control study

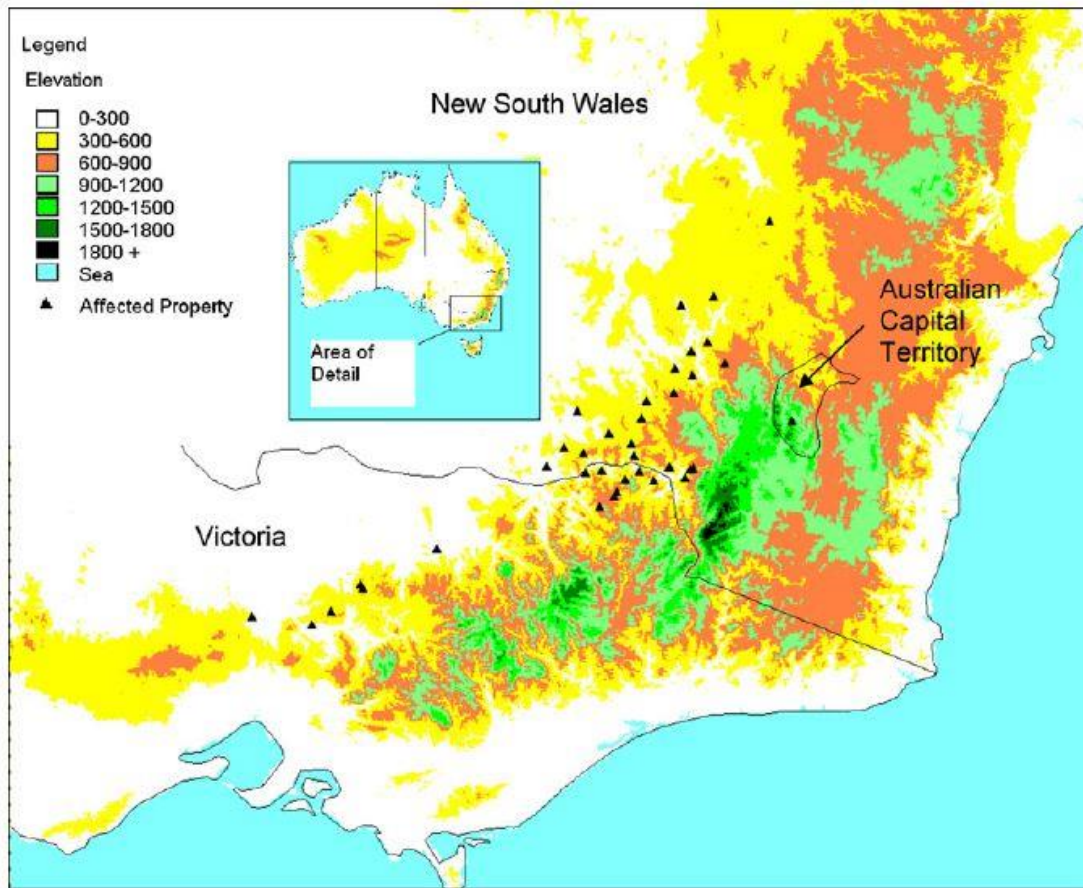


Table 1 Percentage of affected calves per case mob among 66 case mobs from 2002-2007 on 46 farms in south-eastern Australia

Year	Number of case mobs	Number of affected calves	Number of normal calves	Percentage calves affected per mob				
				Minimum (%)	Lower quartile (%)	Median (%)	Upper quartile (%)	Maximum (%)
2002	3	16	140	6.1	-	7.9	-	24.2
2003	15	254	1564	2.1	5.9	26.3	45.7	71.4
2004	24	299	2280	0.7	4.2	12.9	26.4	80.0
2005	9	54	875	0.7	4.0	16.7	28.6	70.5
2006	13	159	360	1.5	13.0	23.1	40.5	80.0
2007	2	17	108	10.0	-	-	-	20.0

Table 2 Contingency tables for categorical management and environment explanatory variables in 66 case mobs and 102 control mobs and for 799 affected calves and 11710 normal calves from case and control mobs born on 46 farms in south-eastern Australia on 46 farms in south-eastern Australia

Variable	Categories	Affected calves (events)	Normal calves	Total calves (trials)	Case mobs		Control mobs	
Dam age at calving	Heifers only	380	1520	1900	27	(79.4%)	7	(20.6%)
	Separate age groups	106	701	807	7	(53.8%)	6	(46.2%)
	Mixed age groups	313	9489	9802	32	(26.4%)	89	(73.6%)
Dam condition decreased during gestation	No	619	9833	10452	53	(38.4%)	85	(61.6%)
	Yes	180	1877	2057	13	(43.3%)	17	(56.7%)
Main breed of dams	Angus	446	5726	6172	33	(37.5%)	55	(62.5%)
	Crossbred	138	2989	3127	13	(41.9%)	18	(58.1%)
	Hereford	107	1166	1273	9	(40.9%)	13	(59.1%)
	Other purebred	108	1829	1937	11	(40.7%)	16	(59.3%)
Spring calving	No	25	939	964	2	(12.5%)	14	(87.5%)
	Yes	774	10771	11545	64	(42.1%)	88	(57.9%)
Trees grazed by dams during gestation	No	419	9380	9799	33	(26.0%)	94	(74.0%)
	Yes	344	2291	2635	31	(79.5%)	8	(20.5%)
Inadequate pasture ^a	No	402	8345	8747	35	(27.6%)	92	(72.4%)
	Yes	361	3326	3687	31	(75.6%)	10	(24.4%)

Dams vaccinated during gestation		No	290	4486	4776	25 (34.2%)	48 (65.8%)
		Yes	509	7224	7733	41 (43.2%)	54 (56.8%)
Dams wormed during gestation		No	490	7185	7675	40 (39.6%)	61 (60.4%)
		Yes	309	4525	4834	26 (38.8%)	41 (61.2%)
Dams moved between paddocks during gestation ^b		No	783	11686	12469	65 (39.2%)	10 (60.8%)
		Yes	16	24	40	1 (50.0%)	1 (50.0%)
Paddock tree cover level		< 5%	348	7810	8158	28 (25.7%)	81 (74.3%)
		> 5%	407	3734	4141	35 (62.5%)	21 (37.5%)
Paddock adjoins forest, crown land or national park		No	561	10636	11197	50 (33.8%)	98 (66.2%)
		Yes	202	1035	1237	14 (77.8%)	4 (22.2%)
Paddock adjoins public road		No	652	8271	8923	53 (37.3%)	89 (62.7%)
		Yes	111	3400	3511	11 (45.8%)	13 (54.2%)
Paddock adjoins neighbour		No	505	7031	7536	41 (35.0%)	76 (65.0%)
		Yes	258	4640	4898	23 (46.9%)	26 (53.1%)
Paddock predominantly hilly to steep terrain		No	226	7507	7933	20 (19.8%)	81 (80.2%)
		Yes	573	4203	4776	46 (68.7%)	21 (31.3%)

Main pasture type in paddock							
	Native	137	6430	6567	36 (85.7%)	6 (14.3%)	
	Mixed	447	2857	3304	18 (42.9%)	24 (57.1%)	
	Improved	179	2384	2563	12 (14.3%)	72 (85.7%)	
Water source							
	Earthen dam	679	8113	8792	52 (46.8%)	59 (53.2%)	
	Creek/River	77	2848	2925	12 (27.3%)	32 (72.7%)	
	Bore/reticulated	30	735	765	1 (8.3%)	11 (91.7%)	
Main aspect of the paddock							
	North	474	3642	4116	28 (39.4%)	43 (60.6%)	
	South	117	2810	2927	15 (46.9%)	17 (53.1%)	
	East	98	3987	4085	14 (34.1%)	27 (65.9%)	
	West	74	1232	1306	7 (31.8%)	15 (68.2%)	
Lime applied to paddock in the last 5 years ^b							
	No	528	7945	8473	59 (36.9%)	10 (63.1%)	
	Yes	271	3756	4027	7 (87.5%)	1 (12.5%)	
Fertilizer applied to paddock in the last 5 years							
	No	528	7945	8473	45 (36.3%)	79 (63.7%)	
	Yes	271	3756	4027	21 (47.7%)	23 (52.3%)	
Supplemental feed provided							
	No	263	4323	4586	20 (27.4%)	53 (72.6%)	
	Yes	536	7387	7923	46 (48.4%)	49 (51.6%)	
Supplemental feed fed from the ground ^b							
	No	82	218	300	2 (50.0%)	2 (50.0%)	
	Yes	454	7169	7623	44 (48.4%)	47 (51.6%)	
Feed type							

Year affected	Nil	263	4323	4586	20 (27.4%)	53 (72.6%)
	Hay	331	5042	5373	30 (51.7%)	28 (48.3%)
	Silage	134	882	1016	10 (50.0%)	10 (50.0%)
	Pellet	71	1436	1507	6 (35.3%)	11 (64.7%)
	2002	16	637	653	3 (33.3%)	6 (66.7%)
	2003	254	3113	3367	15 (39.5%)	23 (60.5%)
	2004	299	4827	5126	24 (38.1%)	39 (61.9%)
	2005	54	1358	1412	9 (42.9%)	12 (57.1%)
	2006	159	1498	1657	13 (40.6%)	19 (59.4%)
	2007	17	277	294	2 (40.0%)	3 (60.0%)

-
- a. Inadequate pasture defined as that not in sufficient quantity to maintain weight of grazing cattle during gestation
b. Rejected for multivariable analysis due to low numbers of data per category or high number of missing data

Table 3 Contingency tables for categorical management and environment explanatory variables associated with mob events-trials based on 799 affected calves and 11710 normal calves born in 66 case mobs and 102 control mobs on 46 farms in south-eastern Australia

Variable	Categories	Affected calves (events)	Normal calves	Total calves (trials)
Dam age at calving	Heifers only	380	1520	1900
	Separate age groups	106	701	807
	Mixed age groups	313	9489	9802
Dam condition score decreased during gestation	No	619	9833	10452
	Yes	180	1877	2057
Main breed of dams	Angus	446	5726	6172
	Crossbred	138	2989	3127
	Hereford	107	1166	1273
	Other purebred	108	1829	1937
Spring calving	No	25	939	964
	Yes	774	10771	11545
Trees grazed by dams during gestation	No	419	9380	9799
	Yes	344	2291	2635
Inadequate pasture ^a	No	402	8345	8747
	Yes	361	3326	3687
Dams vaccinated during gestation	No	290	4486	4776
	Yes	509	7224	7733
Dams wormed during gestation	No	490	7185	7675
	Yes	309	4525	4834
Dams moved between paddocks during gestation	No	783	11686	12469
	Yes	16	24	40
Paddock tree cover level	< 5%	348	7810	8158
	> 5 %	407	3734	4141
Paddock adjoins forest, crown land or national park	No	561	10636	11197
	Yes	202	1035	1237
Paddock adjoins public road	No	652	8271	8923
	Yes	111	3400	3511
Paddock adjoins neighbour	No	505	7031	7536
	Yes	258	4640	4898
Paddock predominantly hilly to steep terrain	No	573	4203	4776
	Yes	573	4203	4776
Main pasture type in paddock	Improved	137	6430	6567

	Native	447	2857	3304
	Mixed	179	2384	2563
Water source				
	Earthen dam	679	8113	8792
	Creek/River	77	2848	2925
	Bore/reticulated	30	735	765
Main aspect of the paddock				
	North	474	3642	4116
	South	117	2810	2927
	East	98	3987	4085
	West	74	1232	1306
Lime applied to paddock in the last 5 years				
	No	528	7945	8473
	Yes	271	3756	4027
Fertilizer applied to paddock in the last 5 years				
	No	528	7945	8473
	Yes	271	3756	4027
Supplemental feed provided				
	No	263	4323	4586
	Yes	536	7387	7923
Supplemental feed fed from the ground				
	No	82	218	300
	Yes	454	7169	7623
Feed type				
	Nil	263	4323	4586
	Hay	331	5042	5373
	Silage	134	882	1016
	Pellet	71	1436	1507
Year Affected				
	2002	16	637	653
	2003	254	3113	3367
	2004	299	4827	5126
	2005	54	1358	1412
	2006	159	1498	1657
	2007	17	277	294

a. Inadequate pasture defined as that not in sufficient quantity to maintain weight of grazing cattle during gestation

Table 4 Final generalized linear mixed model for mob status based 66 case mobs and 102 control mobs on 46 farms in south-eastern Australia

Variables	<i>b</i>	SE(<i>b</i>)	Odds-ratios	LCL (OR)	UCL (OR)	<i>P</i> -Value
<i>Random effects</i>						
Farm ID	0.68	1.36	-	-	-	
Paddock ID	2.19	1.48	-	-	-	
<i>Intercept</i>	-6.41	4.99	-	-	-	
<i>Confounders</i>						
Dam age at calving						0.08
Mixed age groups	-	-	1.0	-	-	
Heifers only	1.66	0.86	5.3	0.9	29.9	
Separate age groups	1.84	1.36	6.3	0.4	98.8	
Year affected						0.8
2007	-	-	1.0	-	-	
2002	1.99	5.12	7.3	<0.1	>999	
2003	2.61	4.94	13.6	<0.1	>999	
2004	2.81	4.91	16.6	<0.1	>999	
2005	3.65	4.98	38.6	<0.1	>999	
2006	2.15	4.95	8.6	<0.1	>999	
<i>Fixed effects</i>						
Main pasture type in paddock						<0.001
Improved pasture	-	-	1.0	-	-	
Native pasture	4.40	1.13	81.4	8.3	798	
Mixed pasture	0.52	0.89	1.7	0.3	10.2	
Paddock predominantly hilly to steep terrain						0.008
No	-	-	1.0	-	-	
Yes	2.20	0.83	8.9	1.7	48.5	
Inadequate pasture ^a						0.001
No	-	-	1.0	-	-	
Yes	2.76	0.77	15.8	2.8	89.1	

Generalised chi-square = 50.8

Generalised chi-square/d.f. = 0.33

a. Inadequate pasture defined as that not in sufficient quantity to maintain weight of grazing cattle during gestation

Table 5 Final generalized linear mixed model for mob events-trials based on 66 case mobs and 102 control mobs on 46 farms in south-eastern Australia

Variables	<i>b</i>	SE(<i>b</i>)	Odds-ratios	LCL (OR)	UCL (OR)	<i>P</i> -Value
<i>Random effects</i>						
Farm ID	1.30	1.18	-	-	-	
Paddock ID	3.43	0.99				
<i>Intercept</i>	-8.68	0.71	-	-	-	
<i>Confounders</i>						
Dam age at calving	-	-	-	-	-	<0.001
Mixed age groups	-	-	1.0	-	-	
Heifers only	1.94	0.57	6.9	2.1	22.2	
Separate age groups	2.36	0.83	10.6	1.9	57.2	
Year affected						<0.001
2007	-	-	1.0			
2002	-0.94	0.46	0.4	0.2	1.0	
2003	1.88	0.43	6.5	2.7	15.7	
2004	1.66	0.37	5.3	2.5	11.2	
2005	1.20	0.45	3.3	1.3	8.3	
2006	1.43	0.34	4.2	2.1	8.4	
<i>Fixed effects</i>						
Main pasture type in paddock	-	-	-	-	-	0.025
Improved pasture	-	-	1.0	-	-	
Native pasture	1.81	0.67	6.1	1.6	23.8	
Mixed pasture	0.97	0.69	2.6	0.6	10.8	
Paddock predominantly hilly to steep terrain	-	-	-	-	-	<0.001
No	-	-	1.0	-	-	
Yes	2.05	0.44	7.8	3.2	18.9	
Feed type						0.019
Nil	-	-	1.0	-	-	
Hay	0.40	0.53	-	-	-	
Pellet	-0.08	0.96				
Silage	-0.56	0.44				
Inadequate pasture ^a	-	-	-	-	-	<0.001
No	-	-	1.0	-	-	
Yes	0.20	0.80	-	-	-	
<i>Interaction Terms^b</i>						
Inadequate pasture x Feed type						
Hay x Inadequate pasture	1.98	1.00	-	-	-	0.004
Pellet x inadequate pasture	3.95	1.40				
Silage x inadequate pasture	2.67	1.01				

Generalised chi-square = 166.67

Generalised chi-square/d.f. = 1.09

a. Inadequate pasture defined as that not in sufficient quantity to maintain weight of grazing cattle during gestation

b. The terms present in interactions with zero *b* are not displayed

Table 6 Descriptive statistics for 23 soil explanatory variables for 125 paddocks surveyed on 46 farms in south-eastern Australia in 2008-2009

Variable	Case Paddocks (n=50)				Control Paddocks (n=75)				Total (n=125)			
	Mean	Std Dev	Minimum	Maximum	Mean	Std Dev	Minimum	Maximum	Mean	Std Dev	Minimum	Maximum
Nitrate nitrogen mg/kg ^a	18.3	14.1	1.5	52.1	23.2	18.3	1.0	80.0	21.3	16.9	1.0	80.0
Magnesium meq/100g	0.9	0.6	0.3	3.5	1.1	0.9	0.3	3.7	1.0	0.8	0.3	3.7
Calcium meq/100g ^b	3.7	2.4	1.0	12.0	4.1	2.6	0.8	14.9	4.0	2.5	0.8	14.9
Sulfate sulphur mg/kg ^a	6.3	3.5	1.8	20.0	8.0	6.6	2.7	38.0	7.4	5.6	1.8	38.0
Phosphorous- Colwell mg/kg	27.1	14.5	9.3	78.0	33.3	18.8	9.2	120.0	30.9	17.5	9.2	120.0
pH (water) ^b	5.4	0.4	4.6	6.7	5.3	0.3	4.7	6.3	5.4	0.4	4.6	6.7
Potassium meq/100g	0.6	0.3	0.2	1.4	0.6	0.2	0.3	1.3	0.6	0.2	0.2	1.4
Organic carbon (%)	2.6	0.8	1.5	6.0	2.7	0.9	1.2	4.9	2.7	0.9	1.2	6.0
pH (calcium chloride)	4.6	0.5	4.0	6.2	4.5	0.3	3.8	5.6	4.6	0.4	3.8	6.2
Phosphorous buffer index ^a	74.8	30.3	21.0	200.0	81.1	32.4	33.0	160.0	78.4	31.5	21.0	200.0
Sodium % of cations (%) ^a	1.1	0.7	0.3	2.8	1.4	1.0	0.2	4.2	1.3	0.9	0.2	4.2
Aluminium % of cations (%) ^a	14.1	14.2	0.6	58.0	11.2	12.0	0.6	43.0	12.3	12.9	0.6	58.0
EC saturation index ^a	0.7	0.3	0.3	1.3	0.7	0.4	0.1	1.9	0.7	0.3	0.1	1.9
Ca:Mg ratio	4.4	2.1	1.8	12.0	4.2	1.8	1.9	9.5	4.2	1.8	1.9	9.5
Cation exchange capacity meq/100g ^b	5.9	2.8	2.9	16.6	6.1	2.9	2.8	19.0	6.0	2.9	2.8	19.0
Manganese mg/kg	35.1	19.6	7.2	84.0	34.9	19.0	9.5	112.0	34.9	19.1	7.2	112.0
Iron mg/kg	184.8	72.1	80.0	402.0	228.9	94.0	55.0	460.0	211.1	88.2	55.0	460.0
Zinc mg/kg	1.3	0.7	0.3	3.4	1.5	1.1	0.3	7.9	1.4	1.0	0.3	7.9
Copper mg/kg	0.6	0.5	0.1	2.4	0.6	0.5	0.1	2.7	0.6	0.5	0.1	2.7
Aluminium meq/100g ^a	0.6	0.6	0.1	2.3	0.5	0.4	0.1	1.8	0.5	0.5	0.1	2.3
Sodium meq/100g	0.056	0.047	0.02	0.26	0.085	0.09	0.01	0.41	0.074	0.077	0.01	0.41
Chloride mg/kg ^a	8.4	5.2	5.0	28.0	10.4	5.9	5.0	26.0	9.6	5.7	5.0	28.0
Electrical conductivity dS/m	0.077	0.044	0.027	0.23	0.091	0.051	0.032	0.28	0.085	0.048	0.027	0.28

a. Rejected for multivariable analysis due high number of missing data

b. Rejected for multivariable analysis due to high collinearity with other variables

Table 7 Contingency tables for categorical environment explanatory variables for 50 case paddocks and 75 control paddocks on 46 farms in south-eastern Australia

Variables	Categories	Case paddock	Control paddock
Paddock predominantly hilly to steep terrain	No	16 (20.8%)	61 (79.2%)
	Yes	34 (70.8%)	14 (29.2%)
Paddock tree cover level	<5 %	22 (26.5%)	61 (73.5%)
	>5 %	28 (66.7%)	14 (33.3%)
Main source of water provided from a creek or river	Dam	38 (44.2%)	48 (55.8%)
	Reticulated/ bore	2 (22.2%)	7 (77.8%)
	Creek/river	10 (33.3%)	20 (66.7%)
Main pasture type in paddock	Improved	9 (14.3%)	54 (85.7%)
	Native	26 (89.7%)	3 (10.3%)
	Mixed	15 (45.5%)	18 (54.5%)
Fertilizer applied to paddock in the last 5 years	No	34 (37.0%)	58 (63.0%)
	Yes	16 (48.5%)	17 (51.5%)
Paddock adjoins forest, crown land or national park	No	33 (31.4%)	72 (68.6%)
	Yes	15 (83.3%)	3 (16.7%)
Paddock adjoins public road	No	41 (39.0%)	64 (61.0%)
	Yes	9 (45.0%)	11 (55.0%)
Paddock adjoins neighbour	No	34 (38.2%)	55 (61.8%)
	Yes	16 (44.4%)	20 (55.6%)
Main aspect of the paddock	North	21 (40.4%)	31 (59.6%)
	South	11 (34.4%)	21 (65.6%)
	East	12 (54.5%)	10 (45.5%)
	West	6 (31.6%)	13 (68.4%)

Table 8 Final generalized linear mixed model for paddock status based on 50 case paddocks and 75 control paddocks on 46 farms in south-eastern Australia.

Variables	<i>b</i>	SE(<i>b</i>)	Odds-ratios	LCL (OR)	UCL (OR)	<i>P</i>-Value
<i>Random effects</i>						
Farm ID	0.54	0.91	-	-	-	
<i>Intercept</i>	-4.50	1.04	-	-	-	
<i>Fixed effects</i>						
Main pasture type in paddock						<0.001
Improved pasture	0.00	-	1	-	-	
Native pasture	3.37	0.87	29.1	5.1	165.1	
Mixed pasture	1.83	0.74	6.2	1.4	27.1	
Paddock predominantly hilly to steep terrain						0.016
No	0.00	-	1	-	-	
Yes	1.64	0.68	5.1	1.3	20.1	
Soil potassium	3.40	1.21	29.9	2.7	331.5	0.006

Generalised chi-square = 101.83

Generalised chi-square/d.f. = 0.93