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Prepared by: Jenny-Ann Toribio, Peter White & Peter Windsor

Farm Animal & Veterinary Public Health, Faculty of Veterinary Science
The University of Sydney

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Studies of the epidemiology and risk factors involved in the pathogenesis of acorn calf disease in Australia

Epidemiology of congenital chondrodystrophy of unknown origin (CCUO) in Australia

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Abstract

Congenital chondrodystrophy of unknown origin (CCUO) is a condition where a calf from a healthy cow is born with disproportionate dwarfism. In Australia CCUO occurs in beef herds principally in south-eastern NSW and north-eastern Victoria. A sporadic condition until 2001, annual occurrence since, involving higher numbers of affected farms and affected calves, with a few farms outside the focus region is evidence that CCUO is now an emerging condition in southern Australia. As yet the pathogenesis of CCUO is not understood but has been postulated to involve manganese deficiency. This project investigated the epidemiology of CCUO in Australia and found that occurrence on affected farms is characterised by a history of maternal nutritional deficiency during gestation in spring calving mobs related to drought (defined as a three-month period in which rainfall is within lowest 10% on record) during the 2nd to 4th months of gestation. An on-farm trial conducted using an injectable multimineral supplement (including manganese, copper, zinc and selenium) did not prevent birth of CCUO calves. At this point extension is needed to support beef producers to recognise, report and prevent CCUO, and further research to delineate the climatic and nutritional conditions that contribute to CCUO occurrence.

Executive Summary

Congenital chondrodystrophy of unknown origin (CCUO) is the condition, commonly known as acorn calf disease based on its first description in the USA, investigated in this project.

The case definition for a calf with CCUO used throughout this project was a calf from a clinically well dam with disproportionate dwarfism showing signs at birth of limb deformities (including bowed legs, shortened limbs, enlarged joints or arthrogryposis), and/or shortening of the upper jaw (with domed head and/or dished face) and/or twisted spinal column (lordosis, kyphosis, scoliosis).

In Australia the birth of CCUO calves was sporadic until 2001, with the exception of a single outbreak in NSW during 1991, and consistently involved beef herds principally located in south-eastern NSW and north-eastern Victoria. However a notable increase in reports of affected calves from 2002 raised serious concern about the cost of this condition to affected producers, and about the possibility of CCUO being an emerging condition.

This project was a direct response to industry request for research to progress understanding of the epidemiology of CCUO in Australia, particularly its geographic and temporal distribution and risk factors associated with CCUO occurrence.

A nation-wide survey of rural veterinarians combined with data from published reports provided the most complete description of the spatio-temporal distribution of CCUO in Australia to date. This demonstrated that since 2002 CCUO occurrence increased with reports each year involving multiple farms and often higher total numbers of affected calves per year. While the majority of these farms were in south-east NSW and north-east Victoria a trend toward more reports outside this focus region was evident with reports from previously unreported locations in Western Australia and South Australia. This survey identified some cases not included in official records indicating that some affected farmers do not report the birth of affected calves. Under-reporting of CCUO means that even this study does not fully depict the level and distribution of affected farms in southern Australia. The estimated direct income loss for farmers arising from the 1081 CCUO calves reported for 2002-2007 totalled AU$818 315 with an average loss per affected calf of AU$757.

A case-control study involving 46 affected farms in the focus region on which all affected mobs from 2002 to 2007 calved during spring found significant associations between the birth of CCUO calves in a mob and four management and environment variables that link either directly or indirectly with poor maternal nutrition during gestation. The birth of CCUO calves was more likely when:

- Dams had grazed on *inadequate pasture*
- Dams had grazed in a paddock with the *main pasture type* being native or mixed pasture compared to improved pasture
- Dams had grazed in a *paddock with predominately hilly to steep terrain*
- Dams were given a *supplement feed* (variable included in one final model).

This last finding was contrary to expectation but most likely reflects that mobs fed a supplement were those experiencing the most extreme nutritional stress and the supplement given was insufficient to meet requirements for normal foetal development.

Similar to the results for mobs, analysis of the case-control data to examine associations between the birth of CCUO calves in a paddock and the environment and soil characteristics of the paddock found significant associations with the two environment variables listed above related to main pasture type and paddock with predominately hilly to steep terrain. This further confirmed the direct or indirect link with poor maternal nutrition during gestation.

The results for paddocks also found a significant association between increased soil potassium and the birth of CCUO calves in a paddock. This finding was unexpected as potassium has not been shown to affect other trace minerals in vivo. However a dietary excess of potassium has been shown to produce mild metabolic alkalosis and affect calcium homeostasis. At this point a potential role for potassium in the development of CCUO calves is not understood.

Notably the investigation of soil variables in this case-control study found no association with soil levels of the suspect trace minerals manganese and zinc or with any other mineral such as iron, that might alter the availability of these trace minerals.

Association of the birth of CCUO calves with periods of drought on the 46 study farms was investigated based on historical climate records using time series analysis. CCUO occurrence was associated with drought five months before calving corresponding to the 2nd to 4th months of gestation for spring calving herds on the 46 farms during 2002 to 2007. Note that drought was defined as a three-month period in which rainfall is within the first decile (that is, the lowest 10% on record) for a given location.

An on-farm trial conducted to investigate the effect of administration of an injectable multimineral supplement (including manganese, copper, zinc and selenium) at intervals during gestation on the birth of CCUO calves found no difference in the proportion of CCUO calves between treatment (22% of calves born had CCUO) and control (20%) groups, and no significant difference in dam liver manganese, zinc or copper levels between groups, although each decreased in both groups over gestation.

The evidence gathered by this project shows that CCUO in Australia is:

- An emerging condition reported since 2002 on an annual basis with outbreaks involving ≥10 farms/year during 5 of 8 years from 2002-2009.
- Predominately a condition reported in beef herds on the south-western slopes of NSW and in north-east Victoria, however, a feature of emergence is a trend toward more reports outside this focus region.
- Associated with maternal nutritional deficiency during gestation that is related to drought during the 2nd to 4th months of gestation.
- A condition that occurs more frequently than is recorded in official records due to non-reporting by some affected farmers.

Although aspects of this project were constrained by the quality of case records, these conclusions definitely apply to affected farms in south-east NSW and north-east Victoria where CCUO occurrence is of concern to the producer.

These findings suggest that industry, government and researchers in southern Australia should either maintain or introduce activities that provide beef producers with information to support recognition, reporting and prevention of CCUO, and continue targeted research to further improve understanding of factors contributing to CCUO occurrence and of the pathogenesis of this condition.

Contents

1 Background

1.1 Background

1.1.1 Global Occurrence

Acorn calf disease was first reported in the USA in the 1940's, describing congenital skeletal abnormalities in calves that were considered to result from the ingestion of acorns by cows during pregnancy (Hart *et al.*, 1947). Although acorn calves were unable to be experimentally induced following the feeding of acorns to pregnant cows, the misnomer acorn calf disease is still a name often used to describe the syndrome.

Since descriptions of congenital chondrodystrophy of unknown origin (CCUO) have been reported in both free range and housed beef cattle in Europe, North America, South Africa, New Zealand and Australia (Gunn *et al.*, 1997; Lindblom and Traven, 2006; Mee, 2001; Ribble *et al.*, 1989; Staley *et al.*, 1994; Valero *et al.*, 1990). Affected calves have been referred to in various publications as acorn calves, bulldog calves, dwarfed deformed calves, chondrodystrophic calves, and calves with congenital joint laxity and dwarfism (CJLD) or congenital spinal stenosis.

The common pathological feature seen in affected calves is failure of bone growth through endochondral ossification due to chondrodystrophic lesions affecting epiphyseal growth plates.

Affected calves display varying degrees of disproportionate dwarfism. Typically calves have shortened and often rotated limbs, and superior brachygnathia. Kyphosis, lordosis or torticollis of the spine may also be present (Cave *et al.*, 2008; McLaren *et al.*, 2007). The proportion of calves born dead or dying soon after birth is thought to be high. Breathing difficulties are often associated with deformities of the nasal turbinates and trachea (McLaren *et al.*, 2007). Mildly affected calves may grow normally but later, still show evidence of the deformities affecting them at birth, such as abnormally shaped or shortened limbs, curved spines and facial defects (Cave *et al.*, 2008). Often calves are not viable and euthanased due to welfare concerns.

Over the decades several causative agents for the condition have been suggested, including plant toxins, chemicals, genetic factors and infectious agents, with minimal evidence to support these hypotheses. The birth of affected calves is highly seasonal and outbreaks have occurred following a period of poor nutritional intake such as extended drought or winter feeding of silage, supporting suggestions that a nutritional factor is involved in acorn calf disease. However the definitive causative factor(s) remain elusive.

A common hypothesis is that the condition may result from a maternal nutritional deficiency between day 45 and 245 of gestation and may involve a deficiency of manganese (Hart *et al.*, 1947; McLaren *et al.*, 2007; Orr and McKenzie, 1981; Staley *et al.*, 1994; Valero *et al.*, 1990).

The potential role of manganese is postulated because it is necessary for numerous enzymatic reactions and for the synthesis of mucopolysaccharides, which are essential for normal cartilage and bone production. However in Australia the condition is unlikely to be due to simple dietary manganese deficiency as soils in the affected areas are often high in manganese and are acidic which favours the uptake of manganese by plants. Other soil minerals may interfere with manganese bioavailability including iron which preferentially binds to manganese receptors. Application of lime, dolomite or gypsum onto pastures may also hinder absorption of manganese by cattle and manganese can also be removed from feed material by leaching. This may account for the expression of the syndrome in calves born to cows fed only silage during periods of gestation. In addition to manganese deficiency, a deficiency of zinc has also been shown to produce skeletal deformities in several other species including rats, mice and sheep and could contribute to the pathogenesis of CCUO.

1.1.2 Occurrence in Australia

In Australia the birth of affected calves was first reported in 1957 to dams of several different beef breeds on seven properties in the Albury district. Occurrence until this decade was sporadic but consistently involved beef herds and the majority were located in south eastern New South Wales (NSW) and north eastern Victoria. A notable increase in reports of affected calves in south eastern Australia since 2002 raised serious concern about the cost of this condition to affected producers, and about CCUO possibly being an emerging condition.

A meeting of animal health personnel was held at Albury in October 2005 to review available information about the occurrence of affected calves in Australia. This meeting agreed upon a case definition for the condition that included:

Dwarf phenotype

- Bow legged
- Limb deformities and arthrogryposis
- Brachygnathia, domed head and dished faced
- Kyphosis, spina bifida, scoliosis.

Epidemiological features of the 2003-2005 outbreaks based on the knowledge of meeting participants discussed at this meeting were:

- Geographical distribution predominantly south eastern NSW and north eastern Victoria
- Seasonal occurring from July to November
- Affected calves born throughout the spring calving season (so point source is unlikely)
- Period of 'intrauterine exposure' from February to April - approximately $2^{nd\ to}$ 4^{th} month of gestation
- No genetic link between affected herds
- All beef cattle breeds appear to be affected
- Similar proportions of male and female calves affected
- No consistent supplementary feeding practice among affected herds except no feeding of grain or pellets
- No consistent pattern of fertilizer application or weed control on affected properties.

Meeting participants concluded that the condition is most likely multi-factorial because affected calves rarely occur in other drought affected areas of Australia and on most affected properties individual dams in a herd not the entire herd give birth to affected calves. Further the aetiology was considered to involve intrauterine manganese deficiency as this is consistent with the clinical and histopathological presentation. A viral aetiology was considered unlikely on the basis of epidemiology, and the lack of serum IgG levels and arbovirus serology results in affected calves. Proposed risk factors worthy of investigation were soil mineral composition and water supply with soil ingestion and the consumption of warm to hot water hypothesised as potentially interfering with manganese uptake.

The meeting concluded that a significant amount of research was required to identify the cause of this syndrome and recommended that Meat and Livestock Australia (MLA) be approached to fund a research project.

This project was a direct response to industry request for research to progress understanding of the occurrence and aetiology of CCUO in Australia.

1.1.3 Name and Case Definition

The condition under investigation in this project is commonly known as acorn calf disease based on its first description in the United States. As outlined above, given the literature reports calves with similar clinical and pathological presentation from multiple countries and both free range and housed beef production systems, we propose and use from this point on the name - congenital chondrodystrophy of unknown origin (CCUO) - to refer to the condition.

The case definition for a calf with CCUO, based on that of McLaren *et al.* (2007), used throughout this project was a calf from a clinically well dam with disproportionate dwarfism showing signs at birth of limb deformities (including bowed legs, shortened limbs, enlarged joints or arthrogryposis), shortening of the upper jaw (with domed head and/or dished face) and/or twisted spinal column (lordosis, kyphosis, scoliosis).

1.1.4 Purpose

The purpose of this project was to improve understanding of the epidemiology of CCUO in Australia particularly its geographic and temporal distribution and risk factors associated with CCUO occurrence.

Currently, due to the lack of knowledge about CCUO occurrence and aetiology, there are no recommendations for CCUO control and prevention available to beef producers in southeast Australia. The intention of this research was to provide findings that could form the basis of recommendations for on-farm control and prevention of CCUO.

1.2 Project Objectives

This project had 4 objectives written in the project contract to be completed by 30 November 2009. As this research progressed 4 additional objectives were added to the work undertaken by Peter White for his PhD. These objectives and the related findings are included in this report to provide a complete presentation of the research that we have undertaken on CCUO.

Objectives	**Activities**
Stated in contract	
1. To undertake a nationwide survey of public and private rural veterinarians involved in examining congenital abnormalities of calves to establish the geographic and temporal distribution of CCUO[1], and to distinguish cases from similar disorders (such as joint laxity, which has been confused with CCUO).	Part 1 - Survey of Rural Veterinarians
2. To undertake a retrospective examination of case records of previous outbreaks to improve documentation of the disorder.	Part 2 - Analysis of Existing Records
3. To undertake a case-control study involving properties affected since 2003 and their neighbours as control properties to identify risk factors associated with disease occurrence.	Part 3 - Case-control study
4. If considered appropriate, intervention studies on affected farms or pen trials to determine whether the disease can be reproduced by testing identified risk factors will be proposed.	See Objective 7 and Recommendations
Additional	
5. To investigate association between rainfall deficit during the gestation period of spring calving herds and the occurrence of CCUO calves	Part 4 - Analysis of Rainfall data

[1] The contract document for MLA B.AH.0004 stated acorn calf disease rather than CCUO.

Objectives	Activities
6. To document a detailed case study of one affected farm	Part 5 – Case Study
7. To investigate the effect of dam trace mineral supplementation on occurrence of CCUO calves on the case study farm	Part 5 – Supplement Trial
8. To document reported occurrences of CCUO calves in southeast NSW and northern Victoria in 2008-2009	Part 6 – CCUO in 2008-2009

2 Methodology

This 6-part study commenced with a national survey of cattle veterinarians and government veterinary officers in 2007 to estimate the prevalence and spatio-temporal distribution of the disorder and estimate the financial cost to producers. Next, responding veterinarians were asked to provide relevant case histories and these were examined in conjunction with the literature to obtain the general features of cases and identify any common animal, mob or farm characteristics that might be considered possible risk factors. This information was used to design a case-control study to identify risk factors associated with CCUO occurrence. The association of CCUO occurrence with periods of drought was examined using historical climate records and CCUO distribution data obtained from the survey of veterinarians. Concurrently, a detailed case study and supplementation trial was conducted on a severely affected farm in southern NSW. In late 2009 a phone interview was conducted with each participant in the case-control study to obtain information on occurrence of CCUO on these affected farms during 2008 and 2009.

For detailed methods please refer to Appendix 1.

2.1 Part 1 - Survey of Rural Veterinarians

A nationwide survey of cattle veterinarians was undertaken in two parts involving a self administered questionnaire and follow up telephone interview. Procedures involving contact with and the collection of information from veterinarians were approved by the University of Sydney Human Ethics Committee (approval number 07-2007/10074). The target population of this study was rural veterinarians who provide services to cattle producers in Australia. With the intention to obtain a representative sample of this target population and to maximise response rate, we used a census-based survey approach and contacted all veterinarians known in 2007 to be cattle veterinarians or government veterinarians. Veterinarians who were members of the Australian Cattle Veterinarians (ACV) special interest group of the Australian Veterinary Association (AVA) were contacted by ACV staff due to privacy regulations. To encourage participation, a questionnaire and information sheet were included in the July 2007 edition of the ACV newsletter along with an article to inform readers about the disorder and the purpose of the research. Follow-up mail-outs to non-responders were sent by ACV staff one month and two months later and consisted of a modified version of the newsletter article, a cover letter and the questionnaire.

State government employed veterinarians were sent the newsletter article, questionnaire, information sheet and subsequent reminders by e-mail to coincide with those sent to ACV veterinarians. At the end of the survey period, one-month after the third mail out and e-mail, all respondents who reported observing calves fitting the case definition for CCUO and consented to provide further information were recruited for the telephone questionnaire. The interview sought to collect details about affected calves born each calving season on an affected property with an emphasis on calves born between 2002 and 2007. The questionnaire used in the telephone interview classified a case as the birth of one or more affected calves on a property in one year.

Hard copies of all case records were analysed and relevant information cross checked with verbal information from telephone interviews. The data on reported occurrences of CCUO from each document or record obtained were entered into a Microsoft Access database (Microsoft Corporation, Redmond, USA) 'case records data' and checked for errors. All descriptive analysis of data was performed using GenStat Release 10.2 (Lawes Agricultural Trust, 2007). The prevalence and spatiotemporal distributions were described to the extent possible based on our analysis of the available data.

Mapping of the distribution of case farms was attempted using property addresses or GPS coordinates. However, due to missing data on farm location, the distribution of case farms was approximated using the postcode of the nearest town or village. This limited the usefulness of this spatial evaluation. However accurate maps based on GPS data were later produced in Part 3 – Case-control study to accurately map the spatial distribution of CCUO in south eastern Australia from 2002 to 2007.

2.2 Part 2 - Analysis of Existing Records

The analysis of existing records involved the collection of data from veterinarians responding to the survey in Part 1 and from a search of the literature. Information collected from surveyed veterinarians was classified into one of the following three categories:

1. Sporadic or isolated cases occurring prior to 2002.
2. Cases occurring during a major outbreak in 1991.
3. Cases occurring during the years 2002 to 2007.

The database created in Part 1 – Survey of veterinarians was used for analysis. When available, further information concerning the management and the environment of affected and unaffected mobs was coded and added to the database for analysis to identify any common features among affected mobs. All descriptive analysis of data was performed using GenStat Release 10.2 (Lawes Agricultural Trust, 2007). In addition, a database containing data from a 1991 government survey was analysed at the univariable level using the UniLogistic Macro for SAS (http://elearn.vetsci.usyd.edu.au/magicmacros) and SAS statistical software (release 9.2, SAS Institute Inc., Cary, NC, USA) to identify any significant associations between variables and the occurrence of CCUO.

An estimate of the financial losses incurred by farmers with CCUO between 2002 and 2007 was estimated using two methods. Firstly, a simple "loss of income" calculation was used to predict income loss in the year following the birth of CCUO calves for the number of cases reported in the survey of veterinarians. The second method involved constructing a gross margin (GM) based on predicted income and variable farm costs based on a typical 100 cow self replacing southern beef farm using the estimates of herd prevalence determined from the survey of veterinarians. Industry standard figures for GM calculations were supplied by NSW Industry and Investment (NSW Department of Primary Industries, 2009a). The GM was calculated for a two-year period following the birth of CCUO calves to include the carry on effect of losses. The calculation did not consider the birth of affected calves in successive years, but assumed a loss of 5 heifer calves and 5 bull calves due to CCUO in the first year. Using a 'worse case' scenario, it was then assumed 10 additional cows without calves at marking were culled. Ten additional heifers were then retained to replace these culled cows leading to fewer calves in the following year.

2.3 Part 3 - Case-control Study

The case-control study was undertaken to identify risk factors involved with CCUO using face to face interviews with beef producers. The study population consisted of beef farms in south eastern Australia affected by CCUO in the years 2002 to 2007 inclusive and that met specific selection criteria. A target sample size of 100 affected mobs with a minimum of 56 affected mobs was required for this study.

The University of Sydney Human Ethics Committee (approval number 02-2008/10640) approved procedures involving contact with and the collection of information from producers. Private and government veterinarians provided names and addresses of property owners or managers with reported affected herds. A small payment was offered to producers to compensate for the time required to conduct an interview in an attempt to improve the participation rate.

During the interview, information about mobs of pregnant cattle present on the farm during years of CCUO occurrence was used to identify case mob/s and control mobs. A case mob was a mob reported by the producer in which at least one CCUO calf was born in a calving season. A control mob was a mob reported by the producer to be free of CCUO calves in the same calving year as the case mob/s. For producers 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.

Soil samples were collected at the time of interview in case paddocks and control paddocks using a standard soil sampling technique. Where available, recent soil test results from a National Association of Testing Authorities (NATA) accredited lab were provided by producers, negating the need to sample soils. Samples were collected using a standard technique, labeled, sealed in plastic bags and held at 4^{o}C before sending to a NATA accredited commercial laboratory (Incitec Pivot Lab Services, Werribee, Victoria) for analysis, usually within the following week. The location and altitude of each paddock was determined using a global positioning system meter (Garmin GPS 205).

Microsoft Access (Microsoft 2003) was used to create a relational database for the entry and management of the questionnaire and laboratory data. For statistical analysis this data was imported into SAS statistical software (release 9.2, SAS Institute Inc., Cary, NC, USA). Two data sets were constructed for analysis. Data set one contained data for management and environment variables for all mobs (n=168). Data set two contained data for soil variables concerning paddocks (n=125). Two separate analyses were conducted using these data sets.

Two outcomes were used in analysis of the mob dataset. First, the outcome variable mob status 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 that were 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.

The explanatory variables in the mob related to farm management and environment variables and included proposed risk factors and confounding factors. In the paddock dataset, the explanatory variables investigated soil and environment variables. Descriptive analyses were performed on the outcome and all explanatory variables.

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 the UniLogistic Macro for SAS (http://elearn.vetsci.usyd.edu.au/magicmacros). Variables with an unconditional association with the outcome at a significance of $P<0.25$ were selected for further investigation. Variables were tested in pairs for collinearity and if found, a variable was selected for inclusion in further analyses based on an opinion of biological plausibility. The remaining explanatory variables with ≤10% missing data were selected for inclusion in the multivariable model.

Separate generalised linear mixed models 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 fixed effects dam age and year affected were then added to the model as confounding variables. 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.

A generalised linear mixed model was 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.

2.4 Part 4 - Analysis of Rainfall Data

The data concerning the number of CCUO calves used for this study was obtained during interviews with 46 producers interviewed as part of the case-control study in Part 3. Producers provided an estimate of the number of CCUO calves born in each month of each calving season from October 2001 to December 2007 during the interviews. This period allowed for inclusion of rainfall data throughout the gestation period of calves born in the years from 2002 to 2007.

Rainfall data for each farm (average monthly total, mm) were obtained using the Australian Bureau of Meteorology website for historical climatic data (www.bom.gov.au/climate/data/weather-data.shtml). A time series of average monthly rainfall was created based on the average of the monthly rainfall values for all farms for the period of interest.

2.4.1 Time Series Analysis

Time series analysis was used to examine the relationships between rainfall occurring during gestation and the occurrence of CCUO (Shumway and Stoffer, 2006). The Applied Statistical Time Series Analysis program (ASTSA, version 2.0. Department of Statistics, University of California, Davis, http://www.stat.pitt.edu/stoffer/tsa2/) was used for all time series analysis. Autocovariance (ACF) and partial autocovariance (PACF) functions of the time-series of cases of CCUO were calculated to confirm the seasonal pattern of CCUO. Autoregression was performed and the best fitting model selected based on the goodness-of-fit criterion (Akaike's corrected information criterion (AICc)).

The time series of monthly average rainfall was used to produce a three-month moving average time series for rainfall. The three-month time frame was used to coincide with the Bureau of Meterology definition of drought. Cross correlation functions of the time series of cases of CCUO with average monthly rainfall and three-month moving average rainfall were calculated and vector autoregression was used to determine significant relationships between the time series of cases of CCUO and rainfall in the previous 12 months. The best fitting model was selected using AICc, and the ACF and PACF of the selected model residuals were checked visually for evidence of trends. The residuals were also examined for independence, constant variance and outliers using the Box-Pierce, cumulative spectrum and *z*-distribution tests, respectively.

2.4.2 Logistic Regression Analysis

The results of time series analysis were used to create a dataset for average monthly and three-month average rainfall deficiency on the 46 farms used in the study. For each farm, an outcome variable month status was created for each calving month (July to October) for each year (2002 to 2007) coded as 1 when affected calves were born during the month and 0 when no calves were born. Explanatory variables were created for rainfall deficiency at the time lags identified from the time series analysis for each month and year on each farm using the long-term first decile available from the Bureau of Meteorology (www.bom.gov.au/climate/data/weather-data.shtml). Descriptive analysis of all data was performed using SAS statistical software (release 9.2, © 2002-08, SAS Institute Inc., Cary, NC, USA) and unconditional univariable associations investigated using the UniLogistic Macro for SAS (http://elearn.vetsci.usyd.edu.au/magicmacros/LogReg/UniLogistic) as described in Part 3 – Case-control study.

A generalised linear mixed model was created and checked using a similar approach to that used in Part 3 – Case-control study. Random-effect variables for farm and year were included in each model to account for clustering of CCUO occurrence within farm and year.

2.5 Part 5 – Case Study and Supplement Trial

The information examined in this case study was collected during Part 3 – Case-control study using the questionnaire described previously to gather farm management and environment data.

Detailed investigation of this one study property (Farm ID NSW44) was possible due to the availability of monthly reports since 2002 written by the same farm manager that provided an accurate history of farm management from 2002 to 2007. The standard of written records for this study farm, offered a unique opportunity to document and to qualitatively evaluate the impact of farm management on CCUO occurrence on a severely affected farm.

Additional information concerning previous investigations on the farm (soil test results, pasture analysis, pathology reports) was requested from the Hume office of the Livestock Health and Pest Authority (LHPA) and the farm manager. All data was entered into the Microsoft Access database used for the case-control study in Part 3 and imported into GenStat Release 10.2 (Lawes Agricultural Trust, 2007) for descriptive analysis.

Soil samples were collected using the methods described in the case-control study from paddocks containing native pastures with a known association with CCUO, and from paddocks containing improved pastures with no history of association with CCUO. Pasture samples were collected from the same paddocks as soil samples for nutrient analysis and were labeled and submitted to a NATA accredited commercial laboratory (Regional Veterinary Laboratories, Benalla, Victoria) for analysis.

2.5.1 Supplementation Trial

The experimental procedures used for the trial were approved by the University of Sydney Animal Ethics Committee (approval number N00/4-2009/1/5005). A randomly selected sample of multiparous (8 to 10 years old) Angus cows (n=20) was chosen from a mob of 100 in March 2009. The cows were pregnancy tested and confirmed in calf before being included in the study. Half of the selected cows (n=10) were randomly assigned to the control group and half to the treatment group (n=10). The cattle had been previously grazing on creek flats and had their calf weaned at the time of selection. Cows in the treatment group were given three injections of Multimin® injectable chelated trace minerals for cattle (Virbac Pty Ltd) at 6 week intervals at the recommended dose rate of 1ml per 75kg body weight. The injections were given approximately 18 weeks, 12 weeks and 6 weeks prior to the estimated calving date (approximately weeks 18, 24 and 30 of gestation). Blood samples were collected and liver biopsies were performed on treatment and control cows at the time of each treatment following identical procedures and again at approximately four weeks post-calving. The time period for treatments was selected based on convenience utilising the manager's availability to muster cattle from the paddock into the yards. Control cows were not treated with the supplement. After the first treatment all cows were placed in a paddock with a history of being grazed by dams that gave birth to CCUO calves over the last 7 years and retained there until the end of calving.

Following liver biopsies on control and treatment cows, cattle were held in yards overnight and walked back to their paddock the following morning to reduce the risk of haemorrhage. Liver biopsy samples were placed in a sealed 1.5mm plastic collection tube and frozen before shipping to a NATA accredited commercial laboratory (Regional Veterinary Laboratories, Benalla, Victoria). Liver content of iron, copper, zinc and manganese was determined by atomic absorption spectroscopy. Blood samples were collected from tail veins into heparinised vacuum tubes designed for trace mineral analysis. Tails were thoroughly cleaned and dried before collection to remove any faecal contamination. Blood samples were frozen for shipment to the laboratory with liver biopsy samples. Packed cell volume, plasma manganese and whole blood manganese levels were determined using standardised procedures. Plasma zinc levels were not measured as rapid changes in circulating zinc may occur over short periods of time, and results at one time in the day may not reflect the true zinc status of the animal.

Statistical analysis of data was performed to compare proportion of affected calves per group using the Fishers exact test and to compare the liver and blood trace mineral levels between groups and treatment times using the MIXED procedure for SAS statistical software (release 9.2, SAS Institute Inc., Cary, NC, USA). For liver and blood trace mineral levels, models were based on the fixed effects of treatment, time and treatment x time interaction. Non significant terms ($P>0.05$) were removed from the model one at a time until all remaining terms were significant with a value of $P<0.05$. A random effect for cow number was included in all models.

2.6 Part 6 – CCUO in 2008-2009

Follow-up phone interviews were conducted in November and December 2009 with farmers recruited for the case-control study to determine the number of CCUO calves born since case-control interviews were completed and to compile a list of the farmer initiated control strategies used to reduce CCUO occurrence.

Farmers were asked:

Did you observe any calves with deformities fitting the description of disproportionate dwarfism (as discussed at the case-control interview)?

1. What management practices, if any, have you changed since the case-control interview?

Further we obtained details of CCUO calves on other farms that were reported to us by government veterinarians.

3 Results

3.1 Part 1 - Survey of Rural Veterinarians

3.1.1 Response to Survey

Questionnaires were posted to 1014 ACV veterinarians, with only 12 being returned in the first month. After the second mailing a further 161 were returned, with the remaining 69 returned after the third mailing. In total 242 (23.9%) questionnaires where received at the close of the survey period one month after posting the third mailing. Of these 242 responses, 56 (23.1%) reported observing signs of CCUO, with 42 (17.4%) reporting observations in the last five years (2002-2007). All Australian States and Territories were represented in the returns with results shown in Table 3-1.

A total of 178 e-mails were sent to government veterinary officers, of which 68 (38.2%), replied. Symptoms of CCUO were reported by 24 (35.3%) of the respondents with 21 (30.9%) reporting cases in the last five years. The remaining 44 (69.2%) reported never having seen cases fitting the case definition. Table 3-2 outlines the responses received based on the state or territory. The return rate for the 68 e-mails sent directly to government veterinarians was 77.9% (n=53) and for the 110 e-mail questionnaires sent via a head office to government veterinarians was 13.6% (n=15). For the government veterinarians who received the email directly, 20 replied after the first email, 41 after the second and seven after the third email. No additional replies resulted from the second and third e-mails to veterinarians contacted via head office.

Telephone interviews were conducted with 53 ACV respondents and 24 government veterinarians. Three ACV respondents failed to be contacted during the survey period and were not included in the study. Of the ACV veterinarians interviewed, 14 had no records after referring all cases to the relevant government veterinarian and a further five had moved employment, no longer had access to records and could not recall any specific details of cases.

3.1.2 Case Reports Prior to 2002

In total, 14 ACV veterinarians and three government veterinarians reported only observing CCUO calves prior to 2002. Contact with these veterinarians, in addition to those detailed above, provided limited, but useful information concerning the occurrence of CCUO prior to 2002. Numerous case records were obtained from previous investigations undertaken during a major outbreak in NSW in 1991 including 22 pathology reports and hard copies of 19 questionnaires and an electronic data set with results of 33 additional questionnaires from the same investigation. In addition, two unpublished papers based on these data sources were obtained (Johnson, 2007). These data will be examined in Part 2 results – Analysis of existing records.

3.1.3 Case Reports 2002-2007

The respondents reported a total of 135 cases for the period 2002 to 2007, 114 (84.4%) cases by government veterinarians and 21 (15.6%) by ACV veterinarians. A total of 1081 affected calves were reported on 117 farms, of which 12 farms experienced affected calves in more than 1 year. Details on the number of breeding cows and normal calves was only available for 55 farms, where the prevalence of CCUO calves ranged from 0.9% to 92 % (mean 12.5%, SD 14.2%). Table 3-3 compares yearly totals for affected calves and farms. The number of calves reaching a saleable age in good condition was not able to be determined from this data.

In 111 (82.2%) of the reported cases, diagnostic tests were not performed, and the provisional diagnosis of CCUO was based on physical appearance and farm location (located in an area of previously reported cases). Only 24 (17.8%) cases reported by government veterinarians had pathology test results, of these 16 (11.9%) cases were tested for akabane and pestivirus, and eight (5.9%) cases had samples submitted for histopathology all of which were positive for chondrodystrophy. None of the cases reported by ACV veterinarians included any pathology results. Two (1.4%) cases were reported where calves were born without forelimbs limbs in an area of known CCUO activity at Euroa, Victoria. On one NSW farm, there was a report of newborn lambs exhibiting the same chondrodystrophic signs as cattle.

In total 26 (19.3%) cases were reported from areas other than those recorded in the literature. Government veterinarians reported eight (30.8%) of these cases, with ACV veterinarians reporting the remaining 18 (69.2%). None of these cases were confirmed with pathology and five of these cases were assumed by the veterinarian to be due to another cause (either from pestivirus infection or hereditary), with no further investigation performed to confirm a diagnosis. There were 25 cases reported for which a specific location was not provided. These cases were recorded under the general area of the Rural Lands Protection Board (renamed Livestock Health and Pest Authorities) in which the farms were located. A summary of all reported cases for the period 2002 to 2007 is provided in Appendix 2: Case reports 2002 to 2007.

Table 3-1 Results of a survey of 1014 rural non-government veterinarians investigating cases of CCUO in cattle herds in Australia between 2002 and 2007

State/Territory	Number questionnaires returned	Number reporting CCUO cases	Number reporting CCUO cases 2002-2007	Number with no report of CCUO cases
NSW and ACT	92	34	24	58
Victoria	61	11	7	50
Northern Territory	3	0	0	3
South Australia	20	5	5	15
Tasmania	4	2	2	2
Queensland	45	2	2	43
Western Australia	17	2	2	15
Total	242 (23.9%)	56 (23%)	42 (17.4%)	186 (77%)

Table 3-2 Results of a survey of 178 rural government veterinarians investigating cases of CCUO in cattle herds in Australian between 2002 and 2007

4 State/Territory	5 Number sent	6 Number returned	7 Number reporting CCUO cases	8 Number reporting CCUO cases 2002-2007	9 Numb with no repo of CCUO cases
10 NSW and ACT	11 43	12 35 (81%)	13 14	14 14	15 21
16 Victoria	17 22	18 16 (73%)	19 6	20 4	21 10
22 Western Australia[a]	23 28	24 2 (7%)	25 2	26 2	27 0
28 Queensland[a]	29 63	30 5 (8%)	31 1	32 1	33 4
34 South Australia[a]	35 13	36 6 (46%)	37 1	38 0	39 5
40 Tasmania[a]	41 6	42 2 (33%)	43 0	44 0	45 2
46 Northern Territory	47 3	48 2 (66%)	49 0	50 0	51 2
52 Total	53 178	54 68 (38.2%)	55 24 (35.3%)	56 21 (30.9%)	57 44 (64.7%)

58 a. E-mail forwarded to government veterinarians by a department officer in these states /territories.

Table 3-3 Number of affected calves and farms reported by year from a survey of government and non government rural veterinarians, 2002-2007

Year	Number of affected calves	Number of affected farms		
		NSW/ACT	Victoria	WA
2002	13	3	-	-
2003	238	17	10	-
2004	555	43	12	-
2005	20	8	2	-
2006	230	28	2	1
2007[a]	25	8	-	1
Total	1081	107[b]	26[b]	2

a. Data concerning affected calves born after 31 October 2007 have not been included in these figures

b. For 5 farms in Victoria and 7 farms in NSW, affected calves were reported for more than 1 year

58.1 Part 2 – Analysis of Existing Records

58.1.1 Sporadic Cases Prior to 2002

Four published papers, three pathology reports and nine case records documenting sporadic CCUO occurrence prior to 2002 were examined. Table 3-4 presents a summary of the sporadic cases reported from 1957 to 2001 with reference to location, number of affected calves born and total number of calves born on farms where known. The majority of these cases occurred in the area of south eastern NSW and northern Victoria. The five reports outside of this area were for two cases in Western Australia (reported in the literature), two cases in Molong, NSW and one case in Scone, NSW. None of these five reports were confirmed with pathology.

The features of CCUO that characterised cases reported prior to 2002 are not clearly defined except for those reported in the literature. The published data does indicate that affected calves were born following exposure of their dams to dry conditions during at least one trimester of their pregnancy (Barry, 1967; Barry and Murphy, 1964; Hawkins, 1994; Peet and Creeper, 1994).

58.1.2 1991 Outbreak in NSW

Officers of the NSW DPI investigated a large CCUO outbreak that occurred in 1991. They designed a questionnaire administered in interviews with producers affected by CCUO. From the completed questionnaires acquired it appears that one person conducted these producer interviews. The case definition for this investigation appears to have included calves with a range of deformities including shortened long bones, enlarged epiphyses and hyperextension of the fetlocks.

The questionnaire had five sections and gathered the following information.

<u>The Farm:</u> data on the farm and all neighbours, with a distance and direction to the nearest town or village used for location. Postal and/or street address was not requested. This section was poorly completed with only approximations to the location being available from the data provided.

<u>History:</u> length of tenure and previous history of deformed calves.

<u>Description of Affected Calves on this Farm:</u> details on the number of calves born alive and dead, and the number with defects including shortened long bones, enlarged epiphyses, bowed front legs, overextension of fetlocks, brachygnathia, unable to rise and unable to suckle. A record of pathology samples was also provided where appropriate. This section was poorly completed with most forms neglecting to detail the number of calves with each deformity.

<u>Breeder Mobs:</u> collected details of each mob on the farm (or at least the case mobs) including age of dams, breed, number, joining details, numbers of normal and abnormal calves and dates of first and last cases. For each trimester of pregnancy the condition score, pasture type and quality, handfeeding details and treatments given were requested. The name of the paddock grazed for each trimester was also recorded. This section was not always completed in detail.

<u>Paddock Description and History:</u> collected information about paddocks where breeder mobs were held during gestation. Information on soil (including any test results), fertilizer and herbicide treatments and water supply were requested. This section was poorly completed.

A total of 52 questionnaires were obtained, nineteen in paper form and 33 as an electronic dataset. These reports included pathology results for 19 properties. All data was combined into Microsoft Excel database, the ‘1991 outbreak’ dataset, with data entered in the fields described previously.

There were 69 case mobs and 20 control mobs on the 52 farms. A total of 538 (11.9%) calves were affected from 4516 calves born. Details for 327 affected calves showed that 72.8% (238) had shortened limbs, 64.5% (211) had enlarged joints, 20.8% (68) had dropped pasterns and 19.3% (63) had superior brachygnathia. Only 1.2% (4) showed signs of spinal deformity.

Following deletion of variables due to large numbers of missing data or low numbers of observations per category, univariable analysis was undertaken on nine variables. The contingency table for variables is provided in Table 3-5. The univariable associations of the explanatory variables investigated with the outcome variable (AFFECTED) are shown in Tables 3-6 and 3-7.

Univariable analysis revealed that the following associations:

- Case mobs were 8.4 times more likely (95%OR 2.5 to 38.7, P<0.001) during second trimester and 3 times more likely (95%OR 1.06 to 8.8, P=0.039) during third trimester to have been handfed than control mobs.
- Case mobs were 5 times more likely (95%OR 1.5 to 20.2, P=0.007) to have grazed inadequate levels of pasture during second trimester than control mobs.
- Case mobs were 12.1 times more likely (95%OR 2.3 to 225, P=0.001) to have been fed pasture hay supplements than control mobs during second trimester.

No significant differences between case and control mobs were detected for the further five variables with sufficient data to perform univariable analysis. The level of missing data prevented any univariable analysis related to breed, animal treatments, soil type, fertilizer application, pesticide usage, soil trace elements and monthly rainfall.

Pathology reports provided liver manganese concentration for 12 affected calves with a mean of 1.83 PPM (median 1.85, range 0.06 – 3.84).

The mean soil pH recorded for 14 paddocks grazed by case mobs was 4.73 (median 4.7, range 4.2 – 5.6) and for 6 paddocks grazed by control mobs was 4.96 (median 5.0, range 4.2 – 5.9).

The level of handfeeding in case mobs (61% of case mobs compared to 15.8% of control mobs) aligned with the very dry weather conditions of 1990/1991 that would have resulted in low availability of pasture and the need for supplement feeding. Historical rainfall data illustrates the very dry conditions in NSW for the periods corresponding to the second trimester of pregnancy for cattle that calved during spring in 1991 (Figure 3-1).

The distribution of the 1991 outbreak case farms based on postcode is shown in Figure 3-2.

58.1.2.1 Pathology Reports

The 22 pathology reports on samples submitted from 20 case farms in 1991 outbreak appear to have formed the basis of the published report by Godwin *et al.* (1992). Reports for six specimens clearly describe a chondrodystrophy with premature closure of the growth plate and disorganisation of the hypertrophic zone of chondrocytes. Tests of samples from five farms were negative to viral aetiologies including Akabane, Aino, Pestivirus and Bluetongue. Three results for liver manganese from affected calves are reported but inconclusive as to a deficiency.

The unpublished paper by Philbey *et al.* (2001) describes the same pathology reports as the Godwin paper. The paper by McLaren *et al*. (2007) confirms the pathology of the 2002-2007 outbreak as being of the same type.

58.1.3 Analysis of 2002-2007 data

The majority of CCUO calves reported between 2002 and 2007 were born in the NSW region of the south western slopes and in north eastern Victoria. There were 3 isolated cases from Western Australia that were also reported. Maps produced based on postcode were not useful for interpretation of spatial distribution. However maps based on GPS data were produced in Part 3 – Case-control study to accurately map the spatial distribution of CCUO in south eastern Australia from 2002 to 2007.

58.1.3.1 Investigation by NSW DPI of 2003/2004 Cases

In 2005 officers of the NSW DPI designed a questionnaire and posted it to producers who had reported CCUO calves in 2003 and/or 2004 with a cover letter requesting producers to complete and return the questionnaire.

Producer inclusion in this survey was based on reported cases of calves with any of the typical signs of CCUO including bent, twisted or shortened limbs, deformed spines and cranial deformities of superior brachygnathia or domed head.

The questionnaire had five sections and gathered the following information.

Owner details: name and phone number, no location was requested. Matching of names to those detailed in other records provided approximate locations for most farms.

Farm Characteristics: farm size, enterprise type, cattle numbers (year not specified), number of normal and abnormal calves in 2003 and 2004, breed and mob details.

Management history: joining and calving dates, paddock locations for each trimester and details on each paddock including soil type, topography, water source, pasture and supplement feeding were requested. Details were only required for the worst-case mob and for control mobs. Other details collected included cattle treatments, fertilizer and herbicide treatments, and average farm rainfall.

The questionnaires from NSW producers identified 16 case mobs and 52 control mobs across 12 farms. However the self administered questionnaires were poorly completed, often with no information for many questions, particularly the questions related to control mobs. The 12 questionnaires were reviewed and data entered into a third Microsoft Excel spreadsheet '2002-2005 outbreak' dataset. Descriptive analysis of variables was undertaken in Excel. Further analysis was not pursued due to very limited information gathered about most of the control mobs.

Evaluation of the questionnaire data from the NSW survey showed that 16 mobs out of a total of 68 mobs produced affected calves on 12 farms. On three farms affected calves were born in 2003 and 2004 and on the remainder affected calves were born only in 2004.

In 2003, 20 (0.8%) affected calves were born from a total of 2489 calves. In 2004, 93 (3.7%) affected calves were born from a total of 2510 calves. There were no details provided on the types of deformities present in affected calves for either year. Supplement feed details were provided for 15 case mobs and 12 control mobs, of which 12 (80%) of the case mobs and 10 (83%) of the control mobs were given supplement feed during gestation.

58.1.3.2 Investigation by DPI Victoria of 2003/2004 Cases

In 2004 a DPI Victoria officer used a form to record information during discussions with producers that had CCUO calves born in 2003 and 2004. Compared to the NSW DPI investigation, this was not a formal investigation rather an attempt to undertake a case study of mobs that produced CCUO calves on case farms. These farms were identified by reported cases of calves with any of the typical signs of CCUO including bent, twisted or shortened limbs, deformed spines and cranial deformities of superior brachygnathia or domed head.

The form had six sections with the following headings:

Farm description
Herd description
Description of case mob
Treatment history of the affected cattle
Management of dams during early gestation
Calf characteristics.

The forms for 26 Victorian farms were reviewed and data entered into the '2002-2005 outbreak' dataset and descriptive analysis of variables undertaken in Excel. As the forms provided no information about control mobs for these farms no investigation of potential risk factors was possible.

Data from the informal investigation in Victoria was insufficient to determine the number of affected calves on the 26 farms. Supplement feeding of case mobs was reported by 12 producers but data on supplement feeding were not provided by the other 14 producers. Details of the deformities present in calves were only completed for 10 farms with twisted limbs being present in all cases. No other descriptive analysis could be undertaken due to high level of missing data.

58.1.3.3 Estimate of Financial Cost

Table 3-8 provides an estimate of the total cost of the 1081 reported cases of CCUO to farmers for the period 2002 to 2007 based on the loss of income in the year when stock would normally be sold. The total cost of AU$818 315 represents an average cost per affected calf of AU$ 757 for the period 2002-2007. Using the mean herd prevalence of 12.5% obtained from the survey data, the estimated GM loss per breeding cow was AU$ 67.80 for the first year after the birth of CCUO calves, and AU$ 69.80 for the second year with a total loss of GM of AU$ 137.60 per breeding cow for the period (Table 3-9). This represents a total estimated GM loss for the two year period of AU$ 13 760 for a typical 100 cow farm, or AU$ 1376 per affected calf (assuming 10 affected calves are born). Using this figure produces an estimate of AU$ 1.488M for the total number of 1081 affected calves reported between 2002 and 2007.

Table 3-4 Sporadic cases of CCUO reported prior to 2002

59 Location	60 Year	61 Number of Farms	62 Number of affected calves	63 Total number of calves
64 NSW Southwestern Slopes[1]	65 1957	66 7	67 31	68 670
69 NSW Southwestern Slopes[1]	70 1958	71 1	72 2	73 179
74 NSW Southwestern Slopes[1]	75 1960	76 3	77 4	78 Not recorded
79 NSW Southwestern Slopes[2]	80 1965	81 8	82 32	83 1353
84 Euroa, Victoria	85 1979	86 1	87 3	88 20
89 Benalla, Victoria	90 1985	91 1	92 6	93 60
94 Uranquinty, NSW[a]	95 1988	96 1	97 9	98 25
99 Wagga Wagga, NSW[a]	100 1988	101 1	102 40	103 150
104 Molong, NSW	105 1988	106 1	107 Not recorded	108 Not recorded
109 Alfredtown, NSW	110 1989	111 1	112 2	113 50
114 Yerong Creek, NSW	115 1990	116 1	117 8	118 72
119 Alfredtown, NSW	120 1990	121 1	122 3	123 50
124 Scone, NSW	125 1990	126 1	127 Not recorded	128 Not recorded
129 Molong, NSW	130 1990	131 1	132 Not recorded	133 Not recorded
134 Western Australia[3, 4]	135 1993	136 2	137 29 (+45 mild)	138 254
139 Wagga Wagga, NSW[b]	140 1996	141 1	142 12	143 13
144 Wagga Wagga, NSW[b]	145 1997	146 1	147 1	148 13

149 a. Pathology results provided indicating chondrodystrophy
150 b. These cases were reported on the same farm in consecutive years
151 1(Barry and Murphy, 1964)
152 2 (Barry, 1967)
153 3 (Hawkins, 1994)
154 4 (Peet and Creeper, 1994)

Table 3-5 Contingency table for explanatory variables investigated for the '1991 outbreak' dataset from surveys conducted by NSW DPI in 1991 for 69 case mobs and 20 control mobs on 52 farms in NSW.

155 Variable	156 Categories	157 Case Mob	158 Control Mob
	160	161	162
159 Quality of pasture in trimester 2	163 Good	164 20	165 11
	166 Poor	167 30	168 6
	170	171	172
169 Quality of Pasture in trimester 3	173 Good	174 24	175 7
	176 Poor	177 26	178 10
	180	181	182
179 Amount of pasture trimester 2	183 Adequate	184 18	185 13
	186 Inadequate	187 28	188 4
	190	191	192
189 Amount of pasture trimester 3	193 Adequate	194 18	195 8
	196 Inadequate	197 28	198 9
	200	201	202
199 Handfeeding trimester 1	203 No	204 58	205 18
	206 Yes	207 9	208 1
	210	211	212
209 Handfeeding trimester 2	213 No	214 26	215 16
	216 Yes	217 41	218 3
219	222	223	224
220 Handfeeding trimester 3	225 No	226 18	227 10
221	228 Yes	229 49	230 9
	232	233	234
231 Pasture hay fed trimester 2	235 No	236 40	237 18
	238 Yes	239 27	240 1
241	244	245	246
242 Pasture hay fed trimester 3	247 No	248 34	249 11
243	250 Yes	251 33	252 8

253

Table 3-6 Univariable associations of explanatory variables with the outcome AFFECTED for the '1991 outbreak' dataset compiled from surveys conducted by NSW DPI in 1991 on 52 farms with 69 case mobs and 20 control mobs, P values <0.25

254 Variable	255 Categories	256 b	257 SE(b)	258 Odds ratio	259 L CL (OR)	260 U CL (OR)	261 P -Value
	263	264	265	266	267	268	269 0 .000
262 Handfeeding trimester 2	270 No	271	272	273 1 .0	274	275	276
	277 Yes	278 2 .13	279 0 .68	280 8 .41	281 2 .51	282 3 8.7	283
284	285	286	287	288	289	290	291
	293	294	295	296	297	298	299 0 .001
292 Pasture hay fed trimester 2	300 No	301	302	303 1 .0	304	305	306
	307 Yes	308 2 .50	309 1 .06	310 1 2.15	311 2 .29	312 2 24.9	313
314	315	316	317	318	319	320	321
	323	324	325	326	327	328	329 0 .007
322 Amount of pasture trimester 2	330 Adequate	331	332	333 1 .0	334	335	336
	337 Inadequate	338 1 .62	339 0 .65	340 5 .06	341 1 .52	342 2 0.23	343
344	345	346	347	348	349	350	351
	353	354	355	356	357	358	359 0 .039
352 Handfeeding trimester 3	360 No	361	362	363 1 .0	364	365	366
	367 Yes	368 1 .11	369 0 .54	370 3 .03	371 1 .06	372 8 .83	373
374	375	376	377	378	379	380	381

Table 3-7 Univariable associations of explanatory variables with the outcome AFFECTED for the '1991 outbreak' dataset compiled from surveys conducted by NSW DPI in 1991 on 52 farms with 69 case mobs and 20 control mobs, P values >0.25

382 Variable	383 Categories	384 b	385 SE(b)	386 Odds ratio	387 L CL (OR)	388 U CL (OR)	389 P -Value
	391	392	393	394	395	396	397 0 .077
390 Quality of pasture in trimester 2	398 No	399	400	401 1 .0	402	403	404
	405 Yes	406 1	407 0	408 2	409 0	410 9	411

		.01	.58	.75	.90	.13	
412	413	414	415	416	417	418	419
	421	422	423	424	425	426	427 0 .290
420 Handfeeding trimester 1	428 No	429	430	431 1 .0	432	433	434
	435 Yes	436 1 .03	437 1 .09	438 2 .79	439 (.48	440 5 3.26	441
442	443	444	445	446	447	448	449
	451	452	453	454	455	456	457 0 .572
450 Amount of pasture trimester 3	458 Adequate	459	460	461 1 .0	462	463	464
	465 Inadequate	466 (.32	467 (.57	468 1 .38	469 (.44	470 4 .28	471
472	473	474	475	476	477	478	479
	481	482	483	484	485	486	487 0 .581
480 Pasture hay fed trimester 3	488 No	489	490	491 1 .0	492	493	494
	495 Yes	496 (.29	497 (.53	498 1 .34	499 (.48	500 3 .85	501
502	503	504	505	506	507	508	509
	511	512	513	514	515	516	517 0 .625
510 Quality of pasture in trimester 3	518 No	519	520	521 1 .0	522	523	524
	525 Yes	526 - 0.28	527 (.57	528 (.76	529 (.24	530 2 .29	

Table 3-8 Estimated total cost to farmers of CCUO cases reported in the survey of veterinarians for the years 2002 to 2007 based on “loss of income” for the following financial year.

531 Year born	532 Year sold	533 EYCI (c/kg)	534 Average price per calf (AU$)[a]	535 Total number reported cases[b]	536 Total loss of income for all reported cases (AU$)
537 2002	538 2003	539 334	540 708	541 13	542 9 204
543 2003	544 2004	545 364	546 772	547 238	548 183 736
549 2004	550 2005	551 374	552 792	553 555	554 439 560
555 2005	556 2006	557 325	558 689	559 20	560 13 780
561 2006	562 2007	563 317	564 672	565 230	566 154 560
567 2007	568 2008	569 329	570 699	571 25	572 17 475
573	574	575	576 Total	577 1081	578 818 315

579 a. The average price assumed half the affected calves were steers and half heifers.

580 b. The number of affected calves assumed all calves born with CCUO were non-viable.

Table 3-9 Comparison of predicted Gross Margin (GM) profit per head for two year period following the birth of affected calves on a hypothetical beef producing farm in south eastern NSW for the financial year 2008/2009.

581	**582** GM (Year Born)	**583** Affected GM (first year)	**584** Affected GM (second year)
585 Cull Cows at AU$ 714	**589** 18 x **592** 12 852	**596** 28 x **599** 19 992	**603** 18 x **607** 12 852
586 Bulls at AU$ 1100	**590** 1 x **593** 1 100	**597** 1 x **600** 1 100	**604** 1 x **608** 1 100
587 Yearlings at AU$ 696	**591** 64 x **594** 44 544	**598** 44 x **601** 30 624	**605** 54 x **609** 37 584
588 Total income (AU$)	**595** 58 496[a]	**602** 51 716[a]	**606** **610** 51 536[a]
611 Variable costs (AU$)	**612** 21 296[b]	**613** 21 296[b]	**614** 21 296[b]
615 GM (AU$)	**616** 37 200	**617** 30 420	**618** 30 240
619 GM/cow (AU$)	**620** 372.00	**621** 304.20	**622** 302.40
623 Reduction in GM/cow for year (AU$)	**624** 0	**625** 67.80	**626** 69.80
627 Reduction in GM for year (AU$)	**628** 0	**629** 6780	**630** 6980

631 a. Income based on 2009 prices neglecting yearly fluctuations.

632 b. Variable cost averaged over period for ease of calculation.

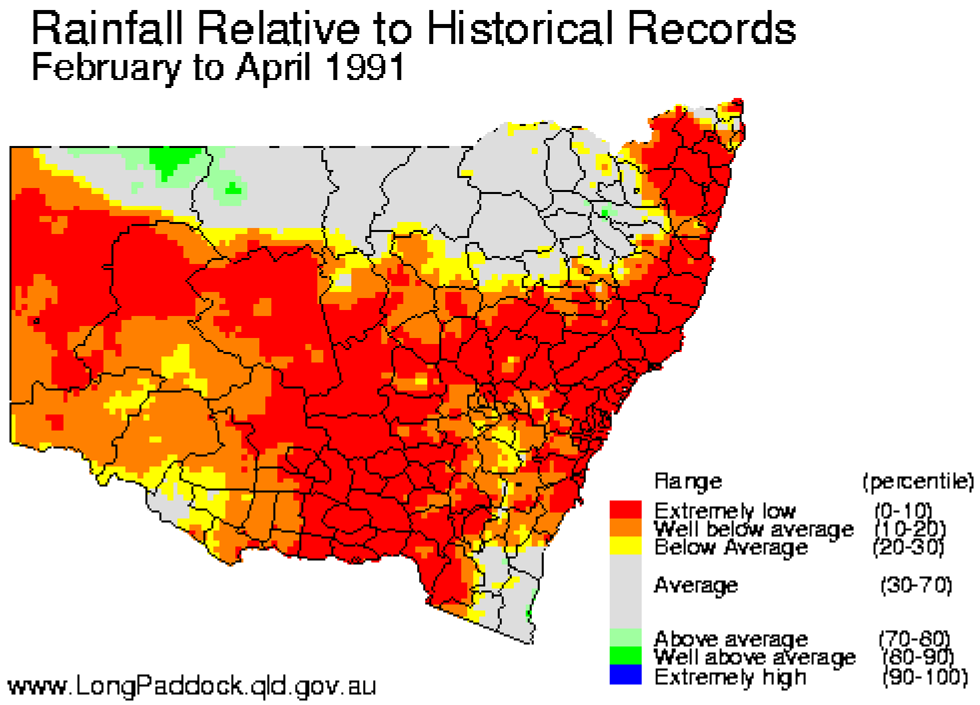

Figure 3-1 Rainfall deficiency for NSW corresponding to the second trimester for CCUO calves born in spring, 1991 (www.LongPaddock.qld.gov.au)

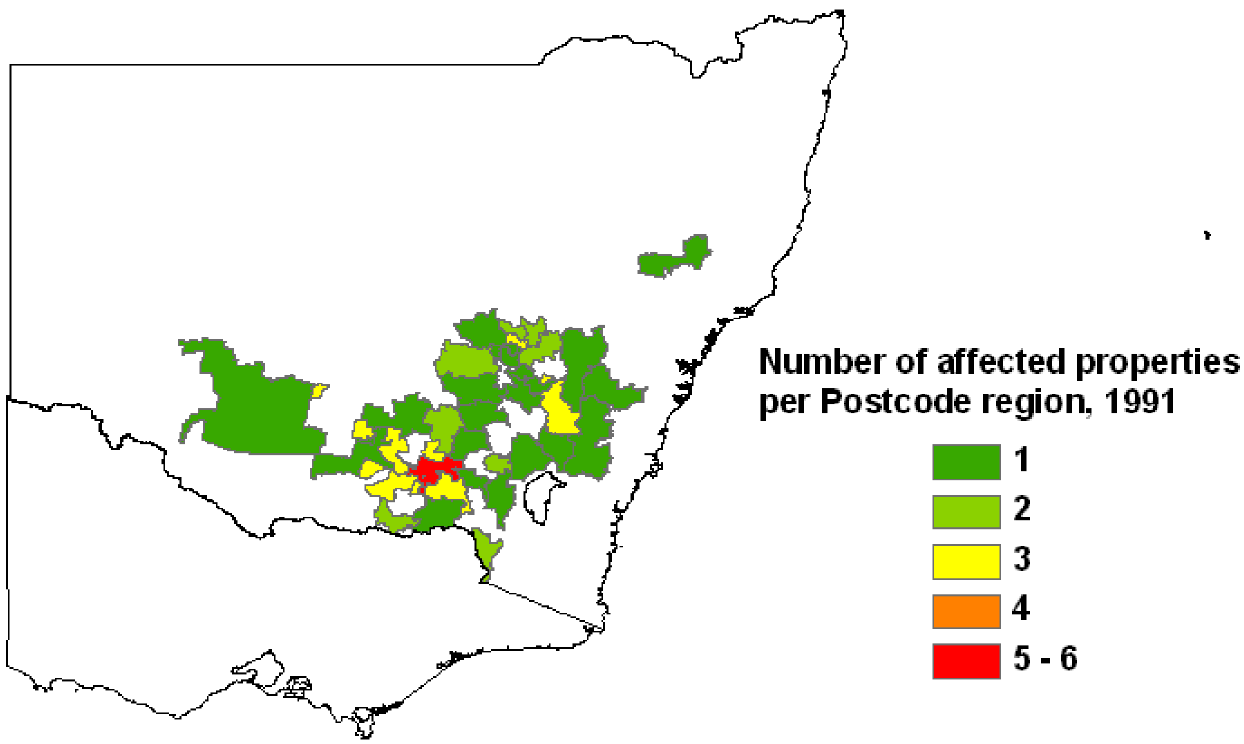

Figure 3-2 Distribution and number of CCUO affected properties based on postcode of the affected property in NSW in 1991

632.1 Part 3 – Case-control study

Of the 98 farm owners or managers reported with affected herds and invited to participate in this study, contact could not be made with 14 (14.2%) producers, eight (8.2%) were excluded based on the description of affected calves provided (no dwarfism present), 19 (19.4%) declined to participate citing a lack of time, memory or interest, and nine (9.2%) had sold their farm or were no longer in cattle production and declined. The remaining 48 (49%) producers were interviewed between March 2008 and February 2009, with a further two (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.

632.1.1 General Farm Characteristics

632.1.1.1 Physical Characteristics

The geographic distribution of farms is shown in Figure 3-3 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).

On 43 of the farms there were areas of flat to undulating terrain adjoining areas of hilly to steep terrain. The topography on the remaining five farms consisted of flat to undulating land with no hilly terrain. In general, areas of steep hilly terrain were present in case paddocks while areas of flat or undulating land were present in control paddocks. On 12 farms, case mobs were reported on the same paddocks over multiple years, with 11 paddocks reporting affected calves for two years, and two paddocks for three years.

632.1.1.2 Case and control Paddocks

A total of 50 case paddocks were identified from the study. These paddocks ranged from 16ha to 250ha (mean 92.3ha) in size and were located on steep land in 37 (74%) cases and contained areas of native pastures in 28 (56%) paddocks and mixed pastures in 13 (26%) paddocks. Soils on these paddocks were all derived from granite (producer reported) and usually contained large granite rocks protruding from the soil. Native pastures were predominantly comprised of *Microlaena spp*, *Austrodanthonia spp* and red grass (*Bothriochloa macra*). The main weed species present included blackberry (*Rubus fruticosis*), thistle (*Onopordum acanthium*, *Carthamus lanatus*) and Pattersons curse (*Echium plantagineum*). Tree species included eucalyptus species, kurrajong and wattle. Cattle in 39 mobs, 31 of which were case mobs, reportedly grazed Stringybark eucalypts, which were generally present in hilly paddocks. 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. Eleven (22%) paddocks adjoined a forest or national park, nine (18%) were adjacent to a public road and 16 (32%) adjoined a neighbouring farm.

Seventy five control paddocks ranging in size from 6ha to 350 ha (mean 68ha) were identified. The topography of 62 (83%) control paddocks was flat to undulating. Fifty-four (72%) paddocks contained improved pasture and 13 (17%) contained mixed pastures. Improved pastures were comprised of perennial and annual ryegrasses, phalaris and clover species with similar types of weeds and tree species to native pastures found on the same farm. Soils were derived from granite in all control paddocks (producer reported). Water in control paddocks was supplied from creeks or rivers in 21 (28%) paddocks, dams in 48 (64%) and reticulated water systems in six (8%) paddocks.

632.1.1.3 Management characteristics

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 mobs and mob sizes ranged from five to 500 breeding cows with a mean of 72. Of the 168 mobs examined, 88 were Angus, 22 Hereford, 31 crossbreed and 27 other purebred (Murray grey, Shorthorn, Limousine, Charolais). On five farms, producers reported observing similar deformities in their lambs, with rotated shortened front limbs. In one case, American Bison (*Bison bison*) were present in a case mob and produced deformed calves. Stocking rate and mob size (number of breeding cows) for case and control mobs is shown in Table 3-10. Movement of stock during gestation was practiced on only 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 loosely coincided with the seasons of autumn or spring. Autumn calves were usually born between February and June, while spring calves were born between July and November. Interviews with producers suggested 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 mobs, but cases were reported for spring calving mobs.

General management practices on farms were similar, and in most cases changed with the seasonal requirements on each farm. Marking and first vaccination coincided with the start of joining. This typically occurred between September and the end of December for spring calving mobs. Weaning on most farms was governed by the quality of the season. Early weaning usually followed a dry summer period and coincided with pregnancy testing to allow for the sale of empty cows. In better years when feed was plentiful calves were left with their mothers up to eight months of age on some farms.

Vaccination was routinely performed on 25 farms, while drenching was only performed regularly on 20 farms. Supplement feeding was provided to 50 (75.6%) of the 66 case mobs and 55 (53.9%) of the 102 control mobs. Silage, straw and hay were the most common supplement used, with pellets only fed to 11 control mobs and seven case mobs. Urea was only supplemented in two case mobs. Grain supplements were not reportedly fed to any mobs.

Fertilizer was applied to 24 (32.0%) control paddocks within five years prior to cases, 13 (9.8%) of these within 12 months prior to the birth of affected calves, on 15 farms. Most fertilizer used was single superphosphate applied at a rate of 125kg/Ha. Lime was applied to seven (10.6%) case paddocks and one (0.9%) control paddock in the five years prior to cases. Herbicide treatment was sporadic and consisted of spot spraying of weeds on most farms. Drought was stated as the limiting factor in both fertilizer and herbicide treatment due to limited farm income and lack of rainfall in recent years.

632.1.2 Calf Deformities

Producers reported a total of 799 affected calves born to dams in the 66 case mobs from 2002 to 2007 (Table 3-11). Between 0.7% and 80% of calves in these mobs were affected by CCUO (median 17.3%, lower quartile 5.8% and upper quartile 35.2%, Table 3-11). Seven producers also recorded incidences of CCUO prior to 2002 and were among the properties reported for the 1991 outbreak by Godwin *et. al.* (1992).

In all case mobs at least one sign of disproportionate dwarfism was present in affected calves (Table 3-12). The predominate signs of shortened legs, superior brachygnathia and bent legs were reported in 64 (96.9%), 60 (90.9%) and 64 (96.9%) mobs respectively. The less commonly reported signs of stiff joints, spinal deformities and swollen joints were present in 49 (74.2%), 36 (54.5%) and 36 (54.4%) mobs respectively. While all producers mentioned that pasterns of some affected calves with limb deformities appeared lax at birth, this was always noted to resolve within a few days.

Histopathology results for 8 case mobs demonstrated that the calves sampled were suffering from a chondrodystrophy consistent with the findings of previous cases of CCUO in Australia. Pathology results for BVD and Akabane virus were performed on 8 other mobs and found to be negative, ruling out these viral agents in affected calves.

632.1.3 Other health issues

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 was related to limb deformities in affected calves, in particular a varus 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 producers reported an increased incidence of bloat in affected calves, with one producer losing 20 calves at eight-months-old to bloat in a one-week period. Three producers 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. These conditions have not previously been recorded in CCUO calves growing to maturity.

632.1.4 Generalised Linear Mixed Models for Mob Status and Mob Events-trials

632.1.4.1 Descriptive and Univariable Analyses

Contingency tables for categorical farm management and environment explanatory variables investigated for the mob status outcome and the mob events-trials outcome are shown in Appendix 4: Descriptive and Univariable Analysis of Variables for Case-control Study.

Of the 24 farm management and environment explanatory variables, three variables were eliminated due to low numbers per category. Of the remainder, 14 variables were unconditionally associated with the outcome mob status at $P<0.25$. The univariable associations of all farm management and environment explanatory variables with mob status are shown in Appendix 4. High correlation detected between supplement feed type (coded 1 to 4) and supplement feed (coded 1,0) provided led to exclusion of supplement feed provided.

After deletion of one highly correlated variable (supplement feed provided), 17 of the explanatory variables were unconditionally associated with the outcome variable mob events-trials at $P<0.25$. The univariable associations of all farm management and environment explanatory variables with mob events-trials are shown in Appendix 4.

632.1.4.2 Multivariable analyses

The final models for mob status and for mob events-trials are presented in Table 3-13 and Table 3-14 respectively.

For the mob status final model there was no suggestion of over dispersion (Generalised chi-square/d.f=0.34) 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.

632.1.5 Generalised Linear Mixed Model for Paddock Status

632.1.5.1 Descriptive and Univariable Analyses

A full description of the soil variables and a contingency table for environment variables are shown in Appendix 4: Descriptive and Univariable Analysis of Variables for the Case-control Study. 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 univariable associations of soil and environment explanatory variables with the outcome variable paddock status are shown in Appendix 4.

632.1.5.2 Multivariable Analyses

The final model for paddock status is shown in Table 3-15. 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.

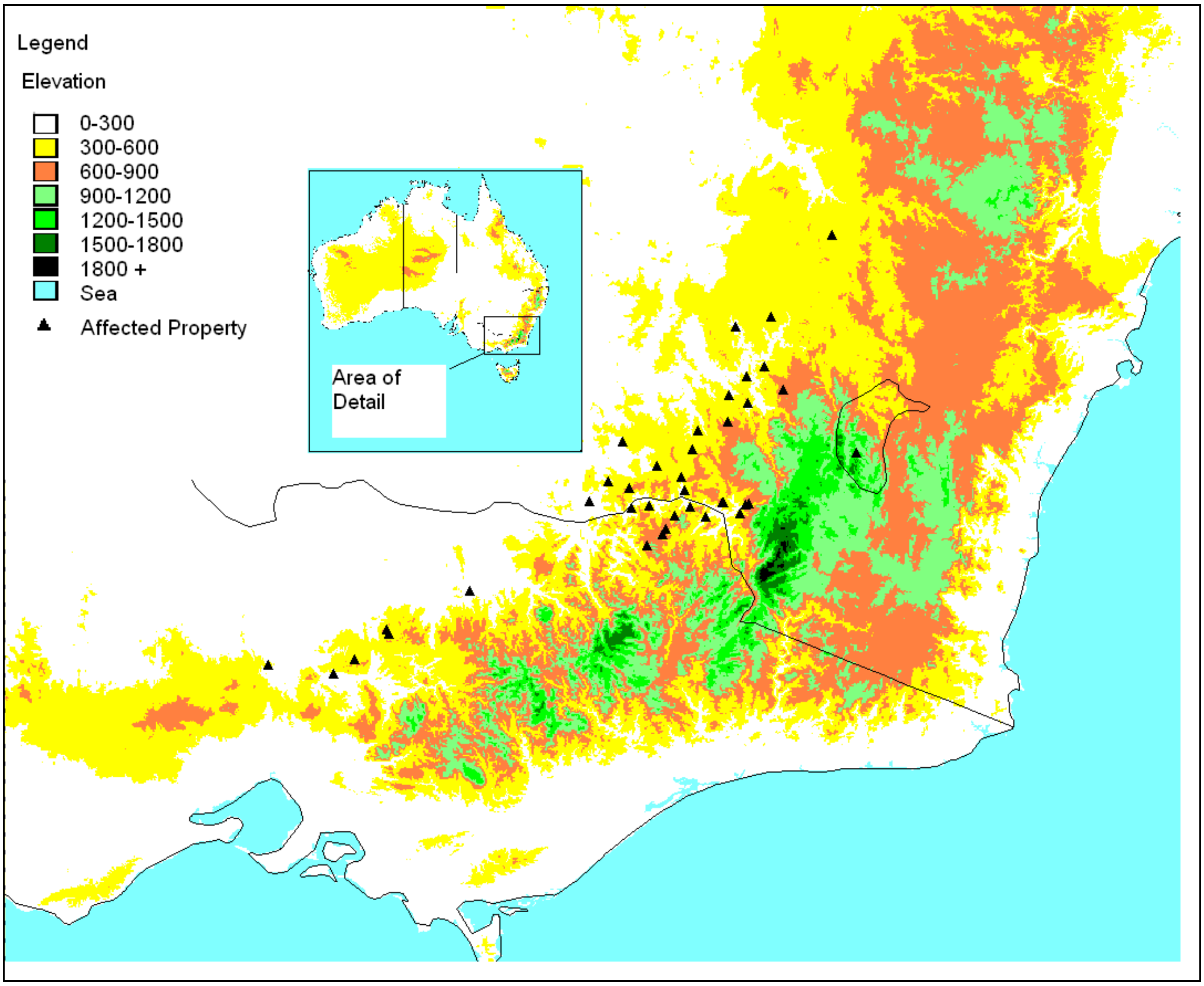

Figure 3-3 Distribution of the 46 farms with affected calves from 2002-2007 in south-eastern Australia that participated in the case-control study

Table 3-10 Descriptive statistics for stocking rate and mob size for 66 case mobs and 102 control mobs from 2002-2007 on 46 farms in south-eastern Australia

Variable		Mean	Std Dev	Minimum	Maximum
Stocking Rate (head/Ha)	Case Mobs	0.9	0.8	0.1	3.8
	Control Mobs	1.0	0.8	0.2	3.4
	Total	0.9	0.8	0.1	3.8
Mob Size (number of breeding dams)	Case Mobs	97.2	115.7	7.0	520.0
	Control Mobs	59.7	35.3	10.0	200.0
	Total	74.5	79.4	7.0	520.0

Table 3-11 Percentage of affected calves per case mob among 66 case mobs from 2002-2007 on 46 farms in south-eastern Australia

				Percentage affected per mob				
Year	Number case mobs	Number affected calves	Number normal calves	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 3-12 Producer reported deformities among affected calves produced by the 66 case mobs from 2002-2007 in south-eastern Australia

Type of deformity exhibited by affected calves						
Shortened legs[a]	Superior brachygnathia[a]	Bent limbs	Spinal deformities	Swollen joints	Stiff joints	% of case mobs
✓	✓	✓	✓	✓	✓	30%
✓	✓	✓	✓			46%
✓	✓	✓				86%
✓						97%
		✓				97%
	✓					91%

a. All mobs experienced either shortened limbs or superior brachygnathia.

Table 3-13 Final generalized linear mixed model for mob status based 66 case mobs and 102 control mobs on 46 farms in south-eastern Australia

Parameters	*b*	SE(*b*)	Odds ratio	LCL (OR)	UCL (OR)	*P*-Value
Random effects						
Farm ID	0.682	1.362				
Paddock ID	2.192	1.479				
Intercept	-6.408	4.993				
Confounders						
Dam age at calving						0.081
Mixed-age cow groups			1.0			
Heifers only	1.663	0.857	5.3	0.9	29.9	
Single-age cow groups	1.843	1.356	6.3	0.4	98.8	
Year Affected						0.816
2007			1.0			
2002	1.986	5.124	7.3	<0.1	>999	
2003	2.609	4.938	13.6	<0.1	>999	
2004	2.814	4.909	16.6	<0.1	>999	
2005	3.652	4.982	38.6	<0.1	>999	
2006	2.151	4.948	8.6	<0.1	>999	
Fixed effects						
Main pasture type in paddock						<0.001
Improved pasture			1.0			
Native pasture	4.399	1.126	81.4	8.3	798	
Mixed pasture	0.524	0.885	1.7	0.3	10.2	
Paddock predominantly hilly to steep terrain						0.008
No			1.0			
Yes	2.195	0.831	8.9	1.7	48.5	
Inadequate pasture[a]						0.001
No			1.0			
Yes	2.759	0.771	15.8	2.8	89.1	

Generalised chi-square = 50.8
Generalised chi-square/d.f. = 0.34
a. Inadequate pasture defined as that not in sufficient quantity to maintain weight of grazing cattle during gestation

Table 3-14 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

Parameters	*b*	SE(*b*)	Odds ratio	LCL (OR)	UCL (OR)	*P*-Value
Random effects						
Farm ID	1.298	1.178				
Paddock ID	3.426	0.994				
Intercept	-8.683	0.712				
Confounders						
Dam age at calving						<0.001
Mixed-age cow groups			1.0			
Heifers only	1.936	0.573	6.9	2.1	22.2	
Single-age cow groups	2.361	0.828	10.6	1.9	57.2	
Year affected						<0.001
2007			1.0			
2002	-0.938	0.461	0.4	0.2	1.0	
2003	1.881	0.428	6.5	2.7	15.7	
2004	1.663	0.370	5.3	2.5	11.2	
2005	1.202	0.451	3.3	1.3	8.3	
2006	1.428	0.343	4.2	2.1	8.4	
Fixed effects						
Main pasture type in paddock						0.025
Improved pasture			1.0			
Native pasture	1.809	0.668	6.1	1.6	23.8	
Mixed pasture	0.965	0.694	2.6	0.6	10.8	
Paddock predominantly hilly to steep terrain						<0.001
No			1.0			
Yes	2.051	0.435	7.8	3.2	18.9	
Supplement feed type						0.019
Nil			1.0			
Hay	0.395	0.529	-	-	-	
Pellet	-0.079	0.956	-	-	-	
Silage	-0.561	0.438	-	-	-	
Inadequate pasture[a]						<0.001
No			1.0			
Yes	0.201	0.797	-	-	-	
Interaction Terms[b]						
Inadequate pasture x Feed type						
Hay x inadequate Pasture	1.979	1.003	-	-	-	0.004
Pellet x inadequate pasture	3.950	1.399	-	-	-	
Silage x inadequate pasture	2.667	1.011	-	-	-	

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 3-15 Final generalized linear mixed model for paddock status based on 50 case paddocks and 75 control paddocks on 46 farms in south-eastern Australia.

Parameters	*b*	SE(*b*)	Odds ratio	LCL (OR)	UCL (OR)	*P*-Value
Random effects						
Farm ID	0.542	0.910				
Intercept	-4.498	1.044				
Fixed effects						
Main pasture type in paddock						<0.001
Improved pasture			1.0			
Native pasture	3.371	0.871	29.1	5.1	165.1	
Mixed pasture	1.826	0.739	6.2	1.4	27.1	
Paddock predominantly hilly to steep terrain						0.016
No			1.0			
Yes	1.639	0.683	5.1	1.3	20.1	
Soil potassium	3.399	1.206	29.9	2.7	331.5	0.006

Generalised chi-square = 101.83
Generalised chi-square/d.f. = 0.93

632.2 Part 4 – Analysis of Rainfall Data

All 799 cases of CCUO included in analysis were diagnosed between the months of July and October, with 94.9% (n=759) of cases occurring in July to September. A total of 32 weather stations were selected to represent rainfall data on the 46 farms. The stations were located between one and 20 km (mean 9.6km, standard deviation 4.6km) from their respective farms. Seven of the stations were used for two or more farms. The time-series of cases of CCUO and average monthly rainfall for the period October 2001 to December 2007 are shown in Appendix 5: Time Series Analysis Figures and Tables.

632.2.1 Time Series Analysis

The linear trend in the time series cases of CCUO (b= -0.0395, t=-0.286, P=0.776) was not statistically significant. All analysis was performed using the original time series for cases of CCUO. There were significant positive autocorrelations (P<0.05, r>0.2248) for cases of CCUO at a lag of 1, 11, 12, 13 and 24 months. No significant negative autocorrelations were detected (Appendix 5). Significant positive partial autocorrelations were detected at one, three and 11 months (P<0.05, r>0.2248) and negative at 2, 4, 13 and 24 months (P<0.05, r<-0.2248; Appendix 5). These results confirm the yearly seasonal pattern of cases of CCUO. Based on this 12 month cycle, the best fitting autoregressive model of cases of CCUO included the cases diagnosed in the previous two months (i.e. at lags of t-1 and t-2) as shown in Table 3-16.

There were a number of significant positive and negative cross correlations between the number of cases of CCUO and the time series for monthly average rainfall as shown in Appendix 5. The strongest negative correlation was at a lag of five months (r=-0.2952), corresponding to the middle of the second trimester of pregnancy in cows giving birth to CCUO calves. For three-month moving average rainfall, the most significant negative cross correlations with the number of CCUO calves were at a lag of five and six months (r=-0.3723 and r=-0.3508, Appendix 5).

The best fitting regression model using cases of CCUO and monthly rainfall included cases of CCUO at one and two months lag and average monthly rainfall at five month's lag (Table 3-17). The ACF and PACF of the residuals of this regression model appeared stationary with the exception of an autocorrelation at a lag of 12 months (Appendix 5). Residuals appeared independent with constant variance. An outlier was detected at t=22 (z=4.95, P<0.01).

The best fitting model for cases of CCUO and three-month moving average rainfall included cases of CCUO at one and two months lag and three-month average rainfall at five months lag (Table 3-18). For this model, inclusion of rainfall at 12 months lag improved model fit. The ACF and PACF of the residuals appeared stationary with no significant correlations (Appendix 5). Examination of residuals revealed an outlier at t=22 (z=4.67, P<0.01) however residuals appeared independent with constant variance. Model fit was not improved by incorporating the average monthly rainfall at five months lag into this model.

632.2.2 Logistic Regression Analysis

Two explanatory variables were created for rainfall deficiency based on the results of the time series analysis. Unconditional associations of the two explanatory variables (monthly rainfall and three-month average rainfall at 5 months lag within the first decile) with the outcome month status are shown in Appendix 5: Time Series Analysis Figures and Tables. The final multivariable model including random effect and fixed effect variables is shown in Table 3-19. There was no suggestion of over dispersion (Generalised chi-square/d.f=0.95) and standardised conditional residuals were normally distributed with no unusual influence of any observation. The ICC for the random effect farm was calculated as 0.04 and that for year as 0.14.

Table 3-16 Best fitting autoregressive model (AICc=6.89) of cases of CCUO in spring calving herds on 46 farms in south-eastern Australia from October 2001 to December 2007

633 Lag (months)	634 *B*	635 S.E.(*b*)	636 *t*-ratio	637 P-Value
638 constant	639 5.805	640 2.322	641 2.500	642 0.015
643 1	644 0.927	645 0.103	646 8.947	647 <0.001
648 2	649 -0.485	650 0.103	651 -4.679	652 <0.001

Table 3-17 Best fitting regression model (AICc=6.95) of cases of CCUO on 46 farms in south-eastern Australia from October 2001 to December 2007, and average monthly rainfall

653 Variable	654 Lag (months)	655 *b*	656 S.E.(*b*)	657 *t*	658 P-Value
659 Constant	660	661 12.844	662 4.289	663 2.994	664 0.004
665 Cases of CCUO	666 1	667 0.844	668 0.109	669 7.715	670 <0.001
671 Cases of CCUO	672 2	673 -0.434	674 0.107	675 -4.060	676 <0.001
677 Monthly rainfall	678 5	679 -0.135	680 0.072	681 -2.120	682 0.038

Table 3-18 Best fitting regression model (AICc=6.93) of cases of CCUO on 46 farms in south-eastern Australia from October 2001 to December 2007, and 3-month moving average of monthly rainfall

683 Variable	684 Lag (months)	685 *b*	686 S.E.(*b*)	687 *t*	688 P-Value
689 Constant	690	691 7.252	692 7.569	693 0.958	694 0.341
695 Cases of CCUO	696 1	697 0.730	698 0.114	699 6.365	700 <0.001
701 Cases of CCUO	702 2	703 -0.443	704 0.106	705 -4.149	706 <0.001
707 Three-month average rainfall	708 5	709 -0.072	710 0.033	711 -2.155	712 0.035
713 Three-month average rainfall	714 12	715 0.083	716 0.034	717 2.444	718 0.017

Table 3-19 Final generalized linear mixed model for month status demonstrating association with three-month average rainfall deficiency at a lag of five months on 46 farms in south-eastern Australia for the calving months July to October 2002-2007

719 Parameters	720 *b*	721 SE(*b*)	722 Odds ratio	723 LCL (OR)	724 UCL (OR)	725 P-Value
726 *Random effects*	727	728	729	730	731	732
733	734	735	736	737	738	739
740 Farm ID	741 0.014	742 0.119	743	744	745	746
747 Year	748 0.539	749 0.340	750	751	752	753
754	755	756	757	758	759	760
761 Constant	762 -1.530	763 0.334	764	765	766	767
768	769	770	771	772	773	774
775 *Fixed effects*	776	777	778	779	780	781
782	783	784	785	786	787	788
789 Three-month average rainfall lagging 5 months	790	791	792	793	794	795 < 0.001
796 No	797	798	799 1.0	800	801	802
803 Yes	804 1.183	805 0.294	806 3.3	807 1.8	808 5.8	809
810	811	812	813	814	815	816

817 Generalised chi-square = 443.7

818 Generalised chi-square/d.f. = 0.95

818.1 Part 5 – Case Study and Supplement Trial

818.1.1 Case Study

818.1.1.1 Physical Characteristics of the Farm

The case study farm is a 2900 Ha beef enterprise located in the upper Murray Valley of NSW on the south western slopes of the Great Dividing Range, approximately 140km east of Albury and 10km north of the Victorian border. The property borders a public road to the north east, the Kosciusko National Park to the east and other properties on the remaining boundaries. The property is divided into approximately 24 paddocks fenced primarily on the basis of topography and convenience. Paddocks on the eastern side of the property rise to the national park and are hilly to steep and moderately timbered with stands of trees containing *Eucalyptus* spp. (including stringybark), *Acaia* spp., and *Brachychiton populneum*. It was noted by the manager that during feed shortages, pregnant dams were often noted grazing the bark of stringybark Eucalypts. Earthen dams are used for watering stock in these paddocks. Similar steep paddocks occur on the western side of the property but with reduced tree cover, where a large hill rises over 200m from the base. The flatter regions of the farm follow a creek through the centre of the property and contain few shade trees. A permanent creek and earthen dams water these paddocks.

Soils on the farm consist of granite derived sandy loams. Soil tests conducted in 2006 by the Livestock Health and Pest Authority and in 2009 as part of a case-control study are recorded in Appendix 6: Case Study Tables. Soils in paddocks associated with CCUO were similar to those in paddocks never associated with CCUO. Fertilizer use in the latter probably resulted in higher levels of phosphorous and sulphur for the 2006 values in Suckers paddock. The paddocks with the lowest pH levels also have the highest levels of iron and manganese, with the 2006 value of manganese lowest in Mocattas paddock. Zinc is relatively low in all paddocks. There is little difference between paddocks for other significant nutrients.

The rainfall recorded for this farm varied from year to year with the highest monthly rainfall usually occurring in the winter months from May to October (Bureau of Meteorology, 2009). The long term average annual rainfall collected on the farm is 778mm. This yearly total has not been reached since 2002 and has averaged only 577mm for the seven year period from 2002-2007 (Appendix 6). The years that produced the highest annual prevalence of CCUO calves to date (2004, 2006 and 2009) did not receive the lowest yearly rainfall. However, these years did experience very dry months in February and March. In 2009, there was a two month period between January 16th and March 20th when no rain fell on the farm.

818.1.1.2 Pasture management

On this farm 17 paddocks contain improved pastures and 7 paddocks have native pastures. The results of a plant survey conducted in 2006 by a Livestock Health and Pest Authority veterinarian are provided in Appendix 6; along with the results of pasture analyses performed in October 2008 and March 2009. The differences in pasture nutrient content between October 2008 and March 2009 in the improved pasture are a result of the complex relationship between soil and plants and the effects of season. The dry March pastures are lower in all nutrients with the exception of calcium, copper and manganese. Comparison of the improved and native (unimproved) pastures for March shows that the native pasture was considerably lower in all nutrients with the exception of molybdenum and calcium. All pastures tested were below the recommended level of intake for copper. Zinc and manganese were lowest in the native pasture results.

Fertilizer, herbicide and pesticide use are performed on an ‘as needed’ basis on the farm. Fertiliser use has been restricted in recent years due to the high cost associated with superphosphate fertilisers. Routine testing of soil levels of basic nutrients are conducted before the decision to fertilise is made. Single super phosphate and molybdenum fortified super phosphate is used on improved pastures. Fertilisers had not been applied to native pastures since 2002, and there was no reliable history on application before 2001. Spot spraying of weeds is frequently performed as necessary throughout the year using Igran (terbutryn), Grazon (triclopyr and picloram), MCPA and Brushoff (metsulfuron methyl).

818.1.1.3 Herd structure and management

The self replacing beef herd consists of approximately 880 Angus and Angus cross Hereford breeding cows, 30 bulls (Angus and Wagyu), 350 steers and 450 weaner heifers. Small Wagyu bulls are used on heifers in an attempt to increase calving ease through smaller calves. Breeding cows are divided into 10 to 11 mobs based on age and are paddock joined with selected bulls. Once pregnancy tested (usually at weaning in March), cows are moved to a gestation paddock where they remain until calving.

From 2002 to 2004, cattle were calved in both spring and autumn, with a gradual shift to spring calving in 2005 as shown in Appendix 6. Since 2005, joining has occurred from late October until late December, with calving commencing in late July and ending in late September.

Marking of calves commences in late October and weaning may commence in February or March, depending on pasture availability. If pasture is in low supply, early weaning is used to make more pasture available for pregnant dams. Calves are weaned onto a mixture of silage and pellets before being moved to fattening pastures, feedlots or saleyards.

Silage is the main supplement feed used on the farm and is grown on the property and stored buried in pits. It is used to supplement cattle when pasture is minimal. Not all mobs receive the supplement due to the distance between pits and some paddocks. The paddocks with a history of CCUO occurrence receive supplement feeds less often than other paddocks closer to the silage pits.

The cattle suffer from very few health problems with the exception of dystocia associated with CCUO calves. Cows are treated with a variety of anthelmintics and annually vaccinated with a generic 7 in 1 vaccine.

818.1.1.4 History of CCUO on the property

Chondrodysplastic calves were first reported by the manager to the local district veterinarian (Hume) in 2003, with more reported in 2004, 2005, 2006, 2007 and 2009 as detailed in Table 3-20. A number of mildly affected calves from previous years have been retained by the manager and exhibit a range of deformities including shortened and bent limbs, kyphosis, dished faces and spastic paresis of one or both hind limbs. Some deformed cows have given birth to normal calves in subsequent years. The birth of affected calves on this property has coincided with drought conditions and the location of cows during gestation, with three paddocks identified by the manager linked to producing affected calves.

818.1.1.5 Previous interventions

On advice from nutritionists and the district veterinarian, the manager has implemented the use of supplements in an attempt to ameliorate the number of CCUO calves born on the property. Use of manganese sulphate mixed with salt as a lick, and an oral mineral drench (Maximin, Virbac) for suspect mobs of cattle grazing paddocks associated with the condition commenced in 2004. The oral drench was given once each year to all cows at the time of calf weaning and the manganese salt supplement was fed out in troughs sporadically during the gestation period with no set timing. Use of the manganese lick ceased after the 2006 calving period, however use of the oral drench continued. These measures have failed to prevent the birth of affected calves in years of low rainfall (2008 experienced a wetter summer with ample pasture). No other preventative measures have been implemented on this farm, except avoiding grazing pregnant cattle in the suspect paddocks. The dry conditions and lack of pasture since 2002 have forced the manager to use all three paddocks in most years, resulting in the birth of affected calves.

818.1.2 Supplement trial

The trial commenced in March 2009 and concluded in October 2009. The first treatment was given in mid March, with follow up treatments in early April and mid June. Cows started calving in the second week of August and had all calved by the start of October. Of the 20 cows selected for inclusion in this study, one from the treatment group went missing after the first treatment and another three cows (two from the treatment group and one from the control group) were missing at the post-calving muster and subsequently found during marking. Two cows from the control group and one from the treatment group gave birth to live deformed calves, and one cow from the treatment group gave birth to a dead deformed calf. No difference in proportion of CCUO calves was found between study groups (Fisher's Exact Test 2 tail P=1). The dead affected calf was not found in time to perform useful pathology tests and the live calves were considered viable and not sacrificed for pathology testing. In any case, testing of calves post suckling is unlikely to reflect the mineral status in utero. At the final treatment, all cows except for one from the control group had dry rough coats with excessive dander. All cows were otherwise in general good health with similar body condition score to when the trial began.

Changes were observed in liver trace mineral levels and whole blood manganese levels measured in this trial (Table 3-21). There were significant decreases in the values of liver iron ($P<0.001$,Table 3-22), zinc ($P<0.001$, Table 3-23) and manganese ($P=0.009$, Table 3-24) in both the control and treatment groups and some significant changes in liver copper. There was a significant time difference in liver copper levels in both groups, first increasing then decreasing overall ($P<0.001$, Table 3-25) and a significant treatment x time interaction in the liver copper model ($P<0.001$, Table 3-25). There were no significant differences between control and treatment groups for any variables, although liver copper values in treatment group were higher than in the control group at the third treatment and at the follow-up biopsy post-calving.

Results of the post-calving liver biopsies showed that three cows from the control group and two from the treatment group were low in zinc (<0.3 mmol/kg wet wt) and three from the control group were low in copper (<0.08 mmol/kg wet wt). An additional test of liver glutathione peroxidise showed that all cows were deficient in selenium (GPX<3 U/g wet wt).

Table 3-20 Yearly cattle numbers at NSW44 2002-2009

Year		Number bulls	Number breeding cows	Number mobs	Number affected mobs	Number affected calves	Annual prevalence (%)[a]
2002	Autumn Spring	22	600 331	9	0	0	0
2003	Autumn Spring	24	452 573	10	0 1	0 44	4.3
2004	Autumn Spring	20	140 796	9	0 2	0 90	9.6
2005	Spring	27	934	9	1	5	5.3
2006	Spring	31	908	9	1	50	5.5
2007	Spring	29	880	11	1	8	0.6
2008	Spring	30	960	10	0	0	0
2009	Spring	30	1100	12	2	65	5.9

a. Annual prevalence based on number of affected calves divided by total number of breeding cows per year.

Table 3-21 Mean values of liver and blood trace minerals in control cows (n=10) and cows (n=10) treated with a parenteral mineral supplement at different treatment times

Variable	Treatment time[a]	Treated group	Control group
Liver Manganese	1	0.045	0.043
mmol/kg wet weight	2	0.043	0.041
	3	0.042	0.040
	Post-calving	0.049	0.047
Liver Iron	1	1.673	1.964
mmol/kg wet weight	2	1.531	1.720
	3	1.199	1.312
	Post-calving	0.89	1.048
Liver Zinc	1	0.402	0.404
mmol/kg wet weight	2	0.325	0.310
	3	0.302	0.297
	Post-calving	0.307	0.300
Liver Copper	1	0.418	0.484
mmol/kg wet weight	2	0.695	0.510
	3	0.537	0.284
	Post-calving	0.349	0.108
Whole blood manganese	1	97.000	99.200
nmol/L	2	86.560	78.500
	3	89.000	72.000
Plasma manganese	1	-[b]	-[b]
nmol/L	2	27.778	28.600
	3	33.889	33.778

a. The 3 treatments were spaced at 6 week intervals with final treatment 6 weeks pre-calving. Results are also presented for liver biopsy conducted 4 weeks post-calving although no treatment was administered at this time.
b. No values available for this treatment

Table 3-22 Effect of parenteral supplementation of trace minerals on liver iron levels during pregnancy in beef cows (n=20) supplemented at 6 week intervals from mid gestation

Parameters	Estimate	Standard error	95% CI	P (χ^2)
Random effects				
Cow ID	0.02	0.05	0.003, >999	0.65
Intercept	1.79	0.11	1.55, 2.05	
Fixed effects				
Treatment times				<0.001
Treatment 1	0			
Treatment 2	-0.12	0.04	-0.19, -0.04	
Treatment 3	-0.54	0.14	-0.85, -0.23	

Table 3-23 Effect of parenteral supplementation of trace minerals on liver zinc levels during pregnancy in beef cows (n=20) supplemented at 6 week intervals from mid gestation

Parameters	Estimate	Standard error	95% *CI*	*P* (χ^2)

Random effects				
Cow ID	<0.001	<0.001	-	0.9
Intercept	0.41	0.013	0.037, 0.043	
Fixed effects				
Treatment times				<0.001
Treatment 1	0			
Treatment 2	-0.08	0.018	-0.12, -0.05	
Treatment 3	-0.10	0.017	-0.13, -0.07	

Table 3-24 Effect of parenteral supplementation of trace minerals on liver manganese levels during pregnancy in beef cows (n=20) supplemented at 6 week intervals from mid gestation

Parameters	Estimate	Standard error	95% *CI*	*P* (χ^2)
Random effects				
Cow ID	<0.001	<0.001	-	<0.001
Intercept	0.044	0.001	0.042, 0.047	
Fixed effects				
Treatment times				0.009
Treatment 1	0			
Treatment 2	-0.002	0.001	-0.004, -0.002	
Treatment 3	-0.003	0.001	-0.005, -0.001	

Table 3-25 Effect of parenteral supplementation of trace minerals on liver copper levels during pregnancy in beef cows (n=20) supplemented at 6 week intervals from mid gestation

Parameters	Estimate	Standard error	95% *CI*	*P* (χ^2)
Random effects				
Cow ID	0.028	0.011	0.015, 0.069	<0.001
Intercept	0.510	0.060	0.38, 0.66	
Fixed effects				
Treatment Group				0.113
Control	0			
Treated	-0.048	0.091	-0.24, 0.14	
Treatment times				<0.001
Treatment 1	0			
Treatment 2	-0.034	0.047	-0.14, 0.06	
Treatment 3	-0.230	0.043	-0.32, -0.14	
Interaction terms[a]				
Treated x Treatment time				<0.001
Treated x Treatment 2	0.243	0.063	0.11, 0.37	
Treated x Treatment 3	0.301	0.058	0.18, 0.42	

a. Interaction terms with zero estimate not shown.

Table 3-26 Effect of parenteral supplementation of trace minerals on whole blood manganese levels during pregnancy in beef cows (n=20) supplemented at 6 week intervals from mid gestation

1160 Parameters	1161 Estimate	1162 Standard error	1163 95% *CI*	1164 *P* (χ^2)
1165 *Random effects*	1166	1167	1168	1169
1170	1171	1172	1173	1174
1175 Cow ID	1176 0.02	1177 0.05	1178 0.003, >999	1179 0.65
1180	1181	1182	1183	1184
1185 *Intercept*	1186 1.79	1187 0.11	1188 1.55, 2.05	1189
1190	1191	1192	1193	1194
1195 *Fixed effects*	1196	1197	1198	1199
1200	1201	1202	1203	1204
1205 Treatment times	1206	1207	1208	1209 0.009
1210 Treatment 1	1211 0	1212	1213	1214
1215 Treatment 2	1216 -0.12	1217 0.04	1218 -0.19, -0.04	1219
1220 Treatment 3	1221 -0.54	1222 0.14	1223 -0.85, -0.23	1224
1225	1226	1227	1228	1229
1230	1231	1232	1233	1234

1234.1 Part 6 – CCUO in 2008-2009

1234.1.1 Follow-up Interviews

Two farmers who participated in the case-control study reported CCUO calves born in 2008, one from Tintaldra, Victoria and one from The Rock, NSW. Only seven affected calves were born on these properties.

Ten farmers reported the birth of CCUO calves in 2009, five from NSW, and five from Victoria. Approximately 100 CCUO calves were reported, with the worst affected farm reporting 70 calves. One Victorian farmer reported 5 deformed and approximately 100 weak and ataxic calves out of a mob of 134. The cows had been grazing very dry native pastures on hilly terrain for most of gestation.

Six farmers who did not report calves with CCUO reported changes in farm management.

- One NSW farmer had not observed any deformed calves since introducing a rotational grazing system, where cows were rotated through suspect paddocks.
- A Victorian farmer reported avoiding the use of suspect paddocks by downsizing the herd, and had not had any CCUO calves since.
- One other NSW farmer had downsized the herd to reduce grazing pressure on pasture and had increased supplement feeds.
- Three other farmers increased the quality and quantity of supplement feed and maintained cows in good condition score throughout gestation.

1234.1.2 Additional Reports

There were no reports of CCUO calves on other farms in 2008.

In 2009 a farmer from Collie in Western Australia reported the birth of 4 out of 11 calves fitting the description of CCUO. The cows had been fed home-grown paddock hay for most of gestation. The main difference with previous years was that the supplement in 2009 did not contain oats.

A government veterinarian from South Australia reported 4 out of 12 calves fitting the CCUO definition born on a farm in the Adelaide hills. Pathology on calf liver suggested low manganese status. Results of histopathology from one affected calf demonstrated a chondrodystrophy consistent with that reported by McLaren *et al.* (2007) for calves in north eastern Victoria. This farm was reported to have experienced only 0.5mm of rain for the 2-month period of January-February 2009.

In addition, the veterinarians investigating these two cases anecdotally reported other farmers in the same districts who had CCUO calves born in 2009 but did not initiate an investigation.

1235Discussion

The work of this project has advanced our knowledge of the epidemiology of CCUO in Australia. In response to industry request for research on CCUO, it undertook the first nationwide survey of rural veterinarians regarding CCUO, a well-designed case-control study that enrolled sufficient cases and controls to draw valid conclusions, the first application of time series analysis to a condition linked to maternal nutritional deficiency, and the first on-farm trial of an injectable multimineral supplement on pregnant cows on an affected farm in Australia. As a result we now have a more complete picture of the spatiotemporal distribution of CCUO, its characteristic features, and of management and environment conditions associated with occurrence. These findings provide the basis for extension of information to producers outlining the key features of CCUO and general recommendations to aid reduction in occurrence.

This discussion will consider epidemiological features of CCUO in Australia, findings of the supplement trial, and the strengths and weaknesses of various aspects of our work.

1235.1 Case definition

The case definition used in this study based on that of McLaren *et al.* (2007) proved to be robust. It enabled veterinarians to differentiate CCUO-affected calves from calves with similar disorders because it required the clinical signs of disproportionate dwarfism to be present at birth in calves from normal dams of any breed. Predominate signs among affected calves in 66 case mobs from 2002-2007 were shortened limbs and superior brachygnathia. No other known condition causes such consistent disproportionate dwarfism except for genetic disorders that are well recognised and breed specific. Bovine viral diarrhoea, whilst known to produce clinical signs in calves including small size and skeletal deformities similar to CCUO, is consistently accompanied by other reproductive disorders (not a feature of cases identified in this project) and the histopathological changes differ considerably to those found in CCUO calves.

Use of a case definition that did not include histopathology proved essential in this work due to the absence of bone pathology reports for over 94% of cases reported from 2002 to 2007. Whilst we acknowledge concern about the low number of histopathology confirmed CCUO cases, this was an inherent risk in the conduct of a principally retrospective investigation, and we consider the number of farms likely to have been misclassified to be low due to the fact there was no evidence among identified cases of a genetic aetiology or of other signs typical of BVD outbreaks such as increased rates of abortion.

1235.2 Distribution

This project through the analysis of case records provided by veterinarians and published data has provided the most complete description of the spatio-temporal distribution of CCUO in Australia to date. The identification of previously unrecorded cases found that the number of affected farms is higher than stated in official records. Moreover this report documents a marked increase in the frequency of CCUO during the last decade as well as occurrence at new locations in southern Australia. Yet we consider, due to producer under-reporting documented in this work (see Section 4.9), that the spatio-temporal distribution of CCUO occurrence in Australia presented is still an under-estimate of actual occurrence particularly in south-east NSW and north-east Victoria.

1235.2.1 Temporal Distribution

CCUO occurrence from 1957 to 2001 was sporadic (Table 3-4) with 76.5% of cases reported for a given year/location involving only a single farm and occurrence reported at more than one location in a year during 6 (50%) of the 12 years with reported cases. This survey identified 12 cases prior to 2002 not previously reported in the literature.

The exception to this sporadic pattern prior to 2002 was the single outbreak involving 52 farms in NSW during 1991.

From 2002 CCUO occurrence has increased with reports each year involving multiple farms. Compared to the previous 44-year period when CCUO reportedly occurred during only 12 (27.3%) years, affected calves were reported every year from 2002 to 2007 (Table 3-3) with outbreaks (defined for the purposes of this work as involving ≥10 farms in a year) occurring during 4 of these 6 years.

1235.2.2 Spatial Distribution

Since 1957 CCUO was predominately reported in the south western slopes of NSW and north-east Victoria, with the exception of the 1991 outbreak where cases extended north to the central tablelands district of NSW.

For sporadic cases prior to 2002, only 3 (9.3%) farm locations were outside the focus region of south-east NSW and north-east Victoria (Table 3-4), and from 2002-2007, 4 (3.4%) affected farms were located elsewhere (Table 3-3). Thus CCUO is principally a condition seen in beef herds in south east NSW and north east Victoria with occasional cases occurring in south west Western Australia, the central and southern tablelands of NSW, and recently in South Australia. However, the occurrence of cases in Western Australia during three years (37.5%) from 2002-2009 compared to one year (2.3%) from 1957-2001, plus the histopathology confirmed case during late 2009 in South Australia, indicates that in recent years CCUO has occurred more often outside the focus region and extended to previously unreported locations in southern Australia.

1235.3 Prevalence

Numbers of affected calves and total calves born per year needed to calculate CCUO prevalence on affected farms were only available on veterinary records for some cases. A comparison of farm prevalence across time showed median prevalence ranged from 7 to 13% (Table 4-1), however due to the missing data for 4 farms for 1957-2002 and 54 farms for 2002-2007, no conclusion about a change in farm prevalence can be made.

Table 4-1 On-farm prevalence of CCUO based on available records for reported cases in Australia

Time period	Number of farms	On-farm prevalence (%)			
		Minimum	Mean	Median	Maximum
1957-2001	10	1.1	21.0	10.6	92.3
1991 outbreak	52	1.7	18.8	13.3	75.0
2002-2007	55	0.9	12.5	7.2	92.0

1235.4 Financial Cost

Income lost by affected farmers due to the 1081 CCUO calves reported for 2002-2007 was estimated to total AU$818 315 with an average loss per affected calf of AU$757 based on a simple “loss of income” model. The true cost to producers based on gross margin should include the losses associated with the production of the affected calf and the follow on losses. Under this model, the loss per calf almost doubled to AU$1376 per affected calf (Table 3-9). This compares to estimated losses through *Neospora caninum* abortion of NZ $900 (approx AU$750) per calf aborted (Reichel and Ellis, 2006). The estimate for CCUO losses based on gross margin presented here is only approximate and may vary widely between farms due to differences in expenditure and management. Higher costs will be experienced with farmers spending more on pasture improvement and supplemental feed, while farmers who retain rather than cull cows that give birth to CCUO calves will incur lower costs.

1235.5 Management & Environment Variables Associated with Mob Occurrence

From the data on the 1991 outbreak involving 52 farms in NSW, univariable analysis of nine variables showed significant association of case mobs compared to control mobs with three variables related to nutritional management of dams during second trimester of gestation (hand feeding, feeding hay supplement, grazing inadequate pastures) and with one variable related to the third trimester (hand feeding).

The case-control study involving 46 farms in NSW and Victoria with cases from 2002 to 2007, found four variables significantly associated with CCUO occurrence in a mob (three of which were present in both the mob status and mob events-trials models) that all link either directly or indirectly with poor maternal nutrition during gestation (Table 4-2).

1235.5.1 Maternal Nutrition

These results provide evidence of the role of poor maternal nutrition in the aetiology of CCUO and align with previous reports stating involvement of nutritional stress during gestation from Australia (Barry and Murphy, 1964; Peet and Creeper, 1994; Cave *et al.,* 2008) and several other countries. Dam grazing of poor quality pastures has been a feature of outbreaks documented in California USA (Hart *et al.*, 1932) and South Africa (Stayley et al., 1994).

Inadequate pasture was strongly associated with the birth of CCUO calves. 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 supplement feed. The ingestion of large quantities of soil may also occur when 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 (Kincaid, 2000) The effects of soil consumption on trace mineral nutrition in ruminants are largely unknown but may contribute to a deficiency through the interaction of essential trace minerals with other minerals such as iron. A nutritional disturbance in pregnant dams resulting from geophagia may therefore contribute to CCUO. The low availability of pasture was more likely a direct result of rainfall deficiency rather than poor management or over-grazing.

Main pasture type in paddock found that dam grazing of native and mixed pasture, compared to improved pasture, was associated with the birth of CCUO calves. The quantity of nutrients in different pasture types varies and is dependant on several factors including soil (mineral levels, moisture content), temperature, amount of incident sunlight, stage of plant growth, fertiliser history and pasture species. Climatic conditions during gestation may have resulted in native and mixed pastures deficient in essential nutrients. Due to the retrospective nature of this study, it was not possible to analyse the pastures actually grazed in 1991 and 2002-2007 by dams that gave birth to affected calves.

Supplement feed type found that giving a supplement feed to dams was associated with the birth of CCUO calves in the final model for the outcome mob events-trials. This finding is contrary to expectation and impossible to evaluate further given that the quantity and nutritional content of supplement feeds actually given to dams is not known. The most probable explanation is that mobs feed a supplement were those suffering some of the most extreme nutritional stress and the supplement provided was insufficient to meet nutrient requirements for normal foetal development. This conclusion is supported by the significant associations found also for the supplement feed given at univariable analyses for both mob status and mob events-trials outcomes. The indication that risk of CCUO calves was higher for dams fed silage or pellets than those feed hay, whilst of interest, should not be over-interpreted given the limited information available on supplements feed to dams gathered by the retrospective case-control study. The finding for silage may be more informative given that feeding of silage is a more common management practice (not only occurring during extreme conditions) and an association with grass-silage only feeding has been demonstrated in Canada (Hidiroglou *et al.*, 1992; Proulx and Ribble, 1992; Ribble *et al.*, 1989). During drought periods, many hay and silage supplements are of poor quality and may not contribute significantly to dam nutrition. Although the addition of grain or hay to the diet was found to reduce or prevent CCUO occurrence in North America and Europe (Gunn *et al.*, 1997; Mee, 2001; Ribble *et al.*, 1989), no producers in the case-control study reported feeding grain supplements in years affected by CCUO.

Dam grazing in a *paddock with predominately hilly to steep terrain* was strongly associated with the birth of CCUO calves. These paddocks due to their terrain often contain native rather than mixed or improved pastures, receive less fertilizer, and experience runoff during rainfall that results in drier soils. As a result the pastures on such paddocks are more likely to provide inadequate nutrition for pregnant cattle. A link with dam grazing of undulating to hilly country was also reported by Cave *et al.* (2008).

1235.5.2 Spring Calving

A dominant feature of affected mobs was the birth of CCUO calves in the spring calving season with all case mobs on 52 farms in the 1991 outbreak and all case mobs on the 46 study farms with occurrence from 2002-2007 having calved during the spring season. Further Cave *et al.* (2008) found that all CCUO calves reported in Victoria and NSW were born in the spring seasons of 2003 to 2005.

Although this management variable was not included in the final multivariable models for mob status or mob events-trials, it was strongly associated with the birth of CCUO calves at the univariable level for both outcomes. This finding suggests a seasonal influence on dam nutritional status resulting in CCUO occurrence that concurs with the strong link to poor maternal nutrition during gestation evident in the variables in the final models discussed above.

A seasonal pattern to the occurrence of this condition with affected calves being born during spring is also frequently reported in other countries with recorded outbreaks (Barry and Murphy, 1964; Cave *et al.*, 2008; Gunn *et al.*, 1997; Ribble *et al.*, 1993; Ribble *et al.*, 1989). Spring calving in winter housed cattle is documented to correspond with feeding of hay or silage during mid pregnancy.

The fact that autumn calving mobs on the case-control study farms did not produce CCUO calves, due to the usual seasonal differences in rainfall for specific trimesters of pregnancy between autumn calving and spring calving dams, suggests that low rainfall during a certain stage of gestation may play a significant role in CCUO pathogenesis through its impact on maternal nutrition.

Consideration of these findings led to recognition that rainfall deficit during gestation was worthy of further investigation in this project.

1235.5.3 Control of Confounding

The two factors considered likely to act as confounders, year and dam age at calving, were forced into multivariable models and found to be significantly associated with the mob events-trials outcome.

Pregnant cattle on study farms were run either as heifer only mobs, mobs containing cows of the same age (or year of birth) or as mobs of mixed age cows. This grouping of dams into age groups may have influenced the location in which pregnant dams were located during gestation. Many farmers reported placing heifer only or older, single age cow groups on to poorer hilly terrain. In the case of heifers this was an attempt to keep unwanted weight gain to a minimum and to improve the fitness for first calving. In the case of older age groups, this was done as a last resort when pastures were scarce. This may account for the increased association between heifer groups and single age cow groups with the birth of CCUO calves. An increased risk for heifers compared to mature cows has been noted previously in Canada (Proulx and Ribble, 1992; Ribble and Janzen, 1987).

Table 4-2 Description of the effect on mob level of CCUO occurrence of the 4 variables included in one or both of the 2 final multivariable models presented in this report

Variable	Number of models where variable present	Effect on outcome variables	Description of trend in effect
Inadequate pasture	2	Detrimental	Greater risk of CCUO calves when dams grazed pasture that was not sufficient to maintain body weight during gestation
Main pasture type in paddock	2	Detrimental	Greater risk of CCUO calves when dams grazed native pasture
Paddock predominately hilly to steep terrain	2	Detrimental	Greater risk of CCUO calves when dams kept in a paddock with predominately hilly to steep terrain
Supplement feed type	1	Detrimental	Greater risk of CCUO calves when dams given pellets or silage and to a lesser extent hay as a supplement feed compared to dams not given any supplement feed.

Table 4-3 Description of the effect on pasture CCUO status of the 3 variables included in the final multivariable model presented in this report

Variable	Effect on outcome variable	Description of trend in effect
Main pasture type in paddock	Detrimental	Greater risk of CCUO calves when dams grazed native pasture and to a lesser extent mixed pasture compared to dams that grazed improved pasture
Paddock predominately hilly to steep terrain	Detrimental	Greater risk of CCUO calves when dams kept in a paddock with predominately hilly to steep terrain
Soil potassium	Detrimental	Greater risk of CCUO calves when dams kept in a paddock with increase in soil potassium level

1235.6 Soil and Environment Variables Associated with Paddock Status

The results of the final model for the paddock status outcome further reinforce the previous finding of a direct or indirect link with poor maternal nutrition during gestation by demonstrating strong associations of the two environment variables pasture type and hilly terrain with paddock status for CCUO. These two variables influence dam nutrition in the same manner as outlined previously.

In contrast the findings based on analysis of soil samples in the case-control study were less revealing and did not demonstrate association with the suspect trace minerals manganese and zinc. The univariable associations of the 23 soil variables investigated did not suggest any significant difference between case and control paddocks for 20 (87%) of the variables. Across the 125 study paddocks, soil zinc levels below 3mg/kg were common, being found in 113 (90%) paddocks. In comparison, soil manganese levels below 30mg/kg were only found in 54 (43%) paddocks.

The one soil variable included in the final model for paddock status was soil potassium and the association suggested that an increase in soil potassium level in a paddock was strongly associated with the birth of CCUO calves. An excess of potassium has been shown to induce a mild metabolic alkalosis in ruminants through an anion-cation imbalance which is believed to interfere with calcium homeostasis (Goff, 2000). However, no other dietary interactions have been demonstrated to occur with potassium, and apart from altering acid-base balance, it is unlikely to contribute to CCUO (Whitehead, 2000).

1235.7 Relationship to Occurrence of Drought

This project provides the first definitive evidence of an association between the birth of CCUO calves and rainfall deficit during a specific period in the gestation of affected calves.

Although low rainfall during gestation was a feature noted in reports of the occasional outbreaks in California USA and South Africa (Hart *et al.*, 1932; Stayley *et al.,* 1994), and of the 1957 and 2003-2005 outbreaks in Australia (Barry and Murphy, 1964; Cave *et al*., 2008), the work undertaken in Part 4 – Analysis of climatic data in this project is important in producing strong evidence that drought during the 2nd to 4th months of gestation contributed to the birth of CCUO calves on 46 farms from 2002 to 2007. This work was also novel in applying time series analysis to investigate a link between rainfall and the occurrence of a condition related to maternal nutrition deficiency, rather than the more common application of relating rainfall and other meteorological factors to the occurrence of vector-borne diseases.

The Climate Change in Australia Technical Report (Hennessey and Mpelasoka, 2007) defined agricultural drought as any three-month period in which rainfall is within the first decile (lowest 10% on record). This is the same definition as that used by the Bureau of Meteorology in Australia to describe a drought (Bureau of Meterology, 2009). The variable, three-month average rainfall at five months prior to calving within the first decile, found to significantly increase the risk of CCUO calves by 3.3 times (95%CI 1.8; 5.8) after accounting for random effects (Table 3-19), clearly meets this definition for drought. Thus we can state that a period of drought five months before calving was strongly associated with the birth of CCUO calves on study farms in south-east NSW and north-east Victoria from 2002 to 2007.

The identified risk period for drought - the 2nd to 4th months of gestation – whilst it corresponds with the periods of poor maternal nutrition from 3rd to 6th months and 3rd to 7th months extrapolated previously for outbreaks in pasture-fed cattle in Australia (Barry and Murphy, 1964; Cave *et al.*, 2008) and South Africa (Stanley *et al.*, 1994) respectively, is shorter and starts earlier in pregnancy. Naturally a period of rainfall deficit might not have an immediate effect on pasture as moisture reserves in the soil and/or plant may maintain growth for a while. These longer postulated risk periods based on veterinary observation therefore most probably reflect a carry-over effect into the 5th and 6th months of gestation arising from the long-term effects on pasture growth of low rainfall. In addition when rainfall arrives to end a time of drought it may result in a flush of pasture that is actually low in nutrients and insufficient to compensate the dam nutrient deficiency.

Identification of this risk period for drought aligns with conclusions from the case-control study about dam exposure to inadequate pastures and supplement feeding during gestation, and a seasonal effect seen with spring calving. Low rainfall toward the end of summer is normal in south-eastern Australia, and restricts pasture growth and causes drying of summer pastures. A deficiency in rainfall at this time will further restrict pasture growth and may result in low levels of pasture cover and supplement feeding leading subsequently to a period of restricted feed intake for pregnant cows. Drought may also alter the nutrient uptake of pasture species further affecting maternal nutrition (Whitehead, 2000). These periods of deficiency occurring late in summer correspond with the second trimester of pregnancy at a time when foetal growth is high. In addition, low pasture availability may lead to increased ingestion of soil and reduced water levels in earthen dams, with an increase in the contamination of water with mud and faeces. The ingestion of soil by cattle has been demonstrated to decrease the availability of some trace minerals through antagonism, while increasing the availability of others (Reid and Horvath, 1980; Whitehead, 2000). These interactions may combine with a deficiency in protein to contribute to a maternal deficiency resulting in the development of CCUO calves. High temperatures experienced through the daytime during summer may result in heat stress, exacerbating any nutritional deficiency through reduced feed intake and possibly resulting in a temporary metabolic alkalosis.

The birth of affected calves by pasture-fed dams in reasonable body condition as indicated by the case definition and recorded for cases of CCUO (such as the 1965 case reported by Barry (1967) plus 63 of 66 case mobs on study farms in the case-control study and all study cows in the supplement trial conducted by this project) shows that the period and nature of maternal nutrition deficiency does not have to be extensive in length nor comprehensive in terms of dietary nutrients involved. Thus whilst CCUO in Australia appears linked to drought (rainfall deficit over 3 month period) over a period involving late summer, the effect of this drought period on quantity and quality of pasture and supplement eaten by dams does not have to be sufficient to decrease dam body condition. For example, dams in only 3 of 66 case mobs in the case-control study were reported to have had body condition scores of less than 2.5 out of 5 at the time of calving. This clearly suggests that the dam experiences a nutrient deficiency leading to a time of inadequate nutrient supply to support normal bone development in the foetus but that does not impact on her own body condition, or if weight is lost mid-gestation it is quickly regained later with pasture growth following rains. It is probable that the variation in severity of clinical signs seen among calves in affected mobs are due to this nutrient deficiency occurring at differing points during mid-gestation and for varying lengths of time, thereby affecting the foetus at differing stages of development. Further the presence of growth arrest lines in the bones of CCUO calves provides evidence of intermittent foetal malnutrition. The rapid fall in plasma zinc levels seen in rats, mice and ewes when fed zinc deficient diets suggests that body stores of zinc are tightly bound and not easily mobilized. A deficiency of circulating zinc therefore develops rapidly in these species and also possibly in cattle. This may reduce the availability of zinc to the developing foetus. In addition to this, a zinc deficient diet reduces appetite and feed intake, further reducing the intake of nutrients including trace minerals and protein.

1235.8 Supplement Trial

The supplement trail was conducted on the case study farm, a farm seriously affected by CCUO with affected calves born in 6 of 7 years from 2003 to 2009. The characteristics of CCUO on this farm correspond closely to key features identified in this project all affected calves being born in spring; all three paddocks involved are hilly and have native pasture; and years with highest CCUO prevalence experienced drought conditions during late summer.

In this trial CCUO calves were born in both the treatment and control groups and decreases in liver iron, zinc and manganese were similar between groups. A fall in maternal manganese status during gestation has been previously described in dams that produced deformed calves (Hidiroglou *et al.*, 1990). Hidiroglou *et al.* (1990) demonstrated a fall in maternal serum manganese levels in hay and silage fed cows, which was not statistically significant but only silage fed cows went on to produce deformed calves. In other studies where dams were provided a nutritionally balanced diet, there was no change in maternal mineral status (Gooneratne and Christensen, 1989; Graham *et al.*, 1994). The reduction in all liver trace mineral levels (with the exception of copper in the treatment group) demonstrated in this trial may reflect the poor quality pasture diet available to the gestating cows during gestation.

At the commencement of the trial in mid-March 2009, there had been a two month period of no rainfall on the property so conditions were met for the risk period for drought linked with CCUO occurrence. Prior to enrolment in the trial, the pasture which study cows grazed was of adequate supply but consisted of dried mixed pasture. For the duration of the trial, study cows in the trial paddock grazed native pasture with low levels of zinc, copper and selenium (Table 9-24), which are reflected in the final liver trace mineral results obtained one month after calving (Table 3-21). This native pasture was dry and sparse in March and rainfall in late March and early April resulted in a flush of pasture growth which rapidly dried following further dry weather. For the 4th to 6th months of gestation (from mid-March to the end of May), study cows relied totally on the mostly dried native pasture in the trial paddock for nutrition. These cows received no supplement feed throughout pregnancy and so ingested only dried pasture for the vast majority of gestation. Nonetheless dam body condition score was maintained to calving although coat condition deteriorated in all but one cow.

The outcomes of this project, like all research activities, were at points enhanced and at other points constrained due to the nature of the research methodologies implemented. The retrospective nature of most methods used in this work placed limits on some aspects of our work due largely to our prior expectations about the completeness and depth of case records kept by veterinarians and of farm records kept by affected producers. These issues and others related to study methods are now discussed.

1235.9 Farmer Reporting

Knowledge of calf deformities in Australia is reliant upon the ability of cattle producers to observe and recognise deformed calves and then upon farmer reporting of deformities to private or public veterinarians. Farmer failure to recognise and report calf deformities limits knowledge about all types of congenital conditions including CCUO.

This project gathered anecdotal evidence that suggests CCUO may occur on more farms and be more widespread than this report reflects as many veterinarians who participated in the Part 1 - Survey stated that affected producers often reported knowledge of other producers in their area who had calves born with similar deformities. Similarly some producers interviewed in Part 3 – Case-control study commented on affected producers that were not included in the list of compiled from official records. This suspected under-reporting of CCUO has been documented previously in some reports of outbreaks in this country (Barry, 1967; Cave *et al.*, 2008).

An examination of farmer attitudes about disease reporting in Western Australia indicated that farmers make reporting and biosecurity decisions based on the perceived risk to their enterprise (Palmer *et al.*, 2009). A key contributing factor to the perceived risk was trust in government departments and representatives. In Western Australia it was found that scientific institutions linked to government suffered from a lack of trust, which may also be the case in the eastern states. There may be many other factors contributing to the under-reporting of CCUO such as:

1. Producers may feel that reporting deformed calves in their herd will negatively affect their reputation as a quality producer or lead to restrictions.

Producers may not know who to report the occurrence of deformed calves to.

Producers may consider a small number deformed calves born on their farm as a normal occurrence.

Producers may fail to observe affected calves due to poor management (lack of observation) or extensive farming practices. In these cases, deformed calves may die and suffer from predation before being observed.

Inexperienced or less observant producers may fail to notice mild deformities in some affected calves.

Producers may feel that the cost of investigation of deformed calves is not justified when only small numbers are affected on rare occasions.

As a result of the under-reporting indicated by veterinarians and producers who participated in our research it is likely that some affected farmers in south-east NSW and north-east Victoria do not report affected calves. Consequently we consider the actual number of affected farms in this region to be higher than the figures presented in this report. In defence of the non-reporting farmers, it is likely that on most of the non-reported farms CCUO occurrence to date was mild (in terms of number of affected calves and severity of deformities) and thus did not raise undue producer concern about the condition or the related financial loss.

Given that this project was based on information obtained from veterinarians and official records it is acknowledged that there is an inherent selection bias due to exclusion of non-reported farms. Whilst the result is an unavoidable under-estimate of the spatio-temporal distribution of CCUO in Australia, in terms of the investigation of risk factors to advance understanding of the aetiology of the condition and development of recommendation for CCUO prevention, the bias toward enrolment of more severely affected farms in the case-control study and the time series analysis may have in some ways enhanced ability to define management and environment factors linked to CCUO.

1235.10 Veterinary Documentation and Investigation

Retrospective investigation of cases in Parts 1 and 2 of this project was limited due to the unexpectedly low number of cases with written records of adequate quality.

A large number of cases were reported by veterinarians in the Part 1 survey for which records were not available. This was due to either a lack of formal records being made by the veterinarian, or due to the veterinarian no longer having access to previous records because of the length of time since case occurred or because the veterinarian had moved jobs and was no longer in the practice or office holding the records. In many cases, reporting veterinarians had never recorded the cases, claiming that the sightings of affected calves were usually incidental, occurring whilst attending another problem that was the reason for the farm visit (such as a dystocia) and for which a medical record was written. In relation to cases that had been recorded but several years ago, variations in the veterinary regulations between states concerning record keeping need to be considered. The New South Wales Veterinary Practice Regulation 2006 and the Queensland Veterinary Surgeons Regulation 2002 state the mandatory keeping of medical records for a minimum three years. Guideline 11 of the Veterinary Practitioners Registration Board of Victoria requires retention of medical records for a minimum of 2 years. Other states and territories do not have mandatory record keeping requirements written into legislation. Essentially, any records older than three years are not required by law to be kept in any state or territory in Australia.

In cases involving low numbers of affected calves, many veterinarians appeared to take little action, offering clients investigative procedures but usually being declined. It is apparent from our work that most private veterinarians only referred cases on to the local government veterinarian for a full investigation during severe outbreaks. Thus many cases of suspected CCUO were not formally investigated at all, and for only a few cases did the veterinarian conduct tests in order to rule out viral aetiologies such as Akabane virus and BVD or submit bone samples to confirm the nature of the pathology involved. Further the investigations of cases during outbreaks undertaken by government veterinarians varied in terms of the depth and completeness of data obtained. Consequently our investigation of data available from the 1991 outbreak and 2003-2005 outbreaks in NSW and Victoria was restricted to conduct of univariable analysis on a small number of variables and mapping by postcode of only the 1991 outbreak.

Of note, among the investigated cases, was a distinct decline in the submission of pathology samples for cases between 2002 and 2007 compared to cases from the 1991 outbreak. This change may result from the recognition of CCUO as a distinct condition in some areas of NSW and Victoria, with veterinary diagnoses being based in the later outbreaks solely on clinical signs. Other factors that may have played a role in the reduction of pathology submissions, particularly in NSW, are the successive closure of rural veterinary laboratories since 1996 and the introduction of a “user pays” system at government veterinary laboratories. The effect of these changes on sample submission rates is worthy of further investigation and discussion.

The absence of histopathology results for over 94% of cases presented in this report raises concern that some cases included in this research, although they fit the CCUO case definition, may have been deformities resulting from a known aetiology (such as BVD or an inherited disorder). Such cases of misdiagnosis would represent misclassification acting to inflate case figures, and potentially acting to introduce information bias in the form of misclassification of the outcome under investigation in the case-control study and time-series analysis. Whilst we are concerned about the low number of confirmed CCUO cases, this was an inherent risk in the conduct of a retrospective study, and we believe the potential number of misclassified cases to be low. This conclusion is based on the fact that inherited genetic deformities are well recognised and no cases examined indicated evidence of a genetic aetiology. Likewise in relation to BVD no cases examined documented signs, aside from the calf deformities, typical of BVD outbreaks such as increased rates of abortion.

1235.11 Response Rate

When the response rate for a study is low and the responders differ significantly from the non-responders in terms of characteristics or practices related to the outcome of interest, then a form of selection bias known as responder bias has occurred and will act to reduce the internal validity of the study (that is, the extent to which findings based on the study participants are true for the target population).

The response rates were low for both Part 1 – Survey of rural veterinarians and Part 3 – Case-control study in this project.

For Part 1 - the overall response rate achieved in this survey was 26%, with a 23.9% return rate from non-government veterinarians and a 38.2% return rate from government veterinarians. However, the return rate for government veterinarians contacted via their respective head office was only 13.6%, which may reflect that the veterinarians who received a forwarded email considered the invitation to participate to be of lesser relevance or importance. The overall low response rate of 26%, while comparable with other studies involving cattle veterinarians where response rates of 12% and 25% were obtained (Hovi and Kossaibati, 2002; Steiner, 2003), may result from the fact that congenital defects in cattle are uncommon and that those veterinarians that had not observed CCUO calves felt no compulsion to return the questionnaire despite requests to the contrary. It is clear veterinarians more likely to respond were those with an interest in CCUO due to frequent exposure. Government veterinarians from NSW and Victoria, the states where CCUO is well known, provided the highest responses at 81% and 73 % respectively. It is very unlikely that not providing self-addressed postage paid return envelopes with the questionnaire posted to ACV veterinarians, a decision based on cost constraints and advice from ACV staff that facsimile return of forms was preferred by their members, was a factor that impeded replies from these veterinarians. This conclusion is supported by the fact that all but three ACV respondents returned the completed questionnaire by e-mail or fax rather than by post.

As the response rate for government veterinarians in NSW and Victoria were above the 70% response rate considered to be satisfactory response to a postal questionnaire (Thrusfield, 2007), and given that these veterinarians were responsible for virtually all documented investigations of suspect calves, we are satisfied that this survey captured the vast majority of reported cases of CCUO in these states. Clearly selection bias present in this survey arises from 1) the exclusion of cases that were not reported to veterinarians (and therefore could not be captured in this survey) and 2) veterinary misdiagnosis of mild cases or rare cases particularly outside NSW and Victoria.

For Part 3 - the low response rate of 49% reflected the attitude of many beef producers during a difficult period where continued drought produced hardship. For many affected producers invited to participate in the case-control study, the problems associated with CCUO were negligible compared to other difficulties they experienced such as lack of income and sourcing adequate feed for stock. In particular, producers who experienced low numbers of CCUO calves seemed most likely to refuse to participate as they considered CCUO as a minor problem. In contrast, producers who had experienced the birth of high numbers of CCUO calves in their herds appeared most likely to participate. The use of a payment to producers for participation in the study provided enough incentive for some producers to take part and increased the overall participation rate.

The case-control study may be subject to selection bias due to 1) the source of the list of affected producers invited to participate (based on veterinarian report) and 2) the response of invited producers. This bias toward affected producers who reported CCUO cases, and of those, toward producers more concerned about CCUO requires consideration during interpretation of the results. Further, given study farms are not a representative sample of all affected farms in south-eastern Australia, the findings of case-control study should be extrapolated to other farms in this region with caution. The same care is required when considering the results of Part 4 – Analysis of rainfall data as the study farms were the same 46 farms enrolled in the case-control study.

1235.12 Case-control Study

The case-control study conducted by this project is an advance in terms of design and thus validity of findings upon prior attempts to investigate risk factors for CCUO in Australia.

Previous investigations conducted by DPI officers in 1991 and 2005 failed to collect adequate information for thorough descriptive and analytical analyses to be undertaken. The emphasis of these previous investigations appears to have been identification of any possible risk factors associated with the management of case mobs or the environment in which they were kept during gestation. However, each investigation failed either to consider all potential risk factors (e.g. trace element levels in soils of paddocks grazed by pregnant dams) or to collect complete information about a sufficient number of case and control mobs to allow analysis.

The case-control study approach is recognised as a robust epidemiological method to investigate the association of range of potential risk factors with a disease outcome using a comparatively low number of study subjects (Elwood, 2007). It is often undertaken retrospectively as was done in this project. A retrospective case-control study will provide the most definitive outcomes when the most common forms of bias to adversely affect this type of study are minimised. In terms of selection bias this requires clear designation of the source population for cases and ensuring that controls are selected from the same population such that they reflect the distribution in exposure status present in that population (Dohoo *et al.,* 2003). For information bias, the sensitivity and specificity of outcome diagnosis need to be high so that researchers are certain about the designation of cases and controls. Also data on exposure to potential risk factors needs to be sourced from reliable records or other credible information sources. In case-control studies, when exposure status is based on memory alone differential misclassification resulting from recall bias can occur due to recall about cases being more accurate than recall about controls (Dohoo *et al.,* 2003).

This case-control study faced challenges in relation to selection bias and information bias. The type of selection bias and implications for study findings were discussed in detail previously. In general, the bias toward enrolment of more severely affected farms is not considered to be detrimental given the purpose of this study was to investigate potential risk factors in order to provide advice to aid prevention of CCUO on affected farms.

Misclassification bias related to the outcome was also mentioned earlier. Control mobs were designated on affected farms rather than neighbouring farms because farmer under-reporting was known to impact knowledge of CCUO occurrence and no documented cases for a neighbour was not a guarantee of CCUO freedom due to the potential for farmer failure to recognise the birth of a few CCUO calves or to disclose occurrence of CCUO on his/her farm when invited to participate in this study. Of the 98 affected farms approached for this study, eight were excluded to avoid misclassification as farmers described calves with bent legs but were unsure if signs of dwarfism were present, and only one or two calves were born on each farm. It is possible that these farms experienced another condition not associated with CCUO such as BVD. The restriction of study farm location to south-east NSW and north-east Victoria and of the time period to 2002-2007 were used to increase the likelihood that farms included in the study were suffering from the same condition and to favour farmer recall about case mobs and their management.

On the 46 study farms, designation of mob status was based on the clinical presentation alone for 58 case mobs with bone pathology examined and confirmed for only 8 case mobs. In spite of this low percentage of histopathology confirmation, we consider that misclassification of case mobs was very unlikely as the 46 study farms present no evidence suggestive of known genetic or infectious aetiology and more than 1 affected calf was present in 65 case mobs. Misclassification of a small number of control mobs, while possible due to farmers missing the occasional mildly deformed calf or not observing an affected calf killed and consumed by wild or feral dogs, is also unlikely as these affected producers were familiar with the clinical signs of CCUO and many were keen to assess their losses related to CCUO.

Information bias impact on some potential risk factors is a concern given farm records on many study farms were found to be minimal and of poor quality. Most farmers did record 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 mobs including parity, calf mortality and supplemental feeding. Explanatory variables based solely on farmer recall were potentially affected by differential misclassification bias to varying degrees. We sought to minimise recall bias in this study through the use of personal interview conducted by a single interviewer and of carefully worded questions to avoid misinterpretation of questions, the elimination of answers that appeared to be guesses or were unclear, and use of facts from case records provided by investigating veterinarians to enhance farmer recall of events during years that CCUO appeared on their farm. Several management variables and all environment variables related to practices or farm characteristics were unlikely to change over the study period and were thus not be affected by farmer recall.

1235.13 Analysis of Rainfall Data

In Part 4 of this project we sought to investigate the relationship between rainfall deficit and CCUO occurrence using time series analysis with the intention to provide information that could warn farmers when weather conditions occur during gestation that are more likely to concur with the birth of CCUO calves. This attempt to develop an early warning system for a condition resulting from a suspected maternal nutrition deficiency is, to our knowledge, original.

The development of early warning systems using time series analysis is common for infectious diseases in veterinary and human health disease, particularly vector borne diseases (Bi *et al.*, 2009; Zhang *et al.*, 2008b). Zhang *et al*. (2008a) concluded that most studies using time series analysis for vector borne diseases are limited by a failure to include other factors possibly related to disease transmission such as population density and existing health status. In our work, episodes of maternal nutrition deficiency in pasture-fed cattle may result from pastures grown under drought conditions (Whitehead, 2000). Other factors contributing to maternal nutrition that may affect the expression of CCUO (e.g. supplement feeding) will also be influenced by periods of drought and as such, are more likely to be predicted by the model presented in Table 3-19 than to contribute independently to CCUO occurrence. Therefore, inclusion of other non-climate variables was deemed unnecessary in our work.

The limitations of this work arising from selection bias held in common with the case-control study were discussed previously.

A further potential limitation specific to Part 4 relates to the rainfall data used. While most producers maintained their own rainfall records, these were not used in the study due to a lack of quality control, missing observations and insufficient data to calculate long-term deficiencies. The rainfall data used, sourced from the Australian Bureau of Meteorology, presented some limitations that could potentially introduce information bias. The distance between farms and the nearest weather station ranged from one to 20km and thus may not accurately reflect rainfall on study farms given that localised rain events such as thunderstorms can result in variable amounts of rainfall over relatively short distances. However, the use of an average value for rainfall determined by averaging the rainfall for each farm provided a fair estimate of rainfall deficiency compared to the long term averages for the geographical region concerned. It is also possible that extended periods of low rainfall may occur without being revealed in monthly figures. For example, a high rainfall storm event could occur at the beginning of the month, delivering the monthly total on one occasion, with extended dry periods either side of this one event. However the use of three-month moving average rainfall is more likely to detect rainfall deficiency than monthly values, and was the variable found to be significant in this study.

1235.14 Supplement Trial

The interpretation of results from the supplement trail as conducted is limited due to the 6-week period between the administration of treatments. Monthly or fortnightly treatments and sampling would have been more suitable in this trial. Closer monitoring of cattle throughout gestation would have given a better indication of changes in trace mineral status. It would also have been preferable to start measuring baseline trace mineral levels before joining to obtain an annual cycle of trace mineral status. During the time of the trial it would have also been useful to examine trace mineral status in a mob on good quality pasture. The number of study cows and the treatment times were restricted by the availability of the manager to perform mustering tasks and assist with sample collection as required.

Another factor affecting this study was the 4 to 6 week spread of calving in the mob. Realistically, throughout the trial each cow was at a different stage of gestation during treatment and sample collection, which makes comparison of the data difficult.

This trial was an opportunistic inclusion in this project based on the initiative of PhD researcher Peter White and the willingness of the farm manager. Given that the climatic conditions during the risk period for drought were met in early 2009 on this farm, it was entirely appropriate to attempt this trial and see whether or not injectable multimineral supplement would alleviate or prevent CCUO on a case paddock on this property. Unfortunately CCUO prevention was not achieved. This opportunistic observation supports the hypothesis that CCUO may be multi-factorial in nature and provides guidance that prevention on affected farmers may require more than mineral supplementation alone.

1236 Success in Achieving Objectives

The contracted and additional objectives of this project were achieved. Key findings and comments related to each objective are summarised below.

1236.1 Objective 1 - To undertake a nationwide survey of public and private rural veterinarians involved in examining congenital abnormalities of calves to establish the geographic and temporal distribution of CCUO, and to distinguish cases from similar disorders (such as joint laxity, which has been confused with CCUO)

Part 1 – Survey of rural veterinarians - the first nationwide survey of veterinarians to investigate CCUO occurrence in Australia was conducted with cases reported by 24 government and 56 non-government veterinarians. It, in combination with Part 2 – Analysis of existing records – and examination of published data, has produced the most complete description of the spatio-temporal distribution of CCUO in Australia to date. We are confident that use of a clear and specific definition in this survey enabled the responding veterinarians to differentiate CCUO-affected calves from calves with similar disorders.

1236.1.1 Temporal Distribution

CCUO occurrence was found to be sporadic from 1957 to 2001 (Table 3-4) with the exception of a single outbreak involving 52 farms in NSW during 1991. Among the cases prior to 2002 included in the survey results are 12 cases not reported previously in the literature.
In contrast since 2002 CCUO occurrence has increased and been annual with reports every year involving multiple farms.

1236.1.2 Spatial Distribution

CCUO was found to be principally a condition of beef herds in south-east NSW and north-east Victoria with occasional cases in beef herds in south-west Western Australia, the central and southern tablelands of NSW, and in South Australia for the first time during 2009. However there is a trend in recent years for CCUO calves to be reported more frequently outside south-east NSW and north-east Victoria and to be reported at new locations in southern Australia, that is, on farms that have not previously seen CCUO calves.

1236.1.3 Under-reporting

We consider the spatio-temporal distribution of CCUO occurrence in Australia described to be an under-estimate of actual occurrence particularly in south-east NSW and north-east Victoria.
On the basis of comments from surveyed veterinarians it is likely that some affected farmers in south-east NSW and north-east Victoria do not report affected calves. Veterinarians therefore could not provide information on all actual cases of this condition and the total number of affected farms in this region from 2002 to 2007 is likely to be considerably more than stated in this report. However we expect that most unreported cases involved a low number of affected calves and involved mild deformity.

1236.2 Objective 2 - To undertake a retrospective examination of case records of previous outbreaks to improve documentation of the disorder

Part 2 – Analysis of existing records – and of related publications where available was undertaken but did not provide the expected additional information on CCUO prevalence and related risk factors with the exception of the 1991 outbreak. Our investigation was severely limited by the fact that written records did not exist or were incomplete for cases not reported in publications.

1236.2.1 Prevalence

Absence of data for some cases on the number of affected calves and total calves born per year required to calculate CCUO prevalence limited our investigation of on-farm prevalence. While it is clear that prevalence can range from 1% up to over 90% with a median usually around 10%, no conclusion about a trend toward an increase or decrease in on-farm prevalence could be drawn from this work.

1236.2.2 Risk Factors

Univariable analysis of data on nine variables from the 1991 outbreak involving 52 farms in NSW showed significant association of case mobs compared to control mobs with three variables related to nutritional management of dams during second trimester of gestation (hand feeding, feeding hay supplement, grazing inadequate pastures) and with one variable related to the third trimester (hand feeding).

1236.2.3 Estimate of Financial Cost

Income lost by affected farmers due to the 1081 CCUO calves reported for 2002-2007 was estimated to total AU$818 315 with an average loss per affected calf of AU$757 based on loss of income, or AU$1.488M with an average of AU$1376 per affected calf born based on a gross margin model.

1236.2.4 Lack of Veterinary Documentation and Investigation

The low number of well documented cases was an unexpected constraint to our investigation of case records from previous outbreaks.

It appears that detailed records and further investigation (including pathology) were undertaken only when the numbers of affected farms or calves were of concern to the producer and/or private veterinarian. A reduction in pathology submissions was noted between the investigations undertaken for cases in 1991 and for cases from 2002 to 2007. Following farmer reporting or veterinary referral, investigations undertaken by government veterinarians varied in terms of the depth and completeness of data obtained such that our analyses were restricted to the 1991 outbreak data and to conduct of univariable analysis on a small number of variables, and mapping by postcode.

The absence of pathology reports for the vast majority of cases raises concern that some deformed calves may have resulted from a known aetiology (such as BVD or an inherited disorder). Whilst we acknowledge concern about the low number of confirmed CCUO cases, we consider the number of farms likely to have been misclassified to be low because there was no evidence of a genetic aetiology or of BVD infection (aside from bone deformities similar to that seen with BVD) present in case records.

1236.3 Objective 3 - To undertake a case-control study involving properties affected since 2003 and their neighbours as control properties to identify risk factors associated with disease occurrence

Part 3 – Case-control study was successfully completed through the enrolment of 48 farms that experienced the birth of CCUO calves from 2002 to 2007 in south-east NSW and north-east Victoria. These farms provided a dataset with 66 case mobs and 102 control mobs to investigate risk factors related to CCUO occurrence in a cattle mob, and 50 case paddocks and 75 control paddocks to investigate risk factors related to paddock status for CCUO.

Farms affected in 2002 were included in this study to ensure that an adequate number of farms would be enrolled. From 2002 to 2007 drought periods were experienced throughout south eastern Australia so low rainfall was a common feature for the entire period of interest in this study. Case and control mobs were identified on each of the enrolled farms rather than neighbours being enrolled as control properties as stated in the contracted objective. The decision to use control mobs rather than neighbouring control farms was based on identification of under-reporting in Part 1 of this project as an issue impacting our knowledge of CCUO occurrence. We considered the fact that a neighbouring farm had not reported CCUO was insufficient to ensure that a farm was actually CCUO-free due to the likelihood that the farmer may not have recognised the birth of a few CCUO-affected calves or may not disclose occurrence of CCUO on his/her farm when approached to enrol as a control farm in this study. In contrast, we were confident that a producer who had reported CCUO and was willing to enrol in this study would be able to identify control mob/s with lower risk of misclassification because of producer familiarity with the clinical signs of CCUO and producer concern about the level of CCUO in his/her herd.

1236.3.1 Variables Associated with Mob Occurrence

All four variables significantly associated with CCUO occurrence in a mob provide evidence of either a direct or indirect link with poor maternal nutrition during gestation.

The final multivariable models show, after adjusting for the effects of dam age and year and other variables in the model, there is greater risk of CCUO calves when:

- Dams grazed pasture that was not sufficient to maintain body weight during gestation
- Dams grazed native pasture
- Dams were kept in a paddock with predominately hilly to steep terrain
- Dams were given pellets or silage and to a lesser extent hay as a supplement feed compared to dams not given any supplement feed.

The two factors considered to be potential confounders, dam age and year, were significantly associated with the mob events-trials outcome. Age based grouping of dams may influence farmer decision on paddock allocation during gestation with many farmers stating heifer only or single age cow groups were kept on poorer hilly terrain. It is likely that such management differences explain the increased association of heifer groups and single age cow groups with the birth of CCUO calves.

1236.3.2 Variables Associated with Paddock Status

Similar to the above, two variables related to pasture type and hilly paddock terrain were significantly associated with CCUO occurrence in a paddock and thus further confirm the direct or indirect link with poor maternal nutrition during gestation.

Although increased soil potassium was also found to be associated with CCUO occurrence in a paddock there is little known to implicate it in the pathogenesis of CCUO at this point. A dietary excess of potassium has been shown to produce mild metabolic alkalosis and affect calcium homeostasis, but has not been shown to affect other trace minerals in vivo.

Notably we found no association with soil levels of the suspect trace minerals manganese and zinc or with any other mineral such as iron, that might alter the availability of these trace minerals.

1236.4 Objective 4 - If considered appropriate, intervention studies on affected farms or pen trials to determine whether the disease can be reproduced by testing identified risk factors will be proposed

During the final stage of this project this objective was addressed in part under additional objective 7 through conduct of Part 5 – Supplement trial. This trial was performed on the case study farm with history of the birth of CCUO calves every year from 2003 to 2007.

The purpose of this supplement trial was to evaluate the effect of administration of an injectable multimineral supplement (including manganese, zinc, copper and selenium) at regular intervals during gestation on the birth of affected calves and on dam liver and blood trace mineral levels. The hypothesis being that low trace mineral levels (particularly manganese and zinc) may be associated with the birth of CCUO calves. Trace mineral supplementation via injection was used to determine if this form of supplementation would increase trace mineral status in the cow.

The hypothesis investigated in this trial, rather than being based on the findings of Part 3 - Case-control study, was based on evidence in the current literature that CCUO occurs when the foetus is exposed during gestation to manganese or zinc deficiency.

Further to this supplement trial, Sections 8.3 and 8.4 describe two intervention studies seeking to further investigate the role of dam supplementation in an on-farm trial and of specific mineral deficiency in a pen-based trial.

1236.5 Objective 5 - To investigate association between rainfall deficit during the gestation period of spring calving herds and the occurrence of CCUO calves

The findings of Part 2 concerning the 1991 outbreak and of Part 3 of this work concurred with prior reports of the birth of affected calves during drought and when dams experienced poor nutrition during gestation. This focused our attention on the role of rainfall deficit during gestation and led to addition of Objective 5 and conduct of Part 4 – Analysis of rainfall data in this project. Data gathered in the case-control study on 46 affected farms and obtained from the Bureau of Meteorology were utilised to successfully undertake this work.

The time-series and regression analyses clearly demonstrated an association between the birth of CCUO calves and a period of drought five months before calving corresponding to the 2nd to 4th months of gestation for spring calving herds on the 46 study farms during 2002 to 2007.

1236.6 Objective 6 - To document a detailed case study of one affected farm

The monthly farm reports available for one farm enrolled in the case-control study provided opportunity to undertake a detailed case study of an affected farm with occurrence of CCUO over consecutive years. This led to addition of Objective 6 and conduct of Part 5 – Case study in association with the supplement trial on the same study farm. Data gathered in the case-control study about this study farm and obtained from the farm manager and LHPA Hume office were utilised to successfully undertake this work.

The case study farm, a self-replacing beef enterprise on the south western slopes of NSW, had experienced the birth of CCUO calves during spring calving in 6 years from 2003 to 2009. These calves were produced by dams that grazed one of three case paddocks during years characterised by low annual rainfall with the highest proportion of affected calves born in the years 2004, 2006 and 2009 that had very low rainfall during February and March (coinciding with the second trimester of gestation for pregnant dams). Cattle kept in the affected paddocks were reported to receive less supplement feed than other paddocks. Provision of oral mineral drench and/or lick to cattle grazing in affected paddocks from 2004 did not prevent birth of affected calves. In 2008 good rainfall provided ample pasture on farm and allowed a reduced use of affected paddocks. As a result, no affected calves were observed in 2008.

Soil tests conducted in 2006 and 2009 found a similar soil mineral profile in paddocks with and without history of affected calves. Pasture was found to be deficient in several nutrients including selenium, copper and zinc in both improved and native pastures on the farm.

1236.7 Objective 7 - To investigate the effect of dam trace mineral supplementation on occurrence of CCUO calves on the case study farm

This additional objective was included in the project when we recognised the potential to conduct an opportunistic trial on the case study farm to investigate the effect of dam trace mineral supplementation on occurrence of CCUO calves. This objective was achieved through conduct of Part 5 – Supplement trial on the case study farm during 2009.

The purpose of this supplement trial was to evaluate the effect of administration of an injectable multimineral supplement (including manganese, copper, zinc and selenium) at regular intervals during gestation on the birth of affected calves and on dam liver and blood trace mineral levels.

In this trial involving only 20 study animals, two cows out of 10 in the control group gave birth to CCUO calves (20.0% - both born live) and two cows out of nine in the treatment group gave birth to CCUO calves (22.2% - one born live and one dead). No difference in proportion of CCUO calves between study groups was found in this trial (Fisher's Exact Test 2 tail *P*=1).

Further significant decreases in liver iron (P<0.001,), zinc (P<0.001,) and manganese levels occurred in both the control and treatment groups over the trial. For liver copper a significant decrease was observed in the control group and a significant increase in the treatment group throughout the trial. Post-calving, dam liver manganese levels had increased and liver iron and copper levels had decreased in both groups (Table 3-21). All cows post-calving were found to be deficient in selenium based on liver glutathione peroxidise levels.

1236.8 Objective 8 - To document reported occurrences of CCUO calves in southeast NSW and northern Victoria in 2008-2009

This objective was added to enable this report to include as complete a record of CCUO occurrence to time of project end as possible based on farmer reporting and was achieved via conduct of Part 6 – CCUO in 2008-2009.

For 2008 two previously affected farms reported the birth of a total of seven affected calves.

For 2009 10 previously affected farms reported the birth of approximately 100 affected calves plus CCUO calves were reported at two new locations – Collie in Western Australia and Harrogate in South Australia (this case was confirmed by histopathology).

1237Impact on Meat and Livestock Industry – now & in five years time

1237.1 Emerging Condition

This study provides definitive evidence that CCUO should be considered an emerging condition of concern to the beef industry in southern Australia. Emerging condition, based loosely on the OIE definition of emerging disease[2], refers in this context to the increase in number of affected farms (populations) with higher total annual numbers of affected calves, and to occurrence in previously unreported geographic locations.

Compared to the sporadic occurrence seen prior to 2002, emergence of CCUO since 2002 to the present is evident in the annual occurrence of CCUO and in the annual numbers of affected farms that include outbreaks involving ≥10 farms/year during 5 of 8 years from 2002-2009.

Whilst the vast majority of affected farms since 2002 were located in the known focus region for CCUO of the south-western slopes of NSW and north-east Victoria, 8 (6.7%) affected farms were located elsewhere with 2 of these farms being new locations. Our findings demonstrate that in recent years CCUO has occurred more often outside the focus region than previously and extended to previously unreported locations in southern Australia.

CCUO occurrence since 2002 has coincided with an extended period of drought in south-east NSW and north-east Victoria and in other areas of southern Australia. This work provides objective evidence of a link between drought during the 2nd to 4th months of gestation for spring calving herds and poor maternal nutrition during pregnancy with the subsequent birth of CCUO calves on affected farms in the focus region. Whilst the specific maternal nutritional deficiency and the pathophysiological mechanism by which it operates remain unknown, it is clear that dam exposure to drought conditions coincides with CCUO occurrence. Anecdotal reports indicate that farmers who implemented changes to improve dam nutrition on a few previously affected farms produced no affected calves during 2009 despite drought conditions.

We expect further occurrence of CCUO calves at spring calving during future drought periods in south-east NSW and north-east Victoria when farmers graze pregnant cattle on hilly–to-steep paddocks with low levels of pasture consisting primarily of native species inadequate to maintain body condition and do not provide sufficient supplementary feed. Furthermore it is likely that isolated cases of CCUO will occur at other locations, and perhaps an increasing range of locations in southern Australia, if drought conditions persist over the next few years.

The potential impact of global warming on rainfall patterns in southern Australia suggests that drought might become a more common phenomenon facing beef producers (Hennessey and Mpelasoka, 2007). In the event that the specific dam nutritional deficiency is not identified in the near future then CCUO can be expected to result in an annual loss of affected calves possibly on a greater number of farms.

The current financial cost of CCUO to affected producers depends on the number of affected calves lost from production on a farm and thus varies from farm to farm. For a small number of producers in south-east NSW and north-east Victoria, CCUO results in an annual loss due to repeated occurrence over several spring calving seasons. Whilst the total estimated cost of AU$818 315 based on 1081 affected calves for the period 2002-2007 does not impact on

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[2] Emerging disease defined as “a new infection resulting from the evolution or change of an existing pathogenic agent, a known infection spreading to a new geographic area or population, or a previously unrecognized pathogenic agent or disease diagnosed for the first time and which has a significant impact on animal or public health.” (OIE, 2008)

industry return, the impact on return for individual enterprises may be substantial. For example a hypothetical beef herd of 100 head with a prevalence of 12.5% of calves affected in one year is estimated to experience a total GM loss for the two year period of AU$ 13 760 (Table 3-9). An impact on industry return would be realised if CCUO occurrence was to substantially increase, with rises in numbers of affected farms and affected calves per farm, as a result of prolonged drought over the next several decades.

1237.2 Farmer Reporting and Veterinary Investigation

This project has identified that farmer failure to recognise affected calves or failure to report occurrence to a veterinarian means current knowledge of CCUO is constrained by an undefined level of under-reporting. Thus the spatio-temporal distribution of CCUO occurrence in Australia described in this report is considered to be an under-estimate of actual occurrence particularly in south-east NSW and north-east Victoria.

This work also revealed a lack of documentation and investigation of calf deformity by veterinarians arising from the limited time available to private and government veterinarians to conduct investigations, and more noticeably for outbreaks in the last decade, from the reduction in funds and regional laboratories to support pathology submissions. This finding flags a serious issue about veterinary failure to adequately investigate apparently low incidence conditions in beef herds that could seriously impact the industry if it leads to a lengthy delay in recognition of an emerging disease or condition that does not have a spectacular acute clinical presentation, such as was seen in the sheep industry with ovine Johne’s disease.

1238Conclusions and Recommendations

The work undertaken in this project has advanced our knowledge of CCUO in Australia by:

- The compilation of the most complete description of the spatio-temporal distribution of CCUO in Australia to date.
- The conduct of a well-designed case-control study that investigated associations between the birth of CCUO calves on affected farms and a set of management, environment and soil variables
- The conduct of time series and regression analyses to investigate the relationship between the birth of CCUO calves on affected farms and rainfall deficit
- The conduct of an on-farm trial to investigate the effect of administration of an injectable multimineral supplement (including manganese, copper, zinc and selenium) at intervals during gestation on the birth of CCUO calves and on dam liver and blood trace mineral levels.

It is clear from the evidence presented in this report that CCUO in Australia is:

- An emerging condition reported since 2002 on an annual basis with outbreaks involving ≥10 farms/year during 5 of 8 years from 2002-2009.
- Predominately a condition reported in beef herds on the south-western slopes of NSW and in north-east Victoria, however, another feature of emergence is a trend toward more reports outside this focus region.
- Associated with maternal nutritional deficiency during gestation that is related to drought[3] during the 2nd to 4th months of gestation.
- A condition that occurs more frequently than is recorded in official records due to non-reporting by some affected farmers.

Although aspects of this work were constrained by features discussed extensively in Section 4, these conclusions definitely apply to affected farms that have reported CCUO occurrence sufficient to be of concern to the producer in the focus region for CCUO of south-east NSW and north-east Victoria.

On the basis of these findings we consider that industry, government and researchers in southern Australia should either maintain or introduce activities that provide beef producers with information to support the recognition, reporting and prevention of CCUO; and continue targeted research to further improve understanding of factors contributing to CCUO occurrence and of the pathogenesis of this condition. An outline of recommendations related to specific extension and research activities follows.

1238.1 Extension to Beef Producers and Rural Veterinarians

It is clear from the under-reporting identified in this work that farmers in the focus region of south-east NSW and north-east Victoria need to be supported in terms of their understanding about the clinical signs and the epidemiology of CCUO. Improved knowledge about the condition is one factor that will encourage discussion about CCUO among farmers themselves and with the veterinarians in their district. The provision of avenues for communication between farmers and local animal health authorities may engender more trust in relation to this condition in particular. It is not an infectious disease so mandatory requirements such as quarantine and trade restriction, that farmers may be fearful of, do not apply to CCUO control and prevention. The link with climatic conditions makes CCUO more an act of nature than mismanagement or poor genetic selection. Again this should help to reduce farmer concern about adverse impact on their

[3] Drought being defined as a three-month period in which rainfall is within the first decile (lowest 10% on record) for a given location.

reputation as beef breeders and producers. Extension activities and resources focused on calf deformities in general and the link to drought preparedness and management, plus CCUO in particular, would assist with farmer education. The trend toward increased occurrence outside the focus region in previously unreported locations indicates that extension should be provided to beef producers throughout southern Australia particularly in more newly affected areas.

Following a farmer report of suspect calves, the veterinarian contacted must be equipped to conduct an adequate investigation or equipped with knowledge of CCUO that will ensure rapid referral to another veterinarian better able to conduct an appropriate investigation. Thus education of veterinarians is an important facet to ensuring that future cases receive sufficient investigation and documentation. Consideration should also be given to provision of funds and laboratory support so that veterinarians are able to submit samples from suspect calves for pathology to confirm diagnosis on previously unaffected farms, particularly at new locations and preferably at no cost to the farmer.

Recommendation 1: Extension of MLA B.AHE.0004 to undertake extension

- To conduct a field day[4] focused on congenital deformities in livestock involving producers, veterinarians and other animal health personnel, and researchers.
- To present findings of this project at a veterinary conference in Australia during 2010.

1238.2 Investigation of Association with Temperature and Evaporation

An association between CCUO occurrence and rainfall deficit has already been demonstrated. Following on from this, an investigation into the association of other climatic factors with CCUO may contribute to the understanding of the causes of the disorder. An evaluation of the historical temperature and evaporation records on affected farms using time series analysis may reveal an association of weather anomalies with CCUO cases. Temperature and evaporation rates, in combination with rainfall, affect the growth of pastures and the availability of feed to grazing cattle. Extended periods of high temperature, high evaporation and low rainfall may severely affect the quantity and quality of available feed. Lower temperatures and evaporation rates may have less of an effect on feed during periods of drought.

Temperature may also have an effect on the metabolism of cattle. Heat stress is known to lead to reduced appetite in cattle and as such it may restrict intake of essential nutrients. Reduced feed intake reduces ruminal activity and increases transition time of ingesta. Periods of heat stress have also been demonstrated to create episodes of metabolic (and ruminal) alkalosis in cattle due to panting (Schneider *et al.*, 1988). The diurnal pattern of alkalosis created by hot days and cool nights has an unknown effect on the nutrition of grazing cattle, however Calamari *et al.* (2007) found that heat stressed cattle have lower plasma magnesium and zinc levels compared to thermoneutral cattle. Blood flow to the uterus, and other organs is also reduced during heat stress which may have an effect on the developing foetus (Roman-Ponce *et al.*, 1978). An association between high temperature during gestation and the birth of CCUO calves may provide evidence for further study into the effects of temperature on gestation.

Further investigation of climatic conditions associated with CCUO would be wise prior to use of climatic conditions to forecast CCUO. Compilation of rainfall, temperature and evaporation data (if all are found to be associated with CCUO occurrence) would provide a more comprehensive basis for development of an early warning system than rainfall alone. The conduct of further research on climatic factors is recommended to underpin the establishment of an early warning system based on climatic conditions suitable for use by beef producers in affected regions of Australia.

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[4] This field day would replace and extend upon the producer meeting at project end, involving principally farmers that participated in the research project, funded under current project contract.

Recommendation 2: Extension of MLA B.AHE.0004 to conduct research

To investigate the associations between temperature and evaporation during the gestation period of spring calving herds and the occurrence of CCUO calves using time series analysis

1238.3 On-farm trial to Investigate Effect of High Quality feed Supplement

As stated previously the on-farm trail undertaken in this project was opportunistic and investigated specifically the effect of dam trace mineral supplementation on occurrence of CCUO calves on the case study farm.

To direct on-farm prevention of CCUO on affected farms, another trial is warranted to investigate the hypothesis that high quality supplementation adequate to meet dam nutritional needs will prevent the birth of CCUO calves during spring on previously affected paddocks.

It is suggested that this trial involve a single farm with a history of CCUO occurrence over several years on more than one paddock. The farm should be able to supply four mobs of cattle, preferably of 20 to 40 cattle each of similar age and breed. Two mobs to be placed in two separate paddocks with history of CCUO calves and two mobs in two separate paddocks with no history of CCUO calves and adequate mixed or improved pastures. The treatment, a high quality supplement including grain, would be provided to one mob on an affected paddock and one mob on an unaffected paddock. Each mob would be bred by artificial insemination to ensure that blood and tissue samples would be collected at the same stage of gestation.

The trial would commence at the end of the joining period when dams would be allocated randomly to treatment/paddock mobs and then placed onto their allocated paddock until calving. Baseline blood trace mineral and liver trace mineral levels should be taken along with pasture samples for analysis. Between months 2 and 4 of gestation, liver and blood samples would be taken from a representative sample from each mob. After month four, monthly samples would be taken. Likewise, pasture samples would also be collected and any supplement fed to treatment groups would be analysed. The amount of supplement fed to treatment groups would need to be based on the available pasture and body condition of the dams.

The outstanding risk with such as on-farm trial is dependence on climatic conditions conducive to CCUO prevailing throughout the trail. As the trial would commence prior to the at risk period for drought in late summer, it is not possible to ensure that the period of low rainfall known to be associated with production of CCUO calves would eventuate. The unpredictability of the climate is a major factor in opposition to a farm based trial.

Recommendation 3: Future research – On-farm trial

To investigate the effect of high quality supplementation adequate to meet dam nutritional needs on the birth of CCUO calves during spring on an affected farm.

1238.4 Pen-based Trial to Investigate Effect of Zinc and Manganese Deficiency

To thoroughly investigate the pathological effects of specific mineral deficiencies on foetal bone development in cattle would require a pen-based trial. Such a trial would permit the investigation of the pathological and biochemical changes in calves associated with a maternal manganese or zinc deficiency in cattle for comparison with the pathology findings from CCUO calves. This will assist to either include or exclude these trace minerals in the pathogenesis of CCUO.

For a manganese deficiency trial, cattle (or sheep or goats) would be placed on a low manganese diet for the duration of gestation. For a zinc deficiency trial, cattle (or sheep or goats) would be placed on intermittent or short duration zinc deficient diets at different times during gestation to correspond with the risk period for CCUO calves (3rd to 6th month of gestation in cattle, corresponding period in sheep and goats). Offspring would be euthanized at birth and examined for changes to growth plates and other tissues. Dams would be monitored throughout pregnancy to assess mineral status.

Recommendation 4: Future research – Pen-based trial

To investigate the effect of low manganese and intermittent low zinc maternal diets in ruminants on their offspring and to examine pathological changes that result.

1238.5 Surveillance of Livestock Deformities

It has been reported that approximately 300 different congenital abnormalities occur in cattle, with a number of deformities poorly investigated or still to be diagnosed despite evidence that they occur in Australia (Windsor and Agerholm, 2009). We have identified under-reporting of CCUO, with anecdotal evidence that farmers often do not report deformities in lambs and calves to their veterinarian or government animal health professional. The reasons for this may include a lack confidence by farmers in the value of an investigation, and the introduction of a “user pays” system for laboratory services.

During Part 1 - Survey of rural veterinarians, additional information offered by 13 responding veterinarians showed that 5 had observed calves with arthrogryposis, 2 had observed calves with additional limbs, and the remaining 6 had observed other birth defects including limb deformities. One veterinarian reported calves without front limbs in Euroa, Victoria, an area with a previously documented report of this condition (Harbutt *et al.*, 1965). In the case control study (Part 3), 6 producers reported observing signs of CCUO and other deformities in sheep on their property. Losses due to livestock deformities appear common, however, at present the costs associated with these losses is difficult to estimate due to incomplete knowledge about the range and frequency of deformities that occur.

It is proposed that a surveillance program similar to the LIDA program used for companion animals (www.vetsci.usyd.edu.au/lida/) would help identify and diagnose congenital conditions in Australian livestock. It is evident that many such conditions may not have been described, and the use of a recording system may provide information valuable to livestock health and production in Australia.

To be successful, the establishment of a surveillance program would need to consist of:

- The creation of a database of known inherited and non-inherited congenital diseases/syndromes in Australian livestock
- The promotion of the program and recruitment of veterinarians from around the country to act as reporting veterinarians
- The development of a reporting system to align with information on the database
- The assessment of reported cases for diagnosis/mis-diagnosis by appropriate experts
- The reporting of collated prevalence information to stakeholders including producers, breed societies and veterinarians.

The benefits to livestock health and production from this surveillance program would arise from a greater understanding of congenital deformities in livestock and the cost of these to industry. The system may also act as an early warning for newly acquired disorders that may occur. There are numerous examples of the rapid spread of genetic disorders arising from the overuse of genetic material in artificial insemination programs. Rapid detection of such disorders could be possible if adequate surveillance was undertaken.

1239Bibliography

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1240 Appendices

1240.1 Appendix 1: Detailed Methodology

1240.1.1 Part 1 – Survey of Veterinarians

A nationwide census based survey of cattle veterinarians was undertaken in two parts, involving a self administered questionnaire and follow up telephone interview. Procedures involving contact with and the collection of information from veterinarians were approved by the University of Sydney Human Ethics Committee (approval number 07-2007/10074).

1240.1.1.1 Participant Recruitment

The target population of this study was rural veterinarians who provide services to cattle producers in Australia. With the intention to obtain a representative sample of this target population and to maximise response rate, a census based survey approach was used and all veterinarians known in 2007 to be cattle veterinarians or government veterinarians were contacted. These two groups of veterinarians were recruited using different methods due to their respective work environments and availability of contact details.

1240.1.1.2 Non-Government Veterinarians

Veterinarians who were members of the Australian Cattle Veterinarians (ACV) special interest group of the Australian Veterinary Association (AVA) were contacted by ACV staff due to privacy regulations. To encourage participation in the survey, an article on ACD was published in the July 2007 edition of the ACV newsletter, and a copy of the questionnaire and information sheet enclosed with the newsletter for veterinarians to complete and return either by mail or facsimile. Follow-up mail-outs to non-responders were sent by ACV staff one month and two months later and consisted of a modified version of the newsletter article, a cover letter and the questionnaire.

1240.1.1.3 Government Veterinarians

State government employed veterinarians were sent the questionnaire and information sheet by e-mail in the same week that the July ACV newsletter was distributed. E-mail addresses for the New South Wales, Northern Territory and Victorian government veterinarians were readily available on the respective state government websites. E-mails to South Australian, Western Australian, Tasmanian and Queensland government veterinarians were sent via a senior state government officer due to the unavailability of e-mail addresses for recipients. Follow-up e-mail messages were sent to non-responders at one month and two months after the first.

1240.1.1.4 Telephone Interview

At the end of the survey period, one-month after the third mail out and e-mail, all respondents who reported observing calves fitting the case definition and consented to provide further information were recruited for the telephone questionnaire.

1240.1.1.5 Questionnaire Development

1240.1.1.5.1 Postal and Email Questionnaire

A one page questionnaire was designed for use with an introductory letter and participant information sheet describing the purpose of the study. A brief questionnaire was used to encourage participation and maximise the response rate, being limited to three questions with a yes/no response and a request for contact details. Respondents were asked:

- if they had observed deformed calves fitting the case definition for CCUO,
- whether or not these calves had been observed in the last five years (2002-2007), and,

- if they agreed to a follow up telephone interview to gather more information and case records about the reported cases.

The case definition used was as follows:

Calves from clinically well dams exhibiting disproportionate dwarfism with:

- Limb deformities including bowed legs, shortened limbs and enlarged joints
- Shortening of the upper jaw, with domed head and/or dished face
- Twisted spinal column including lordosis, kyphosis or scoliosis
- Fixation of the limbs (arthrogryposis) at birth.

1240.1.1.5.2 Telephone Interview

A telephone questionnaire was conducted with all respondents reporting calves fitting the case definition and consenting to further contact. The interview sought to collect details about affected calves born each calving season on a case farm from 2002 to 2007 and for earlier years where information was available. The questionnaire used in the telephone interview classified a case as the birth of one or more affected calves on a farm in one year and obtained the following information for each case:

- Farm location and/or district.
- Year and calving season (autumn, spring).
- Approximate numbers of affected calves and unaffected calves or the number of affected calves reported.
- Clinical signs present in affected calves, in particular those of disproportionate dwarfism.
- Results of pathology tests performed to confirm a diagnosis (either copies of reports or the location of the lab used for testing).
- Copies of any medical records involving cases (with consideration being given to the privacy of producers).
- Any other data relevant to the investigation

All interviews were conducted by one researcher over a four week period. During this time repeated attempts were made to contact each respondent until an interview was completed or at least four messages were left requesting a return phone call.

1240.1.1.6 Data Management and Analyses

All data collected were entered into a purpose built database (Microsoft® Access, 2002) and checked for data entry errors. Standard descriptive analyses were then undertaken using GenStat Release 10.2 (Lawes Agricultural Trust, 2007).

1240.1.2 Part 2 – Analysis of Existing Records

A summary of information acquired from the 2007 survey of rural veterinarians is listed in **Appendix 3: Case Records**. This information was classified into one of the following three categories:

- Sporadic or isolated cases occurring up to 2001.
- Cases occurring during a major outbreak in 1991.
- Cases occurring during the years 2002 to 2007.

The data on reported occurrences of CCUO from each document or record obtained were entered into a Microsoft Access database (Microsoft Corporation, Redmond, USA) ‘case records data’ and checked for errors. Data were entered for the following fields where available: Year, Location, Number of farms, Number of affected calves, Total number of calves, Clinical features of CCUO and Results of pathology investigation. Most of the retrieved information documented CCUO during a year at a district level rather at a farm level.

The spatial distribution of the farms involved with these cases was mapped using ESRI® ArcGIS™ software (ArcGIS Professional 8.0, MapInfo Corporation, USA) based on the postcode of the nearest town or village due to lack of data on exact farm location.

1240.1.2.1 Sporadic Cases to 2001

Data on sporadic CCUO occurrence prior to 2002 were collated from a search of the literature and of laboratory pathology reports and case records requested from the 17 veterinarians who reported cases of CCUO prior to 2002 in the 2007 survey of rural veterinarians.

1240.1.2.2 NSW Outbreak of 1991

Data concerning cases of CCUO in NSW during a major outbreak in 1991 were obtained from a survey conducted by the NSW DPI, pathology reports and two unpublished papers. The survey information was provided both in the form of an electronic spreadsheet and hard copies of surveys. The hard copy information was added to the electronic spreadsheet to form the database ‘1991 outbreak’ to allow analysis of all data together.

Descriptive analysis of variables in this dataset was undertaken in Excel. Variables considered to be potential risk factors for CCUO that had data for >10 observations per category were identified for further investigation.

The dataset was exported to and univariable analysis of the identified variables was undertaken using logistic regression. This statistical approach was implemented although there were only 20 control mobs compared to 69 case mobs in the dataset.

Historical rainfall data for NSW were reviewed graphically using the mapping functions of the Long Paddock website (Queensland Government Environmental Protection Agency, 2008) for the time periods corresponding to the first and second trimesters of pregnancy for spring calving cattle in 1991.

1240.1.2.3 Cases in NSW and Victoria 2002-2007

The 2007 survey of rural veterinarians identified 135 cases of CCUO that occurred from 2002 to 2007. During the telephone interview with each veterinarian who reported CCUO from 2002-2007 we requested copies of case records, pathology reports and other information on file for these cases. Subsequently we obtained documents from two separate surveys conducted by state DPI officers in NSW and Victoria, pathology reports and handwritten notes and diary entries for some cases.

Data from the two surveys were entered into an Excel spreadsheet ‘2002-2005 outbreak’. Descriptive analysis of variables in this dataset was undertaken in Excel. Variables considered to be potential risk factors for CCUO that had data for >10 observations per category were identified for further investigation as for the dataset ‘1991 outbreak’.

Historical rainfall data for 2004 in NSW and Victoria were reviewed graphically using the mapping functions of the Long Paddock website (Queensland Government Environmental Protection Agency, 2008) for 3-month periods corresponding the trimesters of gestation for spring and autumn calving in this year.

1240.1.2.4 Estimates of the Financial Cost of CCUO

The financial losses incurred by farmers with CCUO between 2002 and 2007 were estimated using two methods. Firstly, a simple "loss of income" calculation was used to predict income loss in the year following the birth of CCUO calves for the number of cases reported in the survey of veterinarians. An estimate of the price of yearling cattle based on the yearly average value of the Eastern Young Cattle Index (EYCI) (Meat and Livestock Australia, 2009) was used in conjunction with an estimate of the average carcass weight for yearling steers and heifers of 212 kg (NSW Department of Primary Industries, 2009b). The estimate for carcass weight was based on the farmer selling progeny between 12 and 18 months of age at an average of 400kg live weight and assuming an average of 53% dressing (NSW Department of Primary Industries, 2009b).

The second method involved constructing a gross margin (GM) based on predicted income and variable farm costs. This was used to estimate the reduction in GM for a typical 100 cow self replacing southern beef farm using the estimates of herd prevalence determined from the survey of veterinarians to estimate the number of affected calves in a typical herd, and using industry standard figures for 2009 for GM calculations supplied by NSW Industry and Investment (NSW Department of Primary Industries, 2009a). The variable costs included in the model account for pasture management, veterinary costs and the costs associated with selling livestock. This model assumes a weaning rate of 86%, conception rate of 92% and mortality rate of 2 %. An average year would therefore yield 42 steers and 22 heifers for sale as yearlings, 18 cull cows and one bull, with 20 heifers retained as replacements.

The GM was calculated for a two year period following the birth of CCUO calves to include the carry on effect of losses. The calculation does not consider the birth of affected calves in successive years, but assumes a loss of 5 heifer calves and 5 bull calves due to CCUO in the first year. Using a 'worse case' scenario, it was then assumed 10 additional cows without calves at marking were culled. Ten additional heifers were then retained to replace these culled cows leading to fewer calves in the following year.

1240.1.3 Part 3 – Case-control Study

1240.1.3.1 Selection of Case and Control Mobs

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 NSW or north eastern Victoria.

The University of Sydney Human Ethics Committee (approval number 02-2008/10640) approved procedures involving contact with and the collection of information from producers. Names and addresses of farm owners or managers with reported affected herds were provided by private and government veterinarians. Contact with producers 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 producer willingness to participate in the study. A small payment was offered to producers to compensate for the time required to conduct an interview in an attempt to improve the participation rate. When a producer 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 mob/s and control mobs. A case mob was a mob reported by the producer in which at least one CCUO calf was born in a calving season. A control mob was a mob reported by the producer to be free of CCUO calves in the same calving year as the case mob/s. For producers 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 Episcope 2.0 (http://www.clive.ed.ac.uk) it was determined that a sample size of 56 cases and 113 controls 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 (Table 9-1).

Table 9-1 Estimates of sample sizes required for the number of case mobs and control mobs to be used in the case-control study

Minimum odds ratio to be detected significant	% of controls with factor	Ratio of cases:controls	
		1:1	1:2
3	5%	139,139	99,198
3	10%	78,78	56,113
5	5%	54,54	38,76
5	10%	32,32	23,45

1240.1.3.2 Producer Interview

A questionnaire was used to collect information from each enrolled producer 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 with three producers in NSW during March 2008. As these changes to the questionnaire did not alter the quality or quantity of information collected, data collected from these three producers were included in the study.

1240.1.3.3 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 producer. 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^{o}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 (NATA). 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. The location and altitude of each paddock was determined using a global positioning system meter (Garmin GPS 205).

1240.1.3.4 Data Management

Microsoft Access (Microsoft 2003) was used to create a relational database to manage the questionnaire and laboratory data. Data concerning fertilizer history, supplement 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).

1240.1.3.5 Statistical Analyses

1240.1.3.5.1 Farm Characteristics and Calf Deformities

The general features of study farms 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.

1240.1.3.5.2 Data Sets

Two separate data sets were constructed for analysis. The mob dataset contained data on farm 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.

1240.1.3.5.3 Outcome Variables

Two outcomes were used in analysis of the mob dataset. First, the outcome variable mob status coded as 1 for case mobs and 0 for control mobs. Second, the outcome variable mob prevalence consisted of the number of affected calves in a mob divided by the total number of calves born in a mob. In the paddock dataset, the outcome variable paddock status was coded 1 for case paddocks and 0 for control paddocks.

1240.1.3.5.4 Explanatory Variables

The explanatory variables in the mob dataset were 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.

1240.1.3.5.5 Descriptive Analysis

Descriptive analyses were performed on all explanatory variables. Frequency distributions, bar charts and bar charts with outcome were determined for categorical variables. Mean, standard deviation and range were determined for continuous variables, and histograms and box plots with outcome were produced. All descriptive analyses were performed using SAS statistical software and UniLogistic Macro (http://elearn.vetsci.usyd.edu.au/magicmacros/LogReg/UniLogistic).

1240.1.3.5.6 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 (http://elearn.vetsci.usyd.edu.au/magicmacros). Variables with an unconditional association with the outcome at a significance of $P<0.25$ were selected for further investigation. First, these variables 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.

1240.1.3.5.7 Multivariable Analysis

1240.1.3.5.7.1 Model Building for Mob Status and Mob Prevalence

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 dam age, year affected and mob size were then added to the model one at a time to check for significant changes (>10%) in the estimates of variables in the model. If confounding was present, or if the variable was significant, 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.

1240.1.3.5.7.2 Model Building for Paddock Status

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.

1240.1.4 Part 4 – Analysis of Rainfall Data

1240.1.4.1 Data Source

The data concerning the number of CCUO calves used for this study was obtained during interviews with 46 producers interviewed as part of a case-control study investigating risk factors for CCUO. These farms were all located on the south-western slopes of NSW and in north-eastern Victoria. All farms included in the study were reported as affected by a veterinarian and had experienced the birth of CCUO calves between 2002 and 2007. An estimate of the number of CCUO calves born in each month of each calving season was provided by producers during the interviews. Due to the rarity of CCUO cases, the time series was constructed using case numbers rather than the incidence for the time period of interest from October 2001 to December 2007. This period allowed for inclusion of rainfall data throughout the gestation period of calves born in the years from 2002 to 2007.

Rainfall data (average monthly total, mm) were obtained using the Australian Bureau of Meteorology website for historical climatic data (www.bom.gov.au/climate/data/weather-data.shtml). The GPS coordinates of each affected farm were used to generate a list of all weather stations within a radius of 20km. The closest weather station with the most complete set of data (minimum 10 years) was selected and data downloaded into Microsoft® Office Excel. A time series of average monthly rainfall was created based on the average of the monthly rainfall values for selected weather stations for the period of interest (October 2001 to December 2007).

1240.1.4.2 Data Analysis

1240.1.4.2.1 Time Series Analysis

Time series analysis was used to examine the relationships between rainfall occurring during gestation and the occurrence of CCUO on 46 farms between 2002 and 2007 over a total of 75 months (Shumway and Stoffer, 2006). The Applied Statistical Time Series Analysis program (ASTSA, version 2.0. Department of Statistics, University of California, Davis, http://www.stat.pitt.edu/stoffer/tsa2/) was used for all time series analysis.

The time series for cases of CCUO was transformed using linear regression to remove any long-term trend in the time series. Autocovariance (ACF) and partial autocovariance (PACF) functions of the time-series of cases of CCUO were calculated to identify the seasonal pattern of CCUO. Autoregression was performed to determine if a value in the time-series of cases of CCUO at time *t* could be predicted by cases at a previous time (*t*-1, *t*-2….*t*-12). Selection of the best fitting model was based on the goodness-of-fit criterion (Akaike's corrected information criterion (AICc)).

The time series of monthly average rainfall was used to produce a three month moving average time series for rainfall. Cross correlation functions of the time series of cases of CCUO with average monthly rainfall and three-month moving average rainfall were calculated to identify significant correlations between the series. Vector autoregression was used to determine significant relationships between the time series of cases of CCUO and rainfall in the previous 12 months. The best fitting model was selected using AICc, and the ACF and PACF of the selected model residuals were checked visually for evidence of trends. The residuals were also examined for independence, constant variance and outliers using the Box-Pierce, cumulative spectrum and *z*-distribution tests, respectively.

1240.1.4.2.2 Logistic Regression Analysis

The results of time series analysis were used to create a dataset for average monthly and three-month average rainfall deficiency on the 46 farms used in the study. For each farm, an outcome variable month status was created for each calving month (July to October) for each year (2002 to 2007) coded as 1 when affected calves were born during the month and 0 when no calves were born. Explanatory variables were created for rainfall deficiency at the time lags identified from the time series analysis for each month and year on each farm. Long-term first decile was used for categorisation of rainfall deficiency, as these figures were readily available from the Bureau of Meteorology (www.bom.gov.au/climate/data/weather-data.shtml). Explanatory variables for monthly and three-month average rainfall were coded 1 where rainfall for the lag period identified was within the first decile, and 0 when above the first decile. Descriptive analysis of all data was performed using SAS statistical software (release 9.2, © 2002-08, SAS Institute Inc., Cary, NC, USA) and the UniLogistic Macro for SAS developed by Dhand (http://elearn.vetsci.usyd.edu.au/magicmacros/LogReg/UniLogistic). The association between each explanatory variable and the outcome variable was investigated on an individual basis using the likelihood-ratio chi-square test implemented in the UniLogistic Macro for SAS (http://elearn.vetsci.usyd.edu.au/magicmacros).

Variables with an unconditional association with the outcome at a significance of $P<0.25$ were selected for further investigation. First, these variables were tested in pairs for collinearity using Spearman rank correlation and highly correlated variables (coefficient > |0.70|) were then evaluated using either chi-squared tests or Fisher exact tests (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.

The SAS GLIMMIX procedure and a manual stepwise approach was used to build the multivariable model (Dohoo *et al.*, 2003). Random-effect variables for farm and year were included in each model to account for clustering of CCUO occurrence within farm and year. 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 the random effect terms.

1240.2 Appendix 2: Cases Reports 2002-2007

Tables 9-2 to 9-7 Description of CCUO cases reported in the period 2002 to 2007 by government veterinarians and veterinary members of the ACV special interest group of the AVA surveyed in 2007.

Table 9-2 Reports of affected calves in 2002 – 13 affected calves on 3 properties

1241Case Number	1242Location	1243Number affected	1244Pathology
1	ACT	1	Nil
2	Gundagai RLPB, NSW	5	Nil
3	Dandaragan, WA	7	Nil

Table 9-3 Reports of affected calves in 2003 – 238 affected calves on 27 properties

1245Case Number	1246Location	1247Number affected	1248Pathology
4	Cooma RLPB, NSW	3	Nil
5	Adaminiby, NSW	1	Nil
6	Bongarby, NSW	2	Serology ruling out viral cause
7	Gundagai RLPB, NSW	2	Nil
8	Gundagai RLPB, NSW	1	Nil
9	Gundagai RLPB, NSW	18	Nil
10	Gundagai RLPB, NSW	1	Nil
11	Gundagai RLPB, NSW	23	Pathology confirming chondrodystrophy
12	Tooma, NSW	30	Nil
13	Lankeys Creek, NSW	2	Nil
14	Mannus, NSW	2	Nil
15	Ournie, NSW	4	Nil
16	Hume RLPB, NSW	2	Nil
17	Ournie, NSW	1	Nil
18	Mannus, NSW	1	Nil
19	Narrandera, NSW	14	Serology ruling out viral cause
20	Harden, NSW	5	Nil
21	Burrowye, VIC	1+	Serology ruling out viral cause
22	Euroa, VIC	30	NB cases of amelia
23	Strath Creek, VIC	2	Nil
24	Violet Town, VIC	3	Nil
25	Nariel Valley, VIC	9	Nil
26	Cudgewa, VIC	8	Nil
27	Wodonga, VIC	3	Nil
28	Wadonga, VIC	4	Nil
29	Tallangatta, VIC	60	Nil
30	Corryong, VIC	6	Nil

Table 9-4 Reports of affected calves in 2004 – 555 affected calves on 55 properties

1249Case Number	1250Location	1251Number affected	1252Pathology
31	Tullibigeal, NSW	10	Nil
32	Adaminiby, NSW	8	Serology ruling out viral cause
33	Adaminiby, NSW	1	Serology ruling out viral cause
34	Adaminiby, NSW	25	Serology ruling out viral cause
35	Adaminiby, NSW	1	Serology ruling out viral cause
36	Cooma RLPB, NSW	5	Nil
37	Gundagai RLPB, NSW	12	Nil
38	Gundagai RLPB, NSW	20	Nil
39	Gundagai RLPB, NSW	1	Nil
40	Gundagai RLPB, NSW	17	Nil
41	Gundagai RLPB, NSW	4	Nil
42	Gundagai RLPB, NSW	9	Nil
43	Gundagai RLPB, NSW	5	Nil
44	Gundagai RLPB, NSW	12	Serology ruling out viral cause
45	Gundagai RLPB, NSW	15	Nil
46	Brungle, NSW	8	Pathology confirming chondrodystrophy
47	Gundagai RLPB, NSW	5	Nil
48	Gundagai RLPB, NSW	3	Nil
49	Gundagai RLPB, NSW	12	Pathology confirming chondrodystrophy
50	Hume RLPB, NSW	5	Nil
51	Mannus, NSW	5	Nil
52	Humula, NSW	8	Nil
53	Carabost, NSW	5	Nil
54	Tooma, NSW	4	Nil
55	Mannus, NSW	3	Nil
56	Cookadinia, NSW	20	Nil
57	Hume RLPB, NSW	20	Nil
58	Hume RLPB, NSW	50	Nil
59	Wymah, NSW	5	Nil
60	Walla, NSW	4	Nil
61	Talmalmo, NSW	10	Nil
62	Mannus, NSW	2	Nil
63	Tumburrumba, NSW	2	Nil
64	Tumburrumba, NSW	2	Nil
65	Tumburrumba, NSW	2	Nil
66	Ournie, NSW	10	Nil
67	Ournie, NSW	4	Nil
68	Lankeys Creek, NSW	2	Nil
69	WaggaWagga RLPB,NSW	3	Nil
70	Holbrook, NSW	1	Serology ruling out viral cause

Table 9-4 (continued) Reports of affected calves in 2004 – 555 affected calves on 55 properties

1253 Case Number	1254 Location	1255 Number affected	1256 Pathology
71	Bowra, NSW	3	Serology ruling out viral cause
72	Young, NSW	3	Nil
73	Tooma, NSW	60	Nil
74	Tooborac , VIC	6	Nil
75	Euroa, VIC	13	Pathology confirming chondrodystrophy
76	Walwa, VIC	1+	Nil
77	Seymour, VIC	5	Nil
78	Ruffy, VIC	2	Nil
79	Seymour, VIC	8	Serology ruling out viral cause
80	Glenrowan West, VIC	6	Nil
81	Tintaldra, VIC	8	Nil
82	Wodonga, VIC	1+	Nil
83	Wodonga, VIC	30	Nil
84	Walwa, VIC	27	Serology ruling out viral cause
85	Seymour, VIC	12	Pathology confirming chondrodystrophy

Table 9-5 Reports of affected calves in 2005 – 20 affected calves on 10 properties

1257 Case Number	1258 Location	1259 Number affected	1260 Pathology
86	ACT	3	Nil
87	Adaminiby, NSW	1+	Pathology confirming chondrodystrophy
88	Gundagai RLPB, NSW	2	Serology ruling out viral cause
89	Lankeys Creek, NSW	2	Nil
90	Humula, NSW	2	Nil
91	Moree, NSW	2	Embryo transfer calves
92	Crowther, NSW	3	Nil
93	Young, NSW	1	Nil
94	Ruffy, VIC	3	Nil
95	Euroa, VIC	1	NB case of amelia

Table 9-6 Reports of affected calves in 2006 – 230 affected calves on 31 properties

1261 Case Number	1262 Location	1263 Number affected	1264 Pathology
96	ACT	1	Nil
97	Bombala, NSW	2	Serology ruling out viral cause
98	Bombala, NSW	2	Nil
99	Vittoria, NSW	1	Nil
100	Adelong, NSW	40	Nil
101	Cootamundra, NSW	11	Nil
102	Hay, NSW	1+	10% calves affected, total number unknown
103	Humula, NSW	8	Nil
104	Ournie, NSW	3	Nil
105	Talmalmo, NSW	4	Nil
106	Woomargama, NSW	5	Nil
107	Holbrook, NSW	3	Nil
108	Holbrook, NSW	4	Nil
109	Lankeys Creek, NSW	3	Nil
110	Cookardinia, NSW	2	Nil
111	Lankeys Creek, NSW	6	Nil
112	Holbrook, NSW	3	Nil
113	Lankeys Creek, NSW	2	Nil
114	Holbrook, NSW	2	Nil
115	Ournie, NSW	5	Nil
116	Molong, NSW	3	Nil
117	Narrandera, NSW	5	Nil
118	Narrandera, NSW	25	Nil
119	Wagga Wagga, NSW	6	Serology ruling out viral cause
120	Weejasper, NSW	20	Not reported to District Veterinarian
121	Young, NSW	6	Nil
122	Young, NSW	2	Pathology confirming chondrodystrophy
123	Tooma, NSW	14	Nil
124	Tallangatta, VIC	1	Serology ruling out viral cause
125	Kingston, SA	40	Calves believed to be Mn deficient
126	Burracoppin WA	1	Nil

Table 9-7 Reports of affected calves in 2007 – 25 affected calves on 9 properties

1265Case Number	1266Location	1267Number affected	1268Pathology
127	Gundagai RLPB, NSW	2	Nil
128	Ournie, NSW	9	Nil
129	Cookardinia, NSW	1	Nil
130	Cookardinia, NSW	1	Nil
131	Wymah, NSW	1	Nil
132	Nyngan, NSW	1	Nil
133	Cootamundra, NSW	1	Nil
134	Tooma, NSW	8	Pathology confirming chondrodystrophy
135	Burracoppin, WA	1	Nil

1268.1 Appendix 3: Case Records

Table 9-8 Case records obtained concerning cases of CCUO prior to 2002

Data source	Type of report	Year of cases	Number of cases involved	Location
Private veterinarians and government veterinarians	Anecdotal reports and diary entries of sporadic cases of CCUO reported during telephone interviews.	1979-1997	12	NSW
NSW DPI	Two pathology reports concerning sporadic cases	1988	2	NSW
NSW DPI	Questionnaires from interviews with 52 producers-incomplete details on all forms	1991	52	NSW
NSW DPI	Excel spreadsheet containing data from interviews with 33 of the 52 producers above	1991	33 (included in above)	NSW
NSW DPI	22 pathology reports confirming chondrodystrophy	1991	22	NSW
Private veterinarians	2 unpublished papers concerning 1991 cases in NSW, one paper based on questionnaire results and one paper based on pathology reports.	1991	-	NSW

Table 9-9 Case records obtained concerning cases of CCUO from 2002 to 2007

Data source	Type of report	Year of cases	Number of cases involved	Location
Private veterinarians and government veterinarians	Case records, diary entries and anecdotal reports of cases of CCUO reported during telephone interviews.	2002-2007	91	NSW, Victoria, ACT and Western Australia
NSW DPI	12 postal questionnaires from producers	2003/2004	12	NSW
Victorian DPI	Questionnaires from interviews with 26 producers	2003/2004	26	Victoria
Victorian and NSW DPI	Pathology reports confirming chondrodystrophy	2003-2007	8	NSW/Victoria
Victorian and NSW DPI	Pathology reports excluded viral aetiology in cases	2003-2006	16	NSW/Victoria

1268.2 Appendix 4: Descriptive and Univariable Analysis of Variables for Case-control Study

Table 9-10 Contingency tables for categorical management and environment explanatory variables in 66 case mobs and 102 control mobs on 46 farms in south-eastern Australia

Variable	Categories	Case mobs	Control mobs
Dam age at calving	Mixed age groups	32	89
	Heifers only	27	7
	Single age cow groups	7	6
Dam condition decreased during gestation	No	53	85
	Yes	13	17
Main breed of dams	Angus	33	55
	Crossbred	13	18
	Hereford	9	13
	Other purebred	11	16
Trees grazed by dams during gestation	No	33	94
	Yes	31	8
Inadequate pasture[a]	No	35	92
	Yes	31	10
Dams vaccinated during gestation	No	25	48
	Yes	41	54
Spring Calving	No	0	14
	Yes	66	88
Dams wormed during gestation	No	40	61
	Yes	26	41
Dams moved between paddocks during gestation[b]	No	65	101
	Yes	1	1
Paddock tree cover level	< 5%	28	81
	> 5 %	35	21
Paddock adjoins forest, crown land or national park	No	50	98
	Yes	14	4
Paddock adjoins public road	No	53	89
	Yes	11	13
Paddock adjoins neighbour	No	41	76
	Yes	23	26

Table 9-10 (continued) Contingency tables for categorical management and environment explanatory variables in 66 case mobs and 102 control mobs on 46 farms in south-eastern Australia

Variable	Categories	Case mobs	Control mobs
Paddock predominantly hilly to steep terrain	No	20	81
	Yes	46	21
Main pasture type in paddock	Native	36	6
	Mixed	18	24
	Improved	12	72
Water source	Earthen dam	52	59
	Creek/River	12	32
	Bore/reticulated	1	11
Main aspect of the paddock	North	28	43
	South	15	17
	East	14	27
	West	7	15
Lime applied to paddock in the last 5 years[b]	No	59	101
	Yes	7	1
Fertilizer applied to paddock in the last 5 years	No	45	79
	Yes	21	23
Supplement feed provided	No	20	53
	Yes	46	49
Supplement feed fed from the ground[b]	No	2	2
	Yes	44	47
Supplement feed type	Nil	20	53
	Hay	30	28
	Silage	10	10
	Pellet	6	11
Year affected	2002	3	6
	2003	15	23
	2004	24	39
	2005	9	12
	2006	13	19
	2007	2	3

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 9-11 Contingency tables for categorical management and environment explanatory variables associated with mob prevalence 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	Normal calves
Dam age at calving	Mixed-age groups	313	9489
	Heifers only	380	1520
	Single-age cow groups	106	701
Dam condition score decreased during gestation	No	619	9833
	Yes	180	1877
Main breed of dams	Angus	446	5726
	Crossbred	138	2989
	Hereford	107	1166
	Other purebred	108	1829
Trees grazed by dams during gestation	No	419	9380
	Yes	344	2291
Inadequate pasture[a]	No	402	8345
	Yes	361	3326
Dams vaccinated during gestation	No	290	4486
	Yes	509	7224
Spring Calving	No	0	769
	Yes	799	10941
Dams wormed during gestation	No	490	7185
	Yes	309	4525
Dams moved between paddocks during gestation[b]	No	783	11686
	Yes	16	24
Paddock tree cover level	< 5%	348	7810
	> 5 %	407	3734
Paddock adjoins forest, crown land or national park	No	561	10636
	Yes	202	1035
Paddock adjoins public road	No	652	8271
	Yes	111	3400
Paddock adjoins neighbour	No	505	7031
	Yes	258	4640

Table 9-11 (continued) Contingency tables for categorical management and environment explanatory variables associated with mob prevalence 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	Normal calves
Paddock predominantly hilly to steep terrain	No	226	7507
	Yes	573	4203
Main pasture type in paddock	Improved	137	6430
	Native	447	2857
	Mixed	179	2384
Water source	Earthen dam	679	8113
	Creek/River	77	2848
	Bore/reticulated	30	735
Main aspect of the paddock	North	474	3642
	South	117	2810
	East	98	3987
	West	74	1232
Lime applied to paddock in the last 5 years[b]	No	528	7945
	Yes	271	3756
Fertilizer applied to paddock in the last 5 years	No	528	7945
	Yes	271	3756
Supplement feed provided	No	263	4323
	Yes	536	7387
Supplement feed fed from the ground[b]	No	82	218
	Yes	454	7169
Supplement feed type	Nil	263	4323
	Hay	331	5042
	Silage	134	882
	Pellet	71	1436
Year Affected	2002	16	637
	2003	254	3113
	2004	299	4827
	2005	54	1358
	2006	159	1498
	2007	17	277

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 9-12 Univariable associations of farm management and environment variables with mob status in 168 mobs on 46 farms in south-eastern Australia, P values <0.25.

Variables	Categories	b	SE(b)	Odds ratio	LCL	UCL	P-Value
Inadequate pasture[a]	No	1.0					<0.001
	Yes	2.09	0.42	8.12	3.70	19.16	
Trees grazed by dams during gestation	No	1.0					<0.001
	Yes	2.40	0.45	11.04	4.81	28.03	
Dam age at calving	Mixed-age groups	1.0					<0.001
	Heifers only	2.37	0.47	10.73	4.26	26.92	
	Single-age cow groups	1.18	0.59	3.25	1.03	10.34	
Paddock consists of predominantly hilly to steep terrain	No	1.0					<0.001
	Yes						
Main pasture type in the paddock	Improved	1.0					<0.001
	Native	3.56	0.54	35.0	12.9	110.1	
	Mixed	1.45	0.44	4.25	1.8	10.4	
Paddock tree cover level	<5 %	1.0					<0.001
	>5 %	1.62	0.36	5.06	2.55	10.34	
Paddock adjoins forest, crown land or national park	No	1.0					<0.001
	Yes	1.93	0.59	6.86	2.32	25.19	
Spring calving	No	1.0					<0.001
	Yes	13.98	335.40	>999	4.98	>999	
Mob size (number of breeding dams)	Constant	-0.96	0.25	.	.	.	0.002
	Number of dams	0.01	0.00	1.01	1.00	1.01	
Water Source	Earthen dam	1.0					0.003
	Creek/River	-0.85	0.39	0.43	0.20	0.92	
	Bore/reticulated	-2.27	1.06	0.10	0.01	0.82	
Supplement feed provided[b]	No	1.0					0.005
	Yes	0.91	0.33	2.49	1.31	4.85	

Table 9-12 (continued) Univariable associations of farm management and environment variables with mob status in 168 mobs on 46 farms in south-eastern Australia, P values <0.25.

Variables	Categories	b	SE(b)	Odds ratio	LCL	UCL	P-Value
Supplement feed type	Nil	1.0					0.026
	Hay	1.04	0.37	2.84	1.38	5.96	
	Silage	0.97	0.52	2.65	0.96	7.43	
	Pellet	0.37	0.57	1.45	0.45	4.35	
Paddock adjoins neighbour	No	1.0					0.153
	Yes	0.49	0.35	1.64	0.83	3.24	
Fertilizer applied to paddock in the last 5 years	No	1.0					0.185
	Yes	0.47	0.35	1.60	0.80	3.22	
Dams vaccinated during gestation	No	1.0					0.240
	Yes	0.38	0.32	1.46	0.78	2.76	

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

b. eliminated due to high correlation with other variables

Table 9-13 Univariable associations of farm management and environment variables with mob status in 168 mobs on 46 farms in south-eastern Australia, P values >0.25

Variables	Categories	b	SE(b)	Odds ratio	LCL	UCL	P-Value
Paddock adjoins public road	No	1.0					0.432
	Yes	0.35	0.44	1.42	0.59	3.40	
Stocking Rate	Constant	-0.42	0.26				0.462
	Head/Ha	-0.16	0.22	0.85	0.54	1.30	
Dam condition score decreased during gestation	No	1.0					0.618
	Yes	0.20	0.408	1.23	0.55	2.73	
Main aspect of the paddock	East	1.0					0.634
	North	0.23	0.41	1.26	0.57	2.85	
	South	0.53	0.48	1.70	0.66	4.45	
	West	-0.11	0.56	0.90	0.29	2.68	
Dams wormed during gestation	No	1.0					0.917
	Yes	-0.03	0.32	0.97	0.51	1.82	
Main breed of dams	Angus	1.0					0.968
	Crossbred	0.19	0.43	1.20	0.52	2.76	
	Hereford	0.14	0.49	1.15	0.43	2.97	
	Other	0.14	0.45	1.15	0.47	2.75	
Year affected	2002	1.0					0.997
	2003	0.27	0.78	1.30	0.30	6.93	
	2004	0.21	0.75	1.23	0.30	6.26	
	2005	0.41	0.83	1.50	0.30	8.67	
	2006	0.31	0.79	1.37	0.30	7.41	
	2007	0.29	1.15	1.33	0.12	13.46	

Table 9-14 Univariable associations of farm management and environment variables with mob events-trials in 168 mobs on 46 farms in south-eastern Australia, P values <0.25.

Variables	Categories	b	SE(b)	Odds ratio	LCL	UCL	P-Value
Inadequate pasture[a]	No	1.0					<0.001
	Yes	0.81	0.08	2.25	1.94	2.61	
Trees grazed by dams during gestation	No	1.0					<0.001
	Yes	1.21	0.08	3.36	2.89	3.90	
Dam age at calving	Mixed-age groups	1.0					<0.001
	Heifers only	2.03	0.08	7.58	6.47	8.89	
	Single-age cow groups	1.52	0.12	4.58	3.62	5.77	
Spring Calving	No	1.0					<0.001
	Yes	15.17	263.1	>999	29.16	>999	
Paddock consists of predominantly hilly to steep terrain	No	1.0					<0.001
	Yes	1.51	0.08	4.53	3.87	5.32	
Main pasture type in the paddock	Improved	1.0					<0.001
	Native	1.99	0.10	7.34	6.05	8.97	
	Mixed	1.26	0.12	3.52	2.81	4.43	
Paddock tree cover level	<5 %	1.0					<0.001
	>5 %	0.87	0.08	2.39	2.06	2.77	
Paddock adjoins forest, crown land or national park	No	1.0					<0.001
	Yes	1.31	0.09	3.70	3.11	4.39	
Mob size (number of breeding dams)	Constant	-2.26	0.05	.	.	.	<0.001
	Number of dams	0.00	0.00	1.00	1.00	1.00	
Water Source	Earthen dam	1.0					<0.001
	Creek/River	-1.13	0.12				
	Bore/ reticulated	-2.63	0.05				
Supplement feed type	Nil	1.0					<0.001
	Hay	0.08	0.09	1.08	0.91	1.28	
	Silage	0.92	0.11	2.50	2.00	3.11	
	Pellet	-0.23	0.14	0.80	0.61	1.04	

Table 9-14 (continued) Univariable associations of farm management and environment variables with mob events-trials in 168 mobs on 46 farms in south-eastern Australia, P values <0.25.

Variables	Categories	b	SE(b)	Odds ratio	LCL	UCL	P-Value
Paddock adjoins neighbour	No	1.0					<0.001
	Yes	-0.26	0.08	0.77	0.66	0.90	
Dam condition score decreased during gestation	No	1.0					<0.001
	Yes	0.42	0.09	1.52	1.28	1.81	
Main breed of dams	Angus	1.0					<0.001
	Crossbred	-0.52	0.10	0.59	0.49	0.72	
	Hereford	0.16	0.11	1.18	0.94	1.46	
	Other	-0.28	0.11	0.76	0.61	0.94	
Year affected	2002	1.0					<0.001
	2003	1.18	0.26	3.25	2.01	5.64	
	2004	0.90	0.26	2.46	1.53	4.28	
	2005	0.46	0.29	1.58	0.92	2.88	
	2006	1.44	0.27	4.22	2.59	7.40	
	2007	0.89	0.36	2.44	1.21	4.95	
Paddock adjoins public road	No	1.0					<0.001
	Yes	-0.88	0.10	0.41	0.34	0.51	
Stocking Rate	Constant	-1.87	0.07				<0.001
	Head/Ha	-0.82	0.06	0.44	0.39	0.50	
Main aspect of the paddock	East	1.0					<0.001
	North	1.67	0.11	5.30	4.26	6.65	
	South	0.53	0.14	1.69	1.29	2.23	
	West	0.89	0.16	2.44	1.79	3.32	

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

Table 9-15 Univariable associations of farm management and environment variables with mob events-trials in 168 mobs on 46 farms in south-eastern Australia, P values >0.25

Variables	Categories	b	SE(b)	Odds ratio	LCL	UCL	P-Value
Dams vaccinated during gestation	No	1.0					0.256
	Yes	0.09	0.08	1.09	0.94	1.27	
Fertilizer applied to paddock in the last 5 years	No	1.0					0.283
	Yes	0.08	0.08	1.09	0.93	1.26	
Dams wormed during gestation	No	1.0					0.986
	Yes	0.00	0.08	1.00	0.86	1.16	

Table 9-16 Descriptive statistics for 23 soil explanatory variables for 125 paddocks surveyed on 46 farms in south-eastern Australia in 2008-2009

	Case Paddocks (n=50)				Control Paddocks (n=75)				Total (n=125)			
Variable	Mean	Std Dev	Min	Max	Mean	Std Dev	Min	Max	Mean	Std Dev	Min	Max
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	0027	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 9-17 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	61
	Yes	34	14
Paddock tree cover level	<5 %	22	61
	>5 %	28	14
Main source of water provided from a creek or river	Dam	38	48
	Reticulated/bore	2	7
	Creek/river	10	20
Main pasture type in paddock	Improved	9	54
	Native	26	3
	Mixed	15	18
Fertilizer applied to paddock in the last 5 years	No	34	58
	Yes	16	17
Paddock adjoins forest, crown land or national park	No	33	72
	Yes	15	3
Paddock adjoins public road	No	41	64
	Yes	9	11
Paddock adjoins neighbour	No	34	55
	Yes	16	20
Main aspect of the paddock	North	21	31
	South	11	21
	East	12	10
	West	6	13

Table 9-18 Univariable associations of soil and environment variables with paddock status for 125 paddocks surveyed on 46 farms in south-eastern Australia in 2008-2009, P values <0.25

Variables	Category	*b*	SE(*b*)	Odds ratio	LCL (OR)	UCL (OR)	*P*-value
Paddock predominantly hilly to steep terrain	No	0					<0.001
	Yes	2.23	0.42	9.26	4.14	21.93	
Paddock tree cover level	<5%	0					<0.001
	>5%	1.69	0.42	5.40	2.42	12.52	
Main pasture type in paddock	Improved pasture			1.0			<0.001
	Native pasture	3.95	0.71	52.00	14.75	253.51	
	Mixed pasture	1.61	0.50	5.00	1.91	13.85	
Soil iron		-0.01	0.00	0.99	0.99	1.00	0.003
Paddock adjoins forest, crown land or national park	No			1.0			0.017
	Yes	1.57	0.71	4.80	1.31	22.86	
Soil sodium		-6.99	3.61	<0.001	<0.001	0.44	0.024
Soil potassium		1.64	0.82	5.18	1.08	28.00	0.040
Soil phosphorous Colwell		-0.02	0.01	0.98	0.96	1.00	0.114
Water source	Earthen dam	0					0.127
	Creek/River	-0.46	0.44	0.63	0.26	1.5	
	Bore/reticulated	-1.71	1.09	0.18	0.02	1.5	
Soil magnesium		-0.33	0.27	0.72	0.40	1.19	0.204
Soil electrical conductivity		-5.21	4.32	0.01	<0.001	17.77	0.213

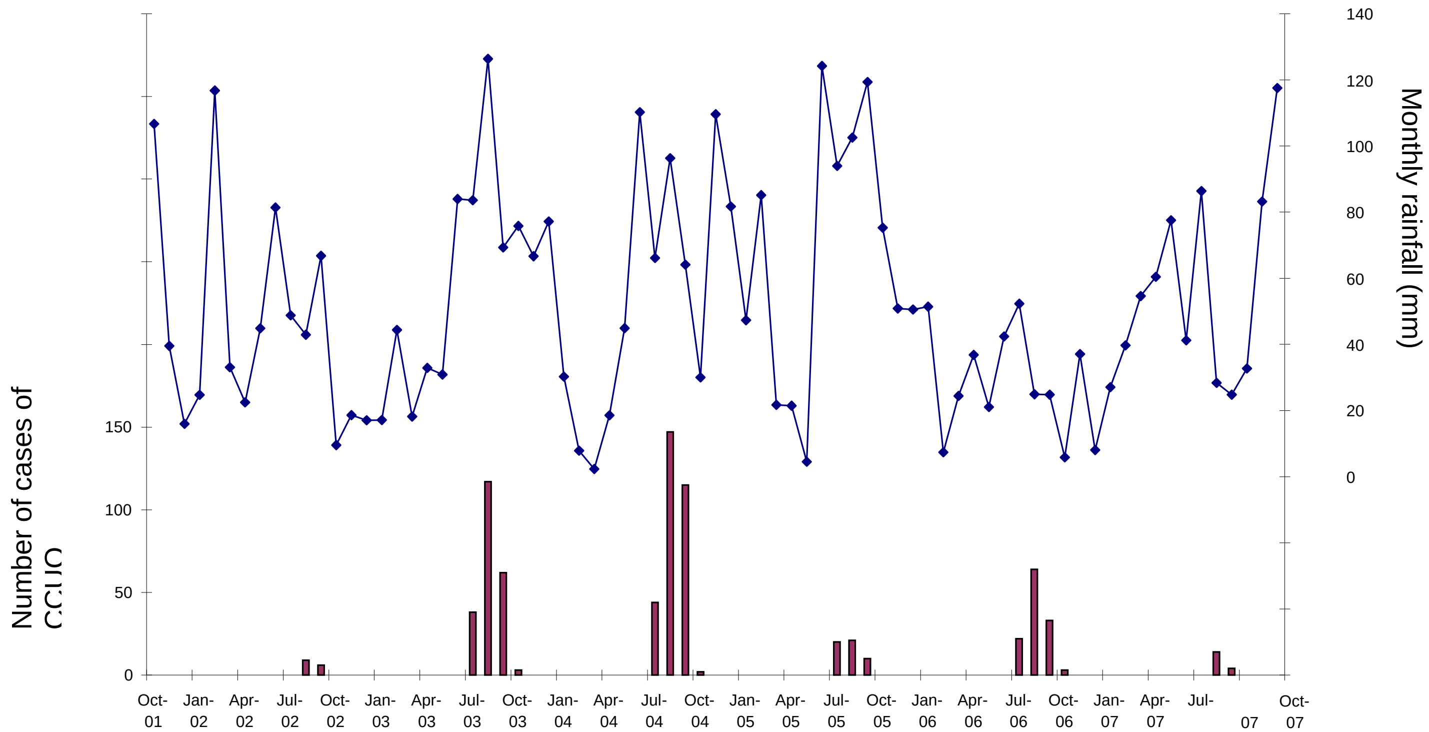
Table 9-18 (continued) Univariable associations of soil and environment variables with paddock status for 125 paddocks surveyed on 46 farms in south-eastern Australia in 2008-2009, P values <0.25

Variables	Category	*b*	SE(*b*)	Odds ratio	LCL (OR)	UCL (OR)	*P*-value
Fertilizer applied to paddock in the last 5 years							0.249
	No	0					
	Yes	0.47	0.41	1.61	0.72	3.60	

Table 9-19 Univariable associations of soil and environment variables with paddock status for 125 paddocks surveyed on 46 farms in south-eastern Australia in 2008-2009, P values >0.25

Variables	Category	*b*	SE(*b*)	Odds ratio	LCL (OR)	UCL (OR)	*P*-value
Soil zinc		-0.22	0.22	0.80	0.50	1.20	0.295
Paddock adjoins neighbour	No			1			0.378
	Yes	0.36	0.41	1.44	0.64	3.24	
Soil organic carbon		-0.17	0.22	0.84	0.53	1.29	0.435
Soil copper		-0.31	0.39	0.73	0.32	1.53	0.4416
Calcium:magnesium ratio		0.06	0.10	1.06	0.87	1.30	0.541
Paddock adjoins public road	No						0.552
	Yes	0.29	0.49	1.34	0.50	3.53	
Main aspect of the paddock	East			1			0.580
	North	0.32	0.48	1.38	0.55	3.59	
	South	0.79	0.57	2.20	0.72	6.91	
	West	0.43	0.62	1.54	0.45	5.27	
Soil pH (calcium chloride)		0.16	0.48	1.17	0.45	3.03	0.746
Soil manganese		0.00	0.01	1.00	0.98	1.02	0.949

Figure 9-1 Time series for total number of CCUO calves born per month (bars) and average monthly rainfall (line) on 46 farms in south-eastern Australia between October 2001 and December 2007



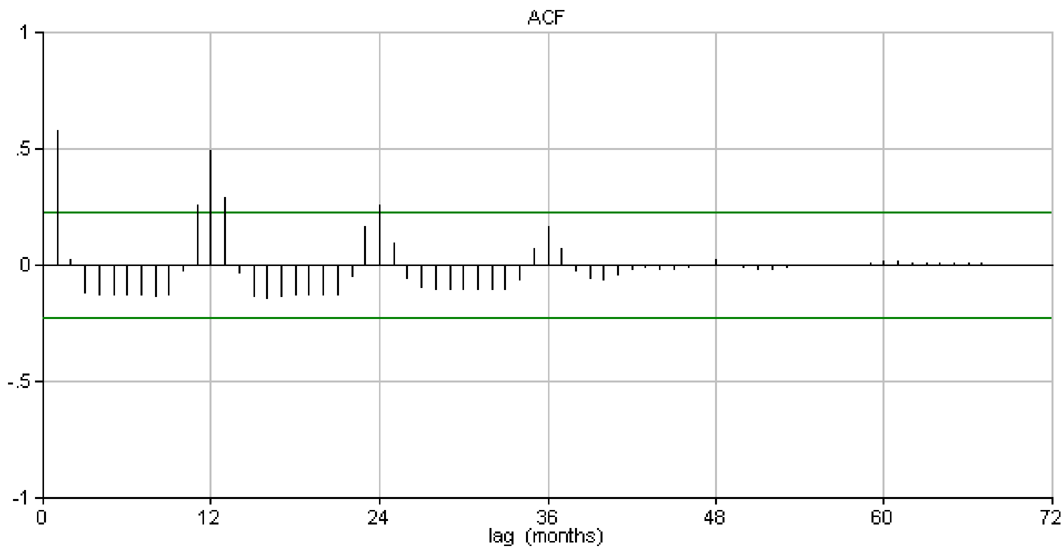


Figure 9-2 (a) Autocorrelation function for the time series for cases of CCUO from October 2001 to December 2007 on 46 farms in south-eastern Australia and 95% confidence limits

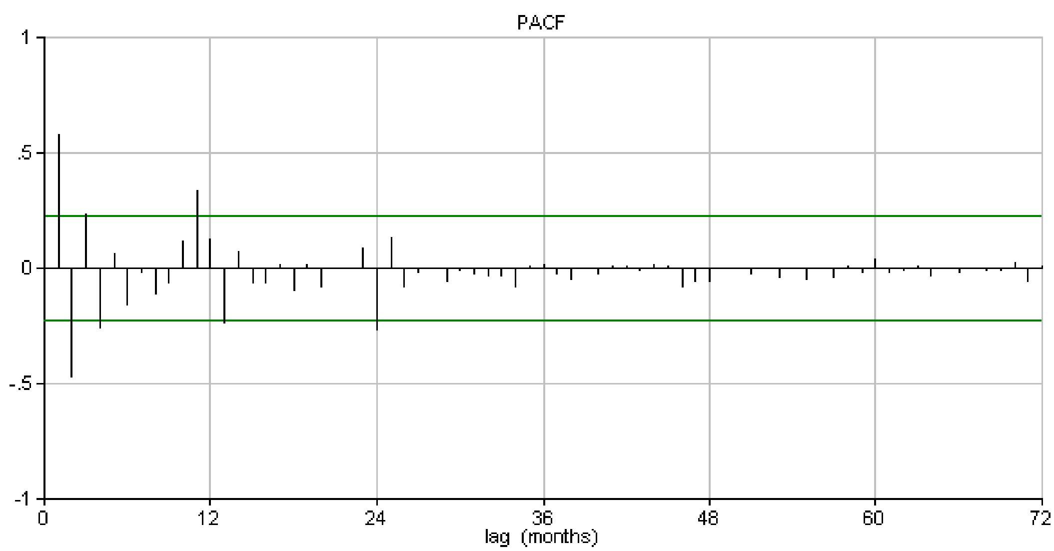


Figure 9-2 (b) Partial autocorrelation function for the time series for cases of CCUO from October 2001 to December 2007 on 46 farms in south-eastern Australia and 95% confidence limits

Table 9-20 Cross correlation between cases of CCUO on 46 farms in south-eastern Australia from October 2001 to December 2007, and average monthly rainfall and three month moving average rainfall. Values with *P*<0.05 in bold

1269 Lag in months	**1270**CCF (*r*) Average monthly rainfall	**1271**CCF (*r*) three month moving average rainfall
12720	**1273****0.276**	**1274****0.320**
12751	**1276****0.319**	**1277****0.391**
12782	**1279**0.244	**1280****0.279**
12813	**1282**0.034	**1283**0.023
12844	**1285**-0.213	**1286**-0.236
12875	**1288****-0.295**	**1289****-0.372**
12906	**1291****-0.263**	**1292****-0.351**
12937	**1294**-0.169	**1295**-0.233
12968	**1297**-0.053	**1298**-0.101
12999	**1300**0.022	**1301**0.007
130210	**1303**0.056	**1304**0.133
130511	**1306**0.211	**1307****0.269**
130812	**1309****0.312**	**1310****0.398**

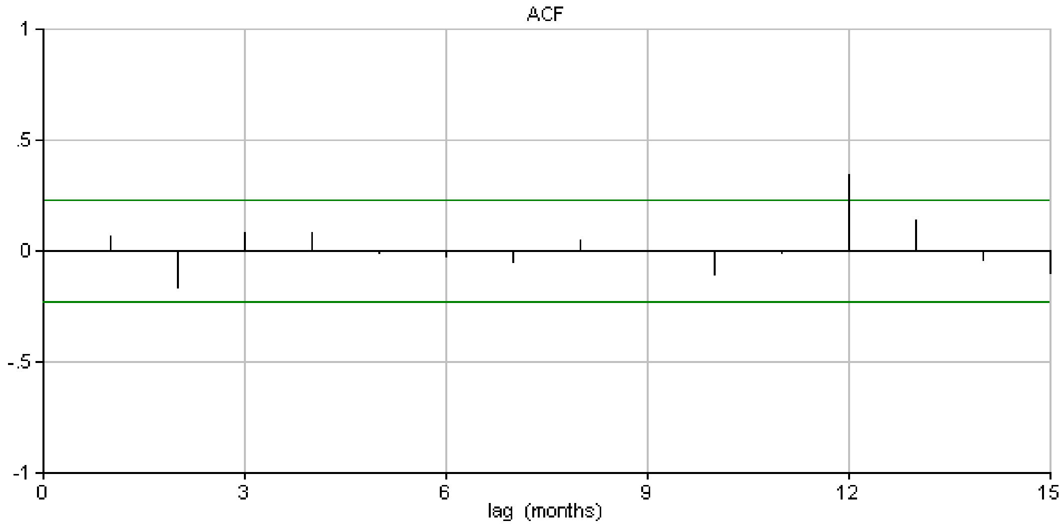


Figure 9-3 (a) Autocorrelation function (ACF) for residuals of the model shown in Table 3 for cases of CCUO on 46 farms in south-eastern Australia from October 2001 to December 2007, and average monthly rainfall and 95% confidence limits

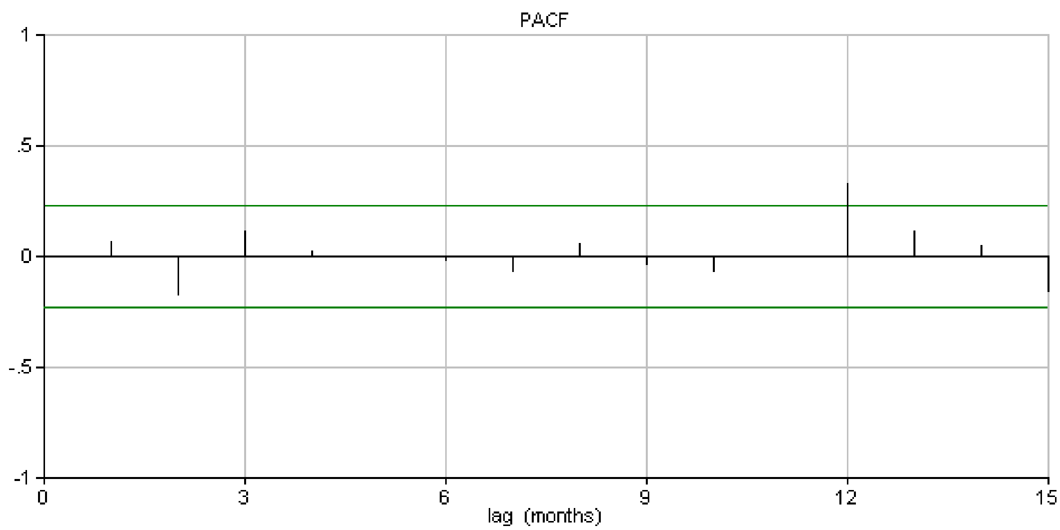


Figure 9-3 (b) Partial autocorrelation function (PACF) for residuals of the model shown in Table 3 for cases of CCUO on 46 farms in south-eastern Australia from October 2001 to December 2007, and average monthly rainfall and 95% confidence limits

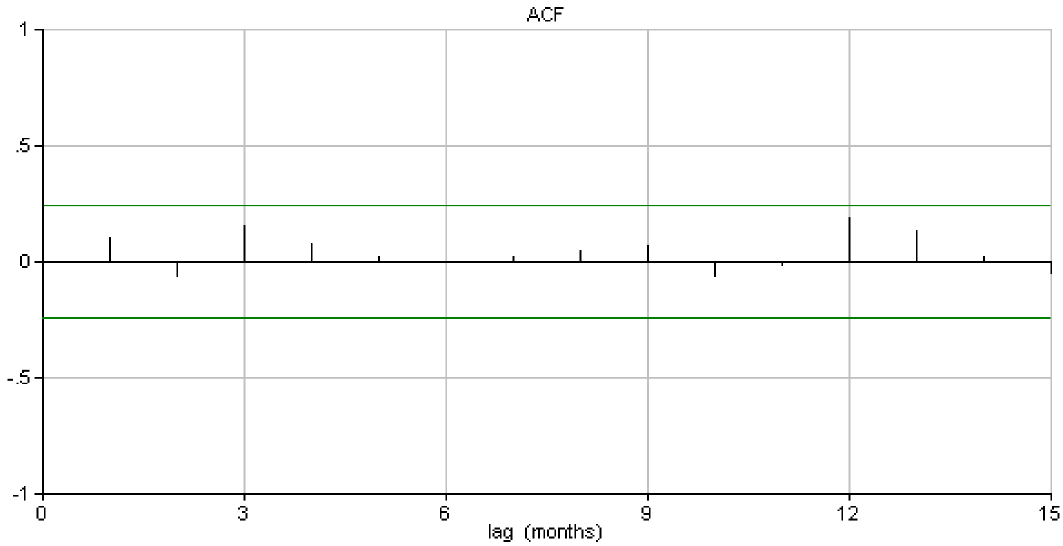


Figure 9-4 (a) Autocorrelation function (ACF) for residuals of the model shown in Table 4 for cases of CCUO on 46 farms in south-eastern Australia from October 2001 to December 2007, and three month moving average rainfall and 95% confidence limits

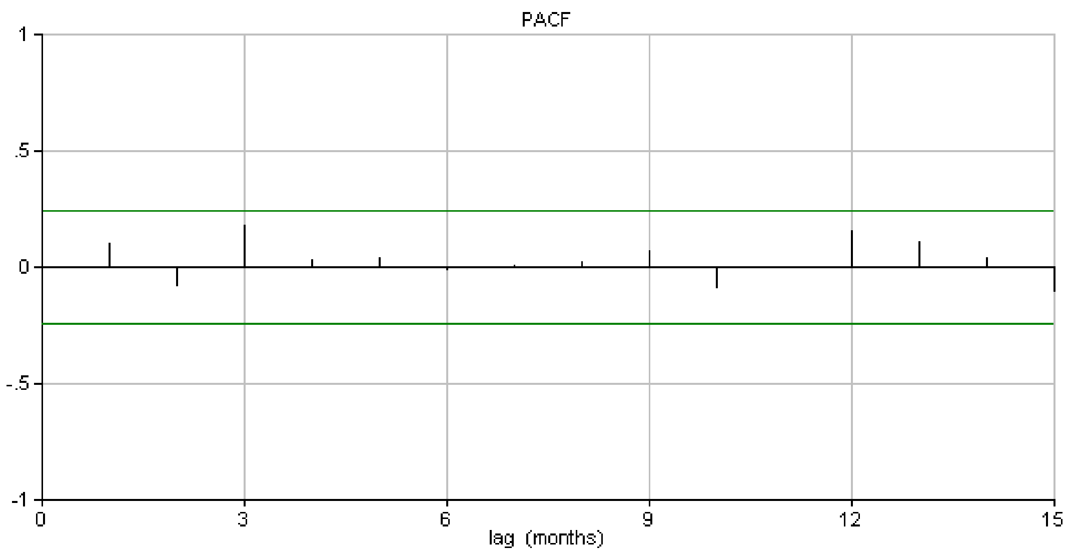


Figure 9-4 (b) Partial autocorrelation function (PACF) for residuals of the model shown in Table 4 for cases of CCUO on 46 farms in south-eastern Australia from October 2001 to December 2007, and three month moving average rainfall and 95% confidence limits

1310.1 Appendix 6: Case Study Tables

Table 9-21 Soil test results for two paddocks on NSW44 conducted in 2006 by the LHPA veterinarian in 2009 during a site visit

Test	Macottas (Native) 2006	Macottas (Native) 2009	Suckers (Improved) 2006	Kerry's (Improved) 2009
1311 pH (calcium chloride)	1312 4.9	1313 4.9	1314 4.5	1315 4.2
1316 Ph (water)	1317 5.9	1318 5.7	1319 5.2	1320 4.9
1321 Electrical Conductivity (dS/m)	1322 0.04	1323 0.07	1324 0.11	1325 0.11
1326 Phosphorous Colwell	1327 30	1328 38	1329 53	1330 39
1331 Phosphorous Buffer Index (mg/kg)	1332 46	1333 52	1334 48	1335 67
1336 Aluminium Saturation (%)	1337 1.5	1338 1.3	1339 2.4	1340 8.6
1341 Organic carbon (%)	1342 2.1	1343 3.1	1344 2.4	1345 3.4
1346 Calcium (meq/100g)	1347 5	1348 6	1349 4.6	1350 3.1
1351 Magnesium (meq/kg)	1352 0.99	1353 1.1	1354 1.1	1355 0.68
1356 Sodium (meq/kg)	1357 0.02	1358 0.02	1359 0.033	1360 0.07
1361 Potassium (meq/kg)	1362 0.6	1363 0.69	1364 0.76	1365 0.52
1366 Calcium:magnesium ratio	1367 5.1	1368 5.5	1369 4.2	1370 4.6
1371 Copper (mg/kg)	1372 0.61	1373 0.26	1374 0.62	1375 0.52
1376 Iron (mg/kg)	1377 87	1378 100	1379 170	1380 300
1381 Manganese (mg/kg)	1382 17	1383 41	1384 48	1385 120
1386 Zinc (mg/kg)	1387 1.0	1388 2.2	1389 2.0	1390 2.0
1391 Sulfur (KCL) (mg/kg)	1392 2.7	1393 3.7	1394 27	1395 8.3
1396 Nitrate Nitrogen (mg/kg)	1397 NA	1398 7.4	1399 NA	1400 33

Table 9-22 Monthly rainfall recorded by the manager for NSW44 in south eastern NSW from January 2002 to August 2009

Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total
14012 002	**1402** 8	**1403**1 06	**1404**4 5	**1405**2 9.5	**1406**4 2	**1407**9 1	**1408**5 2	**1409**5 9	**1410**5 2.5	**1411**8 .5	**1412**1 9	**1413**6	**1414**5 48.5
14152 003	**1416** 0	**1417**3 4	**1418**3 4	**1419**1 3.5	**1420**2 1	**1421**6 9.5	**1422**5 1	**1423**1 08	**1424**6 2.5	**1425**7 9	**1426**4 7	**1427**8 8.5	**1428**6 18
14292 004	**1430** 4	**1431**3	**1432**0	**1433**1 7	**1434**1 9	**1435**8 1	**1436**3 9	**1437**8 6.5	**1438**5 0	**1439**2 2	**1440**1 42.5	**1441**7 1	**1442**5 55
14432 005	**1444** 3	**1445**5 3	**1446**2 3.5	**1447**4 0.5	**1448**0	**1449**1 15.5	**1450**1 07	**1451**7 9	**1452**1 04	**1453**5 4	**1454**4 2.5	**1455**3 6	**1456**6 98
14572 006	**1458** 6	**1459**0	**1460**2 6	**1461**3 8.5	**1462**2 3.5	**1463**4 6	**1464**3 2.5	**1465**4 4	**1466**1 6	**1467**7 .5	**1468**3 4.5	**1469**9	**1470**3 43
14712 007	**1472** 1.5	**1473**6 7	**1474**6 1	**1475**6 4.5	**1476**6 2	**1477**4 8	**1478**8 9.5	**1479**2 6	**1480**2 2.5	**1481**3 0.5	**1482**7 7.5	**1483**1 25.5	**1484**6 85
14852 008	**1486** 5.5	**1487**2 6	**1488**4 2	**1489**4 6.5	**1490**2 0.5	**1491**3 6.5	**1492**9 9.5	**1493**4 1	**1494**4 7	**1495**3 0	**1496**1 05	**1497**5 0.5	**1498**5 90
14992 009	**1500** 9.5	**1501**0	**1502**1 3.5	**1503**9 4	**1504**1 0.5	**1505**7 6.5	**1506**6 2.5	**1507**1 05	**1508**-	**1509**-	**1510**-	**1511**-	**1512**-
1513L ong term average[a]	**1514** 2	**1515**4 4	**1516**4 7	**1517**5 2	**1518**7 5	**1519**6 0	**1520**7 6	**1521**8 4	**1522**7 8	**1523**8 0	**1524**6 6	**1525**5 4	**1526**7 78

a. Long term average determined from farm records

Table 9-23 Pasture species identified in improved and unimproved pasture on NSW44 in a 2006 pasture survey conducted by the LHPA veterinarian

Improved pasture species	Native pasture species
Philaris spp	*Microlaena* spp
Lolium spp (ryegrass)	*Bothriochloa macra* (red grass)
Trifolium spp (clover)	*Austrodanthonia* spp (wallaby grass)
Rubus fruticosis aggregate (blackberry)	*Lolium* spp (ryegrass)
Carthamus ianatus (saffron thistle)	*Carthamus ianatus* (saffron thistle)
Onopordum acanthium (scotch thistle)	*Hypericum perforatum* (St Johns Wort)
Silybum marianum (Variegated thistle)	*Echium plantagineum* (Patterson's curse)
Arctotheca calendula (cape weed)	*Xanthium spinosum* (Bathurst burr)
Echium plantagineum (Patterson's curse)	*Arctotheca calendula* (cape weed)
Xanthium spinosum (Bathurst burr)	*Rumex acetose* (Sorrel)
Rumex acetose (Sorrel)	*Rubus fruticosis aggregate* (blackberry)
Taraxicum officinale (dandelion)	*Rosa rubiginosa* (Briar)
Brachychiton populneum (Kurrajong)	*Geranium* spp (native geranium)
Eucalyptus spp	*Conyza* spp (Fleabane)
Acacia spp (wattle)	*Plantago turrifera* (small sago weed)
	Acacia spp (wattle)
	Eucalyptus spp
	Brachychiton populneum (Kurrajong)

Table 9-24 Comparison of pasture analysis results for the farm NSW44 performed on a pasture improved paddock and unimproved paddock in 2006 and 2009

Test conducted	Improved Pasture 2008 (October)	Improved Pasture 2009 (March)	Native Pasture 2009 (March)
1527 Calcium g/kg	1528 3.5	1529 4.6	1530 4.7
1531 Chloride g/kg	1532 NA	1533 1.5[a]	1534 1.2[a]
1535 Cobalt mg/kg	1536 <0.4	1537 0.29	1538 0.21
1539 Copper mg/kg (available)	1540 3.1[a]	1541 5.0[a] 1542 0.32	1543 3.1[a] 1544 0.2[a]
1545 Iron mg/kg	1546 551	1547 343	1548 173
1549 Magnesium g/kg	1550 1.5	1551 1.1[a]	1552 0.8[a]
1553 Manganese mg/kg	1554 152	1555 167	1556 62
1557 Molybdenum mg/kg	1558 NA	1559 0.22	1560 1.51
1561 Nitrate mg/kg	1562 NA	1563 215	1564 42
1565 Phosphorous g/kg	1566 1.9	1567 1.3[a]	1568 1.1[a]
1569 Potassium g/kg	1570 12.4	1571 5.4	1572 4.4 [a]
1573 Selenium mg/kg	1574 <10	1575 0.03[a]	1576 0.02[a]
1577 Sodium g/kg	1578 0.3[a]	1579 0.3[a]	1580 0.1[a]
1581 Sulphur g/kg	1582 1.4	1583 0.7[a]	1584 0.4[a]
1585 Zinc mg/kg	1586 24.9	1587 18.3[a]	1588 16.7[a]
1589 Crude Protein g/kg	1590 NA	1591 81.9	1592 42.5
1593 Total Nitrogen g/kg	1594 NA	1595 13.1	1596 6.8
1597	1598	1599	1600

1601 NA=not available

1602 a. Below recommended levels for gestating cows ((Australian Agricultural Council. Ruminants Subcommittee., 1990)