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Lamb rib fractures preliminary investigation

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Abstract

Objective

A preliminary investigation into the cause of bone fractures in lambs.

Design/procedure

Thirty-seven properties with or without a history of rib fractures detected in lambs at slaughter from across Kangaroo Island and south east South Australia were enrolled in an on-farm survey to study factors associated with the incidence of rib fractures. Participants were required to collect rib, humerus and liver samples from stillborn and mismothered lambs. These samples as well as pasture samples were processed to establish micromineral and vitamin status of lambs as well as bone strength as indicated by density and cortical thickness. The correlation of the mineral and vitamin status of lambs and bone strength, adjusted for property of origin, was estimated.

An additional copper supplementation study was conducted on one property with a history of rib fractures to see if copper supplementation reduced the incidence of rib fractures.

Results

Results correlating lamb liver mineral status and bone density found that lambs lower in copper had a greater bone density ($p<0.05$). There was no difference between bone density or cortical thickness in lambs from properties with or without a history of rib fractures. There were associations between pasture mineral content and properties with a history of rib fracture incidence, but this was not evident in liver samples from the same properties. Liver mineral status in neonatal lambs had no bearing on the presence or absence of rib fractures. In addition, copper supplementation had no impact on the prevalence of rib fractures.

Conclusions

The results indicate that copper deficiency is not a principal cause of bone weakness in neonatal lambs that may ultimately lead to rib fractures. A larger sample size is required to improve the statistical significance of these findings.

Executive Summary

Rib fractures in lambs is a costly condition that results in a significant economic loss estimated to be $3 million per annum to producers and processors and raises significant welfare concerns. Incidence in lambs has been reported by some processors for decades but it was not until the introduction of the Enhanced Abattoir Surveillance (EAS) program by The Department of Primary Industries and Regions of South Australia (PIRSA) in 2007 that its true prevalence was established.

Whilst previously considered a consequence of primary trauma due to poor husbandry it is now postulated that these fractures are secondary to pathologically weakened bones. With a greater understanding of neonatal and juvenile animal bone pathology the cause of these fractures may be identified and potentially eliminated.

The objective of this study was to investigate possible predisposing factors on-farm leading to rib fractures diagnosed in lambs at slaughter, concentrating on the potential role of copper (Cu) deficiency. Using the EAS data 37 properties with and without rib frcature histories were enrolled from the south east of South Australia and Kangaroo Island in a study to examine the mineral content of pasture as well as the liver and bones of stillborn and mismothered lambs. A copper supplementation trial was also conducted on a property with a history of rib fracture tracebacks from the abattoirs.

Lambs lower in liver copper had a greater bone density ($p<0.05$). There was no difference in bone density or cortical thickness between those properties with or without a history of rib fractures. There were associations between pasture mineral content and properties with a history of rib fracture incidence, but this was not evident in liver samples from the same properties. Liver mineral status in neonatal lambs had no bearing on the presence or absence of rib fractures. In addition, the copper supplementation trial had no impact on the prevalence of rib fractures.

Although areas in South Australia with the highest incidence of rib fractures are often also recognised as copper deficient areas, there did not appear to be a direct association between copper deficiency and predisposition to bone fractures based on this preliminary study.

An investigation at the individual animal level may produce more definitive results. Ideally, collecting results at slaughter and assessing the mineral status and bone strength of individual animals may give a greater correlation between nutritional status and bone health.

While seasonal conditions were exceptionally dry this year, the combination of the on-property survey and the supplementation trial does discount the hypothesis that rib fractures are primarily a copper responsive condition.

This study also highlights the important role of the EAS program to establish statistically sound data on which to base research into the incidence of conditions such as rib fractures.

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1 Background

1.1 Enhanced Abattoir Surveillance Program

The Enhanced Abattoir Surveillance (EAS) program was introduced in 2007 by the Department of Primary Industries and Regions of South Australia (PIRSA) Animal Health in order to monitor health conditions in South Australian sheep (Matthews, 2014). The program provides feedback on 21 health conditions to all sheep producers who send sheep to export abattoirs owned by Thomas Foods International (TFI) at Lobethal and Murray Bridge (Matthews, 2014). Data on 14 health conditions were already being collected for the Australian Quarantine and Inspection Service (AQIS) National sheep health monitoring scheme. The remaining seven conditions are recorded specifically for South Australian producers and includes the prevalence of rib fractures. Surveillance programs in other States indicate rib fractures have been evident at slaughter across southern Australia for many years (AGRIC WA, 2001). Data collected through the EAS in South Australia have established approximately 1% of lines of sheep present with rib fractures at slaughter (Fig. 1) and the incidence in affected lines has been as high as 80% (Geary, 2014). An estimated cost of rib fractures to the sheep industry based on the reported prevalence and potential lost productivity for processors and producers is up to $3m annually. (This figure is based on a 2-4 kg trim, depending on if one or both sides of the rib cage have suffered fractures. This equates to a cost of $10-$20 per lamb assuming a carcase value of $5/kg. An average incidence of 0.5% of lambs affected as per the 2014 EAS data has been used).

Up to two thirds of all rib fractures reported in lines of sheep processed at the two SA abattoirs currently participating in the EAS occur in lambs sourced from the South East region of South Australia (Bennett, 2014) with an even greater proportion in lambs processed at JBS, Bordertown (Geary, 2014). This supports earlier reports by AGRIC WA (2001) of fractures of ribs and long bones in sheep at slaughter, especially in those animals originating from the south east of Southern Australia.

There has been a significant increase in the percentage of South Australian producers who consigned lambs with rib fractures and the overall percentage of lambs affected since 2007 (Fig. 1). Statewide in 2014, seven percent of producers who consigned sheep and 0.5% of sheep slaughtered had rib fractures (Matthews and Dickason, 2015).

On a regional basis the overall percentage of sheep slaughtered with rib fractures for Kangaroo Island (KI), the Mid and Lower South East (LSE) and Adelaide Hills/Fleurieu ranged between 1-2%. One in four consignments of sheep from the LSE and KI had sheep with rib fractures in 2014 with an average incidence of 9% within affected lines for these regions. This is similar to the findings over the previous 4 years (Matthews and Dickason, 2015).

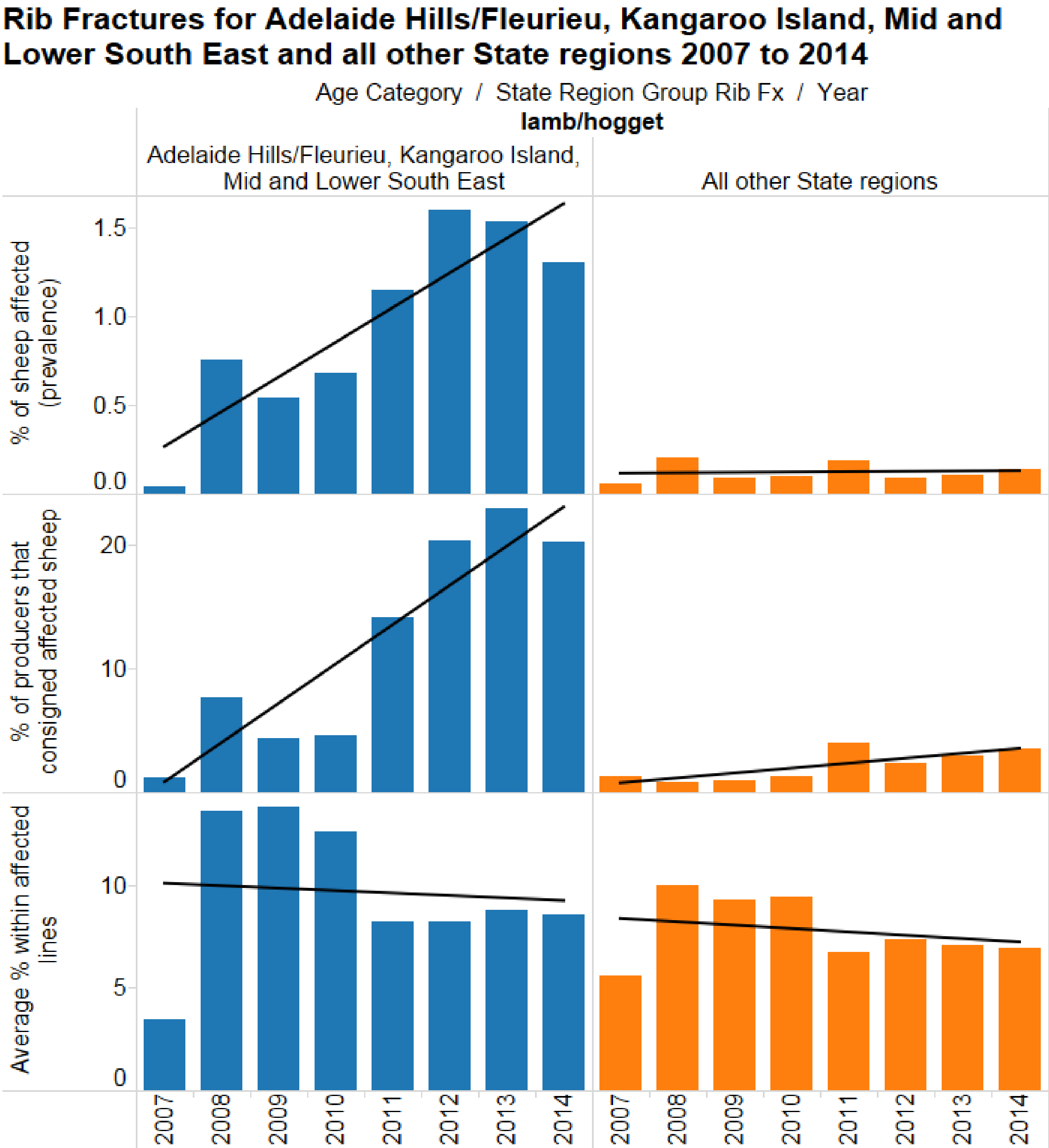

Figure .1 Graphs showing the percentage of sheep affected (prevalence), the percentage of producers who consigned affected sheep and the average percentage of the condition seen within affected lines for Rib Fractures. The graphs represent lamb/hogget data for 2007 to 2014. State regions are divided into two groups: 1) Adelaide Hills/Fleurieu, Kangaroo Island, Mid and Lower South East and 2) All other State regions.

(Matthews and Dickason, 2015)

Figure 2 illustrates the prevalence of rib fractures by month for the period 2007-14 in South Australia based on EAS data. It highlights a marked increased in prevalence in spring indicating predisposition to the condition for lambs born in the late autumn / winter. In contrast lambs born in later winter / spring will not be sold until the following summer / autumn when rib fractures have a much lower prevalence.

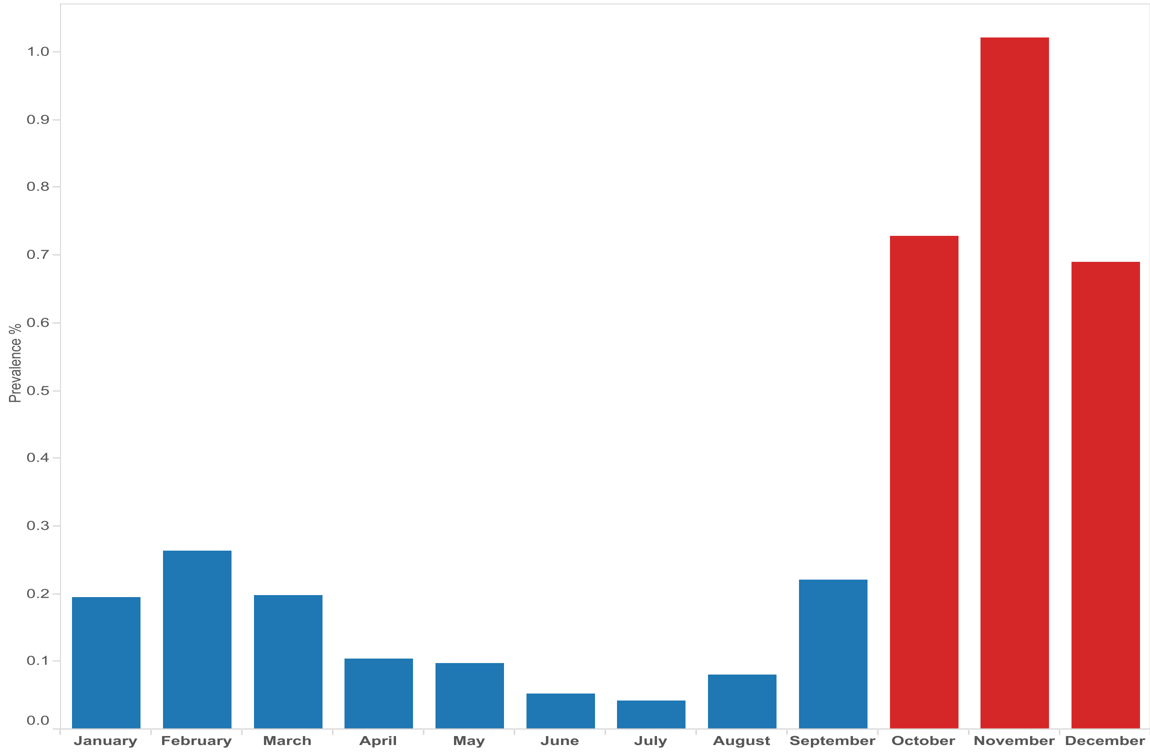

Figure 2: Graph of the prevalence of rib fractures by month for the period 2007 – 14 in South Australia based on EAS data from TFI.

1.2 Literature Review

Eales and Small (2008) reported that rib and bone fractures are not a large contributor to lamb mortality rates in the UK, but they are becoming a more common occurrence in newborn lambs and attributed this to careless handling and mismothering. Rib fractures are difficult to detect and treat in a live animal. Young animals that suffer from rib fractures are usually euthanised when the problem becomes apparent (Eales *et al*., 2004; Eales and Small, 2008; Spurling, 2012). The Australian experience is that rib fractures are only detected at slaughter. There are occasional reports of long bone fractures occurring on farm (Rendell, 2014; O'Sullivan, 2014). The affected ribs and associated tissue are trimmed at the abattoir, and this causes significant loss of profit for the processor and producer (Geary, 2014). As a consequence, there are welfare, production and financial imperatives to elucidate the epidemiology and implement preventative strategies for this condition.

Bone fractures can be caused by excessive force when handling livestock or relatively minor trauma to exceptionally weak bones (Eales and Small, 2008; Foster, 2014). Small and premature lambs are also more susceptible to rib fractures, as their delivery is usually complicated and their skeletal system is not fully formed at the time of lambing (Carley & Radulovacki, 2003; Eales and Small, 2008). Fractures may occur during rough handling or accidental injury inflicted by the dam, which may predispose to disease and death (Charnley & Winter 1999; Carley & Radulovacki, 2003; Winter, 2004).

Abnormal bone development, including the development of fragile bones, is often attributed to malnutrition especially of minerals. Deficiencies tend to have more of an impact on young or pregnant animals due to the increased need for these nutrients during growth and lactation, while mineral deficiencies in older and non-pregnant animals have less of an impact (Underwood & Suttle, 1999). Problems with bone development in young lambs are often due to a calcium or copper deficiency, although it is possible that these problems may arise from a deficiency of other minerals (Watt, 2007).

1.2.1 Macro element deficiencies

Deficiencies of macro elements can cause metabolic bone diseases such as rickets, osteomalacia or osteoporosis (Eales *et al.*, 2004). Rickets is characterised by defective mineralisation at sites of endochondral ossification and of newly formed osteoid (Thompson, 2008). This is therefore a disease of young growing animals that affects the size, shape and strength of bones, and is usually identified by characteristic physeal lesions (Eales & Small, 2008). These problems are more likely to occur during development, as calcium levels remain fairly consistent in fully developed bones. The incidence of rickets has decreased significantly due to increased knowledge of interactions between calcium, phosphorous and vitamin D (Winter, 2004). Osteomalacia is the equivalent disease in adults, whose growth plates have closed. In this case, there is failure of normal mineralisation of osteoid formed during bone remodelling causing bones to become weakened (Thompson, 2008).

Osteoporosis is the most common metabolic bone disease of grazing sheep, particularly lambs, and is characterised by loss of bone density without the loss of normal bone structure. It may go unnoticed unless pathological bone fractures occur (Thompson, 2008). Osteoporosis can be caused by deficiencies in a variety of nutrients such as calcium, phosphorous and copper. It may also be caused by starvation, where there is overall nutrient deprivation (Thompson, 2008), as well as due to chronic gastrointestinal parasitism (Coop *et al*., 1981).

Calcium and phosphorous are primary components in the skeletal structure, along with copper (Suttle, 2010). Approximately 99% of calcium in the body is found in bones, and it is a major contributor in the mineralisation of bone (Suttle, 2010; Masters & White, 1996). Phosphorous plays a significant role in bone formation within the body, and maintaining a balanced ratio between calcium and phosphorous is essential to normal growth and development. Copper is integral to lysyl oxidase essential for the cross-linking of collagen and elastin to provide bone strength (Masters & White, 1996) and the activity of this cuproenzyme appears directly influenced by the amount of available dietary copper (Rucker et al 1998). Deficiency in ruminants is recognised as a cause of osteoporotic-like lesions (Howell & Davison 1959). Other trace elements, such as zinc, manganese and sulphur, are also essential for bone development and maintenance (Hidiroglou, 1980).

Calcium absorption is not only dependent on the availability of calcium in the diet, but also the level of phosphorous intake and the presence of vitamin D (Eales & Small, 2008; Matthews, 2014). It is possible for lambs to receive insufficient amounts of calcium in their diet, such as if a lamb does not get enough milk in the first 12 weeks of life (Caple *et al*., 1988). Usually calcium deficiencies occurring in the lamb are a result of a calcium deficient ewe during pregnancy or lactation that can be corrected through nutritional intervention or medical treatments (Cole, 1980; Masters & White, 1996).

It is much more common for calcium to be sufficient in the diet but not sufficiently absorbed due to an imbalance in the calcium-phosphorous ratio, which is generally around 2:1 (Underwood & Suttle, 1999). When this ratio is out of balance, most commonly through increased levels of phosphorous, it can severely limit calcium absorption, retention and urinary excretion within the animal (Eales & Small, 2008; Masters & White, 1996). Common feeds that are associated with low calcium or high phosphorous content include cereal grains, grass hays and green oats (Matthews, 2014; Cole, 1980). Calcium deficiency is most likely to affect the lamb during late pregnancy, especially if the ewe's diet consists mostly of cereals (Cole, 1980). This is unlikely to occur in isolation, as sheep and lambs will graze on the same feed and so evidence of bone problems should be evident across the mob (Eales & Small, 2008).

Vitamin D also has a large influence on calcium balance, as calcium cannot be absorbed if the animal does not have adequate levels of vitamin D (Underwood & Suttle, 1999; Eales & Small, 2008; Caple, 1990). A deficiency in vitamin D from a lack of sun exposure that leads to calcium deficiency is less common in Australia, though it has been reported during the winter in Tasmania and New South Wales (Cole, 1980; Caple, 1990; Watt, 2007). Lush green feed can also antagonise the benefits of vitamin D assisting calcium absorption (Matthews, 2014). In addition, there are reported instances of genetic defects in responsiveness to vitamin D, as shown through apparent inherited rickets in Corriedale sheep (Thompson *et al*., 2007).

Phosphorous deficiency in lambs can cause extreme problems in skeletal development and may result in a softening of bones that fracture easily i.e. osteoporosis (Masters & White, 1996). This may occur in lush green pastures, which have a much lower phosphorous content than grain feeds (Spurling, 2012). However, animals can generally tolerate calcium-phosphorous ratios up to 10:1 without any ill effects. There is a much higher tolerance for a lack of phosphorous in the diet (Underwood & Suttle, 1999).

1.2.2 Trace element deficiencies

Soils in the south east of South Australia are naturally deficient in trace elements such as copper, selenium, cobalt, zinc and manganese (Hughes 2012; Judson and Babidge 2002; McFarlane *et al.*, 1990). Copper deficiency is common on calcareous sands, on siliceous sands with low organic matter and on ironstone soils and peats, all of which are found in the region. Figure 3 illustrates the copper deficient areas in South Australia based on extensive survey of cattle liver copper levels across the State. The pH of soils may also indicate the availability of different trace elements, with high pH generally tying up copper, zinc, manganese, boron and iron, while in more acidic soils molybdenum becomes less available, and manganese and aluminium more readily available (Hughes 2012).

Copper deficiency in particular has been linked to the incidence of rib fractures in sheep and has been previously characterised by signs of osteoporosis including light, brittle bones with thin cortices in the humerus, tibia and ribs (Higgs 2004; Hidiroglou 1980). These signs are mostly subclinical and thus undetected copper deficiency may cause economic losses for producers without their awareness (Gooneratne *et al* 1989). Additionally, sheep with trace element deficiencies have slower growth rate, which leads to a longer interval to slaughter and consequent production loss (Spurling, 2012). Trace element deficiencies are therefore both an animal welfare issue and an economic problem.

The low level of copper in the south east of South Australia was first reported in 1938 when a disease known at the time as "coastal disease" was found to respond to treatment with copper and cobalt. The disease got its name due to the affected sheep being primarily located in coastal areas, grazing on highly calcareous dune sands (Lee, 1950). Subsequent research found that copper deficiency was also causing health and productivity issues much further inland (Fig. 3) resulting in the reference to "coastal disease" eventually not being used.

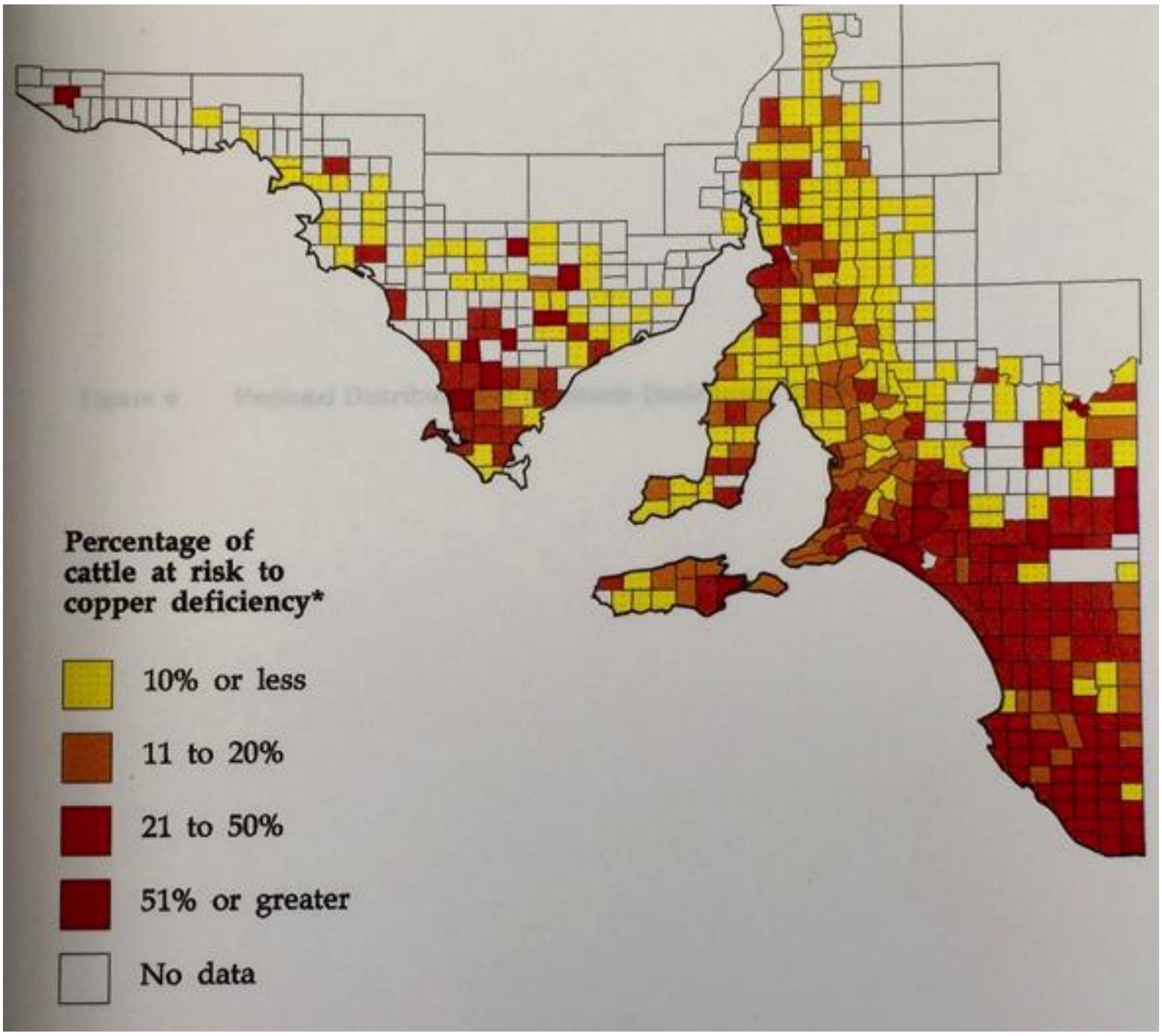

Figure 3: Copper deficient areas of South Australia based on liver sample analysis of cattle at slaughter 1989-91 (Koh *et.al.* 1994)..

Based on EAS data for the spring of 2013, the incidence of rib fractures was found to coincide with acid sand over clay (duplex) soils receiving more than 500 mm rainfall annually (Fig. 4). This is also where copper, cobalt and selenium deficiencies are commonly found in livestock (Koh *et. al.* 1994).

1.2.3 Copper availability in the ruminant

When copper absorption exceeds physiological requirements it is stored in the liver, and accessed when there is a deficiency in the blood and utilised until depleted (ARC 1980). The foetal lamb acquires its copper through the placenta, and is ideally born with high hepatic concentrations, that soon diminish to adult levels (Prohaska 1990). Unfortunately, placental transfer in sheep is not efficient resulting in lambs often born with low liver reserves and making them susceptible to copper deficiency. Colostrum is rich in copper allowing newborn lambs to increase hepatic storage. However, copper concentration of milk during lactation rapidly declines and is insufficient to meet copper requirements of a suckling neonate (Radostits et al, 2006). Milk from normal ewes contains 0.2 – 0.6 mg/L, where normal whole blood copper levels for most species are 0.8 – 1.2 mg/L (Campbell 1983). Newborn calves are protected against neonatal hypocuprosis by adequate concentrations of copper in cow's milk, whereas newborn lambs accept the equivalent copper status as the ewe (Radostits et al, 2006).

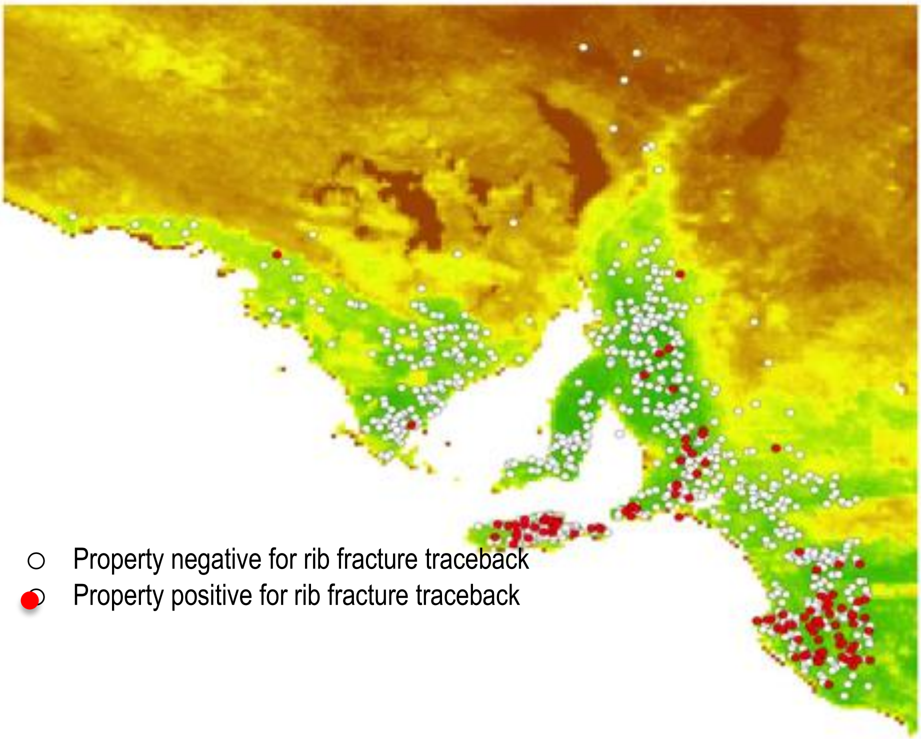

Figure 4: Map indicating the distribution of rib fracture tracebacks during October to December 2013 based on property identification codes (PIC) and EAS trackback data from TFI in South Australia.

An intake of copper equivalent to 10mg/kg dry matter prevents primary copper deficiency, while diets including less than 5mg/kg dry matter will cause hypocuprosis (Campbell 1983). As a general rule, pastures containing < 3mg/kg DM produce signs of deficiency in grazing animals; 3-5mg/kg is regarded as dangerous; and > 5 (preferably 7-12mg/kg) can be considered safe (Radostits et al, 2006) unless other factors (e.g. increased sulphur or molybdenum in the soil) induce secondary deficiency.

Ruminants are much more susceptible to copper deficiencies as they absorb low levels of copper (<1.0-10.0%) compared to non-ruminants (Underwood and Suttle 1999); and this is thought to be due to the complex anaerobic interactions that occur in the rumen (Spears, 2003; Suttle, 1988; Laven and Smith 2008). The finding of copper deficient soils in South

Australia led to many of the deficient areas being top-dressed with copper, greatly reducing the incidence of deficiencies in livestock in these areas. Hannam *et al.* (1982) found that deficient areas treated with 2kg/ha copper fertiliser still had adequate soil levels up to 23 years later. However, McFarlane *et al.* (1990) reported areas in the south east of South Australia grazed by livestock with low serum copper concentrations despite adequate top-dressing with copper. By correlating pasture trace element levels with serum copper concentrations it was concluded that low serum copper was commonly associated with raised molybdenum levels rather than a primary copper deficiency. This supported earlier work that found that the ability to absorb and retain copper is inversely proportional to the intake of molybdenum (Cole, 1980; Hidiroglou *et al*., 1982; Suttle, 1988; Suttle, 2010; Reuter, 2007). There is, however, some evidence that diets high in molybdenum are not detrimental to copper absorption provided sufficient dietary copper is available (Hidiroglou *et al*., 1982). More recent work by O'Sullivan (2014) and Dickson (2015) indicates serum copper status is a very poor indicator of animal copper status based on liver analysis. As such, the copper interaction with molybdenum should ideally be studied using liver rather than serum copper concentration.

Molybdenum forms a triple complex with copper and sulphur in the rumen, known as copper thiomolybdate (Suttle, 1991; Laven and Smith, 2008). This complex reduces copper absorption by the rumen leading to an induced copper deficiency. Similarly, high dietary sulphur may impact the availability of copper in the rumen. Suttle (1991) reported that when these thiomolybdates are absorbed in sufficient quantities, they might affect the action of copper systemically by changing the binding of copper to albumin.

Pasture composition and season can also influence the availability of copper (Gooneratne *et al* 1989; Cole, 1980; Hidiroglou *et al*., 1982; Suttle, 1988). Grasses tend to have lower copper concentration than clovers, and both have decreased levels when pastures are lush and rapidly growing. Availability of copper in pastures increases when plants are mature, due to the different forms of copper that are present and decreased ruminal sulphide production (Underwood, 1981).

Copper is required in sheep for growth of bone and wool, for pigmentation, myelination of nerve fibres and leucocyte function (Underwood, 1977; Arthur *et al.,* 1981). In addition, copper facilitates iron transport (Frieden, 1971). Copper is especially essential for enzymes monoamine oxidase and lysyl oxidase. These enzymes contribute to the cross-linking of bone collagen. Disruption of these enzymes therefore affects collagen solubility and bone strength, and hence bones become fragile and easily broken (Hidiroglou, 1980; Underwood, 1977; Lloyd Davies, 1983; Masters & White, 1996; Sargison, 2008; Eales & Small, 2008). Copper deficiency therefore increases the incidence of fractures in long bones and rib bones (Higgs, 2004; Hidiroglou, 1980).

There is some evidence that genetics play a role in sensitivity to copper deficiency. For example, Scottish Blackface absorb copper at a much lower rate and are more susceptible to deficiency, while Suffolks and Texels are much more efficient and tend to be significantly less susceptible (Aitken, 2007). Although, this in return means that the Suffolks and Texels are more susceptible to copper poisoning while the Scottish Blackface is more resistant (Eales & Small, 2008). Merino sheep similarly display evidence of genetic variation in their ability to metabolise trace elements and copper in particular (Judson et al, 1994). These genetic differences may reflect differences in the efficiency of copper absorption (Suttle 1974).

Copper deficiencies in young lambs are often a result of a copper deficiency in the ewe during pregnancy, similar to calcium deficiency (Suttle & Jones, 1987). It is extremely difficult to treat young lambs that suffer from copper deficiency due to maternal diet. Therefore, their bones are likely to be permanently weakened and deformed (Eales & Small, 2008; Hidiroglou, 1980; Winter, 2004). This can easily lead to bending and fractures of the ribs in lambs, both during and post-lambing (Sargison, 2008; Suttle, 2010; Suttle & Jones, 1987). To ameliorate the problem prevention is paramount.

1.2.4 Other interactions

Deficiency of other trace elements, such as zinc, sulphur and manganese, can cause skeletal problems in developing lambs (Vaughan, 1970). Selenium is also considered essential for bone homeostasis (Zhang *et* al 2014) and growth (Ren *et* al 2007). These are less prominent than calcium and copper deficiencies, as their concentrations vary less significantly between feeds and their absorption is not drastically affected by the presence of other minerals (Bellof & Pallauf, 2007). Zinc deficiencies have been shown to cause dwarfism in lambs, while deficiencies in manganese result in lighter, deformed bones that are easily fractured (Hidiroglou, 1980; Underwood & Suttle, 1999).

Equally, the availability of dietary copper is decreased in animals that have high dietary intake of zinc, iron, cadmium, nitrogen and calcium (Suttle 1988; ARC 1980; Underwood 1977; Reuter, 2007).

Sex, breed and genetics can have an impact on the absorption of these trace elements (Bellof & Pallauf, 2007; Masters & White, 1996; Thompson *et al*., 2007). The type of pasture also has an influence, particularly on grazing animals. Lush as well as dry pastures tend to contribute to zinc deficiencies. Pasture zinc-mineral quality fluctuates depending on the season, similar to several other mineral deficiencies (Masters & White, 1996). All trace elements can be supplemented, though zinc and manganese are usually provided through short-acting drenches, mineral licks or blocks rather than oral capsules as with copper (Masters & White, 1996; Trengove and Judson, 1985).

1.3 Diagnosis

In cases of increased bone fragility and rib fractures in lambs, all possible nutritional causes need to be considered when making a diagnosis. Blood samples should be obtained and assessed for levels of calcium, phosphorous and vitamin D. In cases of suspected copper deficiency, liver samples obtained by biopsy or at slaughter / post mortem are preferred. Copper is stored in the liver and utilised by the sheep in times of dietary deficiency. Therefore, the first sign of copper deficiency will be a decrease in liver storage levels. Prolonged deficiency results in depletion of copper reserves and an eventual fall in plasma copper levels. Plasma levels are measured via ceruloplasmin, a major copper-containing enzyme, which contains 70-90% of plasma copper, but liver copper analysis is considered a much more reliable indicator of current copper status (Allott, 2014). Plasma ceruloplasmin activity is an alternative to plasma copper assay although the latter is more commonly measured in diagnostic laboratories. There are two measures of plasma copper – total copper and TCA soluble copper; the latter excludes any copper-molybdenum complexes.

The selection of animals for sampling should be representative of the group. For example, it is not recommended to sample lambs at slaughter to assess the copper status of the ewes. If blood and/or liver samples indicate a copper deficiency, it is desirable to do pasture testing to establish whether the deficiency is primary, or secondary to other factors such as molybdenum or sulphate excesses or unfavourable pH. It is necessary to check the ratio of all three trace elements to arrive at an accurate diagnosis and treatment strategy. Pasture copper levels at 4 ppm are not deficient so long as molybdenum levels are less than 1.5 ppm (Gartrell *et al* 2004). Liver copper levels at 4 and up to 8 ppm are often deficient (Gartrell *et al.,* 2004).

1.4 Treatment

The treatment of copper deficiency must be based on prior knowledge of the copper levels, as a misdiagnosis or an overdose can very easily lead to copper poisoning (Eales & Small, 2008; Gooneratne *et al*., 1989). Various forms of supplementary copper exist, but some are more effective than others (Judson et al, 1984). Injectable compounds are available, but are not registered for use in sheep in Australia due to various adverse reactions, such as abscess formation at the injection site (Hosking et al, 1986), and risk of copper toxicity due to rapid release from the injection site. However, there are several injectable copper complexes that are considered safe for use in sheep (see Judson in Masters & White 1996). Oral drenches are available but are only practical for primary copper deficiency providing about two months benefit from a single treatment. In secondary molybdenum-induced deficiencies weekly treatments may be required making it impractical (Underwood, 1981). Mineral or salt lick blocks containing copper may be of use, but are generally unreliable as it is unlikely that all animals will access the supplement in correct quantities at regular intervals (Gartrell *et al.,* 2004).

A common method of copper supplementation for both treatment and prevention is the use of soluble gel capsules containing 2.1g of copper oxide (Patten, 2006; Trengove and Judson, 1985). The capsule is administered orally and dissolves rapidly to release copper oxide particles that slowly move from the rumen to the acid environment of the abomasum where they dissolve and the copper is absorbed by the gastrointestinal tract. It is recommended to treat with capsules every 12 months in deficient animals, or every 6 months in clinically deficient animals (Langlands et al, 1986). The use of these capsules in ewes pre-partum has been shown to increase lamb hepatic copper stores (Langlands et al, 1982). It is therefore recommended to dose pregnant animals early in pregnancy, to protect lambs against copper deficiency. Alternatively, other classes of sheep are best dosed on green rapidly growing pasture when deficiencies most commonly develop.

Dissolvable glass bullets containing copper, cobalt and selenium, act similarly to the gelatine capsules, by slowly releasing these trace elements into the rumen as they dissolve (Telfer et al, 1982, Trengove and Judson, 1985). These glass bullets are not commercially available in Australia at this time.

As well as systemic treatment with copper, other management strategies should be implemented. For example, animals should be preferentially grazed on mature pastures rather than lush, rapidly growing pastures, where copper availability tends to be lower (Underwood, 1981). The application of soil amendments such as lime, dolomite and gypsum may also be an option to improve soil balance and encourage the availability of various macro and trace elements in the diet (Trengove, 2004).

1.5 Prevention

The main form of prevention, other than systemically dosing sheep with copper, is top-dressing soils with copper fertiliser, which as previously mentioned, can result in adequate pasture copper levels up to 23 years later (Hannam *et al.*, 1982). However, the use of this relies on the deficiency being a primary one rather than secondary to excessive soil molybdenum or sulphur. This is why pasture testing for copper, molybdenum and sulphur levels should be performed and pastures and/or soils treated accordingly. The amount and frequency of treatment should also be determined based on regular pasture testing (Hosking *et al.*, 1986).

2 Projective objectives

To investigate possible predisposing factors on-farm leading to rib fractures diagnosed in lambs at slaughter, concentrating on the potential role of copper (Cu) deficiency.

2.1 Strategy

The approach was to investigate the prevalence of rib fractures in South Australia based on seven years of data obtained from the Enhanced Abattoir Surveillance (EAS) program. Properties with a history of rib fracture incidence were enrolled in a survey to examine factors that may be associated with the occurrence of this condition. An on farm study was also conducted to see if copper supplementation reduced the incidence of rib fractures.

The ultimate objective is to develop strategies to minimise the prevalence of rib fractures in sheep in south eastern Australia.

2.2 Hypothesis

That rib fractures primarily occur during birth due to fragile bones as a consequence of copper deficiency in the neonatal lamb.

3 Methodology

3.1 On-farm survey

Enrolment of properties to participate in the survey was complicated by privacy laws limiting access to personal details of producers with a history of rib fractures. Contact details were obtained by a veterinarian with Primary Industries and Regions SA (PIRSA Biosecurity) responsible for the management of the Enhanced Abattoir Surveillance (EAS) program. Traceback data through the EAS program were used to identify properties with and without a history of rib fracture traceback. The owners of these properties were then approached to ascertain their willingness to be involved in the study. Additional participants were enrolled through expressing an interest while attending one of seven lamb post mortem workshops run on Kangaroo Island and in the south east of South Australia during April – July 2015.

Properties identified through the EAS 2007-14 database that had greater than or equal to 0.05% carcasses with rib fractures and/or 5% or greater history of positive consignments during 2007 – 2014 were defined as positive for rib fracture traceback. Twenty six properties in the SE and KI with significant rib fracture incidence were allocated to the positive traceback group. Eleven properties from the same regions were defined as negative for rib fracture traceback based on less than 0.05% carcasses with rib fractures and/or a history of less than 5% positive consignments for rib fractures during 2007 - 2014. From the 37 properties surveyed, only 13 were finally included in the final data analysis due to the requirement for complete bone and liver data sets.

A survey questionnaire was developed (Appendix 1) and valid on-farm data were collected by personal interview from 29 of these properties – 20 with a positive history of rib fractures and 9 with a negative history of rib fractures. Data collected included information on farming systems, soil type, fertiliser history, sheep and grazing management practices and weather.

The survey participants were trained to do lamb post mortems and provided with a post mortem kit and instructions on how to sample lambs found dead at or around the time of birth (Appendix II). Samples collected on the participating properties included liver, humerus and ribs from up to seven stillborn or mismothered lambs (119 samples in total from 13 properties) as well as pasture from the paddock where lambs were sampled (20 samples in total).

3.2 Liver sample analysis

All liver samples were sent to Regional Laboratory Services at Benalla for trace element analysis. The following independent tests were used to determine the amount of copper, selenium (glutathione peroxidase test) and vitamin B_{12} in the liver samples. Copper levels were measured by flame Atomic Absorption Spectrometry, after acid treatment of liver homogenate sample (Paynter DI,1982). Glutathione peroxidase was measured on cyanide pre-treated liver homogenates as a rate reaction at 340 nm in a coupled enzyme reaction involving Glutathione Reductase (Paynter DI, 1993). Vitamin B_{12} was determined by performing a modified Roche Elecsys competitive binding assay (Roche Cat # 04745736 190) that involved using a purified porcine intrinsic factor. The liver samples were all pre-treated by steps specifically developed by Regional Laboratory Services to remove the possibility for any interference, which are particularly present in ruminant livers.

3.3 Bone sample analysis

The humerus was chosen to assess bone strength due to being an accessable cortical bone. All associated tissue was removed and measurements were conducted at the same location mid-shaft on all samples. Bone strength was assessed by two techniques: 1) Bone Densitometry scan/duel-energy x-ray absorpitometry/ bone densitometry (DEXA scan); and 2) Computed Tomography (CT) scan. The DEXA scan assessed total bone mineral density (g/cm^3) as a measure of bone strength. This test was conducted at the Bone Densitometry Department, Royal Adelaide Hospital. The CT scan was completed at the South Australia Health and Medical Research Institute (SAHMRI), Gilles Plains. This test assessed cortical thickness (mm) as a measure of bone strength and also provided data in Hounsfield units (HU), which are a quantitative scale for describing radiodensity.

3.4 Pasture sampling

Pasture samples were collected from each of the 12 participating farms on Kangaroo Island and 17 in the South East of South Australia. These samples were collected by traversing one or two paddocks on each farm and using a clean plastic glove to pluck the tops of plants at random every 10 steps until a 10 x 15 cm plastic zip lock bag was full. The paddock(s) sampled coincided with where lambs were also sampled. Care was taken to avoid contaminating the sample with dirt, leaves or roots and to ensure a representative area of the paddock was covered. These samples were immediately refrigerated and delivered to the APAL agricultural laboratory, 489 The Parade, Magill SA as soon as practical.

3.5 Pasture sample analysis

The pasture samples were delivered to the APAL laboratory for mineral analysis including nitrate, nitrogen, phosphorus, potassium, calcium, magnesium, sodium, sulphur, boron, copper, zinc, manganese, iron, aluminum, cobalt, molybdenum, chloride and selenium. All samples were oven dried at 80 degree Celsius and then finely ground. Nitrate was measured using a digested water extract in an auto flow analyser. Nitrogen was measured by combustion of a subsample using the DUMAS method. Chloride concentration was determined by a water extract being titrated with silver nitrate. Selenium was measured by acid digestion of an extract followed by fluorometric analysis (Paynter et al, 1993). All other elements were measured by microwave digestion and ICP-OES analysis.

3.6 Copper supplementation trial

A supplementation trial was set up on a property 25 km south east of Naracoorte on the Victorian border. This property had a history of consigning lambs with rib fractures to JBS at Bordertown over several years. 1,000 Merino ewes were randomly allocated to four equal groups of 250 ewes at joining in December 2014. The treatments were:

1) Control - no treatments given
2) Copper capsule given at joining & no other treatments
3) Copper capsule given at joining followed by a Multimin (including copper) injection pre-lambing & to lambs at marking
4) Multimin only (including copper) given pre-lambing & to lambs at marking

They were run on adjacent paddocks for the next 12 months with similar animal health, grazing, water and fertiliser histories. It was noted upon a visiting the trial site 13.03.15 that the owner had spread copper sulphate fertiliser across the farm in autumn 2014 and had applied copper sulphate to the water troughs in all paddocks on a periodic basis. Fourteen ewes were liver biopsied on 13.03.15 (mid pregnancy) to assess their liver copper status. Digital radiographs were taken of 140 lambs in July including 80 from the control group and 20 from each of the remaining three groups to check for evidence of rib fractures. Seven hundred lambs were followed through slaughter at JBS, Bordertown in early December 2015 and evidence of rib fractures noted as well as sampling of liver and ribs from affected lambs.

3.7 Data analysis

Statistical analysis was conducted using SAS (Statistical analysis software, Cary Inc, USA) version 9.4. The historic information of rib fractures for a particular property was presented as a binomial variable [0 = no fracture tracebacks (Negative); and 1 = rib fracture tracebacks (Positive)]. Averages for liver selenium, vitamin B_{12} and copper plus the 12 minerals analysed in the pasture samples were determined for all of the properties, based on their binominal variable, using a linear mixed model in PROC MIXED. The effect of property was included as a confounder and the level of significance was set at $p<0.05$.

4 Results

4.1 Liver trace element content

A total of 119 neonatal liver samples were submitted for mineral analysis from 28 properties. Ten of these properties had a negative history for rib fractures and 18 had a positive history for rib fractures.

Table 1 shows the average liver copper, selenium and vitamin B_{12} levels for the positive and negative traceback groups. Contrary to expectations copper was higher in the positive traceback group (0.66 IU/g) compared to the negative traceback group (0.47 IU/g), but the result was not significant (p=0.23). Selenium was slightly lower in the positive group, but the difference of 1.1 IU/g was not significant (p=0.97). Vitamin B_{12} was 14 IU/g higher in the positive traceback group, but also was not significant (p=0.77).

Table 1: Average neonatal lamb liver copper, selenium and vitamin B_{12} content for properties with either a positive or negative history of rib fracture traceback based on seven years of surveillance data at Thomas Foods International abattoirs in South Australia.

Liver trace element content	Rib fracture traceback	
	Positive	Negative
Copper (mmol/kg ww)	0.66 ± 0.09	0.47 ± 0.13 ^
Selenium (IU/kg ww)	13.4 ± 2.2	13.5 ± 1.4 ^
Vitamin B_{12} (nmol/kg ww)	462 ± 31	448 ± 58 ^

^ = not significant at p = 0.05

4.2 Bone measurements

A total of 49 lamb humerus' were analysed using DEXA and CT scan.

Table 2 compared the results of DEXA and CT scan for bone density, cortical thickness and Hounsfield units respectively.

Table 2: Average neonatal lamb humerus bone density, cortical thickness and cortical value for properties with either a positive or negative history of rib fracture traceback based on seven years of surveillance data at Thomas Foods International abattoirs in South Australia.

Humerus bone measurements	Rib fracture traceback	
	Positive	Negative
Bone density (g/cm^3)	0.36 ± 0.02	0.34 ± 0.02 ^
Cortical thickness (mm)	1.63 ± 0.14	1.59 ± 0.18 ^
Cortical value (Hounsfield Units)	1555 ± 26	1573 ± 32 ^

^ = not significant at p= 0.05

Table 3 indicates a statistically significant association between bone density ($p<0.001$) and liver copper concentration. A level of 0.22 mmol/kg was used to demarcate between normal/high and low liver copper as advised by D Paynter (2015). Neonatal lambs with normal to high levels of copper were found to have less dense bones (0.366 g/cm^2) than those with low copper (0.375 g/cm^2).

Table 3: Average neonatal lamb humerus bone density compared to either normal/high (> 0.22 mmol/kg) or low (< 0.22 mmol/kg) liver copper concentration for properties with either a positive or negative history of rib fracture traceback based on seven years of surveillance data at Thomas Foods International abattoirs in South Australia.

Neonatal lamb humerus	Liver copper (mmol/kg ww)	
Bone density (g/cm^3)	Normal/high	Low
	0.366 ± 0.001	0.375 ± 0.001 *

* = significant at $p < 0.001$

4.3 Pasture mineral content

Analysis of the pasture results indicate a significant association between most minerals and the property history of rib fracture traceback (Tab. 4). Pasture samples from properties with a history of rib fractures were found to have significantly higher manganese, molybdenum, nitrate, phosphorus, potassium and sulphur; and significantly lower aluminium, boron, calcium, chloride, copper, iron, magnesium, selenium, sodium and zinc compared to properties with no history of rib fractures. There was no significant difference evident for cobalt and nitrogen.

Table 4: Average pasture mineral content for properties with either a positive or negative history of rib fracture traceback based on seven years of surveillance data at Thomas Foods International abattoirs in South Australia.

Plant Mineral	Rib fractures		p value
	Positive ± SE	Negative ± SE	
Aluminium (ppm)	548 ± 40	1032 ± 58*	<0.0001
Boron (mg/kg)	12 ± 0.2	20 ± 0.2*	<0.0001
Calcium (%)	0.64 ± 0.01	0.86 ± 0.01*	<0.0001
Chloride (%)	2.0 ± 0.03	2.6 ± 0.04*	<0.0001
Cobalt (mg/kg)	0.39 ± 0.01	0.42 ± 0.02	0.14
Copper (mg/kg)	6.1 ± 0.1	6.5 ± 0.1*	<0.0001
Iron (mg/kg)	520 ± 26	694 ± 38*	<0.0002
Magnesium (%)	0.25 ± 0.00	0.29 ± 0.00*	<0.0001
Manganese (mg/kg)	85 ± 1	69 ± 2*	<0.0001
Molybdenum (mg/kg)	1.64 ± 0.04	1.51 ± 0.05*	<0.05
Nitrate (mg N/kg)	1019 ± 28	867 ± 41*	<0.002
Nitrogen (%)	4.79 ± 0.02	4.84 ± 0.03	0.23
Phosphorus (%)	0.48 ± 0.00	0.43 ± 0.03*	<0.001
Potassium (%)	2.7 ± 0.02	2.6 ± 0.03	<0.08
Selenium (mg/kg)	0.05 ± 0.00	0.09 ± 0.00*	<0.0001
Sodium (%)	0.68 ± 0.01	0.93 ± 0.02*	<0.0001
Sulfur (%)	0.32 ± 0.00	0.31 ± 0.00*	<0.0001
Zinc (mg/kg)	34 ± 0.3	36 ± 0.4*	<0.0001

* = significant at p < 0.05 to <0.0001; SE = standard error

4.4 Copper supplementation trial

4.4.1 Ewe liver biopsies

Table 5 provides liver and plasma copper, selenium and vitamin B_{12} levels for control and copper capsule treated ewes from the trial.

4.4.2 Lamb slaughter results

Percentage of lambs with rib fractures at slaughter from each treatment is provided in Table 6.

4.4.3 Lamb liver copper & selenium results

Results for liver copper and selenium for lambs from different treatments groups are provided in Table 7.

Table 5: Copper, selenium and cobalt results from trial ewes with low and high laboratory reference ranges included for comparison.

ewe #	Liver Cu	Blood Cu	Liver Se	Blood Se	Liver Co	Blood Co
1	2.48	14.2	7.4	478	664	>3000
2	3.04	13.7	7.5	488	700	>3000
3	2.15	14.7	0.6	312	708	>3000
4	3.21	14.2	5.7	375	960	>3000
5	1.79	14.2	5.3	443	864	>3000
6	1.84	13.2	6	394	828	>3000
7	1.52	10.8	8.8	512	973	>3000
8	3.83	15.7	2.6	555	727	>3000
9	3.26	11.8	2.3	498	755	>3000
10	3.53	13.2	1.3	305	736	>3000
11	2.65	14.2	2.9	476	1010	>3000
12	4.1	12.3	5.3	481	770	>3000
13	2.6	12.8	6	448	930	>3000
14	2.43	14.7	8.4	596	816	>3000
Low	**0.23**	**9**	**2**	**50**	**200**	**400**
High	**3.67**	**25**	**25**	**550**	**1500**	**5000**

#1-7 Controls - only Cu received via water trough & fertiliser in 2014
#8-14 Cu capsules administered at joiniing in Dec'14

Mean 1-7	2.3	13.6	5.9	429	814	>3000
Mean 8-14	3.2	13.5	4.1	480	821	>3000

Table 6. Lambs with rib fractures at slaughter by treatment groups

Treatment Group	Total # lambs slaughtered	# with rib fractures (%)
1. Control	132	3 (2.1)
2. Copper capsule only	236	16 (6.8)
3. Copper capsule & Multimin pre-lambing & marking	128	3 (2.3)
4. Multimin only @ pre-lambing & marking	192	8 (4.2)
Total	698	30 (4.4)

Table 7. Mean liver copper and selenium results with standard error for lambs at slaughter as well as low and high laboratory reference ranges for comparison.

Sample id	Liver copper (mmol/kg)	Liver GSHPx+ (U/g)
1. Control	1.8 ± 0.2	2.6 ± 0.4
1a. Control with rib fracture	2.1 ± 0.3	1.5 ± 0.7
2. Copper capsule	1.9 ± 0.1	4.8 ± 0.3
2a. Copper cap with rib fracture	2.1 ± 0.1	4.3 ± 0.3
3. Copper cap + multimin	1.8 ± 0.2	2.6 ± 0.4
3a. Copper cap + multimin with rib fracture	2.1 ± 0.3	1.5 ± 0.7
4. Multimin	2.3 ± 0.2	2.2 ± 0.4
4a. Multimin with rib fracture	2.3 ± 0.2	2.0 ± 0.4
High	3.6	25
Low	0.2	2.0

+ GSHPx = Glutathione peroxidase as an indicator of selenium status

4.4.4 Lamb digital x ray results

Digital x rays were performed on 140 lambs when they were approximately 3 months old using a mobile x ray unit. This was achieved by restraining each lamb with their legs extended in a horizontal ventro-dorsal position on a table. The lambs were selected at random with a total of 60 from the control group and 20 from each of the treatment groups x rayed. The x rays were viewed during the process and recorded on a USB data card. No evidence of rib fractures were evident in the 140 lambs examined.

5 Discussion

5.1 On-farm survey

The original aim was to include 10 positive and 10 negative properties for rib fracture incidence in the study, but a much larger sample was included to allow for an anticipated drop out rate. This was expected due to the nature of the sampling required. It was logistically and financially impractical to collect the dead lamb specimens personally during the lambing period on the participating properties and so the participants were trained to collect the required specimens. Inevitable variation in diligence and uniformity of specimen collection was a consequent disadvantage of this process resulting in the final inclusion of data from 28 of the original 37 properties enrolled.

The qualitative nature of the survey data completed by all 28 properties meant it was not suitable for statistical analysis. The data provide an in depth understanding of the livestock management on each property, but it was not used in this focus on the relationship between copper deficiency and rib fracture incidence in lambs. The epidemiological associations between the questionnaire data and the quantitative analysis reported in this document is therefore still to be analysed.

5.2 Liver trace elements

The objective of this investigation was to identify possible predisposing factors on-farm leading to rib fractures diagnosed in lambs at slaughter, concentrating on the potential role of copper deficiency. The findings of the liver analysis do not support copper deficiency as a key aetiological influence on the incidence of rib fractures in lambs. The results indicate no statisitical difference in the average copper, selenium or vitamin B_{12} status of neonatal lambs from properties that were positive or negative for rib fracture traceback. This lack of statistical significance may be a result of low study power or it may genuinely reflect that copper is not the major factor in rib fractures in lambs. The copper supplementation study further supports that copper alone is not a major determinant in the incidence of rib fractures as a similar prevalence of rib fractures occurred in both supplemented and unsupplemented groups.

The inclusion of 28 properties in the on-farm survey exceeded the original aim, but still may have been an insufficient sample to produce a statisitically significant result. To replicate this study a much greater sample of lambs on fewer properties collected by the same person would be prefered. It would also be recommended to gather a balanced sample size across an equal number of positive and negative rib fracture traceback properties.

5.3 Bone strength

There was no statistical difference in bone density, cortical thickness or cortical value between properties with or without a history of rib fractures. This indicates a lack of association between bone strength in neonates and past incidence of rib fractures. Seasonal variation may explain this finding as there appeared to be a lower incidence of rib fractures coinciding with an annual rainfall well below the average, in the south east of SA and on Kangaroo Island in 2015. Another explanation could be that bone fractures become apparent at a later stage in lamb growth contrary to the hypothesis tested in this study.

Bone density results from the DEXA scan compared to liver analysis further discounted the hypothesis that copper deficiency predisposes to rib fractures in neonatal lambs. On the contrary, bone density was higher for lambs with lower liver copper status ($p<0.001$). This finding (see Table 3) is unexpected and contradictory to expectations and the literature. Although not fully understood copper is believed to be a cofactor in the enzyme Protein-lysine 6-oxidase production necessary for collagen crosslinking and normal bone development. These results may be due to decreased levels of copper increasing bone mineralisation through an unclear pathway or more likely failure to achieve a statistically significant sample size.

The premise that fractures occur secondary to primary weakening or inadequate development of bone was not supported by the results. A comparison between cortical thickness of the humerus plus Hounsfield Units and the historical incidence of rib fractures (Table 2) revealed no difference in bone strength/health between the control and experimental group. Total bone density analysed via DEXA scan only differed by 0.02 g/cm^2. These results are more likely to support the belief that rib fractures are due to primary mechanical force beyond the bones normal physiological limits (Thompson, 2007), rather than minor trauma to weakened bones due to improper formation (Eales and Small, 2008).

Computed tomography scan results that assessed bone strength through average cortical thickness and a transformed Hounsfield value supported the DEXA scan results. There was no difference in cortical thickness of the humerus on positive or negative rib fracture traceback properties. This also supports the premise by Charnley and Winter (1999) of fractures resulting from excessive force when handling and primary mechanical force as the cause of fractures.

5.4 Pasture analysis

The minerals of particular interest from these results are calcium, copper, iron, manganese, molybdenum, selenium and sulphur due to their role in bone development either independently or in combination. The mean value of copper in pastures from the positive traceback group (6.1 mg/kg) was found to be significantly less ($p<0.0001$) than that of the negative traceback properties (6.5 mg/kg) and fits with the hypothesis. Molybdenum and sulphur can both affect the absorption of copper by changing the binding of copper to albumin (copper sulphide is insoluble and hence not absorbed, Cu-Mo complexes in the blood are probably unavailable). Thus, either high sulphur or molybdenum can cause copper deficiency by changing the binding of copper to albumin (Suttle, 1991). The results for sulphur (0.32%) and molybdenum (1.64 mg/kg) in the pasture sampled from the positive properties were both significantly higher than for negative properties [($p<0.0001$) and ($p<0.05$) respectively) and strongly support the familiar molybdenum-sulphur-copper interaction. This finding indicates that the copper deficiency could be a secondary rather than primary phenomenum. A low average liver copper would be anticipated on rib fracture positive properties based on these pasture findings, but this was not evident in the liver analysis.

Calcium was found to be significantly lower ($p<0.0001$) on rib fracture positive properties and fits with the multiple key roles that calcium has in ossification within the body (Radwinska and Zarczynska, 2014). This finding highlights the need to examine the mineral composition and especially calcium in the bone samples collected from this study. Similarly, the copper content of bones sampled will provide a useful comparison with the liver copper and bone density results. However, the opportunity to have the bones analysed for mineral content is still some months away and so not able to be reported at this stage.

Iron was found to be significantly lower ($p<0.0002$) in pasture from properties positive for rib fractures while manganese was significantly higher ($p<0.0001$). These findings do not appear to have obvious impacts, but the significantly low selenium ($p<0.0001$) at 0.05 mg/kg on rib fracture positive farms does correlate with the important role that selenium plays in bone growth and deficiency contributing to osteoporosis. However, the liver selenium was found to be normal to high across most farms in the study. This may be explained by the liver selenium reflecting dietary intake over the preceeding 2-3 months, whereas the pasture sample reflects the selenium on offer at the time of sampling some weeks after lambing.

A confounding variable that may have affected the pasture results is paddock selection. The paddock sampled was selected by asking the farmers which paddock the sheep lambed in and where lamb samples were collected. It is unlikely that the sheep were in the same paddock for the whole gestation period. Therefore, pasture sampling from the paddock they lambed in is not likely to be an accurate representation of the ewe’s nutrition during gestation.

This study, while not supportive of the hypothesis, does highlight the important role of the EAS scheme to establish statistically sound data on which to base aetiological and epidemiological research into the incidence of conditions such as rib fractures.

Although no variation was seen at the flock level, an investigation at the individual animal level may produce more definitive results. Ideally, collecting results at slaughter and assessing the mineral status and bone strength of individual animals may give a greater correlation between nutritional status and bone health.

The current study only collected samples from stillborn or mismothered lambs to dismiss husbandry activities as the cause of fractures. This would mean that it was the ewe nutrition and poor placenta transport of copper that was responsible for inadequate bone formation in utero. Any fractures sustained would most likely be the result of parturition. Investigation into the prevalence of the rib fractures and mineral deficiency in the juvenile animal will give greater indication of the aetiology of these fractures and allow comparison to the neonatal lamb.

5.5 Supplementation trial

The liver biopsies taken mid pregnancy three months after the trial was set up indicated that both the control and treated groups had adequate copper. This was attributed to the application of copper sulphate to the soil 12 months earlier as well as regular additions of copper to the water troughs of all stock. At this stage there was concern that the trial results may not be informative.

Subsequently, 140 three month old lambs were digitally x-rayed to check for evidence of rib fractures, but none were found. A similar lack of digital x-ray evidence of fractures was found by Rendell (2014) during scanning of 200 lambs at lamb marking on a property near Hamilton, Victoria with a recent history of both rib and long bone fractures.

Following 698 lambs through the abattoir revealed a mixed outcome, that had no clear relation to the treatments given. An overall prevalence of 4.4% rib fractures was in line with expectations, but the lack of correlation with copper treatments further casts doubt over a direct relationship between copper status and the incidence of rib fractures. Interestingly the highest prevalence of rib fractures (6.8%) was in the group that received the copper capsules only, while the control group that received no treatment had only 2.1% rib fractures. The absence of copper deficiency in the control group possibly reflects the seasonal conditions being a relatively dry year but may also reflect the application of copper sulphate to the water troughs over summer and as a fertiliser in 2014. This outcome cautions against drawing too many conclusions from the trial.

6 Conclusions/recommendations

In conclusion, rib fractures in lambs is a costly condition that results in a significant economic loss and raises significant welfare concerns. Although areas in South Australia with the highest incidence of rib fractures are also recognised as copper deficient areas, there did not appear to be a direct association between copper status and predisposition to bone fractures based on this preliminary study in 2015. This study did not find an association between copper deficient lambs at birth and properties with a reported history of rib fractures. However, the preliminary nature of this investigation along with a below average rainfall year and incomplete analysis of bone mineral composition, limits the conclusions that can be made. It is suggested that this study be repeated with a larger sample of lambs on fewer properties with more consistent sampling to assist in gaining statistical signficance.

The copper supplementation trial failed to establish any association between copper supplemenetation and the incidence of rib fractures. Rib fractures were observed at slaughter but with no apparent trend in relation to the supplements given. While seasonal conditions were exceptionally dry this year, the combination of the on-property survey and the supplementation trial does discount the hypothesis that rib fractures are a direct copper responsive condition.

Additional controlled copper supplementation studies on several rib fracture affected properties in an above average rainfall year should clearly establish whether copper deficiency contributes to the incidence of rib fractures. This should be supplemented with further work on predisposing factors including the interaction between elements and identifying when fractures are occurring.

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8 Appendices

Appendix I: Survey

Survey by **Date / /**

MLA RIB FRACTURE PROJECT – Property Survey

Property Owner

Address

Tel: **Mob:**

Email:

Property Location

Hundred: Sections:

General Property information

	Hectares/acres	Comment
Total area managed		
Winter grazed area		
Hay/silage for own use		
Cropped area		
Lucerne		
Native grass pasture		
Area scrub (not grazed)		
Area scrub (grazed)		

<u>Rainfall</u>

Average annual:

Do you have rainfall records for 2007 - 2014 available? Y / N

<u>Cattle Enterprise</u>

Livestock type	Total Number (As of 1 March 2014)	Nos. & descriptions of introduced sheep	Source of introductions
cows			
Heifers			
Weaners			
Bulls			
Steer			

Calving months -

Sheep Enterprise:

Livestock type	Total Number (As of 1 March 2014)	Nos. & description of introduced sheep (incl age, type etc)	Source of introductions (location, genetics)
Ewes			
Rams			
Wethers			
Weaners			
Lambs			

Management

EWE						RAM	
Enterprise (2013/14)	Ewe Nos.	Breed	Joining month	Length of joining	Shearing mth	Ram Nos. (used)	Breed
Merino							
First cross							
Second cross							
British Breed							

Was there any major changes (in livestock and/or management) prior to 2013? Y / N

Details:

Reproductive Efficiency

Enterprise	Scanning %	Marking %	Weaning %
Merino			
First cross			
Second cross			
British Breed			

Ewe Husbandry (2013/14)

	Pre-joining/joining management (6 weeks prior to rams out)		**Early-mid pregnancy** (ram out to 90 days)		**Late pregnancy** (last trimester)		**Lactation** (incl lamb treatments)	
	What	**When**	**What**	**When**	**What**	**When**	**What**	**When**
Number of times handled								
Grazing management incl pasture type and grazing practices (incl supplementary feed types/levels).								
Trace element supplementations								
Vaccinations								
Parasite control								
Condition score								

Any recent change to husbandry or treatments – details?

Lamb Husbandry

Weaning to Abattoir	What	When	Describe type of sheep handling equipment used.
Grazing management incl type of feed, paddock moves			
Number of times handled			
Trace elements given			
Vaccinations			
Parasite control			
Shearing			
Transport			

Any recent change to husbandry or treatments – details?

Weaning practices (age, drafts etc)

Lamb Consignment History in 2014

Date/Month	Number lambs consigned	Rib fracture Incidence	Source (O or I)

Source: O – bred on farm; I – introduced from another farm

Mineral deficiencies in livestock

Are you aware of any current or past mineral / trace element deficiencies associated with your property / livestock? Y / N

Details:

Did you provide any supplements? – details:

Do you have any test results available? – details:

In new born lambs have you ever noticed?

- Leg bone fractures
- Staggering
- Steely wool
- Other signs (describe)

Soil and Pasture Management

Soil testing

Do you have recent soil test results available?

Fertiliser / trace element history (on paddocks/country normally grazed by ewes and lambs)

Year	Paddock detail	**Treatments** (type, rates etc)

Pasture testing

Do you have pasture test results? Details:

Soil modification

What soil modification practices undertaken over the last 5 years? Y / N

Details:

Water Sources

What are the main sources of stock water?

Water test results available?

Have you noticed rib fracture problems occurring in lambs reared on particular country, paddocks etc?

Y / N

Area detail	Comment/observations

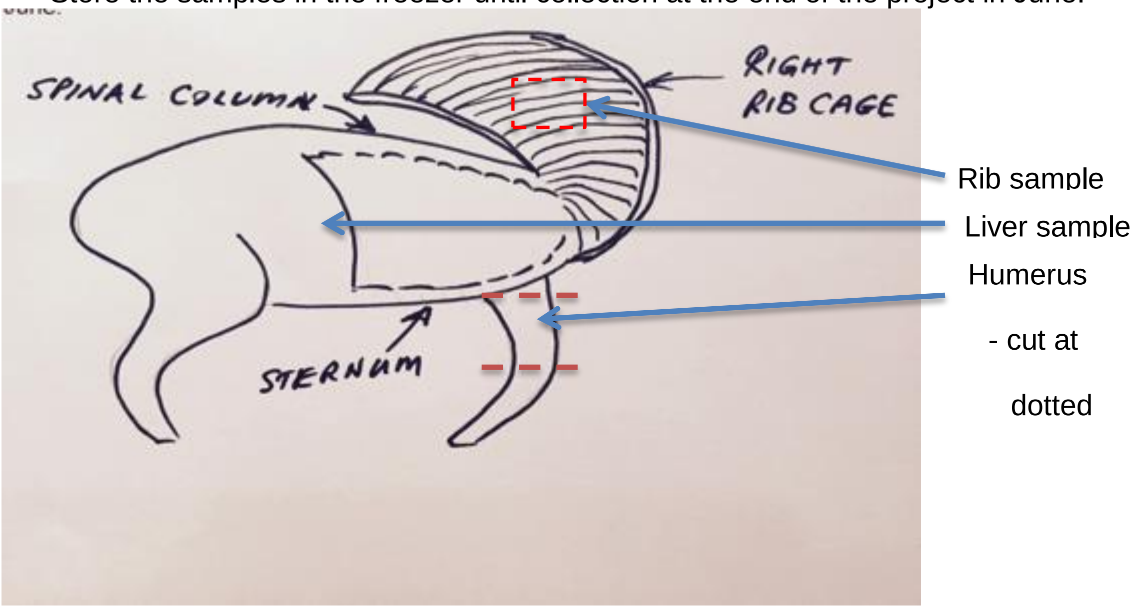
Appendix II: Post mortem instructions

RIB FRACTURE INVESTIGATION IN STILLBORN LAMBS 2015

The purpose of this SOP (standard operating procedure) is to provide a consistent detection procedure for rib fractures in stillborn lambs.

All co-operators are encouraged to examine as many stillborn lambs as possible during the first six weeks of lambing and collect samples from <u>five lambs detected with rib fractures and / or five lambs without rib fractures</u> using the following procedure:

- Record details of each stillborn lamb sampled in the attached table.
- Lay the lamb flat on one side and use a sharp knife cut into the abdomen of the stillborn lamb starting at the tail end of the rib cage and cutting cranially through the sternum and along the spinal column – refer diagram below. Aim to examine the entire rib cage in two halves ie from each side of the carcase.
- Samples required from five lambs in total:
  1. any rib fractures including at least two normal ribs on either side of the fractured rib(s) – total sample size no larger than slice of bread
  2. piece of liver – no larger than match box
  3. one upper foreleg (humerus) –severed at shoulder & elbow joints
- Place samples in plastic bag provided. These samples will be used for mineral analysis.
- Using the permanent marker pen provided record the number of the lamb on the plastic bag corresponding to the number on the recording sheet.
- Store the samples in the freezer until collection at the end of the project in June.



STILLBORN LAMB RIB FRACTURE RECORDING SHEET

PROPERTY NAME: ___________________________

LOCATION:_____________________________

PARAMETER	LAMB #1	LAMB #2	LAMB #3	LAMB #4	LAMB #5
DATE EXAMINED					
MOB / PDK NAME					
AGE OF EWES (yrs)					
MOB SIZE (No. ewes)					
SAMPLES TAKEN - RIBS Y / N - HUMERUS Y / N - LIVER Y / N					
LIST ANY MINERAL SUPPLEMENTS GIVEN TO EWES IN LAST 12 MONTHS?					
ANY COMMENTS / OBSERVATIONS					

NUMBER OF DEAD LAMBS OBSERVED: ______________ NUMBER OF STILLBORN LAMBS EXAMINED: _______________

TOTAL NUMBER OF LIVE LAMBS: ______________ ESTIMATED OR COUNTED?

LAMBING %: _______________

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10 Key messages

This preliminary study discounts the hypothesis that rib fractures are a copper responsive condition.

The key communication messages are:

- The incidence of rib fractures is higher in areas with higher rainfall (> 500mm) on acidic duplex soils
- Prevalence is higher in wet winter years in southern Australia
- The actual predisposing factors are not clearly understood, however it probably involves a complex interaction amongst a number of elements
- Treatment with copper alone is unlikely to stop lambs from exhibiting rib fractures
- Further research is required to unlock the complex interaction between the elements