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An investigation of the potential link between arthritis and tail length in sheep

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Executive summary

Arthritis/polyarthritis caused by bacterial infections is a relatively common condition in lambs and has recently been identified as one of the priority endemic disease of Australian sheep for MLA research investment.

The primary objective of this research project was to investigate if there is an association between tail length and arthritis in lambs. If there is, attention to correct tail length when docking would be a cost-effective method way of reducing the prevalence of arthritis in sheep. The hypothesis was that short docking leads to infected tailing wounds that take longer to heal, with subsequent spread of bacteria through the blood to the joints, resulting in arthritis/polyarthritis.

Arthritic joint samples collected from lamb carcases at an abattoir in South Australia were tested by culture, PCR and immunohistochemistry for the presence of bacteria.

In addition, data from the South Australian Enhanced Abattoir Surveillance Program database were analysed to determine the historical occurrence of arthritis in sheep less than and more than two years of age, including potential correlations with age, region and anonymous property identifier, as well as potential correlations between grass seed infestation and arthritis, and between pneumonia/pleurisy and arthritis.

An association between tail length and bacterial arthritis/polyarthritis in lambs was identified, with shorter tails (one or two coccygeal vertebrae) being a higher risk factor for arthritis/polyarthritis than longer tails (three or more coccygeal vertebrae). *Erysipelothrix rhusiopathiae* was re-confirmed as the most common cause of bacterial joint infections in Australian lambs. In lambs, *E. rhusiopathiae* usually causes a fibrinopurulent arthritis and osteomyelitis after docking or castration.

The project also revealed correlations between arthritis/polyarthritis and pleurisy/pneumonia and between arthritis/polyarthritis and grass seed infestation in sheep both less than and more than two years of age.

The findings of this study also suggest that pneumonia/pleurisy is much larger problem in sheep in southern Australia than previously recognised. Approximately 50 per cent of 227 consignments of lambs examined had at least one carcase affected by pleurisy (the chronic sequel to pneumonia). On average, pleurisy affected 2.2 per cent of carcases within affected lines. Trimming for pleurisy resulted in rib removal in two-thirds of 101 carcases examined, with an average trim weight of 1.0 kg. Trimming for pleurisy is estimated to result in a $6 penalty per carcase to producers and an $8-10 loss per kilo in high value cuts (i.e. ‘frenched’ racks) to the processing sector. A comprehensive survey of risk or protective factors for ovine pneumonia in Australia is recommended, as well as research to establish the contribution of the known primary and secondary pathogens of ovine pneumonia in pneumonia in sheep in southern Australia, with the aim of identifying appropriate control methods.

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1 Background

Arthritis/polyarthritis caused by bacterial infections is a relatively common condition in lambs and has recently been identified as one of the priority endemic disease of Australian sheep for MLA research investment (Lane et al., 2015).

1.1 Impact of arthritis/polyarthritis in lambs

Arthritis/polyarthritis in lambs causes economic losses on-farm through the culling of affected animals and at slaughter through condemnation of all or parts of carcases (Paton et al., 2003). In addition, research conducted in the United Kingdom has also demonstrated that arthritis can substantially delay the turn-off time of affected animals, for example 29-36 days in intensively reared lambs (Green et al., 1995). Arthritis is also a potential welfare issue when affected sheep are held on farm prior to being destroyed (Farquharson, 2007; Farquharson, 2008).

In 1997 it was estimated that 1 per cent of lambs slaughtered in Western Australia had arthritis and that an additional 1.4 per cent were culled on farm because of the condition (Paton et al., 2003).

Data collected in Australian export abattoirs from 2000 to 2006 revealed that nationally 0.02 per cent of carcases were condemned for polyarthritis, with no records kept nationally of carcases that had been trimmed (Farquharson, 2008). The per cent of adult and lamb carcases condemned because of polyarthritis was similar; however, polyarthritis accounted for a larger proportion of condemnations in lambs than adult sheep (18.5 per cent of condemnations in lambs compared to 2.5 per cent of condemnations in adult sheep). Victoria had the highest rate of condemnation of lambs due to polyarthritis, followed by New South Wales and Tasmania. For adult sheep, the highest condemnation rate was also in Victoria, followed by Tasmania and Western Australia.

In the mid-2000s arthritis/polyarthritis was estimated to cost the Australian sheep industry $18-26m annually (Farquharson, 2007; Sackett et al., 2006). However, these estimates probably did not reflect the true cost of the disease because it was assumed that the prevalence of arthritis in lambs in eastern Australia was the same as that in Western Australia. The export abattoir data mentioned above indicates that Western Australia had one of the lowest condemnation rates for polyarthritis in lambs (0.008 per cent compared to 0.037 per cent in Victoria). In addition, the modelling did not consider the potential for delayed turn-off of lambs with arthritis/polyarthritis.

More recently the annual cost of arthritis/polyarthritis to the Australian sheep industry was estimated to be $39m annually (Lane et al., 2015). This estimate included weight loss in affected lambs (estimated to be 3.0 kg, 4.0 kg or 4.5 kg for Merino, dual purpose and prime lambs, respectively), but also did not consider delayed turn-off times. It was assumed that 0.018 per cent of carcases are condemned for arthritis and 0.07 per cent of carcases trimmed, and that the average trim weight is 3.0 kg.

1.2 Aetiology of infectious arthritis/polyarthritis in lambs

In lambs, arthritis/polyarthritis usually occurs secondary to a bacterial infection at a site distant from the joint(s) involved (i.e. umbilicus, marking or castration wounds, gastro-

intestinal tract, lungs) (Thompson, 2007). The synovial membrane has a rich blood supply and is a preferred site for localisation of blood-borne bacteria (Thompson, 2008). Haematogenous spread of bacteria often results in multiple joints becoming infected, although infection may resolve in some joints and persist in others, particularly the large joints of the limbs (Thompson, 2008). Osteomyelitis may develop in adjacent bones, the vertebrae or intervertebral disks (Thompson, 2008).

Inadequate transfer of antibodies in colostrum is thought to predispose to the development of arthritis/polyarthritis in lambs (Piercy, 1974; Thompson, 2007).

A number of different bacteria have been isolated from arthritic joints of lambs, including *Chlamydia pecorum, Erysipelothrix rhusiopathiae, Escherichia coli, Mycoplasma* spp., *Staphylococcus* spp., *Streptococcus* spp., *Arcanobacterium pyogenes*, *Fusobacterium necrophorum* and *Histophilus somni* (Thompson, 2007; Thompson, 2008). Of these bacteria, *E. rhusiopathiae, Streptococcus* spp., *Staphylococcus* spp., *C. pecorum* and *H. somni* are considered the most important pathogens involved in bacterial joint infections in lambs.

C. pecorum and *E. rhusiopathiae* have a strong predilection for the synovial membrane and can cause arthritis/polyarthritis without septicaemia or osteomyelitis.

H. somni usually results in acute septicaemia and death within 12-24 hours. It appears to be most common in lot-fed lambs (Thompson, 2007).

Sample submission rates from cases of ovine arthritis to veterinary diagnostic laboratories in Australia are low, but for cases in which a diagnosis could be established, there was an even spread of *E. rhusiopathiae*, *C. pecorum* and a range of other pyogenic bacteria combined (Farquharson, 2008).

1.2.1 Erysipelothrix rhusiopathiae

Erysipelothrix rhusiopathiae is a small, gram-positive bacillus that is ubiquitous in nature worldwide (Wang et al., 2010). Infection can occur in a wide range of animal species, with the most economically important infections occurring in humans, pigs, calves, ducks, domestic turkeys and sheep.

E. rhusiopathiae is considered to be the most common cause of bacterial arthritis/ polyarthritis in lambs globally (Thompson, 2007). Microbiological culture of ovine arthritic joints collected in an abattoir in Western Australia resulted in a positive result in approximately one third of cases, with *Erysipelothrix rhusiopathiae* present in 100 per cent of joints with a positive culture (Paton et al., 2003).

E. rhusiopathiae usually develops first at a site on the skin i.e. umbilicus, docking and castration wounds, shearing wounds or cuts and abrasions during dipping. Infection can be limited to the skin or spread via the blood to the joints.

In lambs, *E. rhusiopathiae* usually causes a fibrinopurulent arthritis and osteomyelitis after docking or castration and sometimes umbilical infection (Thompson, 2007). Death from septicaemia is rare. The arthritis that develops is subacute or chronic and rarely fatal, with death the result of severe lameness rather than from the infection *per se*. The large limbs of the joints are most commonly affected.

In older animals infection usually occurs after dipping associated with contaminated, non-bactericidal dips (Thompson, 2007). The fetlocks (metacarpophalangeal and metatarsophalangeal joints) are most commonly affected (Thompson, 2007).

The cell wall of *E. rhusiopathiae* is relatively resistant to breakdown by mammalian lysosomal enzymes and subsequent removal by macrophages. As a result, persistent inflammation can develop, even when infection appears to have resolved or bacteria cannot be isolated (Thompson, 2007).

1.2.2 Streptococcus spp.

Streptococcal septicaemia and polyarthritis is regarded to be the second most common form of polyarthritis in lambs globally (Thompson, 2007). It is generally accepted that the umbilicus is the most likely route of infection and that the disease is more common in sucker lambs.

Some lambs will die of acute septicaemia. In others the bacteria will localise in a number of sites including the uvea of the eye, the cerebrospinal meninges, the valvular endocardium, the myocardium, the kidneys and the joints. Localisation may occur in only the joints and persist in the large limb joints, similar to infection with *E. rhusiopathiae*. About 20 per cent of the lambs with polyarthritis will also have valvular endocarditis.

In the United Kingdom *Streptococcus dysgalactiae* is the bacterium most commonly isolated from arthritic lesions in lambs (Watkins and Sharp, 1998).

S. dysgalactiae, an α-haemolytic streptococcus, has been isolated from the vaginal tract of ewes and from the superficial skin surrounding the umbilicus of lambs and it is proposed that carrier ewes may be an important source of environmental contamination (Rutherford et al., 2014). *S. dysgalactiae* has also been isolated from the milk of ewes and ingestion of infected milk and a digestive pathway of infection may also be possible (Lacasta et al., 2008; Rutherford et al., 2014).

Similar to *E. rhusiopathiae*, the cell wall of *Streptococcus* spp. is relatively resistant to breakdown by mammalian lysosomal enzymes and subsequent removal by macrophage and, as a result, persistent inflammation may develop (Thompson, 2007).

1.2.3 Staphylococcus spp.

Coagulase-positive *Staphylococcus* spp. were the third most common type of bacteria isolated from arthritic joints of lambs in the United Kingdom (Watkins and Sharp, 1998). Although staphylococci are considered to be a relatively common cause of polyarthritis in farm animals (Thompson, 2007), infection is thought to be a relatively unimportant cause of arthritis/polyarthritis in lambs in Australia (Farquharson, 2008).

Coagulase-positive *S. aureus,* non-haemolytic coagulase-negative *Staphylococcus* spp. and haemolytic coagulase-negative *Staphylococcus* spp. are part of the normal flora of the skin of sheep (Haarstad et al., 2014).

Chlamydia pecorum

Chlamydia pecorum is an intra-cellular gram negative bacterium that has been associated with polyarthritis in lambs (Everett, 2000; Thompson, 2007). However, many chlamydiae coexist with their host without causing disease, with the animal serving a natural reservoir of infection for other animals (Everett, 2000). Infection is via the oral route, with nonvirulent strains not spreading beyond the mesenteric lymph nodes (Thompson, 2007). Virulent strains spread more widely and can infect the bladder, brain, eye, lymph, joints and prostate (Everett, 2000). Lambs rarely die from infection, even when it spreads beyond the intestinal tract (Thompson, 2007).

Chlamydia spp. have been isolated from the joints of Australian lambs with polyarthritis (Tammemagi and Simmons, 1968). A recent survey of lambs in South Australia, New South Wales, Victoria and Western Australia found that 30.1 per cent of healthy lambs were shedding *C. pecorum* in their faeces, with a higher level of shedding at weaning and post-weaning, and a lower level of shedding at slaughter (Yang et al., 2014b).

1.3 Rationale for a link between bacterial arthritis and tail length in lambs

Arthritis/polyarthritis in lambs is usually secondary to bacterial infection at a site distant from the joint involved, including bacterial infection of lamb marking wounds (Thompson, 2007). *E. rhusiopathiae* is the most common cause of bacterial joint infections globally, including in Australia. *E. rhusiopathiae* usually causes a fibrinopurulent arthritis and osteomyelitis after docking or castration wounds become infected, and less commonly subsequent to umbilical infection (Thompson, 2007).

Currently, the majority of lambs raised for meat production in Australia will be tail docked and most male lambs will be castrated.

Research conducted in the 1940s revealed that tails docked long (fourth joint) or medium long (third joint) healed faster than tails dock medium (second joint) or short (tail stump one-half to one inch long) (Johnstone, 1944). Infection was most common in lambs with the tails docked short (96.4 per cent or 54 of 56 lambs with short tails) and least common in lambs with tails docked long (13.3 per cent or 12 of 89 lambs with long tails). The rate of infection in lambs with medium tails was in between the infection rates of lambs with long or short tails (63.9 per cent or 62 of 97 lambs with medium tails).

A survey of 40 graziers on the central, north-eastern and north-western plains and southern tablelands and south-western slopes of New South Wales during the 1970s revealed that the tails of lambs butted or docked short took approximately 2 weeks longer to heal (Watts et al., 1979).

Currently, many prime lambs breeds are docked short.[1]

The aim of this research project was to investigate if there is a link between tail length and arthritis in lambs. If there is, attention to correct tail length when docking would be a cost-effective method to reduce the prevalence of arthritis in sheep.

[1] Sheep Veterinarians comments on the Sheep Standards and Guidelines 6 May 2013. http://www.animalwelfarestandards.net.au/files/2011/05/Sheep-Veterinarians-Comments.pdf

The hypothesis was that short docking leads to infected tailing wounds that take longer to heal, with subsequent spread of bacteria through the blood to the joints, resulting in arthritis.

In addition, when it was noticed that many of the consignments of lambs being examined for arthritis/polyarthritis were also affected by pleurisy/pneumonia, it was decided to collect prevalence and trim weight data on this condition.

2 Project objectives

The objectives of this project were to:

- Analyse data from the South Australian Enhanced Abattoir Surveillance Program database to determine the historical occurrence of arthritis in sheep less than and more than two years of age, including potential correlations with sex, age, region and anonymous property identifier. In addition, the potential correlation between grass seed infestation and arthritis and between pneumonia/pleurisy and arthritis was investigated.
- Conduct a survey of tail length, arthritis and pleurisy in lambs by collecting slaughter data at one abattoir in South Australia (Thomas Foods International Murray Bridge abattoir).
- Test arthritic joint samples by culture, PCR and immunohistochemistry for the presence of bacteria and *Mycoplasma ovipneumoniae*.
- Make recommendations to extend the results of the project nationally.

3 Methodology

3.1 South Australian Enhanced Abattoir Surveillance Program data

3.1.1 Arthritis and grass seeds

Data collected on the prevalence of arthritis and grass seed infestation at two South Australian abattoirs, January 2011 – December 2013, were extracted from the South Australian Enhanced Abattoir Surveillance Program database into an Excel spreadsheet using the following filters/column headings:

- Order number
- Kill date
- Sex (female, male, male castrate, unknown, not recorded)
- Age (less than 2 years old, more than two years old)
- Owner alias (to ensure anonymity)
- Property alias (to ensure anonymity)
- Number killed
- Per cent with arthritis
- Per cent with grass seeds
- PIC region

Only data from directly consigned lines of sheep were included.

The Enhanced Abattoir Surveillance program is funded by the South Australian Sheep Industry Fund with some national assistance provided by Animal Health Australia. The program provides feedback on conditions and diseases detected at slaughter to South Australian producers. Data is collected for the program by accredited meat inspectors at participating slaughtering establishments. Both direct and market consignments are monitored that include more than 100 animals at one establishment and more than 50 animals at the other. Data recorded is an estimate of carcase prevalence recorded in five per cent increments from five percent within a line/consignment.

Arthritis is recorded when it is estimated that a minimum of five per cent of the carcases within a consignment have one or multiple joints trimmed for arthritis or the carcase is condemned for arthritis.

Grass seed lesions are recorded when carcases are trimmed on the pathology retain rail, a trimming team is required or when the chain is slowed for trimming, regardless of whether the infestation level is light, medium or heavy.

3.1.2 Correlation between between arthritis, grass seed lesion, lung worm and pleurisy/pneumonia

To investigate the potential correlation between arthritis, grass seed lesion, lung worm and pleurisy/pneumonia data for the time period January 2011 – December 2014 were extracted from the South Australian Enhanced Abattoir Surveillance Program database into an Excel spreadsheet using the following filters/column headings:

- Kill date
- State (South Australia only)
- PIC region
- Local government area
- Sex (female, male, male castrate, unknown, not recorded)
- Age (less than 2 years old, more than two years old)
- Owner alias (to ensure anonymity)
- Property alias (to ensure anonymity)
- Number killed
- Per cent with arthritis
- Per cent with pleurisy
- Per cent with grass seeds
- Per cent with lungworm
- Order number
- Split line

Only data from directly consigned lines of sheep were included.

Data for the South Australian Enhanced Abattoir Surveillance Program are collected as described previously (refer Section 3.1.1).

Pleurisy is recorded when a minimum of five per cent of the carcases have pleurisy, regardless of whether the carcase is condemned, the pleura is stripped or all or a part of the rib cage is removed.

Lungworm is recorded in five per cent increments when any lesions characteristic of lungworms (i.e. characteristic inflammatory lesions of the lung tissue) is present.[2] Lungworm results are not currently provided to producers.

3.2 Survey of tail length, arthritis and pleurisy

The survey of tail length, arthritis and pleurisy was conducted at the Thomas Foods International abattoir at Murray Bridge in South Australia. The abattoir data collection was conducted in three phases, December 2014 to January 2015, March 2015 and September to October 2015.

3.2.1 Tail length

The tail length of the carcases was determined by palpating the number of coccygeal vertebrae present in tail of the carcase after skinning but prior to tail removal. Only tails that had not been damaged during processing were assessed. Within a consignment, the tail length of all carcases with arthritis and as many carcases as possible without arthritis was recorded. Tail lengths were recorded manually using a data capture sheet (Appendix 1) and then transcribed to a MS Excel spreadsheet.

3.2.2 Arthritis

Carcases with arthritis were identified by Australian Government Department of Agriculture and Water Resources (DAWR) (formerly AQIS)-accredited meat inspectors in accordance with the Australian Standard for the Hygienic Production and Transportation of Meat and Meat Products for Human Consumption (Anon, 2002). Following trimming of affected joints by the meat inspectors, the joints were collected into plastic bags (one bag per carcase) and stored in buckets on the slaughter floor. At the end of each shift, all the collected joints and associated trim were re-examined grossly and then weighed. The number and which joints were affected and the trim weights were recorded manually using a data capture sheet (Appendix 1) and then transcribed to a MS Excel spreadsheet.

During November 2014 to January 2015 and in March 2015 joints that were still intact were shipped to Gribbles Veterinary Pathology on ice where they were sampled within 18 hours of collection for bacterial culture (swabs from aseptically opened joints). At least 5 g of synovial tissue was collected for PCR and stored at -20 ^{0}C. Synovial tissue samples were also collected into four per cent neutral buffered formalin for histopathology.

[2] This likely to be *Muellerius* sp., rather than *Dictyocaulus filaria*, which resides in the bronchi and trachea.

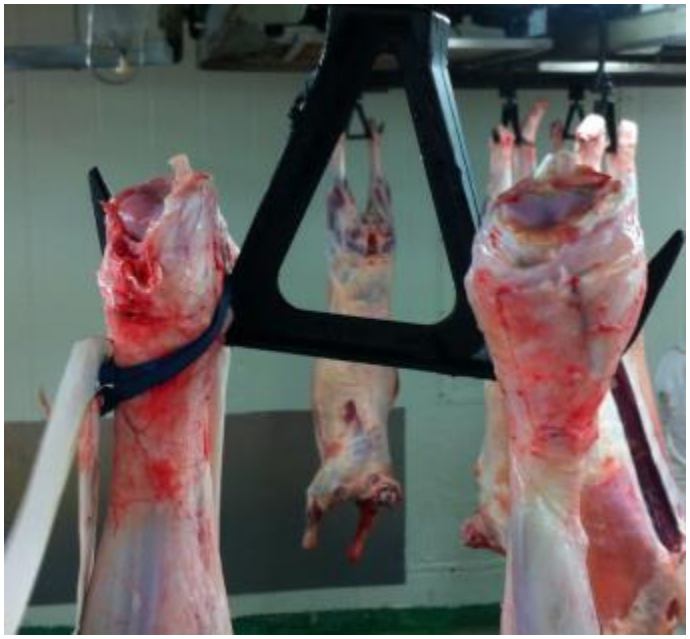

Figure 1. Carcase with one normal and one abnormal tarsal joint. The abnormal joint was cut through during processing so was not sampled for culture, PCR or histopathology. (Source: David Rutley, Thomas Foods International)

3.2.3 Pleurisy

Carcases with pleurisy were identified by DAWR-accredited meat inspectors (**Figure 2**). During January 2015 and in March 2015 only the number of carcases within a consignment with pleurisy was recorded. In September and October 2015 the number of carcases within a consignment with pleurisy was recorded, as well as extent and weight of carcase trimming. The extent and weight of the carcase trimming were recorded manually using a data capture sheet (Appendix 1) and then transcribed to a MS Excel spreadsheet.

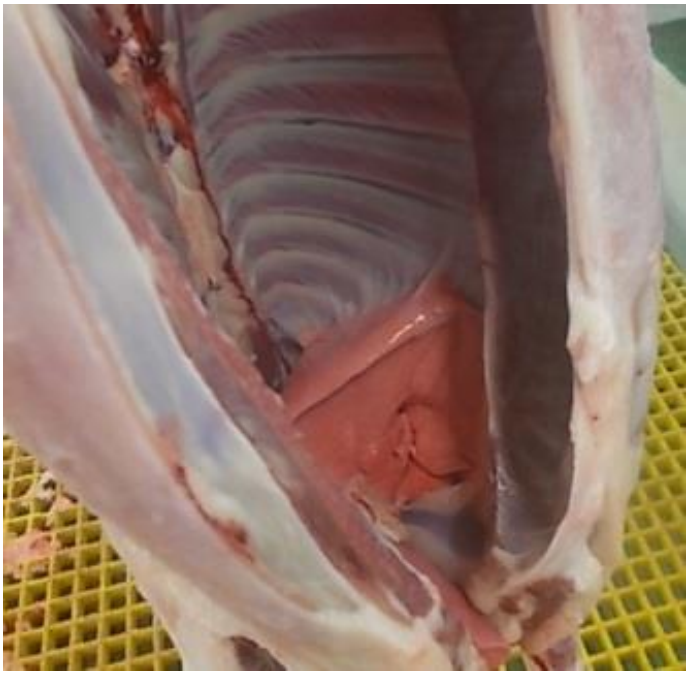

Figure 2. Carcase with pleurisy showing adhesions between visceral and parietal pleura

3.3 Testing of arthritic joint samples by culture, PCR and immunohistochemistry for the presence of bacteria and *Mycoplasma*

3.3.1 Bacterial culture

Joint swabs were cultured for 48 hours aerobically on Horse Blood Agar/MacConkey Agar, Columbia Naladixic Acid Agar, and Chocolate Agar and in CSF Broth, and anaerobically on NEO Agar plates.

After 24 hours the CSF Broth was sub-cultured onto Chocolate Agar and NEO Agar and the plates incubated for a further 48 hours.

Bacterial isolates were identified by a combination of gram stain, catalase, oxidase, kit identification and/or MALDI-TOF.

3.3.2 PCR

Chlamydia pecorum

The testing for *Chlamydia pecorum* was performed at Murdoch University using a previously described method (Yang et al., 2014b) following extraction of DNA from 50 mg of synovial tissue using a QIAamp® Fast DNA Tissue Kit (QIAGEN Pty Ltd, Chadstone Centre, VIC, Australia). A negative control (no synovium sample) was used in each extraction group.

Mycoplasma ovipneumoniae

The testing for *Mycoplasma ovipneumoniae* was conducted at the University of Sydney using the extracted DNA prepared during the testing for *C. pecorum* and a previously described PCR method targeting the P113 gene (Yang et al., 2014a).

Two *M. ovipneumoniae* isolates (Y98 and V339) were provided freeze-dried by the New South Wales Department of Primary Industries for use as positive controls. The two isolates were rehydrated with modified pleuropneumonia-like organism (PPLO) broth (Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Camden NSW). DNA was extracted from 1 mL of broth culture from each isolate using a DNeasy® Blood and Tissue kit (QIAGEN Pty Ltd, Chadstone Centre, VIC, Australia) following manufacturer's instructions for gram-negative bacteria. Purity and concentration of the extracted DNA was assessed by a spectrophotometer (SimpliNano™; GE Healthcare Life Sciences, Silverwater, NSW).

Sequencing at the Australian Genome Research Facility Ltd. confirmed that both isolates were *M. ovipneumoniae.*

The sensitivity of the PCR was assessed using a 10 fold serial dilution series of gDNA starting at 10 ng for each *M. ovipneumoniae* isolate (Y98 and V339) and the PCR targeting the P113 gene. For both isolates the sensitivity was 0.0001 ng of gDNA with an R^2 value of 0.99 and 0.95 for Y98 and V339, respectively.

The specificity of the PCR was assessed against a small panel of *Mycoplasma* species. These included *M. bovis, M. californicum, M. bovigenitalium, M. bovirhinis, M. bovoculi, Acholeplasma laidlawii* and *M. dispar.* All specificity isolates were negative.

All the extracted DNA samples prepared for the screening for *C. pecorum* were tested in a control PCR to ensure that the DNA extraction had been successful, thus ruling out false negatives. To do this, primers that target the Cytochrome B gene were used in a SYBR PCR reaction. These primers are routinely used by the University of Sydney to amplify bovine DNA, but there was uncertainty whether the primers would work on the ovine samples. As a test run, a PCR reaction was set up using the Cytochrome B gene primers and samples 1-5. Interestingly this revealed that there was too much starting DNA, resulting in the fluorescence starting at 100 per cent. The decision was made to quantify all of the samples on a nanodrop to estimate how much DNA was present in each sample. For any sample with more than 40 ng/µl of total DNA, a sub-sample was diluted to approximately 20 ng/µL total DNA.

The ovine samples were then run on the cytochrome B PCR. All samples were positive for Cytochrome B, indicating that the extractions had worked.

All samples were then run on the *M. ovipneumoniae* PCR targeting the P113 gene. Using the amplification and melt curve, samples were determined to be negative, positive or suspect positive.

Suspect samples were run again on a large-scale endpoint PCR and examined on an electrophoresis gel with the intention of sequencing any samples with a positive band.

3.3.3 Immunohistochemistry

Chlamydia pecorum

Sections were cut on silane slides at 4 µm, deparaffinized to water and placed into DAKO® autostainer (DAKO®Autostainer Plus Model LV-1). Slides were then exposed to 3 per cent hydrogen peroxide, *Chlamydia* antibody (anti-Chlamydia mouse monoclonal (Code PG-ACI-C), R-Biopharm® (Caringbah NSW Australia)), DAKO® REAL ™Envision (Code K5007), and DAKO® DAB+ substrate-chromogen solution with Tris Buffered Saline (TBS) rinses in between. Dilutions were at lab conditions. Slides were then removed from autostainer and counterstained with haematoxylin, dehydrated and mounted.

Erysipelothrix rhusiopathiae

Immunohistochemistry for *E. rhusiopathiae* was attempted using sections cut on silane slides at 4 µm as described above, serum from pigs vaccinated with the Eryvac Vaccine (Zoetis Australia Pty Ltd, Rhodes NSW, Australia) and a commercially available rabbit anti-pig horse rabbit peroxidase labelled secondary antibody (Abcam Australian Pty Ltd, Melbourne VIC Australia). Unfortunately the method was found to be subject to background staining, which was beyond the scope of the current project to rectify, so was not pursued.

3.5 Statistical analysis

3.5.1 South Australian Enhanced Abattoir Surveillance Program data

Arthritis and grass seeds

The data extracted from the South Australian Enhanced Abattoir Surveillance Program database were analysed to determine if there were age, regional or temporal effects on the prevalence of arthritis in the sheep slaughtered at the two abattoirs.

For the purpose of the analysis, the carcase data for mixed age and unknown age consignments were excluded because of the difficulties in grouping these to either age category. Sex was also excluded from the analysis because the majority of consignments of sheep less than two years of age were mixed sex and the majority of the consignments of sheep more than two years of age were female, with much fewer numbers of males and male castrate sheep.

The data (proportion of arthritic sheep) were fitted with a generalized linear mixed model with errors assumed to follow a binomial distribution. A logit link function was then used to relate the arthritis proportion to a number of risk factors such as age, regions and time with the seasonal effects represented using a smoothing spline curve in quarterly knots. The mathematical model can be expressed as follows:

Logit(P) = Fixed[age + region + age x region + linear(time) + age x linear(time)] + Random[spline(time; knots=13) + age x spline(time; knots=13) + region x month + age x region x month]

As farms (properties) were nested within regions, examination of farm (property) effects was made in a separate analysis with a similar model.

A residual maximum likelihood (REML) technique was used to estimate all parameters and all analysis was performed either using Genstat version 17 (VSN International Pty Ltd, 2014) or ASReml (Gilmour et al, 2009).

Proportion of arthritic lines was also analysed in a similar fashion.

In addition, the producer (farmer) effect on arthritis was analysed by summarising the data by producer and age categories over the three year period. Data were included in the analysis if there was at least one line affected in both age categories (LT2Y and MT2Y).

Correlation between between arthritis, grass seed lesion, lung worm and pleurisy/pneumonia

Using the data extracted from the South Australian Enhanced Abattoir Surveillance Program database (refer section 3.1.2), the number of carcases with evidence of each disease was calculated from the total number of carcases from each consignment.

Correlation analysis of arthritis, grass seed lesion, lung worm and pleurisy/pneumonia was conducted on (1) all lines and (2) lines that contained carcases with at least one disease condition.

3.5.3 Potential link between arthritis and tail length

Binary data (0 = arthritis absence; 1= arthritis presence) were analysed using a generalized linear model (GLM) with errors assumed to follow a binomial distribution. A logit transformation was used to link the data to risk factors such as Region, Breed, Age and Tail length. A REML technique was used to estimate all parameters and the LR test was used to calculate the deviance values contributed to by the risk factors fitted into the model. A 95 per cent confidence interval was calculated to determine significant differences between levels of each risk factor.

3.5.4 Abattoir survey data – arthritis and pleurisy

The percentages of lambs with arthritis and/or pleurisy from individual lines (consignments) were analysed using a generalized linear model (GLM) with errors assumed to follow a binomial distribution. A logit transformation was used to link the data to risk factors such as Region, Breed and Age. A residual maximum likelihood (REML) technique was used to estimate all parameters and the likelihood ratio (LR) test was used to calculate the deviance values contributed to by the risk factors fitted into the model. A 95 per cent confidence interval was calculated to determine significant differences between levels of each risk factor.

4 Results

4.1 Analysis of the South Australian Enhanced Abattoir Surveillance Program Data

4.1.1 Arthritis and grass seeds

Data were provided from a total of 19,348 directly consigned lines of South Australian sheep, including 14,068 lines of sheep less than two years of age (LT2Y) and 5,234 lines of sheep more than two years of age (MT2Y; **Table 1**). The sheep originated from different PIC regions in South Australia.

Table 1. Percentage lines and sheep slaughtered during 2011-2013 from two South Australian abattoirs

Age categories	Per cent Lines	Per cent sheep	Number of producers
LT2Y	72.71	71.51	3056
MT2Y	27.05	28.27	2439
Mixed ages	0.22	0.21	39
Unknown	0.02	0.02	4
Regions (Origins of sheep)			
Adelaide Hills / Fleurieu	3.29	3.02	145
Barossa / Lower North	5.51	5.54	236
Eyre	19.07	16.74	793
Kangaroo Island	11.75	11.01	241
Lower South East	4.39	6.48	198
Mid South East	6.74	10.81	290
Murray Mallee	14.88	13.67	543
Northern / Pastoral	5.99	6.92	255
Upper South East	5.56	6.68	320
Yorke Peninsula / Mid North	22.82	19.14	764

The majority of the lines were reported to be free of arthritis (**Table 2**). When arthritis was recorded within a line, it was most frequently reported at a low level (i.e. up to 5 per cent of the sheep within a line affected).

Table 2. Percent consignments (lines) within intervals of percent arthritic sheep

	Percent of lines		
Percent of sheep with arthritis within a line	LT2Y	MT2Y	ALL
0	93.745	78.296	89.555
1-5	4.407	16.393	7.657
6-10	1.109	3.611	1.787
11-15	0.185	0.535	0.280
16-20	0.284	0.611	0.373
21-25	0.036	0.153	0.067
26-30	0.085	0.248	0.130
31-40	0.036	0.038	0.036
41-50	0.021	0.038	0.026
51-60	0.028	0.019	0.026
61-70	0.000	0.019	0.005
71-80	0.021	0.000	0.016
81-90	0.014	0.000	0.010
91-100	0.028	0.038	0.031
Total Lines	14068	5234	19302

Age effect

There was a strong evidence that the prevalence of arthritis differed between Age categories (LT2Y and MT2Y, $P<0.001$; Table 3). The infection rate in sheep MT2Y was almost three times of that in sheep LT2Y (Table 4).

In both age categories, the prevalence of arthritis had declined over time ($P<0.001$) (**Table 3**).

Table 3. F values and probabilities of fixed terms and variance components of random terms fitted in the model

		Sheep		Lines	
Fixed terms	Degrees of freedom	F value	P(F)	F value	P(F)
Age Categories	1	252.68	<0.001	477.98	<0.001
Region	9	5.48	<0.01	8.86	<0.01
Age x Region	9	2.11	<0.05	3.13	<0.01
Linear (month)	1	37.63	<0.001	28.48	<0.001
Interaction	1	26.77	<0.001	41.67	<0.001
Random terms		Variance component		Variance component	
Spline (month)	11	0		0	
Age x Spline (month)	22	0.5582		0.5817	
Region x Month	360	0.0387		0.0367	
Age x Region x Month	720	0.3358		0.1215	
Variance	19075	16.93		0.9183	

Table 4. Generalised linear mixed model estimates of the per cent of sheep or consignments of sheep with arthritis for different age categories

	Sheep with arthritis			Consignments with arthritis		
Age Category	Logit	Standard error	Estimate	Logit	Standard error	Estimate
LT2Y	-5.128	0.143	0.0059	-2.557	0.126	0.072
MT2Y	-4.215	0.143	0.0146	-1.120	0.123	0.246
F test	<0.001			<0.001		

The significant interaction between age and time indicates that there were different patterns of infection rates from season to season between the age categories. **Figure 3** and **Figure 5** show there was a small variation in arthritis prevalence from season to season in sheep LT2Y for per cent affected sheep and per cent affected lines. In contrast, in sheep MT2Y the prevalence of arthritis fluctuated more widely between seasons (**Figure 4** and **Figure 6**).

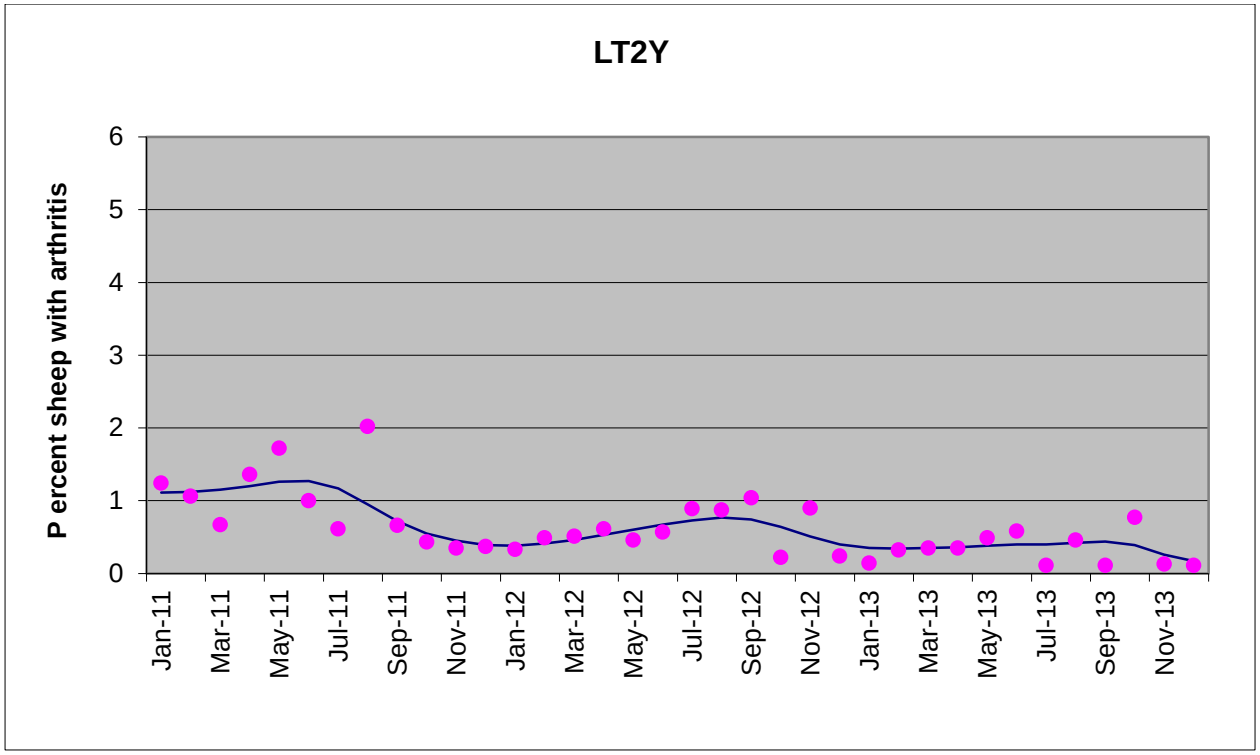

Figure 3. Per cent sheep less than two years of age with arthritis over the three year period 1 January 2011 – 31 December 2013, South Australian Enhanced Abattoir Surveillance Program

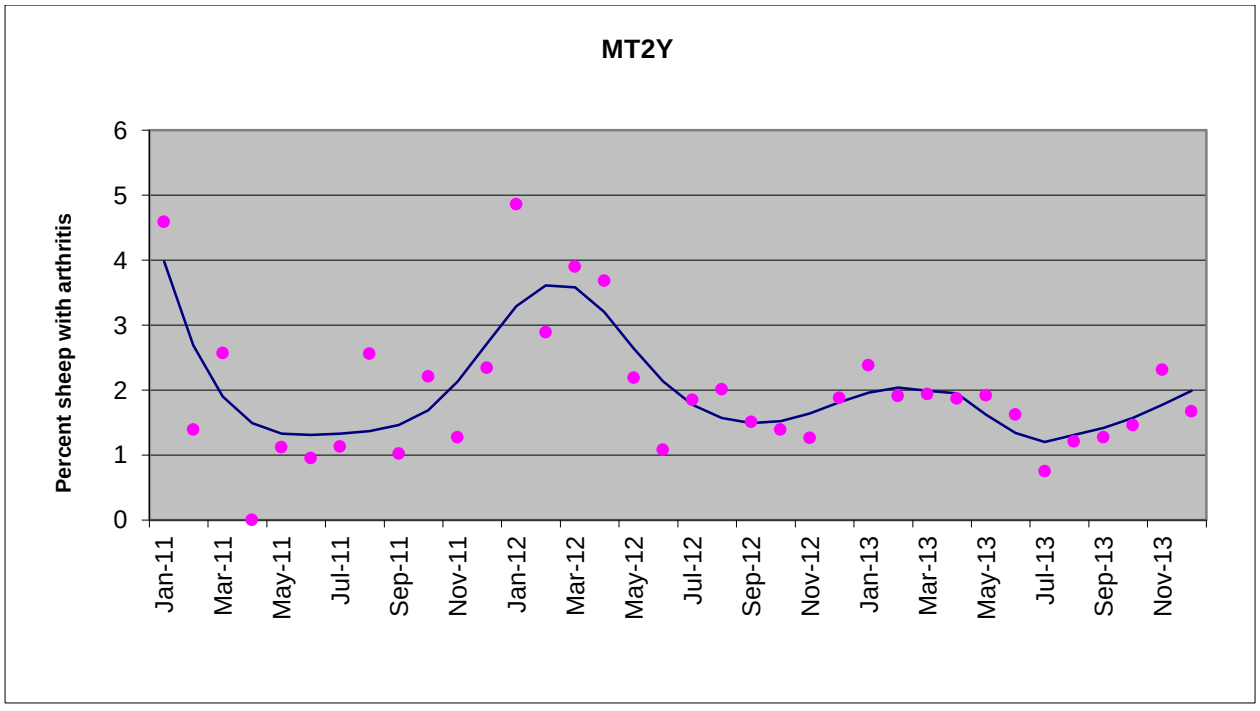

Figure 4. Per cent sheep more than two years of age with arthritis over the three year period 1 January 2011 – 31 December 2013, , South Australian Enhanced Abattoir Surveillance Program

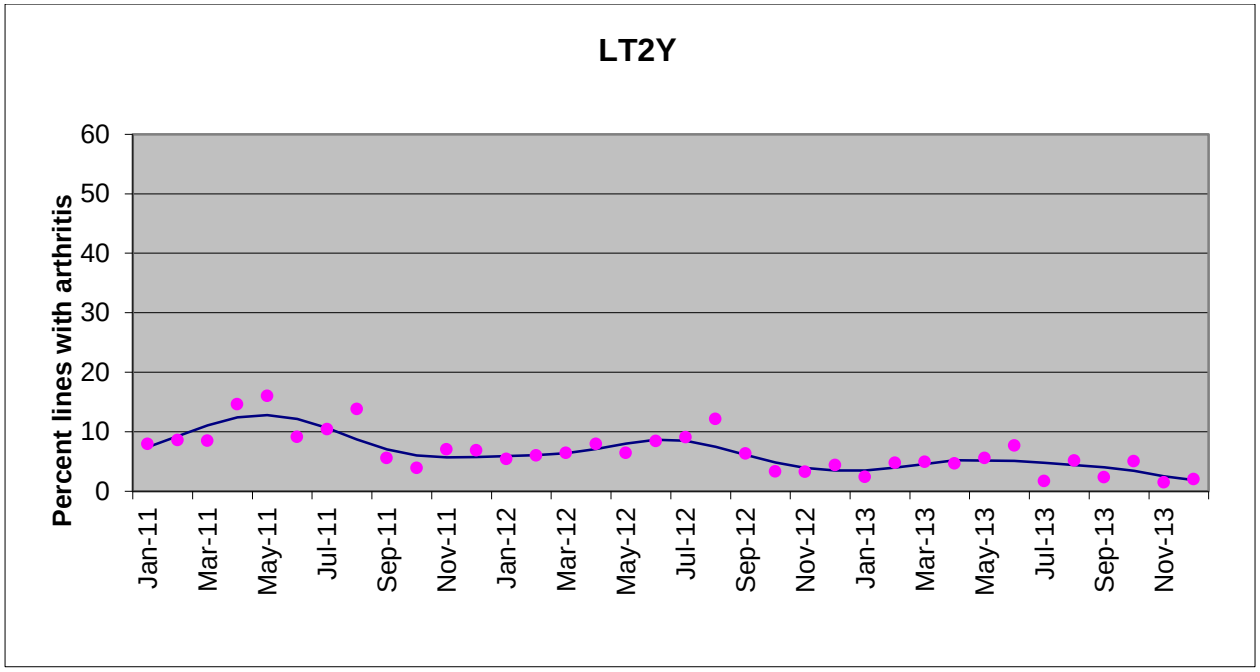

Figure 5. Per cent consignments of sheep less than two years of age with at least five per cent of the line affected by arthritis over the three year period January 2011 – 31 December 2013,South Australian Enhanced Abattoir Surveillance Program

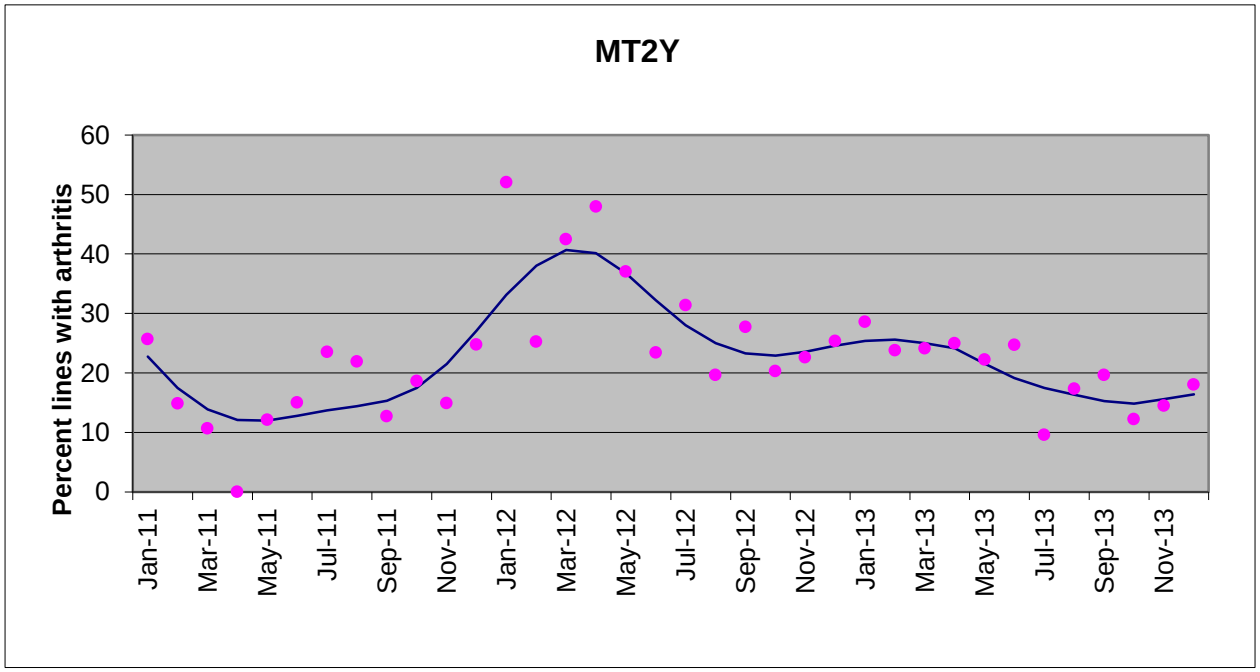

Figure 6. Per cent consignments of sheep more than two years of age with at least five per cent of the line affected by arthritis over the three year period January 2011 – 31 December 2013,South Australian Enhanced Abattoir Surveillance Program

Age by Region

Regional effects and their interaction with age were also significant (*P*<0.01; **Table 3**). The Barossa/ Lower North region had the highest percentage of younger sheep with arthritis, followed by the Murray Mallee and Eyre regions (**Table 5**). In older sheep, the Barossa/Lower North and Upper South were equally high (**Table 5**). Adelaide Hills/Fleurieu region had the lowest arthritis rate in both age groups.

Table 5. Regional effects on the percentage of sheep with arthritis and their interaction with the age of the animals (Back transformed means and 95 per cent confidence limits)

Age	Region	Back transformed mean (%)	Lower 95% confidence limit	Upper 95% confidence limit
LT2Y	Barossa / Lower North	0.0131	0.0090	0.0189
	Murray Mallee	0.0092	0.0062	0.0137
	Eyre	0.0075	0.0048	0.0116
	Kangaroo Island	0.0065	0.0045	0.0095
	Upper South East	0.0056	0.0036	0.0087
	Mid South East	0.0055	0.0035	0.0085
	Northern / Pastoral	0.0051	0.0032	0.0080
	Lower South East	0.0046	0.0031	0.0068
	Yorke Peninsula / Mid North	0.0036	0.0024	0.0053
	Adelaide Hills / Fleurieu	0.0034	0.0017	0.0066
MT2Y	Barossa / Lower North	0.0209	0.0142	0.0307
	Upper South East	0.0202	0.0141	0.0289
	Eyre	0.0166	0.0107	0.0255
	Mid South East	0.0151	0.0101	0.0223
	Lower South East	0.0149	0.0104	0.0213
	Kangaroo Island	0.0142	0.0097	0.0206
	Yorke Peninsula / Mid North	0.0130	0.0089	0.0189
	Murray Mallee	0.0123	0.0082	0.0185
	Northern / Pastoral	0.0114	0.0072	0.0179
	Adelaide Hills / Fleurieu	0.0104	0.0058	0.0185

A similar pattern was also observed when the data were analysed at the level of the consignment (**Table 6**).

Table 6. Regional effects on the percentage of consignments of sheep with arthritis and their interaction with the age of the animals (Back transformed means and 95 per cent confidence limits)

Age	Region	Back transformed mean (%)	Lower 95% confidence limit	Upper 95% confidence limit
LT2Y	Barossa / Lower North	0.1546	0.1150	0.2042
	Eyre	0.1197	0.0842	0.1672
	Murray Mallee	0.0923	0.0678	0.1242
	Kangaroo Island	0.0791	0.0596	0.1042
	Mid South East	0.0765	0.0527	0.1096
	Upper South East	0.0653	0.0448	0.0939
	Northern / Pastoral	0.0605	0.0415	0.0873
	Lower South East	0.0577	0.0428	0.0772
	Yorke Peninsula / Mid North	0.0438	0.0325	0.0587
	Adelaide Hills / Fleurieu	0.0335	0.0176	0.0627
MT2Y	Eyre	0.3114	0.2325	0.4023
	Barossa / Lower North	0.2968	0.2277	0.3760
	Upper South East	0.2844	0.2206	0.3576
	Mid South East	0.2725	0.2075	0.3482
	Lower South East	0.2540	0.2017	0.3141
	Kangaroo Island	0.2301	0.1785	0.2910
	Murray Mallee	0.2175	0.1646	0.2812
	Yorke Peninsula / Mid North	0.2141	0.1663	0.2706
	Northern / Pastoral	0.2108	0.1519	0.2842
	Adelaide Hills / Fleurieu	0.1896	0.1262	0.2742

Region by time

Regionally, the Mid South East region appeared to be the region with the highest percentage of sheep less than two years of age with arthritis consistently over time (**Figure 7**). The Lower South East and Kangaroo Island had highest percentage of sheep less than two years of age affected by arthritis in 2011.

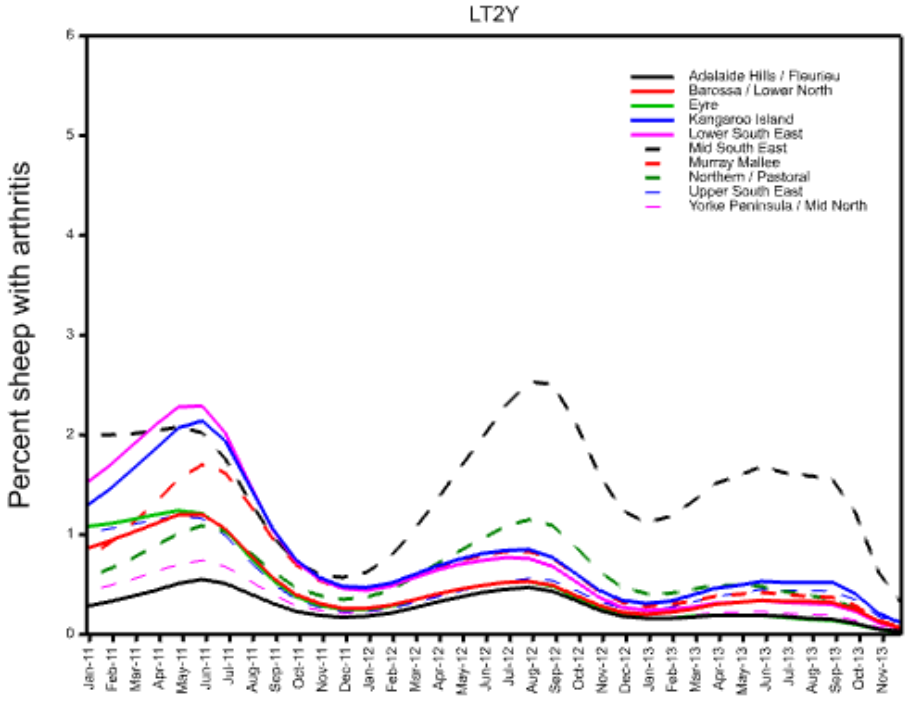

Figure 7. Regional percentage of sheep under two years of age with arthritis, 1 January 20110-031 December 2013, South Australian Enhanced Abattoir Surveillance Program

For sheep more than two years of age the percentage of sheep with arthritis was consistently high in the Mid South East and Northern/Pastoral regions, whereas the lowest percentage of older sheep with arthritis were in the animals sourced from Barossa/Lower North and Adelaide Hills/Fleurieu regions (**Figure 8**).

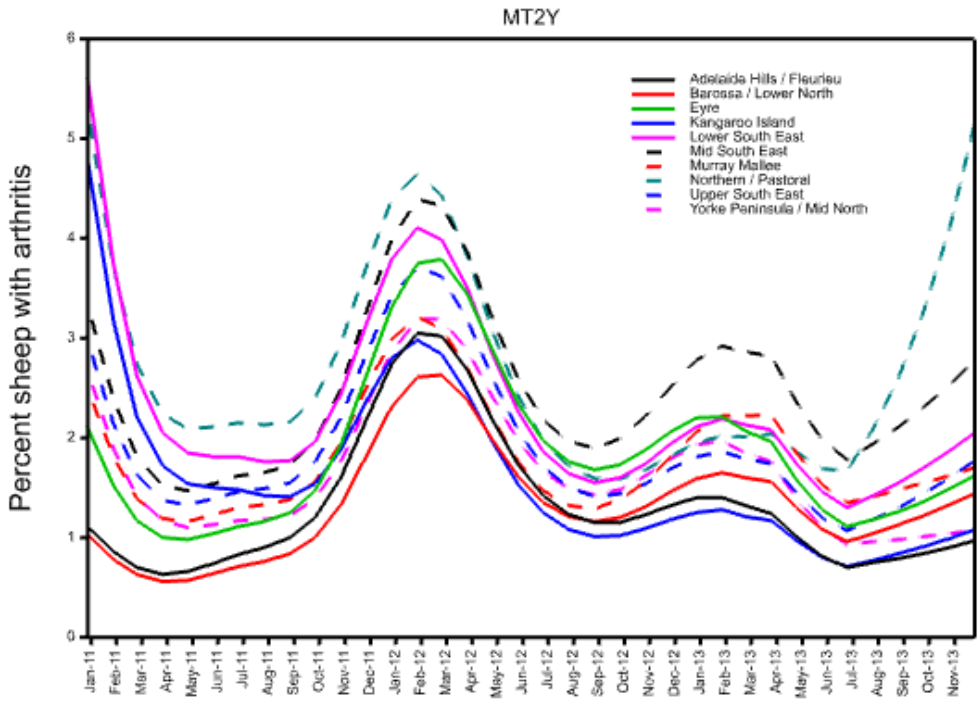

Figure 8. Regional percentage of sheep more than two years of age with arthritis, 1 January 20110-031 December 2013, South Australian Enhanced Abattoir Surveillance Program

When analysed at the level of the consignment, the Mid South East and Lower South East regions had consistently high rates of rates of sheep less than two years of age with arthritis over time (**Figure 9**). In contrast, the percentage of consignments from the Adelaide Hills/Fleurieu with arthritis was consistently low.

A similar pattern of arthritis within lines was found in the group of sheep more than 2 years old (**Figure 10**).

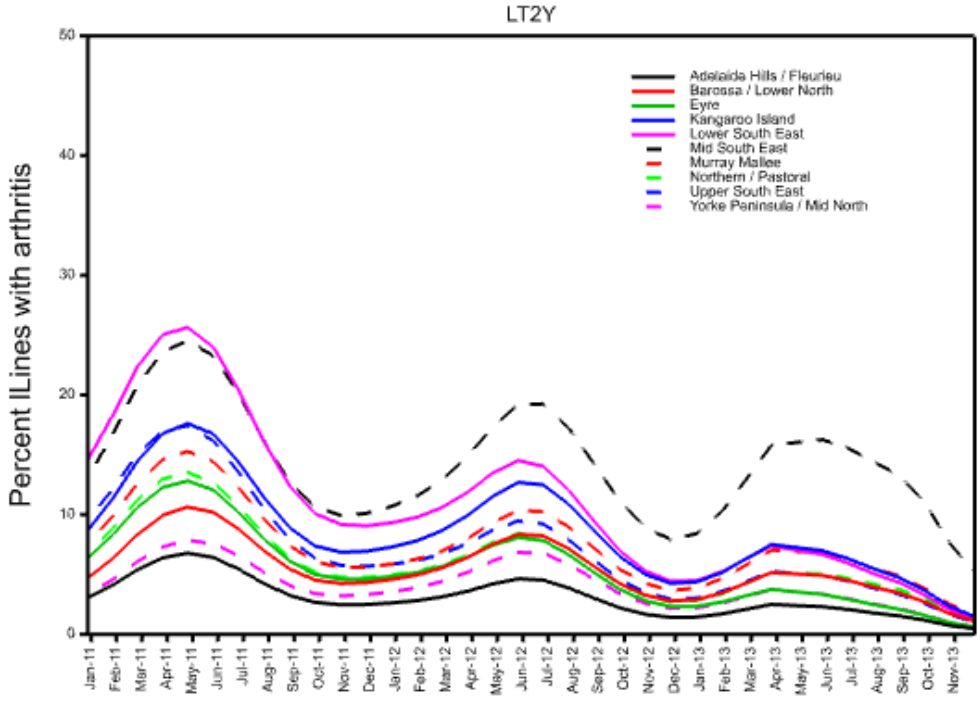

Figure 9. Regional percentage of consignments of sheep less than two years of age with arthritis, 1 January 20110-031 December 2013, South Australian Enhanced Abattoir Surveillance Program

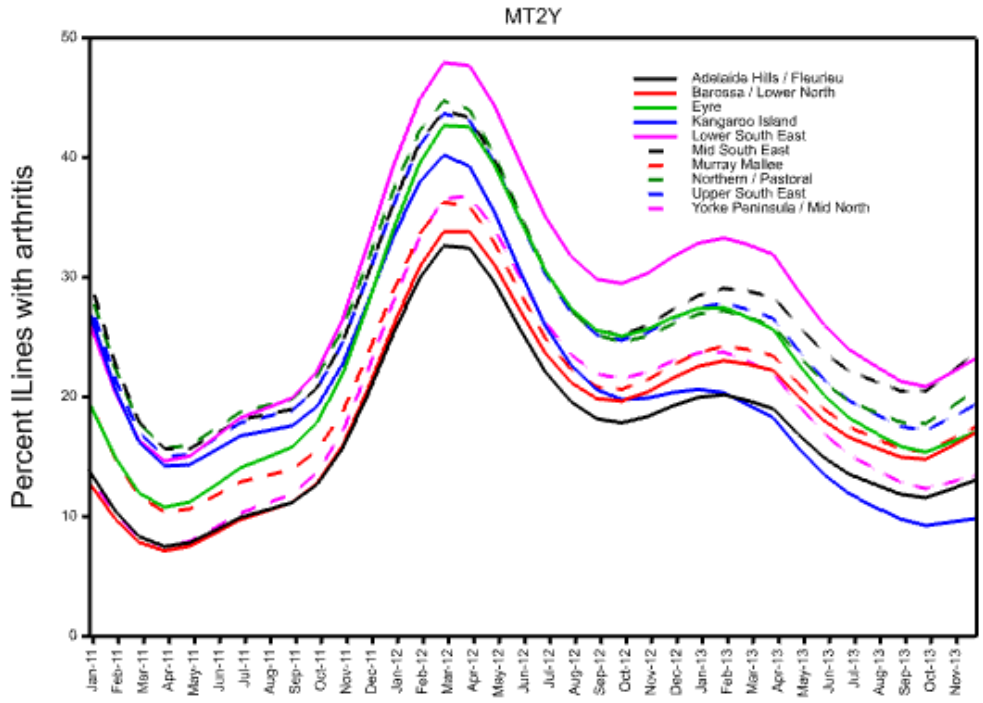

Figure 10. Regional percentage of consignments of sheep more than two years of age with arthritis, 1 January 20110-031 December 2013, South Australian Enhanced Abattoir Surveillance Program

Property/owner effect

There were 1720 producers who sold at least one consignment of sheep less than two years of age and at least one consignment of sheep greater than two years. The majority of these producers sold sheep free of arthritis or with less than five per cent of the line estimated to be affected (51.7 per cent; **Table 7**). Only 10.4 per cent of the producers sold at least one consignment containing animals with arthritis in both age groups, whereas 37.9 per cent of producers sold consignments of sheep with arthritis in either age group.

A Chi square contingency table was used to test the association of the disease between the two age groups of sheep at the producer level. There was a strong association between presence/absence of arthritis between the two age groups (χ^2=21.23; P<0.001).

Table 7. Proportion of the 1720 properties that sold consignments of sheep of different age categories with and without arthritis

		Sheep less than two years of age	
		Arthritis present	Arthritis absent
Sheep more than two years of age	Arthritis present	0.1041	0.1227
	Arthritis absent	0.2564	0.5169

Grass seed lesions

There was strong evidence of an age effect on grass seed lesions in sheep (*P*<0.001; Table 8), with sheep less than two years of age having 10 times the grass seed lesion rate of sheep more than two years of age (**Table 9**). The Murray Mallee and Upper South East were the two regions most commonly affected by grass seeds (**Table 10**). There was no interaction between age and regions (*P*>0.05; **Table 8**).

Table 8. F values and probabilities of fixed terms and variance components of random terms fitted on grass seed lesion

Terms	Degrees of freedom	F values	Prob(F)
Age	1	65.73	<0.001
Region	9	15.35	<0.001
Age x Region	9	1.07	>0.05
Linear (months)	1	10.77	<0.001
Age x linear(month)	1	3.82	>0.05
		Variance	
Spline (month,13)	11	4.50383	
Age by spline (month,13)	22	8.40E-03	
Region x month	360	2.44E-02	
Age x month x region	720	0.907132	
Variance	19057	102.098	

Table 9. Generalised linear mixed model estimates of the per cent of sheep with grass seed for different age categories

Age	Logit	SE	Percent
Sheep less than two years of age	-3.4751	0.1977	3.00
Sheep more than two years of age	-6.0837	0.8848	0.23

Table 10. Regional effects on the percentage of sheep with grass seed lesions and their interaction with the age of the animals (Logit values and back transformed means)

Age	Region	Logit	Standard error	Percent
LT2Y	Murray Mallee	-2.3313	0.2507	8.86
	Upper South East	-2.4252	0.2678	8.13
	Eyre	-2.9733	0.2545	4.86
	Northern/Pastoral	-2.9951	0.2737	4.76
	Yorke Peninsula	-3.3894	0.2567	3.26
	Barossa/Lower North	-3.4365	0.2864	3.12
	Mid South East	-3.6678	0.2893	2.49
	Adelaide Hills	-3.7122	0.3546	2.38
	Kangaroo Island	-4.2192	0.3136	1.45
	Lower South East	-5.6012	0.4426	0.37
MT2Y	Murray Mallee	-3.8724	0.3362	2.04
	Upper South East	-4.1382	0.376	1.57
	Barossa/Lower North	-5.2652	0.5519	0.51
	Yorke Peninsula	-5.2789	0.4148	0.51
	Northern/Pastoral	-5.3982	0.4393	0.45
	Eyre	-5.7623	0.4217	0.31
	Mid South East	-6.3387	0.6229	0.18
	Kangaroo Island	-6.5837	0.6805	0.14
	Lower South East	-6.7571	0.9368	0.12
	Adelaide Hills	-11.4419	0	0

4.1.2 Correlation between arthritis, grass seed lesion, lung worm and pleurisy/pneumonia

Using all the data from the South Australian Enhanced Abattoir Surveillance Program provided by PIRSA for the time period 1 January 2011 - 31 December 2014 (refer section 3.1.2), most correlations between pairs of diseases were highly significant (P<0.01) except the correlation between arthritis and lung worm for sheep less than two years of age (**Table 11**).

Table 11. Correlation coefficient between diseases observed using all the South Australian Enhanced Abattoir Surveillance Program data, 1 January 2011 – 31 December 2014 (* *P*<0.05; ** *P*<0.01)

Sheep less than two years of age				
	Arthritis	Grass seed	Lung worm	Pleurisy/ pneumonia
Arthritis	1			
Grass seed	0.083**	1		
Lung worm	0.068	0.107**	1	
Pleurisy/ pneumonia	0.116**	0.067*	0.149**	1
Sheep more than two years of age				
	Arthritis	Grass seed	Lung worm	Pleurisy/ pneumonia
Arthritis	1			
Grass seed	0.099**	1		
Lung worm	0.131**	0.116**	1	
Pleurisy/ pneumonia	0.370**	0.091**	0.263**	1

Restricting the data to only those consignments that had evidence of at least one disease reduced the significance of the correlations in the sheep less than two years of age with only the correlation between arthritis and pleurisy/pneumonia remaining significant (**Table 12**). In contrast, all the correlations between the diseases for the sheep more than two years remained highly significant, with the strongest correlation between arthritis and pleurisy/pneumonia.

Table 12. Correlation coefficient between diseases observed using only the South Australian Enhanced Abattoir Surveillance Program data where at least one disease condition was present within a consignment, 1 January 2011 – 31 December 2014 (* *P*<0.05; ** *P*<0.01)

Sheep less than two years of age				
	Arthritis	Grass seed	Lung worm	Pleurisy/ pneumonia
Arthritis	1			
Grass seed	0.053	1		
Lung worm	0.008	-0.005	1	
Pleurisy/ pneumonia	0.086**	0.007	0.039	1
Sheep more than two years of age				
	Arthritis	Grass seed	Lung worm	Pleurisy/ pneumonia
Arthritis	1			
Grass seed	0.092**	1		
Lung worm	0.096**	0.107**	1	
Pleurisy/ pneumonia	0.345**	0.079*	0.207**	1

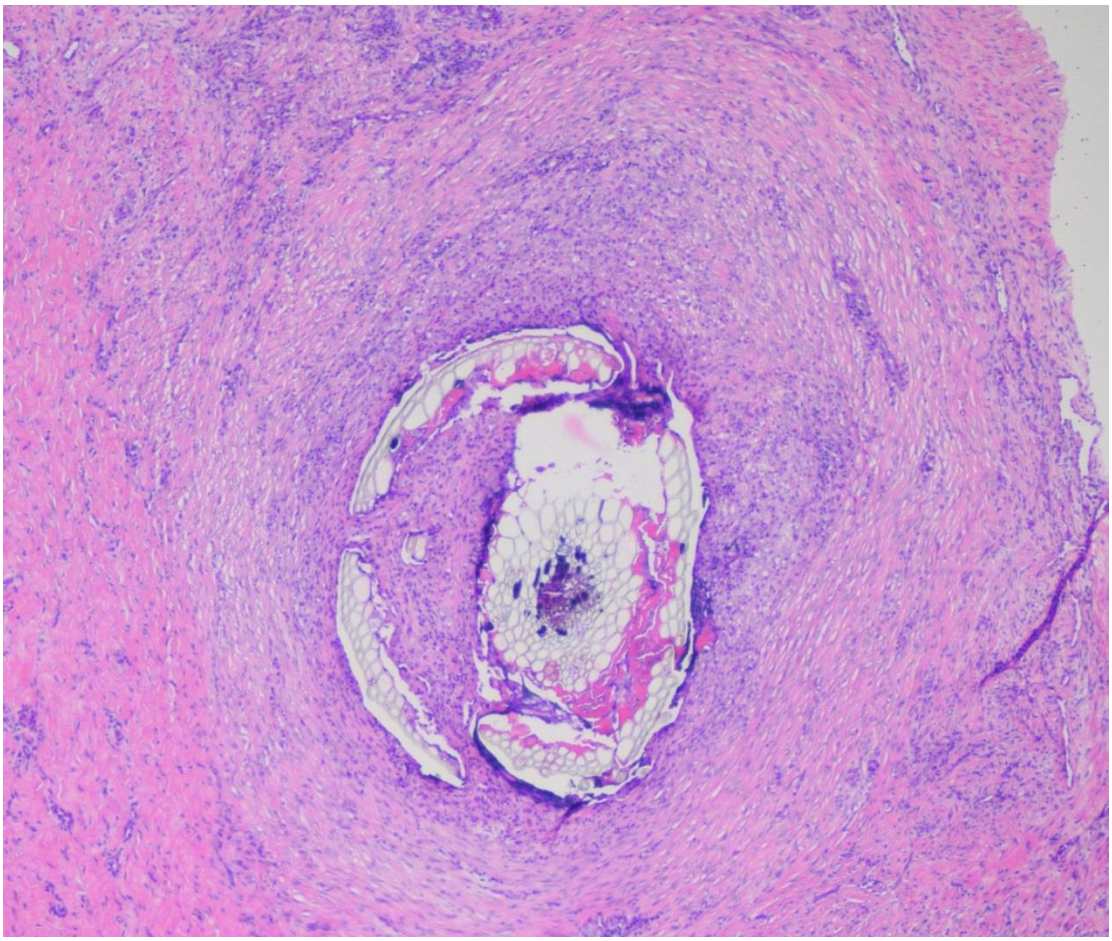

Figure 11. H&E stained section from a crossbred lamb collected on 4 December 2014 showing a vegetable foreign body (grass seed) embedded in the peri-synovial tissues (magnification x 40)

4.2 Survey of tail length, arthritis and pneumonia/pleurisy

The abattoir data collection was conducted in three phases, December 2014 to January 2015, March 2015 and September to October 2015. Data were collected on 354 lines of lambs representing 63,287 carcases (**Table 13**).

The majority of the lines were directly consigned from farm to abattoir (334), with a minority (20) sourced by other means. The average number of lambs within a line was 179 (range 8-969). One hundred and fifty one (42.7 per cent) of the lines included less than 100 lambs. (The South Australian Enhanced Abattoir Surveillance Program does not collect data on lines with less than 100 lambs.)

Table 13. Number of lines and carcases examined

	Total	December 2014 – January 2015	March 2015	September – October 2015
Number of lines	354	165	126	63
Number of directly consigned lines	334	152	120	62
Number of lines consigned by other means	20	13	6	1
Number of carcases included in the lines examined	63,287	34,324	18,389	10,570
Number of directly consigned lambs	60,511	33,517	16,785	10,205
Number of lambs consigned by other means	2,776	807	1,604	365
Average number of carcases within a line	179	208	146	168
Range	8-969	8-969	8-880	15-709
Median	114	133	103	109
Number of lines with <100 carcases	151	63	60	28

The majority of the lines and carcases examined were crossbred lambs, with lesser numbers of Merino and Dorper lambs (**Table 14**). Lambs comprised approximately 80 per cent of the lines and carcases examined.

Table 14. Number of lines and carcases by breed and age categories

	Number of lines (Per cent of total)	Number of carcases (Per cent of total)
Dorper	30 (8.5%)	9,346 (14.8%)
Merino	104 (29.4%)	14,300 (22.6%)
Crossbred	220 (62.1%)	39,641 (62.6%)
Lamb[A]	287 (81.1%)	49,977 (79.0%)
Young lamb[B]	67 (18.9%)	13,310 (21.0%)

[A] Female, castrate or entire male ovine that has zero permanent incisor teeth[3]
[B] Female or castrate male ovine that has zero permanent incisor teeth and no evidence of eruption of permanent upper molar teeth

The regional source of the lines and carcases examined is shown in **Table 15**. Within South Australia, the distribution of the lines was similar to the regional distribution of lines examined under the South Australian Enhanced Abattoir Surveillance Program, with three regions (Eyre, Murray Mallee and Yorke Peninsula/ Mid North) accounting for approximately 50-60 per cent of the lines and carcases examined (Section 4.1).

[3] https://www.ausmeat.com.au/WebDocuments/SheepMeat_Language.pdf

Table 15. Number of lines and carcases by regional source

	Number of lines (Per cent of total)	Number of carcases (Per cent of total)
Adelaide Hills/ Fleurieu	10 (2.8%)	893 (1.4%)
Barossa/ Lower North	11 (3.1%)	1,787 (2.8%)
Eyre	79 (22.3%)	10,665 (16.9%)
Kangaroo Island	21 (5.9%)	1,963 (3.1%)
Lower South East	10 (2.8%)	3,196 (5.1%)
Mid South East	17 (4.8%)	3,984 (6.3%)
Murray Mallee	63 (17.8%)	6,416 (10.1%)
Northern Pastoral	15 (4.2%)	3,999 (6.3%)
Upper South East	13 (3.7%)	2,352 (3.7%)
Yorke Peninsula/ Mid North	42 (11.9%)	7,699 (12.2%)
NSW	21 (5.9%)	8,734 (13.8%)
VIC	38 (10.7%)	9,469 (15.0%)
QLD	1 (0.3%)	768 (1.2%)
Unknown	13 (3.7%)	1,362 (2.2%)
Total	354	63,287

4.2.1 Arthritis

One hundred and sixty nine consignments, or approximately one-half of the consignments, had at least one carcase with arthritis/polyarthritis (**Table 16**).

Table 16. Number of lines and carcases with arthritis/polyarthritis

Number of lines with arthritis/polyarthritis in at least one carcase	169
Per cent lines with arthritis/polyarthritis in at least one carcase	47.8%
Number of carcases with arthritis/polyarthritis	422
Per cent carcases with arthritis/polyarthritis	0.7%
Prevalence of arthritis within affected lines (mean, range)	2.0%, 0.1-17.9%

Four hundred and twenty-two, or 0.7 per cent of the carcases had arthritis/polyarthritis in at least one joint (**Table 16**). When arthritis was present, on average 2.0 per cent of the line was affected (range 0.1-17.9 per cent).

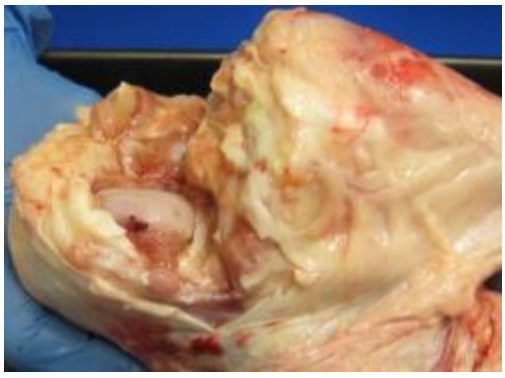

Figure 12. Stifle joint with arthritis showing hyperaemic and oedematous synovium, abnormal joint fluid and erosions on the articular cartilage

Of the carcases with arthritis/polyarthritis, three were condemned for polyarthritis and the remainder of the carcases trimmed. Of the trimmed carcases, most had one joint trimmed for arthritis (280 of the carcases). Eighty-nine of the carcases had two joints trimmed, 26 had three joints trimmed, five had four joints trimmed and two had five joints trimmed. The trimmed joints included 181 tarsal joints, 165 carpal joints, 45 stifle joints and 20 elbow joints. All of the lower limbs joints had been removed prior to the point of inspection so could not be assessed.

The average weight of the trim was 0.747 kg (n=389, range 0.098-4.448 kg, 95 per cent confidence interval 0.672-0.822 kg).

Figure 13. Carcase trimmed for polyarthritis (Source: David Rutley, Thomas Foods International)

4.2.2 Link between arthritis and tail length

A subset of the arthritis data with reliable tail length data was selected for analysis of the potential link between arthritis and tail length. The distribution of carcase samples selected for the analysis is presented in Table 17. The majority of samples were crossbred lambs, with older lambs constituting approximately 86 per cent of the data. Within South Australia the samples were largely came from Eyre (17.41 per cent), the Yorke Peninsula (14.42) and the Murray Mallee (13.72 per cent), similar to the regional distribution of the data overall (refer Section 4.2). Lambs from Victoria constituted around 18.1 per cent of the data.

Table 17. Number of samples selected for further analysis by region, breed and age categories

	Dorper		Merino		Crossbred	
Region	Lamb	Young lamb	Lamb	Young lamb	Lamb	Young lamb
Adelaide Hills/ Fleurieu	0	0	88	0	241	0
Barossa/ Lower North	0	0	196	0	135	120
Eyre	97	0	1346	62	1417	525
Kangaroo Island	0	30	0	0	415	151
Lower South East	0	0	0	0	887	419
Mid South East	0	0	53	26	487	345
Murray Mallee	12	0	1157	25	1423	101
Northern Pastoral	0	0	290	0	317	25
Upper South East	0	0	262	0	355	0
Yorke Peninsula/ Mid North	17	0	1226	0	1523	89
NSW	1220	82	0	0	469	59
Victoria	143	0	290	0	2843	308
Unknown	0	0	0	22	430	76
Total	1489	112	4908	135	10942	2218

Tail length had a significant effect on the prevalence of arthritis in the lambs (*P*<0.001), with shorter tails (one or two coccygeal vertebrae) being a higher risk practice than longer tails (**Table 18**).

Table 18. Percentage of lambs with different tail lengths with arthritis (Means in the same column with different superscripts are statistically different)

Number of coccygeal vertebrae	Per cent arthritis	Total carcases
1	1.949[ab]	513
2	2.247[a]	4007
3	1.485[b]	9503
4	1.544[b]	5246
5	1.310[b]	535

In addition, risk factors such as region (*P*<0.001), breed (*P*<0.001) and lamb age (*P*=0.001) all significantly affecting the incidence of arthritis. The effects of lamb age varied between Regions (p<0.001), however, there was no interaction between Breed and Age (*P*=0.279).

4.2.3 Other risk factors for arthritis – breed, region and age

Using the whole dataset, the percentage of lambs with arthritis from individual lines (consignments) was analysed using a generalized linear model to further investigate the risk factors of region, breed and age. The analysis revealed that both region and breed, but not Age, were significant risk factors for arthritis (**Table 19**).

Table 19. Mean deviance, deviance ratio and approximate probability calculated based on Chi square value of mean deviance

Sources of variation	Degrees of freedom	Mean deviance	P(chi)
Region	12	5.812	<0.001
Breed	2	11.779	<0.001
Age	1	0.752	0.386
Region by Breed	16	1.599	0.06
Region by Age	10	6.245	<0.001
Breed by Age	2	5.777	0.003
Residual	309	2.036	

The regions with the highest prevalence of arthritis were Kangaroo Island, the Murray Mallee, the Mid South East and the Upper South East (**Table 20**).

Table 20. Prevalence of arthritis by region (Back transformed means and 95 per cent confidence limits.)

Regions	Per cent arthritis	Lower 95% confidence limit	Upper 95% confidence limit
Adelaide Hills/ Fleurieu	0.34^{e}	0.11	1.03
Barossa/ Lower North	0.28^{e}	0.12	0.67
Eyre	0.82^{b}	0.66	1.00
Kangaroo Island	1.53^{a}	1.07	2.18
Lower South East	0.59^{bcde}	0.38	0.93
Mid South East	0.90^{ab}	0.65	1.25
Murray Mallee	1.01^{ab}	0.80	1.29
Northern Pastoral	0.75^{bc}	0.53	1.07
Upper South East	0.89^{ab}	0.58	1.36
Yorke Peninsula/ Mid North	0.62^{bcd}	0.47	0.83
NSW	0.34^{de}	0.24	0.49
Victoria	0.42^{cde}	0.31	0.57
Unknown	0.44^{bcde}	0.20	0.97

The prevalence of arthritis was higher in Merino lambs than in Dorper or Crossbred lambs (**Table 21**).

Table 21. Prevalence of arthritis by breed and breed by age. (Back transformed mean and 95 per cent confidence limits.)

Breeds	Per cent arthritis	Lower 95% confidence interval	Upper 95% confidence interval
Dorper	0.38[b]	0.27	0.54
Merino	1.05[a]	0.89	1.23
Crossbred	0.60[b]	0.53	0.68
Breed by Age			
Dorper Lamb	0.31[c]	0.20	0.48
Dorper Young	0.61[bc]	0.36	1.05
Merino Lamb	1.04[a]	0.88	1.22
Merino Young	1.32[a]	0.60	2.90
Crossbred Lamb	0.63[b]	0.54	0.72
Crossbred Young	0.52[bc]	0.40	0.68

4.2.4 Pleurisy

Commencing on 19 January 2015, following the culturing of *Pasteurella* spp. from two arthritic joint samples, data were recorded on the prevalence of pleurisy. In September/October 2015 pleurisy trim data were also collected.

Data on the prevalence of pleurisy were recorded on 227 lines representing 35,839 carcasses (**Table 22**).

Table 22. Number of lines and carcases examined for pleurisy by breed and age categories

	Number of lines (Per cent of total)	Number of carcases (Per cent of total)
Dorper	17 (7.5%)	4,339 (12.1%)
Merino	74 (32.6%)	9,074 (25.3%)
Crossbred	136 (59.9%)	22,426 (62.6%)
Lamb	178 (78.4%)	26,389 (73.6%)
Young lamb	49 (21.6%)	9,450 (26.4%)

Pleurisy was present in 115 of the 227 lines (50.7 per cent) and 334 of the 35,389 carcasses (0.9 per cent) examined. The average prevalence within affected lines 2.2 per cent (range 0.2-12.2 per cent).

Pleurisy trim weight data were collected on 101 carcasses. Thirty-three of the carcasses (32.7 per cent) had the pleura stripped with no rib removal. Thirteen carcasses (12.9 per

cent) had approximately one-quarter of the rib cage removed, 49 (48.5 per cent) had half the rib cage removed and six had three-quarters (1 carcase) or the full ribcage (five carcasses) removed.

The average trim weight when ribs were removed was 1.0 kg (one-quarter of the rib cage 0.50 kg, one-half the rib cage 1.0 kg, three-quarter to the full rib cage 1.9 kg).

The average trim weight including the carcasses from which only the pleura was removed was 0.7 kg (with the weight of the stripped pleura assumed to be zero).

4.2.5 Risk factors for pleurisy

The percentage of carcases with pleurisy from individual lines (consignments) was analysed using a generalized linear model to investigate the risk factors of Region, Breed and Age. The analysis revealed that both region and age, but not breed, were significant risk factors for pleurisy and that there were region by breed and region by age interactions (**Table 23**).

Table 23. Mean deviance and Chi square probabilities of fitted terms to the percentage of carcases with pleurisy from individual lines

Sources of variation	Degrees of freedom	Mean deviance	P(chi)
Region	12	5.05	<.001
Breed	2	2.63	0.072
Age	1	11.56	<.001
Region by Breed	14	2.61	<.001
Region by Age	9	3.72	<.001
Breed by Age	2	2.31	0.100
Residual	186	2.00	

The highest prevalence of pleurisy was in the animals sourced from Kangaroo Island, followed by those sourced from the Murray Mallee (Table 24).The lambs from New South Wales were largely Dorper lambs sourced from the western regions of the State (69.2 per cent).

Table 24. Prevalence of pleurisy by region. (Percent pleurisy and 95 per cent confidence limits.)

Regions	Per cent pleurisy	Lower 95% confidence interval	Upper 95% confidence interval
Adelaide Hills/ Fleurieu	0.87[bcd]	0.42	1.82
Barossa/ Lower North	0.73[bcd]	0.41	1.32
Eyre	0.65[cd]	0.48	0.88
Kangaroo Island	2.41[a]	1.71	3.39
Lower South East	0.96[bcd]	0.56	1.65
Mid South East	0.81[bcd]	0.54	1.20
Murray Mallee	1.24[b]	0.95	1.61
Northern Pastoral	1.00[bcd]	0.66	1.53
Upper South East	0.71[bcd]	0.38	1.31
Yorke Peninsula/ Mid North	0.97[bcd]	0.70	1.33
NSW	0.44[d]	0.29	0.67
Victoria	1.03[bc]	0.74	1.42
Unknown	1.90 ab	1.29	2.80

The prevalence of pleurisy was higher in lambs than in young lambs (Table 25).

Table 25. Prevalence of pleurisy by breed. (Logit transformed means and their standard errors, percent pleurisy and 95 per cent confidence limits.)

Age	Logit(p)	Standard error of the mean	Per cent pleurisy	Lower 95% confidence interval	Upper 95% confidence interval
Lamb	-4.5571	0.0606	1.04[a]	0.92	1.17
Young lamb	-5.053	0.129	0.63[b]	0.49	0.82

4.3 Testing of arthritic joint samples by culture, PCR and immunohistochemistry for the presence of bacteria and *Mycoplasma*

4.3.1 Culture

The majority of the joints could not be submitted for culture because they had been damaged during processing. Joints from 174 carcases were submitted for bacterial culture. For some carcases, more than one affected joint was submitted.

A summary of the culture results is presented in **Table 26**. (Refer to Appendix 2 for the full results.) Positive results were only obtained from the cultures set up in enrichment broth, not by direct plating.

E. rhusiopathiae, alone or in combination, was the bacterial species isolated most frequently (19 of 33 culture positive joints, 57.6 per cent).

Approximately one-half the *E. rhusiopathiae* positive cultures were obtained from young lambs and one-half from lambs (young lambs 9 of 19 *E. rhusiopathiae* culture positive joints, 47.4 per cent; lambs 10 of 19 *E. rhusiopathiae* culture positive results, 52.6 per cent).

Three of the *E. rhusiopathiae* culture-positives were from Merino lamb carcases, 14 from cross-bred lamb carcases and two from Dorper lamb carcases.

Eight of the nineteen carcases had one affected joint trimmed, five had two affected joints trimmed, three had three affected joints trimmed and three had four joints trimmed. The most common joint trimmed was the tarsus, followed by the stifle. None of the joints from which *E. rhusiopathiae* was cultured were from carcases condemned for polyarthritis.

Streptococcus spp. were the next most commonly isolated bacteria (five of 33 culture positive results, 15.2 per cent), including two joints that were positive for α-haemolytic *Streptococcus* spp. Both of the joints culture-positive for α-haemolytic *Streptococcus* spp. were from young lambs and both of the carcases were condemned for polyarthritis with systemic involvement.

Table 26. Bacterial culture results for arthritic joints[4]

Bacterium	Number of joints		
	November 2014 – January 2015	March 2015	All time periods
Pure culture			
Erysipelothrix rhusiopathiae	14	3	17
Alpha-haemolytic *Streptococcus* spp.	2	-	2
Streptococcus spp.	2	1[2]	3
Coagulase-negative *Staphylococcus* spp.	2	-	2
S. warneri	-	1	1
S. epidermidis	1[1]	-	1
Corynebacterium spp.	1	-	1
Micrococcus spp.	1	-	1
Pasteurella spp.	1	-	1
Mixed culture			
Erysipelothrix rhusiopathiae, *Streptococcus* spp.	1	-	1
Erysipelothrix rhusiopathiae, *Staphylococcus* spp.	1	-	1
Micrococcus spp. and coagulase negative *Staphylococcus* spp.	1[2]	-	1
Pasteurella spp. and mixed skin flora	1	-	1
Bacteriologically positive	28	5	33
Bacteriologically negative	59	45	104
Bacteriology not done	127	101	228

[1] Also positive for *C. pecorum* on PCR and IMHC and had an intra-lesional vegetable foreign body

[2] Also positive for *C. pecorum* on PCR and IMHC

[4] Some joint cultures were contaminated with environmental bacteria and these results have been excluded from the table. Refer to Appendix 2 for more information.

Gram Twort staining was performed on the formalin-fixed tissue from all of the joints that had a positive culture result to determine the sensitivity of this method to detect bacteria in culture-negative joints in which marked changes were seen on histopathology. In total, 46 formalin-fixed tissues were stained, with bacteria seen in only 21 of the 46 sections (sensitivity 45.7 per cent; refer Appendix 3 for full results). Of the 19 joint samples that were culture-positive for *E. rhusiopathiae*, 13 of the formalin-fixed tissues were positive for bacteria on Gram Twort stain (sensitivity 68.4 per cent). Because of the low sensitivity of this method to detect bacteria, it was not pursued further.

4.3.2 PCR

Chlamydia pecorum

Synovial tissues from the 148 joints collected in November 2014 – January 2015 and in March 2015 were tested for *Chlamydia pecorum* using PCR. Ten of the 148 synovial tissue samples were positive for *C. pecorum* (6.8 per cent), including tissue from one joint that was also culture positive for *S. epidermidis* and had an intra-lesional foreign body (**Figure 14**), one joint that was culture positive for a *Micrococcus* spp. and a coagulase-negative *Staphylococcus* spp. and one joint that was positive for a *Streptococcus* spp. (**Table 26**).

Four of the PCR-positive samples were from Merino carcases, four from cross-bred lamb carcases and two from Dorper lamb carcases.

Five of the ten carcases that tested positive for *C. pecorum* had one affected joint trimmed and five had two affected joints trimmed. The most common joint trimmed was the tarsus (five of six carcases in which the condemned joint was recorded.) None of the synovial tissue samples that tested positive for *C. pecorum* were from carcases condemned for polyarthritis.

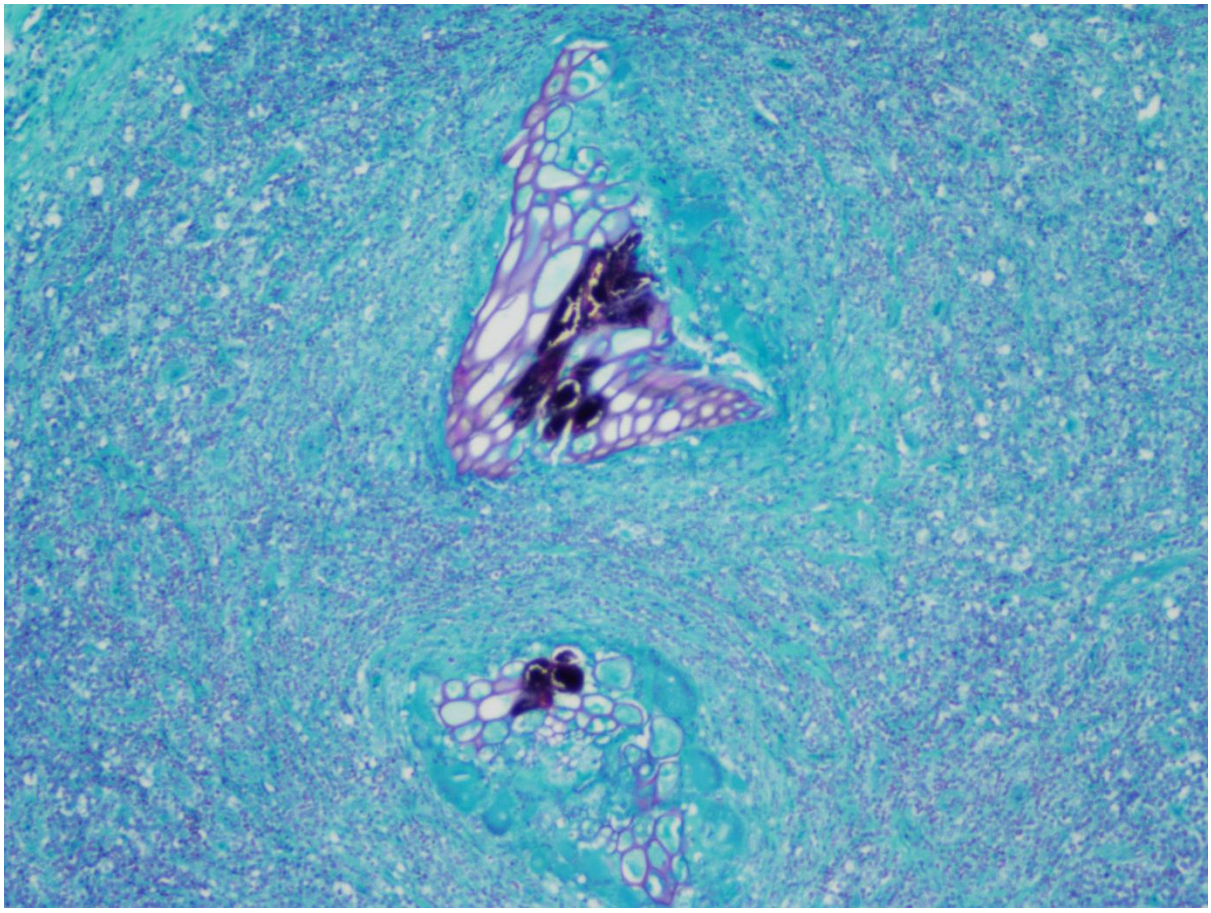

Figure 14. Gram Twort stained section from a cross bred lamb collected on 12 December 2014 showing a grass seed with associated Gram positive bacteria. Samples from this joint were positive for *Staphyloccocus epidermidis* and *Chlamydia pecorum* (magnification x100)

Mycoplasma ovipneumoniae

Synovial tissues from the 148 joints collected from November 2014 to March 2015 were tested for *Myoplasma ovipneumoniae* using PCR. Most samples were negative; however, 42 samples had possible amplification right at the end of the run, with their corresponding melt curve analysis also suggesting a suspected positive.

To confirm if these samples were positive or not, the suspect samples were run again on a large-scale endpoint PCR and examined on an electrophoresis gel. Visualisation of the gel under UV light revealed that all suspect samples were negative.

4.3.3 Immunohistochemistry

Chlamydia pecorum

All of the synovial tissue samples collected in November 2014 – January 2015 and in March 2015 that were positive for *C. pecorum* on PCR were also positive on immunohistochemistry (**Figure 15**.) (Synovial samples that were negative on PCR were not tested by immunohistochemistry.)

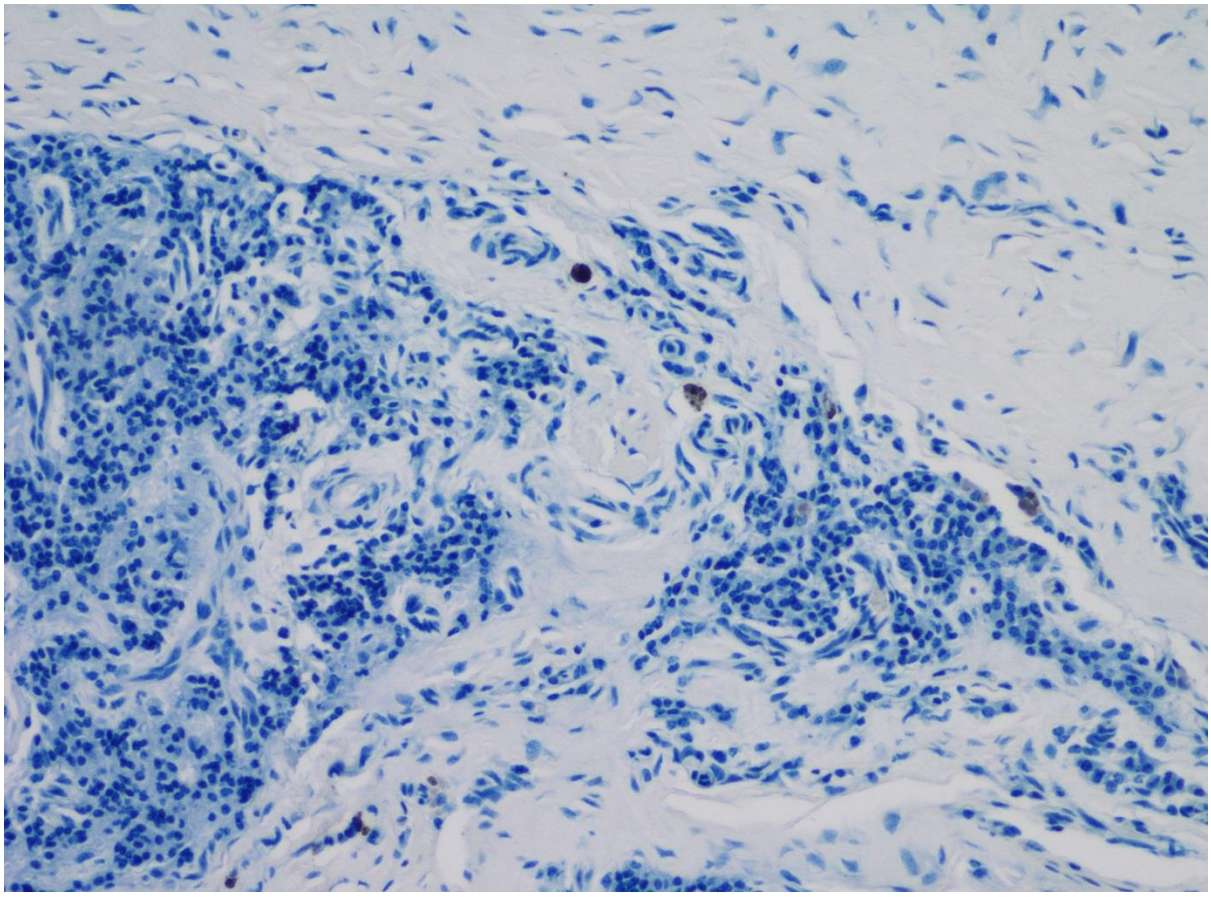

Figure 15. Synovial tissue collected from a Merino lamb carcase on 12 March 2015 showing positive staining for *Chlamydia pecorum* on immunohistochemistry (magnification x200). Synovial tissue was also positive for *C. pecorum* on PCR (3.2×10^4 bacteria per gram of tissue)

5 Discussion

This project has identified an association between tail length and bacterial arthritis/polyarthritis in lambs, with shorter tails (one or two coccygeal vertebrae) being a higher risk factor for arthritis/polyarthritis than longer tails (three or more coccygeal vertebrae). The project has also re-confirmed *Erysipelothrix rhusiopathiae* as the most common cause of bacterial joint infections in Australian lambs. In lambs, *E. rhusiopathiae* usually causes a fibrinopurulent arthritis and osteomyelitis after docking or castration (Thompson, 2007). Secondary joint infection after umbilical infection is less common (Thompson, 2007). Currently many Australian prime lambs are docked short. Therefore, the results of this project suggest that docking prime lambs longer, with a minimum of three palpable coccygeal vertebrae remaining in the tail stump, may help to reduce the prevalence of bacterial joint infections.

Although the project has identified an association between tail length and bacterial arthritis/polyarthritis in lambs, it has not established direct causation *per se*, i.e. that in flocks with an arthritis problem due to *E. rhusiopathiae* and which currently dock tails short, that changing docking practices to leave a longer tail stump will directly prevent joint infections. Further research will be required to establish direct causation. Australian Dorset sheep reportedly have an increased rate of arthritis, which has been attributed to high growth rates putting stress on the joints or due to a concentration of lambs around grain feeders (Farquharson, 2007). However, many Dorset sheep in Australia are docked very short. Dorset breeders may be an appropriate group to work with to establish direct causation between tail length and arthritis/polyarthritis in lambs.

In this study a higher prevalence of bacterial arthritis/polyarthritis was also observed in Merino lambs than in Crossbred or Dorper lambs. Shearing has previously been identified as a risk factor for arthritis/polyarthritis in Australian lambs (Paton et al., 2003) and during March 2015 numerous lines of shorn Merinos were processed by the abattoir. The tails of some of the Merino lambs had been stripped and it is possible that these lambs had been mulesed, which has also been identified as a risk factor for arthritis/polyarthritis (Paton et al., 2003).

Analysis of data from the South Australian Enhanced Abattoir Surveillance Program revealed a higher rate of arthritis/polyarthritis in sheep more than two years of age presented for slaughter than in sheep less than two years of age. A higher rate of arthritis in older Australian sheep at slaughter has been identified previously (Farquharson, 2007). In older sheep there were also significant correlations between arthritis/polyarthritis and a number of other disease conditions, including grass seed infestation, lungworm and pleurisy/pneumonia. The majority of older sheep sent for slaughter have either reached the end of their productive lives or are being culled because of disease. Therefore, the presence of more than one disease condition in these animals makes intuitive sense.

In older sheep infection with *E. rhusiopathiae* usually occurs after dipping associated with contaminated, non-bactericidal dips (Thompson, 2007). The fetlocks (metacarpophalangeal and metatarsophalangeal joints) are most commonly affected (Thompson, 2007). It may be timely for MLA to remind sheep producers of the importance of dip hygiene and of observing the post-shearing interval prior to dipping on product labels. The Northern Pastoral region of South Australia was a region with a consistently high rate of arthritis in older sheep over

time. Traditionally this region has run Merino sheep. As mentioned above, shearing has previously been identified as a risk factor for arthritis/polyarthritis in Australian sheep (Paton et al., 2003). There may be a place for appropriate prophylactic antibiotic treatment of sheep with moderate to severe shearing cuts and this could be another area for future MLA-funded research.

The cell wall of *E. rhusiopathiae* is relatively resistant to breakdown by mammalian lysosomal enzymes and subsequent removal by macrophages. As a result, persistent inflammation can develop, even when infection appears to have resolved or bacteria cannot be isolated (Thompson, 2007). It is possible that some of the cases of arthritis seen in adult sheep are chronic sequelae to joint infections at an early age. With time, a move to longer tail docking may also help to reduce the prevalence of arthritis in older sheep.

Analysis of the South Australian Enhanced Abattoir Surveillance Program also revealed a correlation between arthritis/polyarthritis and pleurisy in sheep both less than and more than two years of age. Approximately 50 per cent of 227 consignments of lambs examined for pleurisy during this study had at least one carcase affected by the condition. This suggests that pneumonia is a much larger problem in sheep in southern Australia than previously recognised.

Pasteurella spp., a common cause of secondary bacterial infection in ovine pneumonia, was isolated from two joint samples during this study. *Pasteurella multocida* has previously been isolated from both pneumonia lung tissues and joints of lambs that presented for locomotor signs and inappetance (Petridou et al., 2011). *Mycoplasma hyopneumoniae* causes both pneumonia and polyarthritis in pigs and *Mycoplasma bovis* can localise in the joints of cattle after primary infection of the lungs (Thompson, 2007). In this study, a PCR for *Mycoplasma ovipneumoniae* failed to detect this bacterium in any of the grossly abnormal, trimmed joint samples.

Trimming for pleurisy is estimated to result in a $6 penalty per carcase to producers and an $8-10 loss per kilo in high value cuts (i.e. ‘frenched’ racks) to the processing sector. Pneumonia is likely to also be contributing to trimming for arthritis/polyarthritis in both young and older sheep. This project has identified a number of properties with endemic ovine pneumonia. There has not been a comprehensive survey of risk or protective factors for ovine pneumonia in Australia. In addition, the contribution of the various respiratory pathogens has not been investigated. Further research in these areas is recommended.

A correlation between arthritis/polyarthritis and grass seed infestation in sheep less than and more than two years of age was also identified through analysis of the South Australian Enhanced Abattoir Surveillance Program data. Vegetable foreign bodies, most likely grass seed remnants, were observed on histopathology in two of the joint samples collected during this project. Bacteria that form the normal bacterial flora of ovine skin (*Corynebacterium* spp., *Micrococcus* spp., coagulase-positive *Staphylococcus* spp., non-haemolytic coagulase-negative *Staphylococcus* spp. and *Streptococcus* spp. including *S. epidermidis*; (Haarstad et al., 2014)) were isolated from several joint samples. Many of the lines of sheep observed in the abattoir had extensive grass seed infestation of the legs and ventral abdomen, with what appeared to be associated mini-abscess formation. It is possible that grass seeds contribute to arthritis/polyarthritis through bacteraemia from grass seed-associated infections at a site distant from the affected joint, as well as direct migration into joints. It is recommended that

MLA consider updating extension materials on grass seeds to include mention of the correlation between grass seed infestation and arthritis/polyarthritis.

Chlamydia pecorum was detected in 6.8 per cent of the joint samples collected during this study. Recently, a longitudinal survey revealed faecal shedding of *C. pecorum* to be relatively common in Australian lambs (30 per cent prevalence). Enteric isolates of *C. pecorum* are not thought to be normally invasive in sheep (Philips and Clarkson, 1998). In addition, many chlamydiae, including *C. pecorum*, are thought to exist in an asymptomatic state within the host (Everett, 2000). These factors could explain the lower prevalence in trimmed, grossly abnormal joints from lambs than detected by screening faecal samples.

One *C. pecorum*-positive joint was normal and bacteria associated with the normal flora of ovine skin were cultured from another two joints. Identification of *C. pecorum* from these joints may have been an incidental finding. Alternatively infestation with grass seeds and subsequent joint infections could have predisposed to systemic invasion of *C. pecorum* (Walker et al., 2015), or systemic infection with *C. pecorum* could have worsened the lesion(s) caused by the invading grass seed(s).

Ten per cent of the joint samples collected during this study had marked to severe changes on histopathology, yet were negative on bacterial culture and in the PCRs for *C. pecorum* and *M. ovipneumoniae*. False negatives on bacterial culture of septic joints are common (Thompson, 2007). Immunohistochemistry for *E. rhusiopathiae* was attempted but found to have significant technical hurdles which were beyond the scope of this project to resolve. Several PCR methods for detecting *Erysipelothrix* spp., including *E. rhusiopathiae*, have been published (Bender et al., 2010; Makino et al., 1994; Pal et al., 2010; Shen et al., 2010; Takeshi et al., 1999; To et al., 2009; Wang et al., 2002; Yamazaki et al., 2013). The frozen synovial tissues collected during this project, as well as the paraffin-embedded formalin fixed tissue, are available and PCR may be a better method of investigating the involvement of *E. rhusiopathiae* or other bacteria in the culture-negative samples.

5.1 Success in meeting project objectives

The project objectives were met.

Objective 1: Analyse data from the South Australian Enhanced Abattoir Surveillance Program database to determine the historical occurrence of arthritis in sheep less than and more than two years of age, including potential correlations with sex, age, region and property identifier. In addition the potential correlation between grass seed infestation and arthritis and between pneumonia/pleurisy and arthritis was investigated.

Three years of data from the South Australian Enhanced Abattoir Surveillance Program database were analysed to determine the historical occurrence of arthritis in sheep less than and more than two years of age, including potential correlations with age, region and property identifier. A potential correlation between arthritis and gender could not be investigated because the majority of consignments of sheep less than two years of age were mixed sex and the majority of the consignments of sheep more than two years of age were female, with much fewer numbers of males and male castrate sheep.

The analysis provided strong evidence of an age effect, with the prevalence of arthritis almost three times greater in sheep more than two years of age than sheep less than two years of age.

There were also regional effects. The Barossa/ Lower North region had the highest percentage of younger sheep with arthritis, followed by the Murray Mallee and Eyre regions. In older sheep, the Barossa/Lower North and Upper South were equally high. Adelaide Hills/Fleurieu region had the lowest arthritis rate in both age groups.

The majority of the producers sold sheep free of arthritis or with less than five per cent of the line estimated to be affected (51.7 per cent), whereas 10.4 per cent of the producers sold at least one consignment containing animals with arthritis in both age groups and 37.9 per cent of producers sold consignments of sheep with arthritis in either age group. At the level of the producer, there was a strong association between the presence/absence of arthritis in the two age groups of sheep.

The analysis also revealed a strong correlation between arthritis and pleurisy/pneumonia and a weaker but still significant correlation between arthritis and grass seeds in sheep less than two years of age.

Objective 2: Conduct a survey of tail length, arthritis and pleurisy in lambs by collecting slaughter data at one abattoir in South Australia (Thomas Foods International Murray Bridge abattoir).

The survey of tail length, arthritis and pleurisy was successfully completed and revealed that arthritis/polyarthritis and pneumonia/pleurisy may be more common in lambs in southern Australia than suggested by abattoir surveillance data, with approximately 50 per cent of 354 consignments of lambs having at least one carcase affected by arthritis and 50 per cent of 227 consignments of lambs examined having at least one carcase affected by pleurisy.

Tail length had a significant effect on the prevalence of arthritis in the lamb carcases, with short tails (one to two coccygeal vertebrae) being a higher risk practice than long tails (three or more coccygeal vertebrae).

Objective 3: Test arthritic joint samples by culture, PCR and immunohistochemistry for the presence of bacteria and *Mycoplasma ovipneumoniae*.

One hundred and forty-eight joint samples were tested by culture, PCR and immunohistochemistry for the presence of bacteria and *Mycoplasma ovipneumoniae.*

The most common bacterium isolated from arthritic joints was *Erysipelothrix rhusiopathiae (*19 joint samples). *Chlamydia pecorum* was detected in 10 joint samples (PCR and immunohistochemistry) and alpha-haemolytic *Streptococcus* spp. in two joint samples. These results are consistent with previously published information on the pathogens known to cause bacterial arthritis/polyarthritis in lambs.

Mycoplasma ovipneumoniae was not detected in any joint samples.

Several joint samples were culture- and PCR-negative, but had marked to severe changes on histopathology. Investigations are continuing in an effort to uncover the possible causative agents in these cases.

Objective 4: Make recommendations to extend the results of the project nationally.

Recommendations to extend the results for the project nationally include:

i) Submitting the project results for publication in peer-reviewed scientific journals.
ii) Presenting the project results at the Australian Sheep Veterinarians annual conference in Dubbo in September 2016.
iii) Presenting the project results at the MINTRAC Quality Assurance and Meat Inspection annual conference on the Gold Coast in October 2016.
iv) Updating MLA extension material on arthritis/polyarthritis and on lamb marking to include the association between short tail length and bacterial arthritis/ polyarthritis in lambs.
v) Funding further research to establish causation between tail length and bacterial arthritis/polyarthritis in lambs. Dorset breeders or other prime lamb producers who dock short and have a problem with arthritis would be appropriate to partner with for this research. Some of the lambs could be docked as normal for that producer (i.e. short) and some docked with at least three joints remaining in the tail stump and the prevalence of arthritis in the two groups compared.
vi) Updating MLA extension material on grass seeds to include the correlation with arthritis/polyarthritis in sheep both less than and more than two years of age.
vii) Updating MLA extension material to include the strong correlation between arthritis/polyarthritis and pleurisy/pneumonia in sheep less than and more than two years of age.
viii) Funding further research on pleurisy/pneumonia in sheep in southern Australia, including a survey to determine risk and protective factors for pneumonia and on-farm/abattoir research to uncover the primary causative agent(s) of pneumonia in Australian sheep.

7 Conclusions/Recommendations

In conclusion, this project has identified an association between tail length and bacterial arthritis/polyarthritis in lambs, with shorter tails (one or two coccygeal vertebrae) being a higher risk factor for arthritis/polyarthritis than longer tails (three or more coccygeal vertebrae), and has re-confirmed *Erysipelothrix rhusiopathiae* as the most common cause of bacterial joint infections in Australian lambs.

The project has also revealed correlations between arthritis/polyarthritis and pleurisy/pneumonia and between arthritis/polyarthritis and grass seed infestation in sheep both less than and more than two years of age.

The prevalence of *Chlamydia pecorum* in grossly abnormal joints trimmed from lamb carcases appears to be approximately four to five times less than the prevalence of faecal shedding of the organism by lambs pre-weaning, post-weaning and at slaughter.

The findings of this study also suggest that pneumonia/pleurisy is much larger problem in sheep in southern Australia than previously recognised.

Recommendations for further research include:

i) Research to establish causation between tail length and bacterial arthritis/polyarthritis in lambs. Dorset breeders or other prime lamb producers who dock short and have a problem with arthritis would be appropriate to partner with for this research. Some of the lambs could be docked as normal for that producer (i.e. short) and some docked with at least three joints remaining in the tail stump and the prevalence of arthritis in the two groups compared.
ii) A comprehensive survey of risk or protective factors for ovine pneumonia in Australia.
iii) Research to establish the contribution of the known primary and secondary pathogens of ovine pneumonia in sheep in southern Australia, with the aim of identifying appropriate control methods.
iv) Further research on the joint samples collected during this study, for example PCR for *Erysipelothrix rhusiopathiae* and other *Mycoplasma* spp.

9 Key Messages

- Arthritis/polyarthritis may be more common in lambs in southern Australia than suggested by abattoir surveillance data, with approximately 50 per cent of 354 consignments of lambs examined at one abattoir in South Australia having at least one carcase affected by the condition. On average, arthritis/polyarthritis affected 2.0 per cent of the carcases within affected lines, less than the five per cent threshold used in the South Australian Enhanced Abattoir Surveillance Program.
- The most common bacterium isolated from arthritic joints was *Erysipelothrix rhusiopathiae.*
- *E. rhusiopathiae* is the most common cause of bacterial polyarthritis in lambs globally. Infection with *E. rhusiopathiae* usually develops first at a site on the skin, most commonly infected docking and castration wounds and less commonly an infected umbilicus. Infection can be limited to the skin or spread via the blood to the joints.
- Previous research indicates that the tails of lambs docked short take longer to heal and are more likely to become infected.
- The hypothesis of this research project was that short docking leads to infected tailing wounds that take longer to heal, with subsequent spread of bacteria through the blood to the joints, resulting in arthritis.
- Tail length had a significant effect on the prevalence of arthritis in the lamb carcases, with short tails (one to two coccygeal vertebrae) being a higher risk practice than long tails (three or more coccygeal vertebrae).
- *Chlamydia pecorum*, either on its own or in association with bacteria normally found on the skin of sheep, was detected in 6.8 per cent of the joint samples collected during this study. The prevalence of *Chlamydophila pecorum* in grossly abnormal joints trimmed from lamb carcases appears to be approximately four to five times less than the prevalence of faecal shedding of the organism by lambs pre-weaning, post-weaning and at slaughter.
- The project also demonstrated associations between arthritis and pleurisy/pneumonia and between arthritis and grass seeds in sheep both less than and more than two years of age.
- Approximately 50 per cent of 227 consignments of lambs examined had at least one carcase affected by pleurisy (the chronic sequel to pneumonia). On average, pleurisy affected 2.2 per cent of carcases within affected lines. Trimming for pleurisy resulted in rib removal in two-thirds of 101 carcases examined, with an average trim weight of 1.0 kg. Trimming for pleurisy is estimated to result in a $6 penalty per carcase to producers and an $8-10 loss per kilo in high value cuts (i.e. ‘frenched’ racks) to the processing sector.

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12 Appendix 1

12.1 Tail length data capture sheet

Lot No.

	0	1	2	3	4	5+	Arthritis	Pleurisy/ pneumonia
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								

12.2 Arthritis data capture sheet

Arthritis	# Joints trimmed	Joints				Trim weight	Trimmed or condemned	Culture	Lot No.	Tail length
		Elbow	Carpus	Stifle	Tarsus					
1										
2										
3										
4										
5										
6										
7										
8										

12.3 Pleurisy data capture sheet

Pleurisy	Trim					Trim weight	Trimmed or condemned	Culture	Lot No.
	Pleura only	¼ ribcage	½ ribcage	¾ ribcage	Full ribcage				
1									
2									
3									
4									
5									
6									
7									
8									

13 Appendix 2

Pathology results table

Date	Lab number	Culture	Chlamydia (bacteria/gram tissue)	Histopathology
27/11/2014	142492698	No growth	4.1×10^5	Mild perivascular plasmacytic synovitis
27/11/2014	142492717	No growth	Negative	Minimal synovial proliferation Mild interstitial mucinous change in peri-synovial ligaments
2/12/2014	142498011	No growth	Negative	Mild fibrinous and neutrophilic synovitis
2/12/2014	142498021	No growth	Negative	Moderate plasmacytic synovitis Moderate to marked fibrinous and neutrophilic synovitis with mild multifocal oedema Mild sub-synovial fibroplasia
2/12/2014	142498020	No growth	Negative	Moderate to marked fibrinous and neutrophilic synovitis Moderate lymphoplasmacytic synovitis Mild synovial hyperplasia
2/12/2014	142498022	*Corynebacterium* species	Negative	Mild synovial hyperplasia
2/12/2014	142498023	*Erysipelothrix rhusiopathiae*	Negative	Marked fibrinous and neutrophilic synovitis with oedema Moderate plasmacytic synovitis Mild synovial hyperplasia
3/12/2014	142498101	No growth	Negative	Mild synovial proliferation
3/12/2014	142498078	*Aeromonas* species	Negative	Marked suppurative and fibrinous synovitis with mild neutrophilic synovitis Mild synovial proliferation
4/12/2014	142498164	No growth	Negative	Moderate lymphoplasmacytic synovitis Moderate synovial proliferation Peri-synovial lymphocytic infiltration and a peri-synovial vegetable foreign body
4/12/2014	142498191	No growth	Negative	Normal
4/12/2014	142498192	No growth	Negative	Mild peri-synovial oedema and fibroplasia (synovial tissue not sampled)
4/12/2014	142498193	No growth	Negative	Marked acute severe suppurative and fibrinous synovitis, with mild fibroplasia
4/12/2014	142498195	No growth	Negative	Marked acute to subacute suppurative and fibrinous synovitis Mild synovial proliferation
4/12/2014	142498194	No growth	Negative	Moderate to marked neutrophilic synovitis with marked lymphoplasmacytic peri-synovitis Mild synovial proliferation
5/12/2014	142498273	*Acinetobacter lwoffi*	Negative	Moderate fibrinous suppurative synovitis; Moderate lymphoplasmacytic synovitis Moderate synovial proliferation
5/12/2014	142498274	*Acinetobacter* species	Negative	Moderate lymphoplasmacytic synovitis
5/12/2014	142498275	No growth	Negative	Normal

Date	Lab number	Culture	Chlamydia (bacteria/gram tissue)	Histopathology
5/12/2014	142498276	No growth	Negative	Mild to moderate neutrophilic synovitis Moderate lymphoplasmacytic synovitis Mild synovial proliferation
5/12/2014	142498277	No growth	Negative	Minimal to mild perivascular plasmacytic synovitis Minimal synovial proliferation
5/12/2014	142498278	No growth	1.4×10^4	Minimal synovial proliferation
5/12/2014	142498279	*Erysipelothrix rhusiopathiae*	Negative	Mild to moderate neutrophilic synovitis Moderate lymphoplasmacytic synovitis Moderate synovial hyperplasia
5/12/2014	142498280	Heavy growth of mixed skin and environmental flora	Negative	Mild peri-synovial oedema (synovial tissue not sampled)
9/12/2014	142498484	*Streptococcus* species	Negative	Severe marked suppurative fibrinous and ulcerative synovitis Mild to moderate synovial proliferation
9/12/2014	142498484	*Bacillus* species and *Streptococcus* species	Negative	Severe marked suppurative fibrinous and ulcerative synovitis Mild to moderate synovial proliferation
9/12/2014	142498483	*Serratia* species	Negative	Marked suppurative synovitis with mild synovial proliferation
9/12/2014	142498485	*Erysipelothrix rhusiopathiae*	Negative	Marked lymphoplasmacytic and suppurative synovitis Mild synovial proliferation
9/12/2014	142498485	No growth	Negative	Marked lymphoplasmacytic and suppurative synovitis Mild synovial proliferation
10/12/2014	142493057	*Escherichia coli, Klebsiella* species and other coliforms	Negative	Moderate plasmacytic synovitis Mild synovial hyperplasia; Mild fibroplasia
10/12/2014	142493058	No growth	Negative	Moderate lymphoplasmacytic synovitis Marked neutrophilic synovitis; Mild synovial hyperplasia
10/12/2014	142493059	Coliform, not identified further	Negative	Moderate lymphocytic plasmacytic synovitis Marked fibrinous and neutrophilic synovitis Mild synovial hyperplasia
10/12/2014	142493060	*Aeromonas* species; joint partially open	Negative	Mild plasmacytic synovitis and mild neutrophilic synovitis Mild to moderate synovial proliferation with mild fibroplasia
10/12/2014	142493061	*Erysipelothrix rhusiopathiae*	Negative	Moderate plasmacytic synovitis Moderate fibrinous and suppurative synovitis Mild synovial hyperplasia
10/12/2014	142493062	Coliform, not identified further; joint partially open	Negative	Moderate neutrophilic synovitis Marked lymphoplasmacytic synovitis Mild to moderate focal neutrophilic choroiditis Mild synovial hyperplasia
10/12/2014	142493109	*Staphylococcus* species	Negative	Mild lymphoplasmacytic synovitis with mild oedema
10/12/2014	142493063	*Erysipelothrix rhusiopathiae*	Negative	Mild lymphoplasmacytic synovitis Mild to moderate fibrinous and suppurative synovitis Mild to moderate synovial oedema

Date	Lab number	Culture	Chlamydia (bacteria/gram tissue)	Histopathology
10/12/2014	14243064	No growth	3.0×10^4	Moderate lymphoplasmacytic synovitis Mild neutrophilic synovitis Mild synovial hyperplasia
10/12/2014	142493066	Alpha haemolytic *Streptococcus* species	Negative	Moderate lymphoplasmacytic synovitis Mild to moderate neutrophilic synovitis Mild to moderate synovial proliferation
10/12/2014	142493067	*Erysipelothrix rhusiopathiae*	Negative	Moderate lymphoplasmacytic and neutrophilic synovitis Mild synovial hyperplasia
10/12/2014	142493068	*Erysipelothrix rhusiopathiae*	Negative	Moderate lymphoplasmacytic synovitis Moderate to marked fibrinous and neutrophilic synovitis Mild synovial hyperplasia
10/12/2014	142493123	*Erysipelothrix rhusiopathiae*	Negative	Within normal limits
10/12/2014	142493065	Alpha haemolytic *Streptococcus* species	Negative	Moderate lymphoplasmacytic synovitis Moderate fibrinous and neutrophilic synovitis Mild synovial hyperplasia
10/12/2014	142493069	*Erysipelothrix rhusiopathiae* and a *Staphylococcus* species	Negative	Moderate to marked lymphoplasmacytic synovitis Mild to moderate neutrophilic synovitis Mild synovial hyperplasia
10/12/2014	142493129	*Bacillus* species	Negative	Moderate lymphocytic plasmacytic synovitis Moderate fibrinous and neutrophilic synovitis Mild synovial hyperplasia
10/12/2014	142493070	No growth	Negative	Mild to moderate lymphoplasmacytic synovitis Moderate to marked fibrinous synovitis
11/12/2014	142493165	No growth	Negative	Moderate lymphoplasmacytic synovitis Moderate neutrophilic and fibrinous synovitis Moderate synovial hyperplasia
11/12/2014	142493166	No growth	Negative	Mild to moderate plasmacytic synovitis
12/12/2014	142493271	*Staphylococcus epidermidis*	2.2×10^4	Moderate lymphoplasmacytic synovitis Moderate fibrinous and neutrophilic synovitis with oedema Focal granulomatous synovitis associated with intra-lesion foreign body
12/12/2014	142493272	No growth	Negative	Mild lymphoplasmacytic synovitis Moderate multifocal fibrinous and suppurative synovitis Mild synovial hyperplasia
12/12/2014	142493273	*Erysipelothrix rhusiopathiae* and a coagulase negative *Staphylococcus* species	Negative	Moderate to marked fibrinous and suppurative synovitis Moderate lymphoplasmacytic synovitis Mild to moderate synovial hyperplasia
12/12/2014	142493274	*Erysipelothrix rhusiopathiae*	Negative	Moderate to marked lymphoplasmacytic, neutrophilic and fibrinous synovitis
12/12/2014	142493275	No growth	Negative	Mild to moderate multifocal lymphoplasmacytic synovitis
12/12/2014	142493276	No growth	Negative	Marked suppurative and fibrinous synovitis Mild lymphoplasmacytic synovitis Mild synovial hyperplasia

Date	Lab number	Culture	Chlamydia (bacteria/gram tissue)	Histopathology
12/12/2014	142493277	No growth	Negative	Mild lymphocytic synovitis Minimal to mild synovial hyperplasia
12/12/2014	142493278	No growth	Negative	Mild neutrophilic synovitis Moderate lymphoplasmacytic synovitis
15/12/2014	142493381	*Erysipelothrix rhusiopathiae*	Negative	Minimal lymphoplasmacytic synovitis Severe, diffuse, per acute, marked, fibrinous neutrophilic synovitis
15/12/2014	142493382	No growth	Negative	Moderate lymphoplasmacytic synovitis Moderate fibrinous and neutrophilic synovitis Mild segmental synovial hyperplasia
15/12/2014	142493383	No growth	Negative	Minimal lymphoplasmacytic synovitis Mild fibrinous synovitis Mild synovial hyperplasia
15/12/2014	142493384	*Erysipelothrix rhusiopathiae*	Negative	Moderate lymphoplasmacytic synovitis Moderate to marked fibrinous and neutrophilic synovitis Mild to moderate synovial hyperplasia
16/12/2014	142493468	No growth	Negative	Minimal plasmacytic synovitis Minimal segmental synovial hyperplasia;
16/12/2014	142493469	No growth	Negative	Multifocal ulcerative and fibro plastic fibrinous synovitis Minimal lymphoplasmacytic synovitis Mild synovial hyperplasia
16/12/2014	142493470	No growth	Negative	Within normal limits
18/12/2014	142495177	No growth	Negative	Minimal synovial hyperplasia
18/12/2014	142495178	No growth	Negative	Minimal lymphocytic synovitis
6/01/2015	155470207	No growth	Negative	Mild lymphoplasmacytic synovitis Minimal neutrophilic synovitis Moderate synovial hyperplasia
7/01/2015	155470285	No growth	Negative	Minimal lymphoplasmacytic synovitis Minimal synovial hyperplasia
7/01/2015	155470286	No growth	Negative	Mild to moderate lymphoplasmacytic synovitis with oedema Mild to moderate synovial hyperplasia
8/01/2015	155470362	No growth	Negative	Minimal to mild lymphoplasmacytic synovitis
8/01/2015	155470363	*Erysipelothrix rhusiopathiae*	Negative	Moderate to marked lymphoplasmacytic synovitis with follicle formation Moderate fibrinous and neutrophilic synovitis with multifocal oedema
8/01/2015	155470365	No growth	Negative	Minimal lymphoplasmacytic synovitis
8/01/2015	155470364	No growth	1.8×10^4	Within normal limits
9/01/2015	155470408	No growth	Negative	Within normal limits

Date	Lab number	Culture	Chlamydia (bacteria/gram tissue)	Histopathology
9/01/2015	155470409	No growth	Negative	Minimal lymphocytic synovitis Deep sub-synovial fibroplasia and mild lymphoplasmacytic peri-synovitis
12/01/2015	155473538	Non-haemolytic *Streptococcus* species	Negative	Severe acute fibrinous necrotising suppurative synovitis Minimal lymphoplasmacytic infiltration Minimal synovial proliferation
12/01/2015	155473539	No growth	Negative	Moderate suppurative synovitis Moderate lymphoplasmacytic synovitis Mild synovial hyperplasia
12/01/2015	155473540	Micrococcus species	Negative	Minimal synovial hyperplasia
14/01/2015	155473683	*Micrococcus* species and coagulase negative *Staphylococcus* species	1.0×10^5	Moderate plasmacytic synovitis Mild neutrophilic and fibrinous synovitis
14/01/2015	155473684	No growth	Negative	Moderate lymphoplasmacytic synovitis Mild neutrophilic synovitis Minimal synovial proliferation
14/01/2015	155473685	No growth	Negative	Mild to moderate neutrophilic synovitis with oedema Marked lymphoplasmacytic synovitis Mild fibroplasia
15/01/2015	155473779	No growth	Negative	Moderate lymphoplasmacytic synovitis Moderate fibrinous and suppurative synovitis Mild to moderate synovial hyperplasia
15/01/2015	155473780	No growth	Negative	Within normal limits
15/01/2015	155473781	No growth	Negative	Mild multifocal lymphoplasmacytic synovitis Mild synovial hyperplasia
15/01/2015	155473782	No growth	6×10^3	Moderate suppurative and fibrinous synovitis Mild to moderate lymphoplasmacytic synovitis Mild sub-synovial fibroplasia
15/01/2015	155473783	No growth	Negative	Moderate to marked suppurative and fibrinous synovitis Mild lymphoplasmacytic synovitis
15/01/2015	155473784	No growth	Negative	Moderate fibrinous and suppurative synovitis Minimal lymphocytic synovitis
15/01/2015	155473785	No growth	Negative	Severe suppurative and fibrinous synovitis Mild lymphocytic synovitis Mild synovial hyperplasia
15/01/2015	155473786	Coagulase negative *Staphylococcus* species	Negative	Within normal limits
15/01/2015	155473787	No growth	Negative	Mild to moderate synovial hyperplasia
19/01/2015	155473989	No growth	Negative	Moderate to marked fibrinous and evasive neutrophilic synovitis Mild to moderate lymphoplasmacytic synovitis Mild fibroplasia
21/01/2015	155470651	No growth	Negative	Mild multifocal lymphoplasmacytic synovitis Mild synovial hyperplasia

Date	Lab number	Culture	Chlamydia (bacteria/gram tissue)	Histopathology
21/01/2015	155470652	No growth	Negative	Moderate multifocal lymphoplasmacytic synovitis Moderate to marked, multifocal, fibrinous and erosive synovitis
21/01/2015	155470653	No growth	Negative	Mild lymphoplasmacytic synovitis
21/01/2015	155470654	No growth	Negative	Severe multifocal fibrinous, necrotising and suppurative synovitis Mild to moderate lymphoplasmacytic synovitis
22/01/2015	155470712	No growth	Negative	Mild to moderate lymphoplasmacytic synovitis; Mild synovial proliferation
22/01/2015	155470713	*Pasteurella* species	Negative	Within normal limits
22/01/2015	155470714	*Pasteurella* species and mixed skin flora	Negative	Moderate multifocal lymphoplasmacytic synovitis Minimal to mild neutrophilic synovitis Mild synovial proliferation
22/01/2015	155470715	*Erysipelothrix rhusiopathiae*	Negative	Moderate lymphoplasmacytic synovitis Marked suppurative synovitis
10/03/2015	155420389	No growth	Negative	Mild synovial proliferation
10/03/2015	155420390	No growth	Negative	Mild synovial proliferation Mild synovial oedema Mild sub-synovial fibroplasia
10/03/2015	155420391	No growth	8.3×10^4	Severe diffuse suppurative synovitis Mild synovial proliferation;
10/03/2015	155420392	*Erysipelothrix rhusiopathiae*	Negative	Moderate to marked suppurative and fibrinous synovitis Mild lymphoplasmacytic synovitis
10/03/2015	155420393	No growth	Negative	Moderate fibrinous and suppurative synovitis Moderate lymphoplasmacytic synovitis Mild synovial proliferation
10/03/2015	155420395	Isolate lost before it could be identified	7.6×10^5	Moderate suppurative synovitis; Moderate to marked lymphoplasmacytic synovitis
10/03/2015	155420396	*Erysipelothrix rhusiopathiae*	Negative	Moderate to marked fibrinous and suppurative synovitis Moderate lymphoplasmacytic synovitis Mild synovial proliferation
10/03/2015	155420397	No growth	Negative	Mild lymphoplasmacytic synovitis with oedema Mild synovial proliferation
10/03/2015	155420398	*Erysipelothrix rhusiopathiae*	Negative	Mild suppurative and lymphoplasmacytic synovitis
11/03/2015	155420452	No growth	Negative	Within normal limits
11/03/2015	155420453	No growth	Negative	Minimal synovial proliferation
11/03/2015	155420454	No growth	Negative	Moderate suppurative synovitis Moderate lymphoplasmacytic synovitis Mild fibroplasia Mild synovial proliferation
12/03/2015	155420561	No growth	Negative	Within normal limits

Date	Lab number	Culture	Chlamydia (bacteria/gram tissue)	Histopathology
12/03/2015	155420562	No growth	Negative	Marked fibrinous and suppurative synovitis Moderate to marked lymphoplasmacytic synovitis Mild synovial proliferation
12/03/2015	155420563	No growth	Negative	Within normal limits
12/03/2015	155420564	No growth	Negative	Mild neutrophilic synovitis Mild to moderate lymphoplasmacytic synovitis Mild synovial proliferation
12/03/2015	155420565	No growth	Negative	Within normal limits
12/03/2015	155420566	No growth	Negative	Minimal plasmacytic synovitis
12/03/2015	155420567	No growth	Negative	Within normal limits
12/03/2015	155420568	No growth	Negative	Marked fibrinous and suppurative synovitis Moderate lymphoplasmacytic synovitis Mild synovial proliferation
12/03/2015	155420569	No growth	Negative	Marked fibrinous and suppurative synovitis; Moderate lymphoplasmacytic synovitis; Mild fibroplasia
12/03/2015	155420570	No growth	Negative	Within normal limits
12/03/2015	155420571	No growth	Negative	Marked suppurative and fibrinous synovitis Moderate to marked lymphoplasmacytic synovitis Mild synovial proliferation
12/03/2015	155420572	No growth	Negative	Within normal limits
12/03/2015	155420573	No growth	Negative	Within normal limits
12/03/2015	155420574	No growth	Negative	Marked fibrinous and suppurative synovitis Mild lymphoplasmacytic synovitis Mild fibroplasia
12/03/2015	155420575	No growth	Negative	Within normal limits
12/03/2015	155420576	No growth	Negative	Within normal limits
12/03/2015	155420577	No growth	3.2×10^4	Marked suppurative and fibrinous synovitis Moderate to marked lymphoplasmacytic synovitis Mild to moderate fibroplasia
12/03/2015	155420578	No growth	Negative	Moderate suppurative and fibrinous synovitis Moderate lymphoplasmacytic synovitis; Mild fibroplasia
13/03/2015	155420607	No growth	Negative	Within normal limits
13/03/2015	155420608	No growth	Negative	Marked fibrinous and suppurative synovitis Moderate lymphoplasmacytic synovitis Mild fibroplasia Mild synovial proliferation

Date	Lab number	Culture	Chlamydia (bacteria/gram tissue)	Histopathology
13/03/2015	155420609	No growth	Negative	Moderate to marked fibrinous and suppurative synovitis Moderate to marked lymphoplasmacytic synovitis Mild fibroplasia Mild synovial proliferation
13/03/2015	155420610	No growth	Negative	Within normal limits
23/03/2015	155421153	*Serratia liquefaciens*	Negative	Minimal plasmacytic synovitis
23/03/2015	155421152	*Serratia liquefaciens*	Negative	Mild to moderate lymphoplasmacytic synovitis Mild to moderate synovial proliferation
24/03/2015	155421215	No growth	Negative	Minimal synovial proliferation
24/03/2015	155421219	No growth	Negative	Marked suppurative and fibrinous synovitis Moderate lymphoplasmacytic synovitis Moderate fibroplasia
24/03/2015	155421217	No growth	Negative	Marked suppurative and fibrinous synovitis Moderate lymphoplasmacytic synovitis Mild sub synovial fibroplasia
24/03/2015	155421218	*Staphylococcus warneri*	Negative	Minimal lymphoplasmacytic synovitis
24/03/2015	155421216	No growth	Negative	Mild plasmacytic synovitis Minimal synovial proliferation
24/03/2015	155421220	No growth	Negative	Moderate to marked neutrophilic and fibrinous synovitis; Moderate lymphoplasmacytic synovitis
25/03/2015	155421317	*Erysipelothrix rhusiopathiae*	Negative	Moderate to marked suppurative synovitis Moderate to marked lymphoplasmacytic synovitis
25/03/2015	155421318	No growth	Negative	Mild lymphoplasmacytic synovitis
25/03/2015	155421319	No growth	Negative	Mild neutrophilic synovitis Mild lymphoplasmacytic synovitis Minimal synovial hyperplasia
25/03/2015	161639	No growth	Negative	Moderate neutrophilic synovitis Moderate to marked lymphoplasmacytic synovitis Moderate synovial proliferation
26/03/2015	155421401	No growth	Negative	Mild synovial proliferation
26/03/2015		No growth	Negative	Mild lymphoplasmacytic synovitis
26/03/2015	155421404	No growth	Negative	Mild to moderate lymphoplasmacytic synovitis Mild synovial proliferation
26/03/2015	155421405	No growth	Negative	Marked suppurative fibrinous synovitis Moderate lymphoplasmacytic synovitis Moderate fibroplasia
26/03/2015	155421406	No growth	Negative	Within normal limits

Date	Lab number	Culture	Chlamydia (bacteria/gram tissue)	Histopathology
26/03/2015	155421407	No growth	Negative	Moderate lymphoplasmacytic synovitis Mild synovial proliferation
26/03/2015	155421408	No growth	Negative	Moderate to marked suppurative synovitis Moderate to marked lymphoplasmacytic synovitis Moderate fibroplasia Mild synovial proliferation

14 Appendix 3

Gram Twort Stain results

Date	Lab number	Culture	Gram Twort
2/12/2014	142498022	*Corynebacterium* spp.	Negative
2/12/2014	142498023	*Erysipelothrix rhusiopathiae*	Gram positive bacteria
3/12/2014	142498078	*Aeromonas* spp.	Negative
5/12/2014	142498273	*Acinetobacter lwoffi*	Gram positive bacteria
5/12/2014	142498274	*Acinetobacter* spp.	Negative
5/12/2014	142498279	*Erysipelothrix rhusiopathiae*	Negative
5/12/2014	142498280	Heavy growth of mixed skin and environmental flora	Negative
9/12/2014	142498484	*Streptococcus* spp.	Negative
9/12/2014	142498484	*Bacillus* spp. and *Streptococcus* spp.	Negative
9/12/2014	142498483	*Serratia* spp.	Gram positive bacteria
9/12/2014	142498485	*Erysipelothrix rhusiopathiae*	Gram positive bacteria
10/12/2014	142493057	*Escherichia coli*, *Klebsiella* spp. and other coliforms	Negative
10/12/2014	142493059	Coliform, not identified further	Negative
10/12/2014	142493060	*Aeromonas* spp.; joint partially open	Gram positive bacteria
10/12/2014	142493061	*Erysipelothrix rhusiopathiae*	Gram positive bacteria
10/12/2014	142493062	Coliform, not identified further; joint partially open	Negative
10/12/2014	142493109	*Staphylococcus* spp.	Negative
10/12/2014	142493063	*Erysipelothrix rhusiopathiae*	Gram positive bacteria
10/12/2014	142493066	Alpha-haemolytic *Streptococcus* spp.	Gram positive bacteria
10/12/2014	142493067	*Erysipelothrix rhusiopathiae*	Gram positive bacteria
10/12/2014	142493068	*Erysipelothrix rhusiopathiae*	Gram positive bacteria
10/12/2014	142493123	*Erysipelothrix rhusiopathiae*	Gram positive bacteria
10/12/2014	142493065	Alpha-haemolytic *Streptococcus* spp.	Gram positive bacteria
10/12/2014	142493069	*Erysipelothrix rhusiopathiae* and *Staphylococcus* spp.	Gram positive bacteria
10/12/2014	142493129	*Bacillus* spp.	Negative
12/12/2014	142493271	*Staphylococcus epidermidis*	Gram positive bacteria associated with an intra-lesional foreign body
12/12/2014	142493273	*Erysipelothrix rhusiopathiae* and coagulase negative *Staphylococcus* spp.	Gram positive bacteria
12/12/2014	142493274	*Erysipelothrix rhusiopathiae*	Gram positive bacteria
15/12/2014	142493381	*Erysipelothrix rhusiopathiae*	Gram positive bacteria
15/12/2014	142493384	*Erysipelothrix rhusiopathiae*	Gram positive bacteria
8/01/2015	155470363	*Erysipelothrix rhusiopathiae*	Negative
12/01/2015	155473538	Non-haemolytic *Streptococcus* spp.	Negative
12/01/2	1554735	*Micrococcus* spp.	Negative

Date	Lab number	Culture	Gram Twort
015	40		
14/01/2015	155473683	*Micrococcus* spp. and coagulase negative *Staphylococcus* spp.	Gram positive bacteria
15/01/2015	155473786	Coagulase negative *Staphylococcus* spp.	Negative
22/01/2015	155470713	*Pasteurella* spp.	Negative
22/01/2015	155470714	*Pasteurella* spp. and mixed skin flora	Negative
22/01/2015	155470715	*Erysipelothrix rhusiopathiae*	Negative
	155420392	*Erysipelothrix rhusiopathiae*	Negative
10/03/2015	155420395	Isolate lost before it could be identified	Negative
10/03/2015	155420396	*Erysipelothrix rhusiopathiae*	Negative
10/03/2015	155420398	*Erysipelothrix rhusiopathiae*	Negative
23/03/2015	155421153	*Serratia liquefaciens*	Negative
23/03/2015	155421152	*Serratia liquefaciens*	Gram positive bacteria
24/03/2015	155421218	*Staphylococcus warneri*	Negative
25/03/2015	155421317	*Erysipelothrix rhusiopathiae*	Gram positive bacteria