Final report

Abattoir survey of ovine pneumonia pathogens in Australian sheep flocks

Project code: P.PSH.2054

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Abstract

The objective of this project was to provide information on the prevalence of the common ovine respiratory pathogens circulating in sheep flocks in south-eastern Australia.

The project was a non-blinded, cross-sectional, observational study with sample collection at sheep abattoirs in Australia. Twenty-four abattoir visits were completed between October 2020 and December 2021.

Polymerase Chain Reaction (PCR) testing for three bacterial pathogens involved in pneumonia in sheep (*Mycoplasma ovipneumoniae, Mannheimia haemolytica* and *Pasteurella multocida*) and two respiratory viruses (ovine Parainfluenza Virus 3 and ovine Respiratory Syncytial virus) was completed on samples collected from 1095 sets of lungs representing 253 abattoir lots.

The project has revealed widespread infection with *Mycoplasma ovipneumoniae* in Australian slaughter sheep, with 64.4% of abattoir lots testing positive for the bacterium. Abattoir lots positive for *Mycoplasma ovipneumoniae* originated in New South Wales, Queensland, South Australia, Tasmania, Victoria and Western Australia.

Mannheimia haemolytica and *Pasteurella multocida* are commensals of the nasal cavities, pharynx and throat of healthy sheep that can overwhelm host defence mechanisms and invade the lower respiratory tract during periods of stress or infections with *Mycoplasma ovipneumoniae* or respiratory viruses. Of the abattoir lots tested, 39.8% were positive for *Mannheimia haemolytica* and 15.3% were positive for *Pasteurella multocida.*

Small numbers of abattoir lots tested positive for ovine Respiratory Syncytial Virus (2.4%) and ovine Parainfluenza Virus 3 (2.0%).

Previously the role of *M. ovipneumoniae* in pneumonia in Australian sheep has been overlooked, with emphasis instead placed on the easier-to-culture bacteria *M. haemolytica* and *P. multocida*. Using new molecular techniques this project has identified *M. ovipneumoniae* as the main pathogen causing pleurisy and pneumonia in Australian sheep. Further research and extension are warranted to help producers manage *M. ovipneumoniae* infection and pneumonia/pleurisy in Australian sheep.

Executive summary

Background

Pneumonia in sheep (Ovine Respiratory Complex, ORC) is a complex disease involving the interaction of pathogen, host and environmental factors. Ruminants are anatomically predisposed to pneumonia through the rumen pressing on the diaphragm, resulting in shallow breathing.

The National Sheep Health Monitoring Project data indicates that up to 50 per cent of Australian sheep flocks have endemic ORC. Data collected as part of MLA Project B.AHE.0238 has also uncovered a high prevalence of Australian sheep flocks with ORC.

During 2018-19 Joan Lloyd Consulting Pty Ltd and Meat & Livestock Australia co-funded pilot research on ORC pathogens circulating in Australian sheep flocks (P.PSH.0814). This project was focussed on method development, including culture and Polymerase Chain Reaction (PCR), but also revealed widespread infection with *Mycoplasma ovipneumoniae* in Australian slaughter sheep sampled at abattoirs in South Australia and New South Wales.

A recommendation from the pilot research project was a larger abattoir survey of respiratory pathogens in Australian slaughter sheep be undertaken.

The current project is an abattoir survey of respiratory pathogens in Australian slaughter sheep. The aim of the project was to build on the research findings of Project P.PSH.0814 and provide further information on the prevalence of the ovine respiratory pathogens circulating in sheep flocks in Australia, including sheep from New South Wales, Queensland, South Australia, Tasmania, Victoria and Western Australia.

Objectives

The objective of this project was to provide information on the prevalence of the common ovine respiratory pathogens circulating in sheep flocks in southern Australia.

Methodology

The project was a non-blinded, cross-sectional, observational study with sample collection at sheep abattoirs in Australia.

Sample collection commenced in October 2020 and concluded in December 2021. Twenty-four abattoir visits to collect samples were conducted, with a minimum of 1500 carcases inspected per abattoir visit. The test system was bronchial swabs, with two swabs collected from each set of lungs.

Pathogens tested for have included *M. ovipneumoniae, Mannheimia haemolytica, Pasteurella multocida,* ovine Parainfluenza Virus 3 and ovine Respiratory Syncytial Virus. Assessment was by polymerase chain reaction (PCR).

Results/key findings

PCR testing for three bacterial pathogens involved in pneumonia in sheep (*M. ovipneumoniae, M. haemolytica* and *P. multocida*) and two respiratory viruses (ovine Parainfluenza Virus 3 and ovine Respiratory Syncytial virus) was completed on samples collected from 1095 sets of lungs representing 253 abattoir lots.

The project has revealed widespread infection with *M. ovipneumoniae* in Australian slaughter sheep, with 64.4% of abattoir lots testing positive for the bacterium. Abattoir lots positive for *M. ovipneumoniae* originated in New South Wales, Queensland, South Australia, Tasmania, Victoria and Western Australia.

M. haemolytica and *P. multocida* are commensals of the nasal cavities, pharynx and throat of healthy sheep that can overwhelm host defence mechanisms and invade the lower respiratory tract during periods of stress or infections with *M. ovipneumoniae* or respiratory viruses. Of the abattoir lots tested, 39.8% were positive for *M. haemolytica* and 15.3% were positive for *P. multocida.*

Small numbers of abattoir lots tested positive for ovine Respiratory Syncytial Virus (2.4%) and ovine Parainfluenza Virus 3 (2.0%).

Benefits to industry

Previously the role of *M. ovipneumoniae* in pneumonia in Australian sheep has been overlooked, with emphasis instead placed on the easier-to-culture bacteria *M. haemolytica* and *P. multocida*. New diagnostic methods based on PCR have allowed this project to detect much wider infection with *M. ovipneumoniae* in Australian sheep than recognised to date.

Pleurisy/pneumonia is common in Australian slaughter sheep. The project finding of widespread infection with *M. ovipneumoniae* in Australian sheep will help sheep producers and their veterinarians put in place appropriate management and treatment strategies to better control outbreaks of disease. Better control and/or prevention of outbreaks of pleurisy/pneumonia will have animal welfare and economic benefits for industry.

In addition, the recently published research from the USA demonstrating impaired lamb growth and productivity from *M. ovipneumoniae* infection, even in the absence of outbreaks of pleurisy/pneumonia, provides incentive for producers to work with their advisors to reduce the prevalence of infection.

Future research and recommendations

To achieve full value from the project, a communications strategy for the key project finding of widespread infection with *M. ovipneumoniae* should be developed. It is recommended that information on the project findings be included on the Meat & Livestock Australia and Animal Health Australia websites, as well as an article in the Meat & Livestock Australia Feedback magazine, and that existing resources on pleurisy/pneumonia in sheep used by Meat & Livestock Australia and Animal Health Australia be updated to include information on *M. ovipneumoniae*.

In the immediate future, a well-designed comparison of the various options to control respiratory disease in feedlot sheep is recommended, with assessment to include clinical, abattoir and meat science parameters. A contract research organisation experienced in conducting pharmaceutical and vaccine efficacy assessments would be best placed to conduct this study for industry.

If, in the future, consideration is given to funding research on autogenous vaccines for *M. ovipneumoniae*, it is recommended this research initially include a well-designed vaccine safety study that includes the assessment of vaccine-induced disease following challenge with virulent *M. ovipneumoniae*. Built into this study should also be an assessment of the immune responses to vaccination, considering research overseas that demonstrated only modest cell mediated and humoral immune responses to four booster vaccinations with autogenous vaccines. It is strongly

recommended that industry work with a contract research organisation and researchers experienced in conducting GLP[i] veterinary drug and vaccine assessments for this work.

In the longer term, consideration could be given to funding research on the feasibility of test and segregation for control of *M. ovipneumoniae* infection in flocks or mobs of sheep. Mycoplasmal infections are notoriously difficult to eliminate with antibiotic treatments, whereas vaccination is complicated by the immunopathological mechanisms that contribute to the disease processes associated with infection. Test and segregation may provide a viable alternative to reduce the prevalence of *Mycoplasma ovipneumoniae* infection in the Australian sheep flock and the significant economic cost the current high prevalence of infection is undoubtably causing. A series of individual farm case studies with highly motived producers is one approach to consider for this work.

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1. Background

Pneumonia in sheep (Ovine Respiratory Complex, ORC) is a complex disease involving the interaction of pathogen, host and environmental factors. Ruminants are anatomically predisposed to pneumonia through the rumen pressing on the diaphragm, resulting in shallow breathing.

The National Sheep Health Monitoring Project data indicates that up to 50 per cent of Australian sheep flocks have endemic ORC. Data collected as part of MLA Project B.AHE.0238 has also uncovered a high prevalence of Australian sheep flocks with ORC.

Data collected as part of MLA Project B.AHE.0238 has uncovered a high prevalence of Australian sheep flocks with pneumonia/pleurisy, with 50 per cent of the lines of lambs examined at an abattoir in South Australia having evidence of pneumonia/pleurisy (Lloyd, et al., 2016). Region and age, but not breed, were significant risk factors for pleurisy, with older lambs at increased risk compared to young or new season lambs.

This finding is consistent with a previous study of the prevalence of pneumonia in South Australian sheep flocks, which also reported that 50 per cent of sheep flocks in that State are affected by pneumonia/pleurisy based on analysis of data from the South Australian Enhanced Abattoir Surveillance Program (Meyer, 2013).

During 2018-19 Joan Lloyd Consulting Pty Ltd and Meat & Livestock Australia co-funded pilot research on ORC pathogens circulating in Australian sheep flocks (P.PSH.0814). This project was focussed on method development, including culture and Polymerase Chain Reaction (PCR), but also revealed widespread infection with *Mycoplasma ovipneumoniae* in Australian slaughter sheep sampled at abattoirs in South Australia and New South Wales.

A recommendation from the pilot research project was a larger abattoir survey of respiratory pathogens in Australian slaughter sheep be undertaken.

The current project is an abattoir survey of respiratory pathogens in Australian slaughter sheep. The aim of the project was to build on the research findings of Project P.PSH.0814 and provide further information on the prevalence of the ovine respiratory pathogens circulating in sheep flocks in Australia, including sheep from New South Wales, Queensland, South Australia, Tasmania, Victoria and Western Australia.

1.2 Pathogens Involved in Pneumonia in Sheep

Pathogens commonly involved in pneumonia in sheep include the bacteria *M. ovipneumoniae, Mannheimia haemolytica* and *Pasteurella multocida*, as well as two respiratory viruses, parainfluenza virus 3 and respiratory syncytial virus.

In Australia, the combined effect of *M. ovipneumoniae, M. haemolytica* and *P. multocida* is often called summer pneumonia (St George, 1972). Hot dry weather, raised dust, summer storms, the first shearing and grain feeding can be stressful for lambs, contributing to outbreaks of the disease.

Internationally it is well recognized that ORC usually becomes evident at critical moments in a lamb's life, for example marking, weaning, first shearing or entry to a feedlot (Navarro, et al., 2019).

Knowing if *M. ovipneumoniae* is present in a mob of sheep allows producers and their veterinarians to put in place appropriate management and treatment strategies to control outbreaks of ORC.

1.2.1 Mycoplasma ovipneumoniae

M. ovipneumoniae was first isolated from a sheep flock in Queensland in the 1960s that had shown poor growth rates and reduced exercise tolerance for some years (Cottew, 1971) (St. George, et al., 1971). It has subsequently been found in most sheep raising countries internationally.

M. ovipneumoniae persists in a flock in chronically infected carrier ewes and rams (Garwood, et al., 2020). Infected ewes pass infection to lambs soon after birth. Lambs may begin showing signs of infection (wheezing, coughing, difficulty suckling, runny nose) from around 1-2 months of age (Sullivan, et al., 1973) (Sullivan, et al., 1973).

The pleura is a thin membrane that covers the outside of the lungs and inside of the chest cavity.

When animals have pneumonia, the pleura can become inflamed. Approximately 20% (1 in 5) sheep that have pneumonia from *M. ovipneumoniae* infection will develop pleurisy (St George, 1972).

Research conducted in the United States of America in the 1990s has demonstrated that immunopathological mechanisms contribute to the disease process associated with *M. ovipneumoniae* infection, similar to mycoplasmal infections in humans and other animal species (Niang, 1996). This complicates the use of vaccination to control mycoplasmal diseases.

A pilot study of test and segregation for elimination of *M. ovipneumoniae* revealed lower average daily gains and lower yield grade carcasses in *M. ovipneumoniae* -exposed lambs, demonstrating the feasibility of test and segregation for control of infection in groups of sheep and that lamb growth and productivity may be impaired even in the absence of respiratory disease (Besser, et al., 2019).

In the United States of America, it is estimated that infection with *M. ovipneumoniae* presence in a flock is associated with a 4.3% reduction in annual lamb production (Manlove, et al., 2019)

1.2.2 Mannheimia haemolytica and Pasteurella multocida

M. haemolytica and *P. multocida* are commensals of the nasal cavities, pharynx and throat of healthy sheep that can overwhelm host defence mechanisms and invade the lower respiratory tract during periods of stress or infections with *M. ovipneumoniae* or respiratory viruses (Dassanayake, et al., 2010) (Loy, et al., 2018) (Garwood, et al., 2020) (Martin, 1996). Once in the lung these bacteria grow and secrete toxins that cause inflammation and lung tissue destruction.

Recent research also indicates that, in low numbers, *M. haemolytica* and *P. multocida* may be part of the microbiota of the ovine lung (Glendinning, et al., 2016). Further supporting this finding, is that *M. haemolytica* isolates from ovine lungs with and without pneumonia are genetically homogenous (Garcia-Alvarez, et al., 2018). The challenge is to interpret the presence of commensal bacteria detected by highly sensitive tools like PCR during disease states.

Vaccinating sheep against *M. haemolytica* or *P. multocida* as a control measure for pneumonia has given mixed results in Europe (Gonzalez, et al., 2019), New Zealand (Goodwin-Ray, 2006) (Zheng, et al., 2015), the United States of America (Sun, 2009) and the United Kingdom (McAuliffe, et al., 2003). In the latter, the sheep were subsequently found to be infected with *M. ovipneumoniae.*

1.2.3 Ovine Parainfluenza Virus 3

Exposure to ovine Parainfluenza Virus 3 is relatively common in Australian sheep (St George, 1972), although disease is usually mild (Martin, 1996).

The virus infects lambs when grouped together for any reason, i.e., weaning, husbandry procedures, and spreads rapidly so that within a few days many or most lambs have clear nasal and ocular discharge and are coughing. Outbreaks can be so mild that no clinical signs are evident. However, serious disease and patchy pneumonia can occur if the animals are stressed for any reason.

1.2.4 Ovine Respiratory Syncytial Virus

Respiratory Syncytial Virus causes severe lower respiratory tract infection in children and calves. Clinical disease in sheep is not well defined (Martin, 1996). However, it is quite likely that the disease in lambs mirrors that in children and calves.

1.3 Economic Cost of Pneumonia in Sheep

In lambs, pneumonia has been associated with mortalities, carcase condemnations, reduced liveweight gain, increased time to reach slaughter weight and reduced carcase quality (i.e., thinner, smaller carcases) (Lacasta, et al., 2019; Green, et al., 1995). Surveys of feedlot lambs in Spain show an association between pneumonia and reduced growth, feed conversion ratio and carcase quality and an estimated average daily loss of 36 g and a 10% delay in lambs reaching slaughter weight (Lacasta, et al., 2019). In New Zealand the principal adverse effects of pneumonia ins lambs have been demonstrated to be mortality, reduced carcass quality, veterinary expenses, reduced average daily bodyweight gain and poor quality of lambs produced (Goodwin-Ray, 2006). In New Zealand the annual cost of pneumonia in lambs to the industry, excluding mortalities was estimated as 1.36 NZD per lamb ((Goodwin-Ray, 2006), whereas in Spain it is estimated as 7% of final lamb value (taking into account carcass condemnation, mortalities, treatments, decreased average daily gain and reduced lamb quality) (Lacasta, et al., 2019).

During MLA Project B.AHE.0238 pleurisy trim data were collected on 101 carcasses (Lloyd, et al., 2016). Approximately half of the carcases (49 of 101, 48.5 per cent) had half the rib cage removed and six had three-quarters (one carcase) or the full ribcage (five carcasses) removed due to pleurisy. 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). Based on these weights, trimming for pleurisy is estimated to result in a $6 penalty per carcase to producers. These losses are highly leveraged to the processor as ‘frenched’ racks are valued at over $25/kg (approx. $40 to 50 per lamb) at wholesale. In addition to lost carcase weight will be the financial penalty to some producers from the trimmed carcase no longer being within specification (discounted price per kg) and the on-floor costs incurred by the abattoir in handling affected carcases.

2. Objectives

The objective of the project was to provide information on the prevalence of the common ovine respiratory pathogens circulating in sheep flocks in southern Australia.

The objective of the project has been achieved.

3. Methodology

3.1 Study Design

The project was a non-blinded, cross-sectional, observational study with sample collection at sheep abattoirs in southern Australia.

Sample collection commenced in October 2020 and concluded in December 2021. Twenty-four abattoir visits to collect samples were conducted, with a minimum of 1500 carcases inspected per abattoir visit.

Sample collection was conducted during four sampling periods (spring, summer, autumn, winter), pending access restrictions from COVID-19, as lambs from different geographic regions of Australia generally turned off at different times of the year.

3.1.1 Experimental unit

For directly consigned lots, the experimental unit was the abattoir lot/consignment/Property Identification Code (PIC).

In abattoirs in which hook tracking allowed identification of the individual carcase in saleyard lines, the experimental unit was the PIC for that carcase. In abattoirs in which hook tracking did not allow identification of the individual carcase in saleyard lines, the experimental unit was the PIC for the saleyard.

3.1.2 Inclusion criteria

Ovine lungs with gross pathological signs consistent with pneumonia, including cranio-ventral consolidation and/or widespread lung mottling and areas of consolidation; thickened pleura.

Five sets of lungs were sampled per abattoir lot/consignment/PIC. The sample size was determined based on the within line prevalence of *M. ovipneumoniae* observed during project P.PSH.0814.

3.1.3 Exclusion criteria

Ovine lungs without gross pathological signs consistent with pneumonia.

3.2 Study procedures

3.2.1 Test system

The test system was bronchial swabs (Copan FLOQ swab or Bacto Transfer swab, viscose tip), with two swabs collected from each set of lungs. One swab was preserved in Liquid Amies Transport Media (2 mL) and one in modified Sucrose Phosphate Glutamate Transport Medium (1 mL) (Atlas & Snyder, 2014).

As much as possible, bronchial swabs were collected on the slaughter floor, with the swabs placed onto wet ice immediately after collection.

The dorsal surface of the trachea was opened with a knife and then the entire bronchial tree swabbed, starting with the right cranial lobe and moving counter-clockwise around the bronchial

tree. The knife was cleaned at an abattoir boiling water station for a minimum of five seconds between each set of lungs.

Swabs in Liquid Amies Transport Media were processed within 24-96 hours of sample collection, depending on the distance between the abattoir and laboratory, and swabs in modified Sucrose Phosphate Glutamate Transport Medium were frozen at -20 °C within 4-6 hours of sample collection. Courier delays with samples sent from Tasmania and Western Australia led to some delays in processing these samples.

When collecting swabs on the slaughter floor was not possible, the right cranial lobe was excised, placed into a sterile Whirl Pak sample bag and frozen at -20 °C.

3.2.2 Assessment of pathogens present

Pathogens tested for have included *M. ovipneumoniae, M. haemolytica, P. multocida,* ovine Parainfluenza-3 virus and ovine Respiratory Syncytial Virus.

Assessment was by PCR (Table 1).

Table 1. PCR methods to be used during the study

Pathogen	Standard Operating Procedure (SOP)	Reference
Mycoplasma ovipneumoniae	GLP00	(Yang, et al., 2014)
Mannheimia haemolytica	GLP031	(Loy, et al., 2018)
Pasteurella multocida	GLP032	(Loy, et al., 2018)
Ovine Parainfluenza-3 virus	GLP033	(Lyon, et al., 1997)
Ovine Respiratory Syncytial Virus	GLP034	(Eleraky, et al., 2001)

Bacterial DNA was extracted from Liquid Amies Transport Media using the QIAamp DNA Mini Kit (Qiagen Australia Pty Ltd) or the MagMax Core Nucleic Acid Purification kit (Life Technologies Australia Pty Ltd) following kit instructions for isolation of bacterial DNA from biological fluids, except bacteria were initially pelleted by centrifugation for 20 minutes at 15,000 x G (Niang, et al., 1999).

Lung tissue was defrosted overnight at 4°C. The surface of the tissue was sprayed with 70% ethanol in water, allowed to air dry and then excised with a sterile scalpel blade. Sterile scissors and forceps were used to collect lung tissue into a sterile stomacher bag, concentrating on abnormal tissue and bronchioles, to which was added 3 mL of sterile phosphate buffered saline. The tissue was homogenized for 1 minute in a paddle homogenizer, allowed to sit for 30 minutes and then re-homogenized. As much fluid as possible was collected from the stomacher bag, leaving the tissue in the bag. Bacterial DNA was extracted from the fluid using the QIAamp DNA Mini Kit (Qiagen Australia Pty Ltd) following kit instructions for isolation of bacterial DNA from biological fluids. except bacteria were initially pelleted by centrifugation for 20 minutes at 15,000 x G (Niang, et al., 1999).

Viral RNA was extracted from modified Sucrose Phosphate Glutamate Transport Medium using the QIAamp MinElute Virus Spin Kit (Qiagen Australia Pty Ltd) or the MagMax Core Nucleic Acid Purification kit (Life Technologies Australia Pty Ltd) following kit instructions. Samples were processed in pools of five concentrated to 200 µL using a Amicon Ultra – 2 or Amicon Ultra-4

Centrifugal Filter Unit (Merck Millipore Australia). In September 2021 it was no longer possible to purchase the Amicon Ultra – 2 Centrifugal Filter Unit because these are in high demand globally for COVID-19 testing.

3.2.3 Data Analysis

Lot-based PCR test data was entered into an Excel spreadsheet, positive/negative for each PCR test. If one or more of the five samples from a lot was positive for a pathogen, the lot was said to be positive.

The PIC was converted to a region/district. Where the PIC for a lot was not available, the post code of the nearest town was used instead.

4. Results

4.1 Abattoir Visits

Twenty-four abattoir visits were completed during the project, including 16 visits to abattoirs in New South Wales, four visits to abattoirs in Tasmania and four visits to abattoirs in Western Australia (Table 2).

COVID 19 travel restrictions made it difficult to access abattoirs in other states. However, the movement of slaughter sheep in eastern Australia meant that samples could be collected from sheep originating in Queensland, South Australia and Victoria through the abattoir visits in New South Wales.

During the abattoir visits, samples were collected from 253 abattoir lots of sheep, including 182 lots of lambs and 71 lots of adult sheep.

Table 2. Abattoir visits completed October 2020 – December 2021

Visit number	Date	Abattoir location	Lungs inspected	Lungs sampled	Per cent sampled	Lots sampled
1	22-Oct-20	New South Wales	1582	20	1.3	4
2	12-Nov-20	New South Wales	1301	40	3.1	9
3	26-Nov-20	New South Wales	1848	40	2.2	13
4	09-Dec-20	New South Wales	1856	50	2.7	21
5	19-Jan-21	New South Wales	2500	40	1.6	8
6	20-Jan-21	New South Wales	2007	40	2.0	8
7	02-Mar-21	New South Wales	2728	57	2.1	11
8	03-Mar-21	New South Wales	4019	65	1.6	14
9	04-Mar-21	New South Wales	2762	50	1.8	10
10	11-May-21	New South Wales	1946	70	3.6	15
11	12-May-21	New South Wales	3369	50	1.5	9
12	13-May-21	New South Wales	2014	70	3.5	20
13	23-Aug-21	Tasmania	1058	24	2.3	9
14	07-Sep-21	Western Australia	2799	50	1.8	10
15	20-Sep-21	Tasmania	1664	19	1.1	10
16	11-Oct-21	Tasmania	1277	50	3.9	10
17	12-Oct-21	Western Australia	3149	50	1.6	10
18	03-Nov-21	Western Australia	2229	45	2.2	10
19	08-Nov-21	Tasmania	2018	45	2.2	9
20	16-Nov-21	New South Wales	2987	50	1.7	10
21	17-Nov-21	New South Wales	2275	50	2.2	10
22	01-Dec-21	Western Australia	3574	50	1.4	10
23	14-Dec-21	New South Wales	2243	35	1.6	7
24	15-Dec-21	New South Wales	1543	30	1.9	6

4.2 PCR Testing

One thousand and ninety-five samples were collected for PCR testing.

4.2.1 Mycoplasma ovipneumoniae

The result of the PCR testing for *M. ovipneumoniae* is shown in Table 3.

M. ovipneumoniae was detected in sampled lots at each abattoir visit (range 28.6% – 100% of sampled abattoir lots).

Across all the abattoir visits, 64.4% of sampled abattoir lots tested positive for *M. ovipneumoniae.*

Table 3. Results of PCR testing for *M. ovipneumoniae* on samples collected October 2020-December 2021

Date	Visit number	Number of samples	Number of lots sampled	Per cent lots positive
22-Oct-20	1	20[A]	4	100
12-Nov-20	2	40[A]	9	77.8
26-Nov-20	3	40[A]	31	69.2
09-Dec-20	4	50[B]	21	47.6
19-Jan-21	5	40[A]	8	100
20-Jan-21	6	40[A]	8	100
02-Mar-21	7	57[A]	11	54.6
03-Mar-21	8	65[A]	14	50.0
04-Mar-21	9	50[A]	10	60.0
11-May-21	10	70[A]	15	46.7
12-May-21	11	50[A]	9	33.3
13-May-21	12	70[A]	20	50.0
23-Aug-21	13	24[A]	9	66.7
07-Sep-21	14	50[A]	10	80.0
20-Sep-21	15	19[A]	10	40.0
11-Oct-21	16	50[A]	10	60.0
12-Oct-21	17	50[A]	10	90.0
03-Nov-21	18	45[A]	10	70.0
08-Nov-21	19	45[A]	9	33.3
16-Nov-21	20	50[A]	10	40.0
17-Nov-21	21	50	10	80.0
01-Dec-21	22	50	10	80.0
14-Dec-21	23	35	7	28.6
15-Dec-21	24	30	6	88.3

[A] DNA extracted from swab in Liquid Amies Transport Media.

[B] DNA extracted from lung tissue stored frozen at -20 ^{o}C.

Positive consignments were from the Braidwood, Central Tablelands, Condobolin, Coonabarabran, Coonamble, Dubbo, Forbes, Goulburn, Gundagai, Hay, Hume, Murray, Narrabri, Narrandera, Northern New England, Riverina, Tamworth , Wagga Wagga, Wentworth, Wilcannia, Yass and Young

regions of New South Wales; the Adelaide Hills / Fleurieu, Eyre and Mid-South-East regions of South Australia; the Benalla, Bendigo, East Gippsland, Hamilton, Indigo, Moira, Swan Hill and Wangaratta regions of Victoria; the St George region of Queensland; the Beaconsfield, Campbelltown, Evandale, Fingal, Kentish, Longford, Oatlands, Ringarooma, Ross and Westbury regions of Tasmania; and the Broomehill, Bruce Rock, Cranbrook, Coomalbidgup, Cuballing, Dardanup, Dumbleyung, Esperance, Gnowangerup, Katanning, Kulin, Jerramungup, Mount Barker, Plantagenet, Quairading, Ravensthorpe, Wagin and York regions of Western Australia.

4.2.2 Mannheimia haemolytica

The result of the PCR testing for *M. haemolytica* is shown in Table 4.

M. haemolytica was detected in sampled lots at all but one abattoir visit (range 0% - 90% of sampled abattoir lots).

Across all the abattoir visits, 39.8% of sampled abattoir lots tested positive for *M. haemolytica.*

Table 4. Results of PCR testing for *M. haemolytica* on samples collected October 2020 – December 2021

Date	Visit number	Number of samples	Number of lots sampled	Per cent lots positive
22-Oct-20	1	20[A]	4	50.0
12-Nov-20	2	40[A]	9	44.4
26-Nov-20	3	40[A]	31	19.4
09-Dec-20	4	50[B]	21	4.8
19-Jan-21	5	40[A]	8	75.0
20-Jan-21	6	40[A]	8	62.5
02-Mar-21	7	57[A]	11	54.6
03-Mar-21	8	65[A]	14	57.1
04-Mar-21	9	50[A]	10	90.0
11-May-21	10	70[A]	15	13.3
12-May-21	11	50[A]	9	0.0
13-May-21	12	70[A]	20	15.0
23-Aug-21	13	24[A]	9	44.4
07-Sep-21	14	50[A]	10	50.0
20-Sep-21	15	19[A]	10	50.0
11-Oct-21	16	50[A]	10	60.0
12-Oct-21	17	50[A]	10	40.0
03-Nov-21	18	45[A]	10	20.0
08-Nov-21	19	45[A]	9	56.0
16-Nov-21	20	50[A]	10	40.0
17-Nov-21	21	50	10	20.0
01-Dec-21	22	50	10	40.0
14-Dec-21	23	35	7	14.3
15-Dec-21	24	30	6	33.3

[A] DNA extracted from swab in Liquid Amies Transport Media.

[B] DNA extracted from lung tissue stored frozen at -20 ^{o}C.

4.2.3 Pasteurella multocida

The result the PCR testing for *P. multocida* is shown in Table 5.

P. multocida was detected in sampled lots in 58.3% of abattoir visits (range 0-64.3 % of sampled abattoir lots).

Across all the abattoir visits, 15.3% of sampled abattoir lots tested positive for *P. multocida.*

Table 5. Results of PCR testing for *P. multocida* on samples collected October 2020 – December 2021

Date	Visit number	Number of samples	Number of lots sampled	Per cent lots positive
22-Oct-20	1	20[A]	4	25.0
12-Nov-20	2	40[A]	9	0.0
26-Nov-20	3	40[A]	31	0.0
09-Dec-20	4	50[B]	21	4.8
19-Jan-21	5	40[A]	8	12.5
20-Jan-21	6	40[A]	8	50.0
02-Mar-21	7	57[A]	11	18.2
03-Mar-21	8	65[A]	14	64.3
04-Mar-21	9	50[A]	10	30.0
11-May-21	10	70[A]	15	6.7
12-May-21	11	50[A]	9	0.0
13-May-21	12	70[A]	20	0.0
23-Aug-21	13	24[A]	9	0.0
07-Sep-21	14	50[A]	10	10
20-Sep-21	15	19[A]	10	0.0
11-Oct-21	16	50[A]	10	0.0
12-Oct-21	17	50[A]	10	40.0
03-Nov-21	18	45[A]	10	20.0
08-Nov-21	19	45[A]	9	0.0
16-Nov-21	20	50[A]	10	30.0
17-Nov-21	21	50	10	0.0
01-Dec-21	22	50	10	40.0
14-Dec-21	23	35	7	0.0
15-Dec-21	24	30	6	16.7

[A] DNA extracted from swab in Liquid Amies Transport Media.

[B] DNA extracted from lung tissue stored frozen at -20 ^{o}C.

4.2.4 Ovine Parainfluenza Virus 3

The result of the PCR testing for ovine Parainfluenza Virus 3 is shown in Table 6.

In total, five of the 253 abattoir lots (2.0%) sampled during the 24 abattoir visits tested positive for the virus, including one abattoir lot from New South Wales, one abattoir lot from Tasmania and three abattoir lots from Western Australia.

Four of the abattoir lots positive for ovine Parainfluenza Virus 3 were comprised of lambs and one of adult sheep.

Table 6. Results of PCR testing for ovine Parainfluenza Virus 3 on samples collected October 2020 – November 2021

Date	Visit number	Number of samples	Number of lots sampled	Per cent lots positive
22-Oct-20	1	20	4	0.0
12-Nov-20	2	40	9	0.0
26-Nov-20	3	40	31	0.0
09-Dec-20	4	50	21	0.0
19-Jan-21	5	40	8	0.0
20-Jan-21	6	40	8	0.0
02-Mar-21	7	57	11	0.0
03-Mar-21	8	65	14	0.0
04-Mar-21	9	50	10	0.0
11-May-21	10	70	15	0.0
12-May-21	11	50	9	0.0
13-May-21	12	70	20	0.0
23-Aug-21	13	25	9	0.0
07-Sep-21	14	50	10	10.0
20-Sep-21	15	19	10	10.0
11-Oct-21	16	50	10	0.0
12-Oct-21	17	50	10	0.0
03-Nov-21	18	45	10	0.0
08-Nov-21	19	45	9	0.0
16-Nov-21	20	50	10	0.0
17-Nov-21	21	50	10	0.0
01-Dec-21	22	50	10	10.0
14-Dec-21	23	35	7	16.7
15-Dec-21	24	30	6	0.0

4.2.5 Ovine Respiratory Syncytial Virus

The result of the PCR testing for ovine Respiratory Syncytial virus is shown in Table 7.

Six of the 253 abattoir lots (2.4%) sampled during the 24 abattoir visits tested positive for ovine Respiratory Syncytial Virus, including three abattoir lots from New South Wales, two abattoir lots from Western Australia and one abattoir lot from South Australia.

Four of the abattoir lots positive for the virus were lambs and two were adult sheep.

Table 7. Results of PCR testing for ovine Respiratory Syncytial Virus on samples collected October 2020 – December 2021

Date	Visit number	Number of samples	Number of lots sampled	Per cent lots positive
22-Oct-20	1	20	4	0.0
12-Nov-20	2	40	9	0.0
26-Nov-20	3	40	31	0.0
09-Dec-20	4	50	21	0.0
19-Jan-21	5	40	8	0.0
20-Jan-21	6	40	8	0.0
02-Mar-21	7	57	11	0.0
03-Mar-21	8	65	14	0.0
04-Mar-21	9	50	10	0.0
11-May-21	10	70	15	20.0
12-May-21	11	50	9	0.0
13-May-21	12	70	20	0.0
23-Aug-21	13	25	9	0.0
07-Sep-21	14	50	10	0.0
20-Sep-21	15	19	10	0.0
11-Oct-21	16	50	10	0.0
12-Oct-21	17	50	10	10.0
03-Nov-21	18	45	10	10.0
08-Nov-21	19	45	9	0.0
16-Nov-21	20	50	10	10.0
17-Nov-21	21	50	10	0.0
01-Dec-21	22	50	10	0.0
14-Dec-21	23	35	7	0.0
15-Dec-21	24	30	6	0.0

5. Conclusion

5.1 Key findings

The project has revealed widespread infection with *M. ovipneumoniae* in Australian slaughter sheep, with 64.4% of abattoir lots testing positive for the bacterium. Abattoir lots positive for *M. ovipneumoniae* originated in New South Wales, Queensland, South Australia, Tasmania, Victoria and Western Australia. Samples collected from young lambs, lambs and adult sheep have all tested positive for *M. ovipneumoniae.*

Throughout the project, travel restrictions and lockdowns associated with COVID-19 impacted the schedule for abattoir visits and sample collection. This prevented us visiting each abattoir during each of the four sampling periods (spring, summer, autumn, winter). Despite this the project was able to collect samples from sheep originating from around Australia and demonstrate widespread infection with *M. ovipneumoniae* in Australian slaughter sheep. However, the concentration of sample collection in some seasons (spring and summer) at the expense of others (autumn and winter) meant that we were unable to investigate seasonal trends in infection status.

In Australia, the combined effect of *M. ovipneumoniae, M. haemolytica* and *P. multocida* is often called summer pneumonia. In this abattoir survey, 39.8% of abattoir lots were positive for *M. haemolytica* and 15.3% were positive for *P. multocida.*

Small numbers of abattoir lots have tested positive for ovine Respiratory Syncytial Virus (2.4%) and ovine Parainfluenza Virus 3 (2.0%). This suggests that the role of respiratory viruses in the pleurisy/pneumonia complex in Australian sheep may be less important in outbreaks of summer pneumonia than underlying infection with *M. ovipneumoniae.*

5.2 Benefits to industry

Previously the role of *M. ovipneumoniae* in pleurisy/pneumonia in Australian sheep has been overlooked, with emphasis instead placed on the easier-to-culture bacteria *M. haemolytica* and *P. multocida*. New diagnostic methods based on PCR have allowed this project to detect much wider infection with *M. ovipneumoniae* in Australian sheep than recognised to date.

Pleurisy/pneumonia is common in Australian slaughter sheep. The project finding of widespread infection with *M. ovipneumoniae* in Australian sheep will help sheep producers and their veterinarians put in place appropriate management and treatment strategies to better control outbreaks of disease. Better control and/or prevention of outbreaks of pleurisy/pneumonia will have animal welfare and economic benefits for industry.

In addition, the recently published research from the USA demonstrating impaired lamb growth and productivity from *M. ovipneumoniae* infection, even in the absence of outbreaks of pleurisy/pneumonia, (Besser, et al., 2019), provides incentive for producers to work with their advisors to reduce the prevalence of infection.

M. haemolytica and *P. multocida* are commensals of the nasal cavities, pharynx and throat of healthy sheep that can overwhelm host defence mechanisms and invade the lower respiratory tract during periods of stress or infections with *M. ovipneumoniae* or respiratory viruses. Recent research also indicates that, in low numbers, these bacteria may also be part of the microbiota of the ovine lung. The challenge for industry and advisors to interpret the presence of commensal bacteria detected by highly sensitive tools like PCR during disease states.

6. Future research and recommendations

To achieve full value from the project, a communications strategy for the key project finding of widespread infection with *M. ovipneumoniae* should be developed. This has commenced in a small way, with presentations to Sheep Connect NSW in April 2021, the Australian Sheep Veterinary Association in June 2021, and regular website and social media updates on the project findings. It is recommended that information on the project findings be included on the Meat & Livestock Australia and Animal Health Australia websites, as well as an article in the Meat & Livestock Australia Feedback magazine.

Existing resources on pleurisy/pneumonia in sheep used by Meat & Livestock Australia and Animal Health Australia should be updated to include information on *M. ovipneumoniae*.

In the 1990s, a respiratory disease of lambs that involved paroxysmal coughing leading to rectal prolapse and reduced weight gain was observed to be widespread in the mid-western USA. *M. ovipneumoniae* was routinely isolated from the respiratory tract of lambs with the disease. Antibodies reactive with ovine respiratory cilia of the upper respiratory tract were detected in the sera of lambs with the disease, but not in lambs without the disease (Niang, et al., 1998). Antibodies to the cilia developed before the onset of the clinical disease, and colonisation of the respiratory tract of the lambs by *M. ovipneumoniae* preceded the production of these antibodies. This finding led the researchers to conclude that an immunopathological mechanism could be contributing to disease caused by *M. ovipneumoniae*, similar to mycoplasma infections in other animal species.

An immunopathological mechanism to the disease caused by *M. ovipneumoniae* was initially suggested in 1979 by researchers working in the United Kingdom who observed different disease expression in conventionally reared and specific-pathogen-free lambs, with the latter less severely affected (Gilmour, et al., 1979). These authors noted that naturally occurring chronic pneumonia in lambs is generally not observed until the animals are over three months of age, although infection with *M. ovipneumoniae* occurs at an earlier age. The authors drew a comparison to atypical pneumonia in humans caused by *M. pneumoniae*, with subclinical infection in infants and disease generally not seen until 5-15 years of age. The authors also cited research in immunosuppressed mice and hamsters, challenged respectively with *M. pulmonis* and *M. pneumoniae*, that developed less severe lesions than controls. They postulated that the minimal development of pulmonary lymphoid tissue observed in the specific-pathogen-free lambs prior to challenge may have resulted in the lack of an ability to recognise mycoplasmas immediately and respond with a chronic pneumonia as do conventional animals.

Abattoir disease surveillance through the National Sheep Health Monitoring Project, the advent of lot-feeding and the introduction of composite breeds of sheep has resulted in respiratory disease in Australian sheep becoming more widely recognised. Overlooked for years, the significance of infection with *M. ovipneumoniae* in Australian sheep is now recognised through the current project

and project P.PSH.0814. Disease is more evident clinically and at abattoirs in feedlot sheep. Clinically the disease resembles the condition found in mid-western USA described above.

Anecdotally numerous options to control pneumonia in feedlot sheep are being used, including individual animal treatments with antibiotics, metaphylaxis with antibiotics and the *Mannheimia haemolytica* vaccines currently registered for use in cattle, without strong scientific evidence to underpin and guide their use. Differing advice is likely leading to confusion amongst producers. It is recommended that a well-designed comparison of the various options to control respiratory disease in feedlot sheep be conducted, with assessment to include clinical, abattoir and meat science parameters. A contract research organisation would be best placed to conduct this study for industry.

Whole-cell, autogenous vaccine for *M. ovipneumoniae* is now available in Australia under permit. Although an old and somewhat crude technology, autogenous vaccines are attractive to the livestock industries because they promise reduced reliance on antibiotics to control infectious disease. Studies conducted overseas (Einarsdottir, et al., 2018), as well as the immunopathological mechanisms of the disease discussed above and the lack of effective mycoplasmal vaccines in most other animals and humans, raises questions about the appropriateness of autogenous vaccines to control of *M. ovipneumoniae*. Recently, lipid-associated membrane proteins of *M. pneumoniae* have been implicated in vaccine-enhanced disease following infection with virulent *M. pneumoniae* (Mara, et al., 2020). To date, vaccine-enhanced disease has prevented development of a vaccine against *M. pneumoniae.*

Whole-cell, autogenous vaccines for *M. ovipneumoniae* will contain these lipid-associated membrane proteins. If, in the future, consideration is given to funding research on autogenous vaccines for *M. ovipneumoniae*, it is recommended this research initially include a well-designed vaccine safety study that includes vaccine-induced disease following challenge with virulent *M. ovipneumoniae*. Built into this study should be an assessment of the immune responses to vaccination, considering research overseas that demonstrated only modest cell mediated and humoral immune responses to four booster vaccinations with autogenous vaccines (Einarsdottir, et al., 2018). It is strongly recommended that industry work with a contract research organisation and researchers experienced in conducting GLP[ii] veterinary drug and vaccine assessments for this work.

During Project B. AHE.0238 we demonstrated a strong association between arthritis and pneumonia in Australian sheep. Inflammatory (reactive) arthritis secondary to mycoplasma infection is well recognised in humans (Goldsmith, 2001). It is highly likely that a similar reactive arthritis occurs in sheep, which would help to explain the association between arthritis and pneumonia. However, given the severity of some of the pneumonias in Australian abattoir sheep and the secondary infections with commensal bacteria of the upper respiratory tract the current study has revealed, another contributing factor could be recurring episodes of sub-clinical low-grade septicaemia with lodgement of bacteria in the joints. During Project B. AHE.0238 we detected *Pasteurella* spp. in trimmed, arthritic joints, supporting this hypothesis. We also detected *Streptococcus* spp. in trimmed joints, the second most detected bacteria after *Erysipelothrix rhusiopathiae*. At the time, 16s rRNA sequencing indicated the *Streptococcus* spp. were most likely to be *S. suis*. Recently, a new *S. suis*-like bacteria, *S. ruminantium* has been described (Okura, et al., 2019). This bacterium has been shown to be a commensal of the tonsils of ruminants and has also been recovered from pulmonary abscesses in sheep. Re-running the blast searches of the 16s rRNA sequencing of the *Streptococcus* spp. isolated during Project B.AHE.0238 indicates these isolates are the new *S. suis*-like bacteria, *S. ruminantium*.

Nephritis is common in Australian slaughter sheep. The condition primarily manifests as white or cream- coloured spots of up to 5 mm diameter spots on the outer surface of the kidney and extending into the renal cortex. This distribution of lesions is highly consistent with emboli lodging within the glomeruli. Animal Health Australia is currently funding pilot research on nephritis in Australian slaughter sheep. It is recommended that MLA monitor the results of this research and, if warranted, consider funding additional research to further investigate nephritis in Australian sheep and the potential association with pneumonia.

In the longer term, consideration could be given to funding research on the feasibility of test and segregation for control of *M. ovipneumoniae* infection. Mycoplasmal infections are notoriously difficult to eliminate with antibiotic treatments, whereas vaccination is complicated by the immunopathological mechanisms that contribute to the disease process associated with them. A series of individual farm case studies with highly motived producers is one approach to consider for this work.

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[i] Good Laboratory Practice

[ii] Good Laboratory Practice