final report

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Impact of bacteria and coccidia on scouring and productivity in sheep

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Executive summary

Intestinal parasites cost the Australian sheep industry an estimated $436 million per annum, yet little is known about the contribution of protozoan parasites and bacteria to scouring and production loss in sheep. This project aimed to:

- Develop a high throughput multiplex quantitative PCR (qPCR) to detect and quantify the pathogen load of 10 pathogens (*Salmonella, Campylobacter, Yersinia, Chlamydia, Eimeria, Cryptosporidium, Giardia, Haemonchus, Teladorsagia and Trichostrongylus)* in sheep faeces across four states; Western Australia (WA), New South Wales (NSW), South Australia (SA) and Victoria (Vic).
- Develop genotyping tools for these pathogens
- Identify any significant associations between pathogens and production parameters
- Develop a qPCR assay for *Escherichia coli* O157:H7 and determine the prevalence and pathogen load of zoonotic pathogens (*Salmonella, Campylobacter, Cryptosporidium, Giardia* and *Escherichia coli* O157:H7) using qPCR in sheep faeces collected from WA sale yards (Muchea and Katanning) and in abattoir effluent (Katanning) (inlet and outlet).

Three multiplex assays were successfully developed, validated and used to determine the prevalence and pathogen load per gram of faeces (g^{-1}) of the ten pathogens (*Salmonella, Campylobacter, Yersinia, Chlamydia, Eimeria, Cryptosporidium, Giardia, Haemonchus, Teladorsagia and Trichostrongylus)* from 3,412 sheep faecal samples collected from 1,189 lambs on three sampling occasions (weaning, post-weaning and pre-slaughter) from eight farms across the four states.

Analysis revealed that there were distinct differences in prevalence between pathogens across the four states. In general, bacterial pathogens were more prevalent in Eastern states than in WA. *Salmonella* had the lowest prevalence of all pathogens across the four states. *Cryptosporidium* and *Giardia* were more prevalent in WA than Eastern states and *Eimeria* was most prevalent in NSW. With the exception of *Haemochus*, the prevalence of strongyle worms was high across most states. *Chlamydia abortus* was not detected in Australian sheep.

Pathogen load and prevalence were determined using the qPCR. Worm egg counts (WEC) were analysed by microscopy. Analysis of any associations between pathogen presence and load with breech faecal soiling (dag score), faecal consistency score (FCS), live weight, hot standard carcase weight (HSCW) and dressing percentage (DP) was conducted using SAS Version 9.2.

No significant main effects for WEC or *Haemonchus* status for live weight, HSCW or DP were observed in this study and only three pathogens (*Cryptosporidium, Giardia* and *Yersinia*) consistently showed significant effects. It should also be noted that lambs in the present study were being raised for slaughter using management typical for each property, including anthelmintic treatments and grazing management. It is possible that the nematode infection pressure and range of WEC observed in these flocks was not sufficient for effects on live weight, HSCW and DP to be observed.

Cryptosporidium shedding was associated with a reduction in DP (1.21-1.25%) and *Giardia* was associated with a reduction in carcase weight (0.594 kg). More specifically, this study identified that shedding of the zoonotic *Cryptosporidium parvum* was associated with reductions in liveweight (2.411-3.457 kg), carcase weight (2.602-2.208 kg) and DP (4.6%). Importantly, this was a longitudinal study, therefore relationships between protozoan shedding at different time points and productivity measures at different time points could be identified. For example, *Giardia* shedding at the post-weaning (but not pre-slaughter) sampling was associated with reduced HSCW (0.594 kg), and *C. parvum* shedding at the post-weaning sampling was associated with reductions in both HSCW and DP, suggesting that protozoan infections can have chronic impacts on carcase productivity.

Pathogenic *Yersinia enterocolitica* shedding was associated with reductions in liveweight (3.4 kg). Furthermore, there was a significant interaction between sampling period (time) and level of shedding category on liveweight, with reductions in liveweight evident at the post-weaning (4.9kg, post hoc P) and pre-slaughter (3.9kg post hoc P) sampling occasions in the lambs classified as high shedding compared to non-shedding lambs. This is the first report of reduced growth of lambs in lambs with higher faecal shedding of pathogenic *Y. enterocolitica* relative to non-infected counterparts.

Significant correlations between WEC and FCS were noted only for two (WA2 and WA3) of the eight flocks, and for these flocks only at one (WA3) or two (WA2) sampling occasions. Of the pathogens identified by qPCR (specifically *Cryptosporidium* spp., *C. parvum, Giardia* spp, *Salmonella* spp., *Campylobacter*. spp., pathogenic *Yersinia* and *C. percorum*), significant associations between pathogen presence and FCS were identified only for *Cryptosporidium* (all species). Lambs shedding *Cryptosporidium* (all species) at weaning and postweaning had higher faecal consistency scores, meaning shedding was associated with looser faecal consistency.

Analysis also identified a complex relationship, with pathogen shedding intensity category not consistently associated with FCS or dag score over the three sampling occasions. High shedding of *C. parvum* was associated with the greatest increase in FCS (relative to lambs in which *C. parvum* was not detected) and the only pathogen with FCS over 3 (soft unformed faeces), but only at post-weaning sampling. WEC was significant as a covariate only for dag score at pre-slaughter sampling.

A set of qPCR primers and probe for detecting and quantitating *Escherichia coli* O157:H7 (an enterohemorrhagic serotype of *E. coli*) was developed and validated. This was then combined in a multiplex qPCR assay to detect and quantitate *E. coli* O157:H7, *Salmonella* and *Campylobacter* in 474 sheep faeces from sale yards (Muchea and Katanning) and from 96 abattoir effluent samples (Katanning). The prevalence and pathogen load of the zoonotic pathogens, *Cryptosporidium* and *Giardia,* in these samples was also determined using the multiplex qPCR previously developed.

All five pathogens (*Campylobacter* spp., *S. enterica, E. coli* O157:H7, *Cryptosporidium* and *Giardia)* were identified in sheep at saleyards in Western Australia and in abattoir effluent. For bacteria, overall prevalences in were generally low (<6%) in sheep at saleyards, but point prevalences ranged considerably in healthy sheep (up to 26% for *E. coli* O157:H7). The overall prevalence of *Cryptosporidium* in sheep at sale yards was 6.5% (31/474), with the zoonotic species *C. parvum* and *C. ubiquitum* accounting for 54.2% of all positives typed.

The overall prevalence of *Giardia* at sale yards was 6.3% (30/474) with the non-zoonotic assemblage E responsible for the majority (81.5%) of positive isolates typed.

Overall, *Campylobacter* has the highest median number of organisms per gram of faeces (g^{-1}) shed (4.7×10^3 organisms g^{-1}) in sheep in saleyards, followed by *Salmonella* (4.5×10^3 organisms g^{-1}), *E. coli* O157:H7 (2.7×10^3 organisms g^{-1}), *Cryptosporidium* and *Giardia* (1.7×10^3 organisms g^{-1} each).

Analysis indicates the following recommendations, which MLA and AWI should consider.

(1) Facilitate validation of a uniform diagnostic qPCR assay across laboratories

MLA and AWI should consider funding efforts to validate and optimise the diagnostic qPCR platform developed as part of this study across different laboratories to maximise time efficiency, accuracy and reliability and ensure that quality diagnostic services are provided for all Australian livestock producers. An investigation into the cost-benefits and practical implications for diagnostic laboratories to use the multiplex qPCR diagnostic should be conducted, combined importantly with a forecasted uptake and probable demand by livestock enterprises. Another alternative would be for Murdoch University to become a reference laboratory for the qPCR platform, similar to the way NSW DPI is the reference lab for PCR testing for Johne's disease. The volume of testing is unlikely to justify rolling out the test across all diagnostic laboratories unless the price is very low because sheep producers are very cost conscious. Another option could be combining the bacterial and protozoal methods with the commercial available PCR test methods for parasitic nematodes already in use in some laboratories in Australia (http://www.ausdiagnostics.com/production-animals.html), or to work with this company to commercialise bacterial and protozoal scours platforms.

(2) More in depth analysis of the production impacts of *Cryptosporidium, Giardia* and *Yersinia* on sheep

A key finding of the present study was that *Cryptosporidium, Giardia* and *Yersinia* had production impacts on sheep. Further-more, controlled studies including controlled feed intake, body condition score and GR knife fat depth are necessary to provide more accurate information regarding the observed production impacts. The study could also include ascertaining whether specific new broad-spectrum anti-protozoal and anti-bacterial chemotherapeutic agents (e.g. inosine 5'-monophosphate dehydrogenase inhibitors) and or re-profiling of approved drugs such as auranofin are cost-effective against *Cryptosporidium, Giardia* and *Yersinia* infections.

(3) Identification of potential risk factors for *Cryptosporidium, Giardia* and *Yersinia* infections in sheep

Little is known about the risk factors for *Cryptosporidium, Giardia* and *Yersinia* infections in sheep. A cross-sectional study should be carried out to identify management factors that may be contributing to disease. This would include a questionnaire being designed and administered to farmers to collect data on demographic, management and health factors hypothesized to be associated with the risk of infection with these pathogens in sheep.

(4) Measure faecal output and pathogen load in lambs over time throughout curfew and lairage

Stress and shedding of potentially zoonotic pathogens (*Giardia, Cryptosporidium, Salmonella,* etc.) has implications beyond the farm gate, particularly for the processing and retail sector, and these have not been well explored to date.

The present study measured prevalence and pathogen load of five pathogens (*Campylobacter* spp., *S. enterica*, *E. coli* O157:H7, *Cryptosporidium* and *Giardia)* in lambs at saleyards and in abattoir effluent. A more in depth study that measures faecal output and pathogen load in lambs over time throughout curfew and lairage is required, bearing in mind that for export abattoirs, such monitoring requires the use of approved tests performed by a registered laboratory. This will provide more meaningful information on risk factors for shedding of zoonotic pathogens at abattoirs with relevance to both carcase contamination and effluent management at the abattoir. These important factors have not been studied in detail and need to be managed/reduced in meat enterprises.

If these actions are taken now, they are likely to enable effective future delivery of improved options in animal health management for Australian livestock producers. Acting now will provide opportunities to minimise production loss and animal health input costs. This will place Australian producers in a better position to respond to future market pressures regarding animal health and welfare issues.

A delay in responding could mean that Australia's producers could be denied the opportunity to adopt new technologies embraced by overseas competitors. Failure to act is likely to result in continuing declines in the provision of commercial tier veterinary diagnostic services for livestock producers.

If the benefits of more accurate qPCR veterinary diagnostic assays can be realised, each producer should be able to control disease more precisely, and have a full knowledge of the suite of disease problems likely to be encountered in a property-specific manner. By combining the results of repeated diagnostic testing with the livestock producer's experience, production data, market information and weather data, it should be both feasible and profitable to implement a precise on-farm animal health management plan.

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Abbreviations

BCS	body condition score
DP	dressing percentage
FCS	faecal consistency score
HSCW	hot standard carcase weight
IAC	internal amplification control
OPG	oocysts per gram
qPCR	quantitative PCR
RSD	relative standard deviation
RSQ	R squared (coefficient of determination)
WEC	Worm egg counts

1 Background

Internal parasites (strongylid gastrointestinal helminths) have been reported to decrease lamb productivity in extensive grazing sheep enterprises. In an animal health and welfare report by Sackett *et al.* (2006), internal parasites were documented to reduce wool staple strength (Barger and Southcott, 1975b), wool fibre diameter and wool growth (Pullman *et al.*, 1991; Larsen *et al.*, 1995; Simpson, 2000) and also to reduce lamb live weights and growth rates (Pullman *et al.*, 1991; McClure *et al.*, 1999; Liu *et al.*, 2005; Louie *et al.*, 2007). Reduced ewe fertility, reduced lambing percentage and increased mortality rates have also been reported (McClure, 2000; Vagenas *et al.*, 2007). The total national economic loss of income (losses in income, combined with treatment and control costs), attributed to internal parasites (strongylid worms) in this report was calculated to be AUD369 million (Sackett *et al.*, 2006).

For meat (prime) lamb (Merino ewes joined with meat breed rams to produce crossbred lambs raised specifically for slaughter) enterprises that utilised internal parasite control programs, Sackett *et al.,* (2006) quoted the internal parasite losses to be AUD4.93/sheep. However for those enterprises with poor or no control programs, the losses per head reached as high as AUD12.08 per head. The reduced income due to internal parasitism in the winter rainfall zones was AUD4.61 per head for sheep enterprises and AUD7.75 per head for specialised meat lamb enterprises. In addition to AUD4.61 per head reduced income, increased sheep husbandry expenses added an extra AUD0.12 per head for crutching (removal of soiled wool prior to wool harvesting) and AUD0.71 per head for anthelmintic treatments (Sackett *et al.*, 2006).

Diarrhoea is a significant management challenge for sheep enterprises and has been reported to be associated with reduced growth rates (Green *et al.*, 1998) and increased risk of breech fleece faecal soiling (commonly referred to as dag, where faeces adhere to the breech of the lambs and sheep; Figure 1) (Larsen *et al.*, 1999; Broughan and Wall, 2007). Although diarrhoea in lambs is routinely attributed to strongylid worms (Besier and Love, 2003; Sargison, 2004), it is a complex, multi-factorial condition, with infectious agents (parasites, bacteria and viruses) and non-infectious agents (nutrition, fungi and environmental stresses) all reported as contributing factors (Larsen *et al.*, 1994; Skirrow, 1994; Eerens *et al.*, 1998; Larsen *et al.*, 1999; Broughan and Wall, 2007; Belloy *et al.*, 2009; Jacobson *et al.*, 2009b; Williams *et al.*, 2010b).

Both diarrhoea and breech fleece faecal soiling also are major risk factors for cutaneous blowfly myiasis (typically referred to as ‘blowfly strike’) (Morley *et al.*, 1976; Hall and Wall, 1995; Broughan and Wall, 2007). Blowfly strike remains the most prevalent ectoparasitic mediated disease of domestic sheep in sheep-rearing countries worldwide and is also an important welfare issue (Wardhaugh and Morton, 1990; Hall and Wall, 1995; Ward, 2001a; Snoep *et al.*, 2002; Bisdorff and Wall, 2008). Furthermore, both diarrhoea and breech fleece faecal soiling in lambs awaiting slaughter increases the risk of carcase contamination by enteric protozoa and bacteria that are associated with meat spoilage and human food poisoning (Greer *et al.*, 1983; Biss and Hathaway, 1996; Hadley *et al.*, 1997; Garcia *et al.*, 2010). In addition, faecal contamination of carcases is associated with trimming of affected carcase tissue, which in turn limits abattoir productivity (Hadley *et al.*, 1997; Gill *et al.*, 1998b).

Scouring is also a common stimulus for the treatment of sheep with anthelmintics. This increased and often unwarranted use of anthelmintic treatments is an unnecessary cost and increased frequency of treatment has been shown to hasten the development of anthelmintic resistance (Wolstenholme *et al.,* 2004; Coles *et al.,* 2006; Woodgate and Besier, 2010), despite the development of new anthelmintics including derquantel (Little *et al.,* 2011) and monepantel (Kaminsky *et al.,* 2008a and b).

Figure 1. Merino sheep showing varying degrees of breech fleece faecal soiling at the breech ('perianal') area.

Little information exists regarding the effects that protozoan parasites (*Cryptosporidium*, *Giardia* and *Eimeria)* and bacterial pathogens (*Campylobacter, Salmonella, Yersinia* and *Chlamydia)*, have on meat lamb production performances. It is likely that these protozoa and bacteria are collectively one of many multiple factors that potentially can contribute to production losses, reduced carcase profitability and pose an increased risk for incidences of diarrhoea in young, growing lambs. This knowledge gap has justified further investigation involving on-farm and abattoir studies across Australia.

1.1 Protozoan causes of scouring

Eimeria: The protozoan disease, coccidiosis, caused by *Eimeria* species is an important enteric disease of sheep, resulting in diarrhoea, inefficient weight gains, and occasionally death (Chartier and Paraud, 2012). Coccidiosis is a widespread, serious economic disease affecting animals who are preweaned (grazing with dam), recently weaned, or in unsanitary, stressful, or crowded conditions. Animals in feedlots are at increased risk (O'Callaghan *et al.*, 1987; Foreyt, 1990; Taylor and Catchpole, 1994; Coop and Wright, 2000). Despite this, little research has been conducted on *Eimeria* in sheep in Australia. Specifically, little information is available on the prevalence of *Eimeria* and their contribution to scouring in sheep in Australia. Prevention and control of coccidiosis in high-risk settings results in significantly greater weight gains and production, whereas disease with or without treatment is likely to result in inefficient production and economic loss to the producer (Chartier and Paraud, 2012).

Cryptosporidium*:* Few studies have been conducted examining the role of *Cryptosporidium* in sheep scouring. One study (Ryan *et al.,* 2005) that examined 1,647 sheep faecal samples from sheep sent for slaughter in Western Australia reported that *Cryptosporidium* were commonly detected in sheep with 11% of lamb lines and 3% of adult lines being positive for *Cryptosporidium* spp. Lines of sheep positive for *Cryptosporidium* were 9.7 times more likely to be scouring than negative lines (odds ratio 95% CI: 2.3-41.6). In a more recent study, data from 2 WA flocks indicates that HSCW and DP, were negatively associated with positive *Cryptosporidium* detection i.e. lambs positive for *Cryptosporidium* at least once had 1.25kg (6.6%) (P=0.029) and 1.46kg (9.7%) (P<0.001) lighter HSCWs compared to lambs never positive for *Cryptosporidium* for both farms. Similarly, DP were reduced by 1.7% (P=0.022) and 1.9% (P<0.001) in *Cryptosporidium* positive lambs on both farms (Sweeny *et al.,* 2011a and b).

Giardia: *Giardia duodenalis (syn. Giardia lamblia, Giardia intestinalis*) is one of the most common protozoan parasites in humans and animals (Feng and Xiao, 2011). Lambs that are infected with *Giardia* spp. usually display clinical signs of diarrhoea, reduced weight gains (Taylor *et al.*, 1993; Olson *et al.*, 1995; Aloisio *et al.*, 2006), an impaired feed efficiency (Olson *et al.*, 1995) and reduced feed intake (Ralston *et al.*, 2003). As with *Cryptosporidium*, sheep commonly become infected with *Giardia* through inadvertently ingesting pasture or supplementary feed contaminated with cysts (Taylor *et al.*, 1993; Olson *et al.*, 1997; O'Handley and Olson, 2006). It is also common for livestock to become infected with *Giardia* via the consumption of contaminated water (Olson *et al.*, 2004; O'Handley and Olson, 2006; Thompson *et al.*, 2008). Livestock infected with *Giardia* display diarrhoea and in some cases reduced growth rates (Olson *et al.*, 1995; O'Handley *et al.*, 1999; Aloisio *et al.*, 2006).

1.2 Bacterial causes of scouring

Salmonellosis: generally occurs in outbreaks with high morbidity and case fatality rates. Salmonellosis is more common in young (weaner) sheep, although sheep of all ages, sexes and breeds are susceptible (Tatavarthy and Cannons, 2010). Acute severe diarrhoea is the predominant sign with fluid, putrid bloody faeces (Plagemann, 1989; Wray *et al.*, 1991; Nesbakken, 2009; Duffy *et al.*, 2010; Tatavarthy and Cannons, 2010). Diagnosis of salmonellosis is based on the acute nature of the outbreak, clinical signs, typical post-mortem findings, isolation of the organism and a history of predisposing factors (Plagemann, 1989; Wray *et al.*, 1991).

Campylobacteriosis & Yersiniosis: (weaner colitis) is most commonly seen in weaner sheep but can cause outbreaks of diarrhoea in all age-groups. Diarrhoea, ill thrift, depression, weakness and deaths are associated with outbreaks of campylobacteriosis and/or yersiniosis and appear to be an emerging problem on farms with higher stocking rates (McSporran *et al.*, 1984; Buddle *et al.*, 1988; Slee and Button, 1990; Bin-Kun *et al.,* 1994; Friedman *et al.*, 2000; Diergaardt *et al.*, 2004; Juste *et al.,* 2009; Okwori *et al.,* 2009; Wilkes *et al.*, 2009). Diagnosis is based on history, clinical signs, histopathological findings, identification of the causative organism from mucosal scrapings from the caecum and colon and culture of the causative organism. Diagnosis is complicated by the fact that carrier animals exist, and thus more complex, expensive and time-consuming post-mortem procedures such as gut mucosal scrapings and histopathology are required, rather than

culture of the causative organism alone, to definitively diagnose the disease (Okwori *et al.,* 2009; Wilkes *et al.*, 2009).

Chlamydia: is mainly associated with arthritis and abortion but has been linked with acute enteritis and may be an undiagnosed cause of scouring and dag in some sheep populations (McCauley *et al.*, 2010; Pantchev *et al.*, 2010).

This project set out to develop a multiplex high throughput quantitative PCR diagnostic technique that would detect and quantify bacteria (*Salmonella, Campylobacter, Yersinia* and *Chlamydia),* and protozoa *(Eimeria, Cryptosporidium* and *Giardia*), in faecal samples, investigate the association between the prevalence and pathogen load of these pathogens with dag score, FCS, live weight, HSCW and DP and develop and extend management strategies which can minimise the risk of scouring.

In addition, we aimed to develop a qPCR assay for *Escherichia coli* O157:H7 and determine the prevalence and pathogen load of zoonotic pathogens (*Salmonella, Campylobacter, Cryptosporidium, Giardia* and *Escherichia coli* O157:H7 using qPCR in sheep faeces collected from WA sale yards (Muchea and Katanning) and in abattoir effluent (Katanning) (inlet and outlet).

2 Projective objectives

2.1 Original project objectives

The original project objectives of this project were to:

1. Develop a high throughput qPCR multiplex assay, [adapting existing primer sequences from the literature and from previous research in our laboratory] for identifying and quantitating *Salmonella, Campylobacter, Yersinia, Eimeria* and *Cryptosporidium* in sheep faeces.
2. Use qPCR and WECs to monitor flocks of sheep on four farms to determine if there is an association between the prevalence of bacteria, protozoans and WEC with scouring
3. Determine if mixed infections of strongyle worms and protozoans/bacteria augment each other such that sheep infected with strongyle worms and protozoans /bacteria suffer more scouring than those with either worms or protozoans /bacterial infections alone
4. Develop molecular tools to examine if scouring and production loss is associated with particular protozoan or bacterial species/genotypes and with particular management systems under Australian conditions.
5. Develop management strategies for sheep to minimise the effects of parasite/bacterial burdens and scouring.
6. Extension of recommendations for management strategies to minimise the risk of bacterial and protozoal scouring in prime lambs to sheep producers

2.2 Revised project objectives

Following discussions with the MLA and as a result of initial data analysis*, the revised objectives were to:

1. Develop a high throughput qPCR multiplex assay, [adapting existing primer sequences from the literature and from previous research in our laboratory] for identifying and quantitating *Salmonella, Campylobacter, Yersinia, Chlamydia*, *Eimeria, Giardia*, *Cryptosporidium, Haemonchus, Teladorsagia and Trichostrongylus* in sheep faeces.
2. Use qPCR and WECs to monitor flocks of sheep on four farms to determine if there is an association between the prevalence of bacteria, protozoans and WEC with scouring
3. Develop molecular tools to examine if scouring and production loss is associated with particular protozoan or bacterial species/genotypes and with particular management systems under Australian conditions.
4. Develop a qPCR assay for *Escherichia coli* O157:H7 and determine the prevalence and pathogen load of zoonotic pathogens (*Salmonella, Campylobacter, Cryptosporidium, Giardia* and *Escherichia coli* O157:H7 in sheep faeces collected from WA sale yards (Muchea and Katanning) and in abattoir effluent (Katanning) (inlet and outlet).
5. Extension of recommendations for management strategies to minimise the risk of bacterial and protozoal scouring in prime lambs to sheep producers.

*For the original objective 3 (determination if mixed infections of worms and protozoans/bacteria augment each other), it was proposed that a study would be conducted on one farm to determine the impact of mixed strongyle and protozoan/bacterial infections on farms. Ewes were to be identified and split into two groups (100 ewes per group) with approximately equal faecal WEC. Group A ewes were to be grazed in their normal paddock and the group B ewes were to be treated with long acting moxidectin and an ivermectin-benzimidazole slow-release rumen capsule and subsequently grazed on a larvae-free paddock with a similar amount of feed on offer as the group A ewes. All animals were to be monitored over 4 months for pathogen prevalence and load as well as production measures.
The success of this section of the study largely rested on being able to generate worm-free paddocks and the ability to maintain a mob of either worm-free sheep or sheep with very low worm burdens in order to determine if sheep exposed to strongyle worms and protozoans/bacteria suffered more scouring than those exposed to protozoa/bacteria alone.
In practice however, attempts at maintaining a worm-free paddock were very difficult. In addition, data from the project revealing the complex nature of the relationships between pathogens with faecal attributes, growth and carcass attributes highlighted several risks associated with performing a single study comparing the effects of protozoal and bacterial pathogens in grazing sheep with and without strongylid challenge. For example, a large number of farm interactions were observed for prevalence and also outcome, so variability between regions, districts or even between paddocks may impact the outcome of a single experiment. Furthermore, shedding of potentially zoonotic pathogens (*Giardia, Cryptosporidium, Salmonella,* etc) has implications beyond the farm gate, particularly for the processing and retail

sector, and these have not been well explored to date. As a result of this, the project objectives and milestones were changed.

2.3 Milestones

The following milestones were agreed on:

Table 1: Milestones for project AHE.0027.

Achievement Criteria		Due Date
1	Contract signing	20-Jun-2010
2	Report on final protocol, RA appointed and all animal ethics approval obtained	02-Mar-2011
3	**GO NO GO Decision by MLA Project Manager** Two multiplex (bacterial and worm) qPCR's developed. If multiplexing not achieved by this point, then project will proceed using partial and simplex qPCRs. qPCR's validated on faecal samples	30-Apr-2012
4	Faecal samples collection completed	01-Sept-2012
5	Analysis of the prevalence of bacteria, protozoa and worms in sheep and associations between scouring using the qPCR assay and WECs	20-Dec-2012
6	Determination of the prevalence and pathogen load of faecal zoonotic pathogens in lambs at sale yards and in abattoir effluent.	30-Nov-2014
7	Data processing and write-up of 6 papers for publication, extension planning on identified risks and modified management practices to sheep farmers and final report preparation	31-May-2015

3 Methodology

3.1 Animals and faecal sample collection (Milestones 1-5)

Faecal samples were collected from cross-bred lambs from 8 different farms (Table 2). Farms were selected to represent a wide range of environmental conditions under which sheep are typically farmed in Australia, including summer dominant, winter dominant and Mediterranean (hot dry summer, cool wet winter) rainfall patterns. Farms were located in 4 states, specifically Western Australia (WA), New South Wales (NSW), Victoria (Vic) and South Australia (SA). Flocks were selected for inclusion in the study before commencement of lambing on the basis that ewe numbers in a single mob were sufficiently large to supply at least 110 lambs for the study from birth until slaughter.

Lambs were born and reared in paddocks for the duration of the study. Lambs were individually identified with ear tags at lamb marking and faecal samples were collected on 3 occasions (i.e. the same animals were sampled on each occasion), specifically weaning (approximately 12 weeks of age), post-weaning (approximately 19 weeks) and pre-slaughter (approximately 29 weeks).

Table 2. Sheep farms sampled during the present study.

Farm	District	Mean annual rainfall (mm)	Farm size (Ha)	Number of sheep	Breed	Commencement of lambing	Winter stocking rate (DSE/Ha)
SA1	Wirrega, SA	430	1040	1800	Suffolk	April	10
SA2	Struan, SA	550	1500	5500	BL/Merino x Suffolk	June	15
Vic1	Rosedale, Vic	620	300	300 [a]	BL/Merino x Dorset and Southdown	July	10
Vic2	Ballarat, Vic	750	1960	7000	Merino x Suffolk	August	13
NSW	Armidale, NSW	495	2958	1000	BL/Merino	May-August	20
WA1	Pingelly, WA	450	1500	1350	Merino x Suffolk	July	12
WA2	West Arthur, WA	500	1250	1750	Merino x Suffolk	August	10
WA3	Frankland, WA	550	560	3300	Merino x Suffolk	July	21

Ha: hectares
DSE: dry sheep equivalent (standard unit used to compare livestock carrying capacity)
SA - South Australia; Vic – Victoria; NSW – New South Wales; WA – Western Australia
BL: Border Leicester

Faecal samples were collected from a combination of Sheep CRC Information Nucleus Flock (INF) and non-INF flocks over 3 sampling periods from 2011/2012-drop lambs. Two mobs (~80 in number) were selected from each flock for sampling (~160 lambs per site). All 160 lambs were to be sampled on 3 occasions (i.e the same animals are sampled on each occasion) at weaning (approx. 12 weeks), post-weaning (1 sample within the postweaning period, when lambs are scheduled for weighing and/or WEC) and pre-slaughter (one week prior to slaughter to avoid stress to the lambs). The main requirement was that each mob needed to be XB lambs that were scheduled for slaughter and recording of carcass weight and DP.

At each sampling occasion, dag score, FCS and live weight of each animal were recorded. Dag score was measured using a scale of 1 (no evidence of breech faecal soiling) to 5 (very severe breech faecal soiling extending down the hind legs to, or below the hocks) used for sheep (Australian Wool Innovation *et al*., 2007). FCS was measured using a scale of 1 (hard, dry pellet) to 5 (liquid/fluid diarrhoea) previously described (Greeff and Karlsson, 1997). Faecal samples were placed on ice until transported to the lab and then stored in the refrigerator (4.0°C). Lambs were slaughtered at commercial abattoirs. HSCW and DP were measured for all lambs. All procedures were approved and monitored by Murdoch University Animal Ethics Committee (approval number R2352/10).

As the main aim of the project was to determine the production impact of bacterial and protozoan infections on sheep, where possible we specifically targeted flocks with a history of scouring and preferably flocks with a history of these infections. Farms sampled in Victoria were Millring (Paulets) in Toongabbie and Lal Lal Estate (Fiskens) in Ballarat as both of these farms had a history of bacterial scouring. [Note that the Paulet lambs were grazed at another block at Rosedale, about 20 km east of their Toongabbie farm]. Similarly, the private flock in SA (Wirrega) also had a history of bacterial scouring. The WA flocks were chosen, as they were known to have a history of protozoan scouring.

3.2 qPCR development and validation (Milestones 1-5)

Genomic DNA was extracted from 250 mg of each faecal sample using a Power Soil DNA Kit (MolBio, Carlsbad, California). A negative control (no faecal sample) was used in each extraction group. An internal amplification control (IAC) consisted of a fragment of a coding region from Jembrana Disease Virus (JDV) cloned into a pGEM-T vector (Promega) was used as previously described (Yang *et al.,* 2013). The IAC primers were JDVF (5'-GGT AGT GCT GAA AGA CAT T-3') and JDVR (5'-ATG TAG CTT GAC CGG AAG T-3') and the probe was 5'- (Cy5) 5'-TGC CCG CTG CCT CAG TAG TGC-BHQ2-3'.

The following terms were used (Table 3).

Table 3. Definitions

Term	Definition
Inclusivity	Measure of sensitivity: Measured as a percentage confirmed (i.e. 10 of 10 positive = 100% inclusive
Exclusivity	Measure of genotyped negative samples not identified by the multiplex. (i.e. 10 of 10 negative = 100% exclusive)
Limit of Detection	Measure of the lowest amount of DNA detected by the multiplex.
Reproducibility	Comparison of Ct value data from the multiplex over several different experiments performed by several different technicians.
IAC	Internal Amplification Control: an internal qPCR control designed to show a positive qPCR result in every sample, therefore controlling for false negatives results in qPCR resulting from inhibition, or qPCR failure. In this case, the IAC amplifies a fragment of non-related DNA Jembrana Disease Virus (JDV) that has been cloned into a plasmid vector and a constant amount added to each PCR.
NTC	No Template Control. A negative qPCR control designed to show that there has been no non-specific amplification in the unknown samples due to DNA contamination.
% RSD	% Relative Standard Deviation. A measure of inter-experiment variability calculated as: % RSD = Average of values /Standard Deviation of values x 100%
Ct Value	Cycle threshold: qPCR cycle at which the amplification threshold is crossed. Used to measure quantitation in qPCR
RSQ (R squared)	The R squared statistic describing the goodness of fit for the log linear regression. Perfect theoretical value 1.0
Efficiency	Amplification efficiency (*E*) was estimated by using the slope of the standard curve and the formula $E = -1+10^{(-1/\text{slope})}$. A reaction with 100% efficiency will generate a slope of −3.32.
rpoB	rpoB is the β subunit of the *Yersinia* RNA polymerase gene.
ompF	*Salmonella* outer membrane protein also known as OmpF porin.
ompA	*Chlamydia* outer membrane protein cell surface antigen gene.
PurA	*Campylobacter* purine biosynthesis gene
GDH	*Giardia* glutamate dehydrogenase gene
Actin	*Cryptosporidium* actin gene
18S	18S ribosomal RNA gene
ITS1	*Haemonchus* ribosomal internal transcribed spacer 1
ITS2	*Teladorsagia* ribosomal internal transcribed spacer 2
FAM, Cy5, Joe, Rox	Flurophores attached to species-specific probes that are "read" by the qPCR machine allowing for detection and quantitation of individual species

Partial multiplexes consisting of the following were designed:

Multiplex 1 to detect C*ryptosporidium, Giardia, Eimeria and Salmonella spp.*

Multiplex 2 to detect *Yersinia, Campylobacter and Chlamydia* and

Multiplex 3 to detect *Haemonchus contortus, Trichostrongylus spp. and Teladorsagia circumcincta* (Table 4).

Note there is currently no IAC in multiplex 1. This is because only 4 channels are available for detection on the Rotor-Gene 6.0. If the protozoan assay were to be offered for use by commercial diagnostic service providers, then the *Salmonella* qPCR would be removed and replaced with an IAC. For the purposes of the current study however, it is not necessary to have an IAC in the multiplex 1 qPCR as the IAC's in the remaining 2 multiplexes are used for determining if the assays are working properly and if there is any PCR inhibitiors in the samples. In the new Rotor-gene 6000, 5 channels are available, called Green (=Fam), Yellow (=Joe), Orange (=Rox), Red (=Cy5) and Crimson. The IAC control can now be incorporated into into Crimson channel for multiplex 1.

Non-ribosomal regions were used for bacterial assays as per published assays. Actin and GDH were chosen for the *Cryptosporidium* and *Giardia* assays respectively as both loci were considerably more specific and reliable than the 18S locus (Table 5).

Note: the loci for *Haemochus, Teladorsagia* and *Trichostrongylus* were originally 18S but this was changed to ITS1, ITS2 and ITS1 respectively following collaborative research on primer sensitivity and specificity with Dr. Peter Hunt at CSIRO (see section 3.2.5 for more detail).

Table 4. Summary of the three multiplex assays to detect *Salmonella, Campylobacter, Chlamydia, Yersinia, Cryptosporidium, Giardia, Eimeria, Haemonchus contortus, Teladorsagia circumcincta* and *Trichostrongylus spp*. in sheep.

	Pathogen/ Internal control	***Targeted gene***	***Fluorescent Channel***
Multiplex I	*Cryptosporidium*	*Actin*	*FAM*
	Giardia	*GDH*	*Cy5*
	Eimeria	*18S rDNA*	*Joe*
	Salmonella	*ompF*	*Rox*
	IAC	*JDV*	
Multiplex II	*Yersinia*	*RpoB*	*FAM*
	Campylobacter	*18S rDNA*	*Joe*
	Chlamydia	*ompA*	*Rox*
	IAC	*JDV*	*Cy5*
Multiplex III	*Haemonchus contortus*	*ITS1 rDNA*	*FAM*
	Teladorsagia circumcincta	*ITS2 rDNA*	*Joe*
	Trichostrongylus spp.	*ITS1 rDNA*	*Rox*
	IAC	*JDV*	*Cy5*

All probes were synthesised using Black Hole Quenchers™ (BHQ™). This is because some commonly used quenchers (such as DABCYL and TAMRA), suffer from a number of

drawbacks, including poor spectral overlap between the fluorescent dye and quencher molecule (DABCYL) or inherent fluorescence of the quencher (TAMRA), resulting in a relatively poor signal-to-noise ratio. Black Hole Quencher™ (BHQ™) molecules have been developed to overcome these drawbacks. They have no native fluorescence, resulting in lower background fluorescence, increase signal-to-noise ratios, providing higher sensitivity, maximize spectral overlap, increasing efficiency of quenching and enable wider choice of reporter dyes for multiplexing assays.

Table 5. Summary of primers and probes used in this project.

Pathogen	Gene	Forward primer	Reverse primer	Probe	Amplicon size
Cryptosporidium	Actin	ATCGTGAAAGAATGACWCAAATTATGTT	ACCTTCATAAATTGGAACGGTGTG	CCAGCAATGTATGTTAATA	161bp
Campylobacter	PurA	CGCCCTTATCCTCAGTAGGAAA	TCAGCAGGCGCTTTAACAG	AGCTCCATTTCCCACACGCGTTGC	121bp
Eimeria	18S rDNA	CGAATGGCTCATTAAAACAGTTATAGTT	CGCATGTATTAGCCATAGAATTACCA	ATGGTCTCTTCCTACATGGA	85bp
Salmonella	ompF	TCGCCGGTCGTTGTCCAT	AACCGCAAACGCAGCAGAA	ACGTGACGACCCACGGCTTTAC	96bp
Giardia	GDH	GGGCAAGTCCGACAACGA	GCACATCTCCTCCAGGAAGTAGAC	TCATGCGCTTCTGCCAG	261bp
Yersinia	RpoB	GGTGCTTCTCTGATTCCATTCTTG	CGCCTGACGTTGCATGTTC	AACACGATGACGCCAACCGTGC	78bp
Chlamydia	ompA	CCATGTGATCCTTGCGCTACT	TGTCGAAAACATAATCTCCGTAAAAT	TGCGACGCGATTAGCTTACGCGTAG	76bp
Haemonchus	ITS1	CATATACATGCAACGTGATGTTATGAA	GCTCAGGTTGCATTATACAAATGATAAA	ATGGCGACGATGTTC *	92bp
Teladorsagia	ITS2*	TCTGGTTCAGGGTTGTTAATGAAACTA	CCGTCGTACGTCATGTTGCAT	TGTGGCTAACATATAACACTGTTTGTCGA	143bp
Trichostrongylus	ITS1	AGTGGCGCCTGTGATTGTTC	TGCGTACTCAACCACCACTA	TGCGAAGTTCCCATCTATGATGGTTGA	114bp

*This probe was prepared by substitution of C-5 propynyl-dC (pdC) for dC and C-5 propynyl-dU (pdU) for dT to enhance base pairing and duplex stability.

3.2.1 Quantitation

For *Cryptosporidium*, target copy numbers detected were converted to numbers of oocysts based on the fact that the actin gene in *Cryptosporidium* is a single copy gene (Kim *et al.,* 1992) and there are 4 haploid sporozoites per oocyst. Therefore, every 4 copies of actin detected by qPCR were equivalent to 1 oocyst.

For *Giardia*, copy numbers detected were converted to cyst numbers on the basis that the *gdh* gene in *Giardia* is a single copy gene (Yee and Denis, 1992) and the fact that there are 4 haploid nuclei per cyst. Therefore, every 4 copies of *gdh* detected by qPCR were equivalent to 1 cyst.

Eimeria oocysts contain eight haploid sporozoites/nuclei and the 18S ribosomal gene is present in 140 copies per haploid genome in *E. tenella* (Lim *et al.,* 2012; http://www.genedb.org/Homepage/Etenella) (i.e. there are 1,120 18S target copies per oocyst). Therefore, for the plasmid detection, the mean number of targets detected by qPCR (for 3 replicate faecal samples) was then divided by 140 and then by 8 (or by 1,120) to equate to number of oocysts detected per 15 µl reaction and then extrapolated up to oocysts per gram (OPG) of faeces. [Note: *Eimeria* oocysts collected directly from the rectum are unsporulated, i.e. diploid (containing two genomes). However due to transit times interstate, microscopic examination revealed that the vast majority of oocysts had sporulated. Hence our decision to base the calculations on sporulated oocysts].

For bacteria, template copy numbers were converted to numbers of organism present on the basis that *purA* (*Campylobacter* spp.), *ompF* (*S. enterica*), *ompA (Chlamydia), rpoB (Yersinia)* are single copy genes (Mollet *et al.,* 1997; Lan and Igo, 1998; Pearson *et al.,* 2007; Tatavarthy and Cannons, 2010; GenBank CP000814) and bacterial genomes are haploid. Therefore the detected plasmid numbers were equivalent to the numbers of *Campylobacter* spp., *S. enterica*, *Chlamydia* and *Yersinia* organisms.

For nematodes, target copy numbers detected were converted to crude estimates of numbers of eggs per gram (epg) of faeces based on the reported copy number of 212 for the ribosomal unit in *H. contortus* (Vierna *et al*., 2013) and a report that eggs contain 90 cells, 4 hours ex host (Veglia, 1913). As samples were at least 48 h old upon receipt by Murdoch University, target copy numbers detected were divided by 228,960 to convert to numbers of eggs per µl and then converted to epg).

3.2.2 Specificity of the qPCR multiplex assays

The qPCR reactions were set up in a DNA free laminar flow with a set of PCR-only pipettes. All the PCR reagents and PCR tubes/racks were set on ice. The DNA concentration varied between 50 to 200 ng / µl for different samples. The concentration of DNA in samples was adjusted so that a uniform amount of DNA (50ng) was added to each PCR.

Conventional end-point PCR with gel electrophoresis and sequencing confirmed the specificity of primer sets, by yielding PCR products of expected size for target species, and no product for non-target species as well as producing the correct sequence as determined by BlastN searches of GenBank. This was further confirmed by qPCR results, showing only one specific PCR product for each set of primers. The specificity of the three multiplex qPCR assays was determined for each individual pathogen using the following range of DNA from bacteria, parasites and strongyle worms from different sources: *Campylobacter jejuni, Campylobacter coli, Salmonella enterica* serovar Typhimurium*, Salmonella enterica* serovar wandsbek II 21:$_{z10:z6}$, *Salmonella enterica* serovar bredeney*, Salmonella enterica* serovar muenchen*, Salmonella enterica* serovar adelaide*, Salmonella enterica* serovar waycross, *Salmonella enterica* serovar infantis, *Chlamydia pecorum*, *Chlamydia abortus*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis, Yersinia intermedia, Yersinia frederiksenii and Yersinia pestis (Department of Agriculture, WA) Streptococcus bovis* (ATCC 33317), *Enterococcus durans* (ATCC 11576), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6633), *Serratia marcescens* (ATCC 14756 pigmented), *Citrobacter freundii* (NCTC 9750), *Enterobacter cloacae* (ATCC 13047), *Coxiella burnetii*, *Giardia duodenalis* assemblages A and E from sheep, *Cryptosporidium muris*, *Cryptosporidium parvum, Cryptosporidium hominis, Cryptosporidium meleagridis, Cryptosporidium felis, Cryptosporidium andersoni*, *Cryptosporidium serpentis*, *Cryptosporidium canis, Cryptosporidium suis, Cryptosporidium bovis, Cryptosporidium fayeri, Cryptosporidium macropodum, Cryptosporidium ryanae*, *Cryptosporidium xiaoi, Cryptosporidium ubiquitum, Cryptosporidium tyzzeri, Cryptosporidium* mouse genotype II, *Cryptosporidium scrofarum*, *Eimeria crandallis, Eimeria weybridgensis, Eimeria ovina, Eimeria ovinoidalis, Eimeria ahsata, Eimeria tilliquae, Eimeria trichosuri, Eimeria sp.* from kangaroos (K2175, K2336 and K2534), *Isospora, Tenebrio, Cyclospora sp., Toxoplasma gondii, Trichostrongylus colubriformis, Teladorsagia circumcincta* and *Haemonchus contortus.*

Only the targeted pathogen was detected from the specific channel. For exclusivity testing, 50 ng of purified DNA from various pathogens was mixed with 50 ng of DNA from faecal

samples and then tested for primer specificity. For inclusivity testing, 10-50 ng of total DNA extracted from known sheep positives were used. All primer sets were 100% exclusive (i.e. only detected the pathogen they were designed to detect) and 100% inclusive (detected all known sheep-derived positives) when tested on reference isolates. No template controls (NTC) produced no amplification and the IAC functions well with 100% of IAC qPCR samples showing a positive qPCR result at the Cy5 Channel. IAC Ct values were shown to have between 1.1%-2.3% RSD and were therefore highly reproducible.

The specificity of the qPCR assays were further tested on faecal samples by sequencing random PCR products to confirm that the correct pathogen had been amplified. The strongyle qPCR assay was tested in collaboration with CSIRO as discussed in section 3.2.4.

3.2.3 Sensitivity of the qPCR multiplex assays

PCR products amplified from the 10 pathogens were cloned into the pGEMT-vector (Promega) and transformed into *E. coli* competent cells. Plasmid DNA for each pathogen was isolated by alkali\SDS lysis followed by column purification using QIAprep Spin Columns (Qiagen) in accordance with the manufacturer's protocol. Plasmid mini-preparations were sequenced using T7 and SP6 sequencing primers (Stratagene, La Jolla, CA, USA) and clones with the correct sequence then used as positive controls for generating a standard curve. The standard curves were used for determining the detection limits of each assay and enumeration of pathogens in stool samples.

The plasmid copy numbers were calculated based on the plasmid size (base pairs) and DNA concentration. 10-fold series dilutions for each of the 10 plasmids were conducted from 10,000 copies down to 1 copy of the plasmid template for sensitivity testing. Plasmid dilutions were then spiked into faecal samples (n=3) that were PCR negative for all pathogens and mean detection limits, RSQ values and % RDS were calculated (Table 6).

Table 6. Mean detection sensitivity and reproducibility of the 10 qPCR assays for 3 faecal samples spiked with plasmid dilutions.

Targeted Pathogen	Mean detection limit per µl	Sample 1 RSQ	Sample 2 RSQ	Sample 3 RSQ	Mean RSQ	% RDS
Cryptosporidium	2 oocysts	1.00	0.99	1.00	0.99	0.15
Giardia	1 cyst	0.98	0.98	0.99	0.98	0.55
Eimeria	0.15 oocysts	1.00	0.97	0.98	0.98	1.10
Campylobacter	5 bacteria	0.99	0.99	0.99	0.99	0.35
Chlamydia	5 bacteria	0.99	0.98	0.97	0.98	0.91
Salmonella	5 bacteria	0.99	1.00	1.00	0.99	0.45
Yersinia	10 bacteria	0.98	0.99	1.00	0.99	0.76
Haemonchus	<1 egg/L1 larvae	0.97	0.97	0.99	0.98	0.93
Teladorsagia	<1egg/L1 larvae	0.99	0.99	0.99	0.99	0.12
Trichostrongylus	<1egg/L1 larvae	0.98	0.97	0.99	0.98	1.01

RSQ (R squared): the square of the coefficient of regression indicates how well the line fits the data. A quantitative real time PCR linear regression should have an R-squared value of > 0.98. The mean RSQ values in the present analysis range between 0.98 to 0.99 indicating that the quantitation is reliable.

3.2.4 Investigation of inhibition and efficiency

The difficulty with applying PCR methods to faeces has been attributed to the difficulty of removing inhibitors. The extraction and purification of DNA from faecal samples largely influences the amount of these inhibitory substances. Previous testing in our laboratory has indicated that the PowerSoil® DNA Isolation Kit (MolBio Cat. No.12888-100), enabled isolation of high quality DNA from faecal samples (data not shown).

Amplification efficiency (*E*) (which is a measure of inhibition), was estimated by using the slope of the standard curve and the formula $E = -1+10^{(-1/\text{slope})}$. A reaction with 100% efficiency will generate a slope of −3.32. A PCR efficiency less than or greater than 100% can indicate the presence of inhibitors in the reaction but reaction efficiencies between 90 and 110% are typically acceptable. To estimate amplification efficiency on faecal samples, serial dilutions of individual DNA samples (undiluted, 1:10, 1:100) were performed and multiple qPCR reactions were conducted on each dilution. The *Ct* values were then plotted versus the log base 10 of the dilution and a linear regression was performed using the Rotor-Gene 3000 software. Amplification efficiency of faecal samples ranged from 88.9% to 110.1%. Mean efficiencies from 3 separate reactions are reported in Table 7.

Inhibition in faecal samples was also measured using the IACs as the IACs were added to all faecal DNA samples to detect any PCR inhibitors present in the extracted DNA. If any inhibition is present in a sample, the IAC will not produce a signal. In our hands, the incidence of PCR inhibition was about 2%. If inhibition was evident, then the sample was diluted and re-amplified.

Table 7. Mean amplification efficiencies of qPCRs on the various pathogens in faecal samples.

Pathogen	Mean Efficiency (%)
Cryptosporidium	108.4
Giardia	95.4
Eimeria	95.3
Chlamydia	94.4
Campylobacter	103.8
Salmonella	97.4
Yersinia	102.3
Haemonchus	107.3
Teladorsagia	94.0
Trichostrongylus	100.5

3.2.5 Optimisation of the strongyle qPCR on faecal samples

This was accomplished in collaboration with Dr. Peter Hunt from CSIRO. 12 faecal samples from Murdoch were sent to CSIRO for testing and Dr Hunt sent 12 samples to Murdoch. Initial testing of 12 samples sent from Murdoch to CSIRO revealed that both the Murdoch *Teladorsagia* and *Haemonchus* probes lacked specificity (Table 8). For example, in Table 8 it can be seen that the Murdoch assay detected 6 *Haemonchus* positives, while CSIRO detected no *Haemonchus* positives. Sequencing of these positives revealed that all 6 were *Trichostrongylus* spp. indicating that the *Haemonchus* primers were cross-reacting with *Trichostrongylus DNA.* As a result of this, new primers and probes were designed for *Haemonchus* at the ITS1 locus and the probe was modified to include C-5 propynyl-dC/dU to

enhance base pairing and duplex stability. A new 5' primer was also designed for *Teladorsagia* at the ITS2 locus and used with the original 3' primer and the probe. Another comparison was conducted on the 12 isolates from CSIRO with the new primers and probes (Table 9).

Table 8. Comparison of amplification of strongyle worms by qPCR between Murdoch and CSIRO on 12 isolates from Murdoch. Te = *Teladorsagia* qPCR, Trich = *Trichostrongylus* qPCR and Hae = *Haemonchus* qPCR. na= no amplification. Note Ct= Cq.

Sample	CSIRO Te Cq	MU Te Ct	CSIRO Trich Cq	MU Trich Ct	CSIRO Hae (Cq)	MU Hae (Ct)
JF1	30.36	28.1	30.71	26.04	na	na
JF10	42.68	32.44	27.35	22.69	na	30.95
JF12	na	na	27.74	22.54	na	30.59
JF14	25.96	23.7	26.03	21.98	na	31.07
JF15	33.53	28.63	25.06	19.62	na	28.55
JF16	na	na	30.16	26.2	na	na
JF17	35.11	28.92	28.53	22.93	na	na
JF22	na	na	28.4	23.54	na	na
JF28	25.42	23.52	25.4	22.73	na	na
JF4	na	na	28.61	22.94	na	30.62
JF9	na	34.12	28.07	21.81	na	30.65
JF64	26.63	25.51	24.65	22.55	na	na

Table 9. Comparison of amplification of strongyle worms by qPCR between Murdoch and CSIRO on 12 isolates from CSIRO. Te = *Teladorsagia* qPCR, Trich = *Trichostrongylus* qPCR and Hae = *Haemonchus* qPCR. na= no amplification.

Sample	Haem MU 1 ul DNA	Haem MU 1ul of 1:10 dltn	*Haem CSIRO* 1:10 dil	Tela MU 1 ul DNA	Tela MU 1ul of 1:10 dltn	*Tela CSIRO* 1:10 dil	Trich MU 1 ul DNA	Trich MU 1ul of 1:10 dltn	*Trich spp. CSIRO* 1:10 dil
CS 1	30.48	34.83	36.26	30.37	31.52	39.3	25.16	29.27	31.64
CS 2	27.18	30.67	31.67	28.28	na	42.2	25.0	28.74	31.78
CS 3	26.35	29.35	29.13	na	29.77	37.59	25.23	29	31.14
CS 4	na	na	na	23.92	28.31	32.38	na	na	na
CS 5	na	na	na	27.21	32.36	38.68	na	na	na
CS 6	na	na	na	28.31	34.53	na	24.12	29.58	29.57
CS 7	28.87	33.41	32.94	na	na	na	na	na	na
CS 8	30.2	35.19	34.8	26.69	31.07	39.03	27.95	33.34	32.4
CS 9	31.82	na	na	25.82	na	na	27.74	32.3	33.79
CS 10	30.66	34.83	41.57	23.92	29.51	33.7	na	na	na
CS 11	33.99	na	na	23.87	31.37	32.27	24.48	28.84	28.59
CS 12	na	na	na	24.62	28.97	33.16	27.19	36.06	31.7

The results of this analysis show that both the Murdoch and CSIRO stronglye qPCRs are comparable.

3.3 Faecal worm egg counts (Milestones 1-5)

Faecal worm egg counts were performed using a modified McMaster technique (Lyndal-Murphy, 1993). Two grams of faeces were used from each sample and each egg counted represented 50 eggs/g (epg) of faeces. Insufficient faecal material was available for some samples collected at the first sampling (weaning) and as a result WEC data were missing for some animals at this time point.

3.4 Genotyping (Milestones 1-5)

For protozoan parasites and bacteria, genotyping was conducted as described below.

3.4.1 Cryptosporidium

All *Cryptosporidium* qPCR positives were also amplified at the 18S ribosomal RNA (rRNA) locus using a nested protocol previously described (Ryan *et al*., 2003). All positives were also screened using a *C. parvum* and *C. hominis* specific qPCR at a unique *Cryptosporidium* specific protein-coding locus previously described (Yang *et al.,* 2009; 2013; Morgan *et al.,* 1997).

Sub-genotyping of *C. parvum* isolates was performed using a two-step nested PCR to amplify a ~832 bp fragment of the *gp60* gene as described (Ng *et al.,* 2008). Subtyping of *C. ubiquitum* was performed using a two-step nested PCR to amplify a ~ 948 bp fragment of the *gp60* gene as described (Li *et al.,* 2014).

3.4.2 Giardia

All *Giardia* qPCR positives were also amplified using assemblage specific primers at the triose phosphate isomerase (*tpi*) locus as previously described (Geurden *et al.*, 2008a). A subset of positives (n=32), were also amplified using a heminested PCR at the *beta-giardin* locus using primers BGexF: 5'- CCCGACGACCTCACCCGCAGT – 3' and BGRev: 5'-GCTCGGCCTTCTCGCGGTCG - 3 for the primary reaction with a predicted PCR product size of 682bp. The forward primer BGinF: 5' – CCTTGCGGAGATGGGCGACACA – 3' was used with BGRev in the secondary PCR with a predicted PCR product size of 380bp. The following cycling conditions were used for both primary and secondary PCRs: 1 cycle of 94 ºC for 3 min, followed by 45 cycles of 94 ºC for 30 sec, 55 ºC for 30 sec and 72 ºC for 1min with a final extension of 72 ºC for 7 min. The same positives were also sequenced at the *gdh* locus using the *gdh* qPCR primers (Table 5).

3.4.3 Eimeria

A subset of 5 positives from each sampling at each farm (n = 120) were amplified at the 18S ribosomal RNA (rRNA) locus using a two-step nested protocol previously described which produced a 497-498 bp product (Pieniazek *et al.,* 1996).

3.4.4 Campylobacter

A subset of five samples that were positive for *Campylobacter* spp. from each sampling on each farm (n = 120) were subjected to PCR for the *Campylobacter* spp. 16S rRNA gene (287 bp amplicon) using primers and PCR conditions described by Lubeck *et al.* (2003).

3.4.5 Salmonella

A subset of five samples that were positive for *S. enterica* from each sampling on each farm (n = 120) were subjected to PCR for the *S. enterica* ompF gene (578 bp amplicon) using primers and PCR conditions described by Tatavarthy and Cannons (2010).

3.4.6 Chlamydia

A *Chlamydia abortus* species specific qPCR, also based on the *ompA* gene, which produces an 86 bp product, was amplified using the forward primer CpaOMP1-F 5'-GCAACTGACACTAAGTCGGCTACA-3', the reverse primer CpaOMP1-R 5'-ACAAGCATGTTCAATCGATAAGAGA- 3' and the probe CpaOMP1-Sb 5'-dFAM-TAAATACCACGAATGGCAAGTTGGTTTAGCG- BHQ-1-3' as described previously (Pantchev *et al.,* 2009).

3.4.7 Yersinia

Pathogenic *Yersinia enterocolitica* were screened in all samples by a qPCR using primers and probe sequences (specific to virulent *Y. enterocolitica yst* gene) as previously described (Ibrahim *et al.,* 1997; Zheng *et al.,* 2007).

A subset of up to 5 qPCR *Yersinia* positives were randomly chosen from each sampling in each farm, (*n* = 111) were amplified at the *rpoB* locus using a nested PCR with the following nested primers (designed for this study using Primer 3); YSNexF: 5'-GGT GAA AGA GTT CTT TGG TTC C-3' and YSNExR: 5'- AAG ATG GAG TCT TCG AAG TTG-3' which produce a PCR product size of 948bp and the internal primers YSNinF: 5'–CAA CCC GTT GTC TGA GAT TAC G-3' and YSNinR 5'-ATT GGC TCA CCC AGA TTC AC-3' which produced a PCR product size of 779 bp. The 25 µl PCR reaction contained 2.5 µl of 10 × Kapa PCR buffer, 1.5 µl 25mM $MgCl_2$, 1 µl 1mM dNTP's, 10 pM of each primer, 1 U of KapaTaq, 1 µl of DNA and 16.9 µl of H_2O. Both primary and secondary PCRs were conducted with the same cycling conditions; 1 cycle of 94 ºC for 3 min, followed by 35 cycles of 94 ºC for 30 s, 58 ºC for 30 s and 72 ºC for 1 min and a final extension of 72 ºC for 5 min.

3.4.8 Sequencing

For sequencing, amplified DNA fragments from the secondary PCR products were separated by gel electrophoresis and purified using an in house filter tip method and used for sequencing without any further purification as previously described (Yang *et al.,* 2013). Purified PCR products were sequenced using an ABI Prism™ Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California) according to the manufacturer's instructions. Nucleotide sequences were analyzed using Chromas lite version 2.0 (http://www.technelysium.com.au) and aligned with reference sequences from GenBank using Clustal W (http://www.clustalw.genome.jp).

3.5 Statistical analysis (Milestones 1-5)

Prevalences were expressed as the percentage of samples positive by PCR, with 95% confidence intervals calculated assuming a binomial distribution, using the software Quantitative Parasitology 3.0 (Rózsa *et al.,* 2000).

3.5.1 Associations between parasites and liveweight, carcase weight and DP

Protozoan parasite shedding for each lamb at each timepoint was categorised as high (faecal oocyst shedding above median for positive samples for the farm/timepoint), low (faecal oocyst shedding below median for positive samples for the farm/timepoint) or not detected (no evidence of faecal oocyst shedding) separately for *Cryptosporidium* spp. (including *C. parvum*), *C. parvum* and *Giardia* spp. Lambs were categorized by frequency of parasite detection (parasite detected on 0, 1, 2 or 3 occasions) separately for *Cryptosporidium* spp. (including *C. parvum*), *C. parvum* and *Giardia* spp. Lambs were also categorised for parasite status (no infection, *Giardia* only, *Cryptosporidium* only, mixed *Giardia* and *Cryptosporidium*) at each sampling occasion. Worm egg count was log transformed for analyses using Log10(WEC+25). Individual sheep were categorised for *Haemonchus* presence/absence for each occasion based on qPCR.

Associations between protozoan parasites with HSCW and DP were analysed using general linear models (SAS Version 9.2, SAS Institute, Cary, NC, USA), with site (i.e. farm locations) and kill group (where sheep in each flock were consigned for slaughter over more than one date) included as fixed effects and WEC included as a covariate. Within this base model, parasite shedding category (high, low or not detected) of either *Cryptosporidium* spp, *C. parvum* or *Giardia* spp. within each of the three different time points (weaning, post-weaning and pre-slaughter) was incorporated as a fixed effect equating to a total of 9 separate models (i.e. 3 parasites x 3 time points). The WEC covariate was not significant (P>0.100) for any model so this was excluded from subsequent models presented in this study.

The associations between trichostrongylid parasites with HSCW and DP were analysed using general linear models (SAS Version 9.2, SAS Institute, Cary, NC, USA) separately for each of the three different time points (weaning, post-weaning and pre-slaughter), with log transformed WEC, *Haemonchus* status (presence/absence), site and kill group included as fixed effects.

Live weight measurements were recorded for each animal at each sampling occasion, so this was analysed in a single linear mixed effects model (SAS Version 9.2, SAS Institute, Cary, NC, USA) for each parasite (i.e. each model was for a specific parasite but included all 3 time points). For protozoan parasites, the linear mixed effects models included the shedding category (high, low or not detected), site (farm location) and sampling occasion (weaning, post-weaning or pre-slaughter) each included as fixed effects, WEC was included as a covariate, and animal identification (ID) included as a random term, to account for the multiple sampling of individuals over time. The WEC covariate was not significant (P>0.100) so this was excluded from subsequent models presented in this study. For trichostrongylid parasites, the linear mixed effects models included log transformed WEC, *Haemonchus* status (presence/absence), site and sampling occasion included as fixed effects and animal identification as a random term.

Associations between HSCW and DP with parasite status were assessed separately for each timepoint using general linear models with parasite status (no infection, *Giardia* only, *Cryptosporidium* only, mixed *Giardia* and *Cryptosporidium*), site (i.e. farm locations) and kill group included as a fixed effects. Associations between liveweight with parasite status were assessed in a single linear mixed effects model with parasite status (no infection, *Giardia* only, *Cryptosporidium* only, mixed *Giardia* and *Cryptosporidium*), site (i.e. farm locations) and timepoint included as a fixed effects, and animal ID included as a random term. Least square means were generated for all general linear models.

Associations between HSCW and DP with frequency of parasite detection were assessed separately for each parasite using linear mixed effects models with frequency of detection (0, 1, 2 or 3 positive occasions) and site (i.e. farm locations) included as a fixed effects. Least square means were generated for all linear mixed effects models.

3.5.2 Associations between *Yersinia* and liveweight

Yersinia shedding for each lamb at each timepoint were categorised as high (faecal bacteria shedding above median for positive samples for the farm/timepoint), low (faecal bacteria shedding below median for positive samples for the farm/timepoint) or not detected (no evidence of faecal bactera shedding) separately for *Yersinia* (all) and pathogenic *Yersinia*. Worm egg count was log transformed for analyses using Log10(WEC+25). *Eimeria* oocyst count (by qPCR) was log transformed for analyses using Log10(OPG+25).

Live weight measurements were recorded for each animal at each sampling occasion, so this was analysed in a single linear mixed effects model (SAS Version 9.2, SAS Institute, Cary, NC, USA) each for *Yersinia* (all) and pathogenic *Yersinia* in two separate models that each included all 3 time points. The linear mixed effects models included the shedding category (high, low or not detected), site (farm location) and sampling occasion (weaning, post-weaning or pre-slaughter) each included as fixed effects, WEC and *Eimeria* OPG were included as covariates, and animal identification (ID) included as a random term to account for the multiple sampling of individuals over time. Least square means were generated for all linear mixed effects models.

3.5.3 Associations between pathogen shedding, FCS and dag.

Associations between WEC (McMaster) and FCS were analysed using bivariate Pearson correlations (two tailed).

Pathogen shedding presence for each lamb at each timepoint was categorised as detected (specific pathogen DNA detected in faeces by qPCR) or not detected (no evidence of pathogen DNA in faeces by qPCR) separately for *Cryptosporidium* spp. (including *C. parvum*), *C. parvum*, *Giardia* spp, *Salmonella* spp., *Campylobacter*. spp., pathogenic *Yersinia* and *C. percorum*.

Pathogen shedding intensity for each lamb at each timepoint was categorised as high (faecal shedding above median for positive samples for the farm/timepoint), low (faecal shedding below median for positive samples for the farm/timepoint) or not detected (no evidence of faecal shedding) separately for *Cryptosporidium* spp. (including *C. parvum*), *C. parvum*, *Giardia* spp, *Eimeria* spp., *Salmonella* spp., *Campylobacter*. spp., *Yersinia* spp. and

pathogenic *Yersinia*. Worm egg count was log transformed for analyses using Log10(WEC+25).

Associations between pathogen detection and FCS were analysed using general linear models (SAS Version 9.2, SAS Institute, Cary, NC, USA), with site (ie farm locations) included as fixed effect and both WEC and *Eimeria* OPG included as covariates. Within this base model, pathogen detection category (detected or not detected) of either *Cryptosporidium* spp. (including *C. parvum*), *C. parvum*, *Giardia* spp, *Salmonella* spp., pathogenic *Yersinia*, *Campylobacter*. spp, and *C. percorum,* within each of the three different time points (weaning, post-weaning and pre-slaughter) was incorporated as a fixed effect equating to a total of 21 separate models (i.e. 7 pathogens x 3 time points). Kill group (where sheep in each flock were consigned for slaughter over more than one date) was included as a fixed effect only for the pre-slaughter timepoint.

Associations between pathogen shedding intensity category (high, low or not detected) with FCS and dag score were analysed using general linear models (SAS Version 9.2, SAS Institute, Cary, NC, USA). Separate models were generated for each dependent variable (FCS and dag score) and timepoint, giving a total of 6 models (i.e. 3 timepoints for FCS and 3 timepoints for dag score). Site (ie farm locations) and pathogen detection category each for *Cryptosporidium* spp. (including *C. parvum*), *C. parvum*, *Giardia* spp, *Eimeria* spp., *Salmonella* spp., *Campylobacter*. spp., *Yersinia* spp. and pathogenic *Yersinia* were included as fixed effects, and Log10(WEC+25) included as a continuous covariate. Kill group (where sheep in each flock were consigned for slaughter over more than one date) was included as a fixed effect only for the pre-slaughter timepoint.

3.6 Faecal and effluent sample collection (Milestone 6)

Faecal samples were collected from lines of lambs going through Muchea and Katanning over 4 sampling periods (Table 10). Ten faecal samples/swabs were taken where possible from 6 lines of sheep selected at random at Muchea and Katanning saleyards (60 samples/yard/month), except that preference for sampling was given to lines showing evidence of scouring. A line of sheep was defined as a group of 50 or more sheep consigned from an identified source. A total of 474 faecal samples were collected from Muchea (n=236) and Katanning (n=238) saleyards over a 4-month period (April – July, 2014).

Table 10. Summary of sheep faecal samples collected from lines of sheep selected at random at Muchea and Katanning saleyards (60 samples/yard/month) over a 4 month period.

Location	Sampling date	Number
Katanning	09, April 2014	60
	08, May 2014	60
	08, June 2014	58
	07, July 2014	60
	Sub-total	238
Muchea	05, May 2014	58
	06, June 2014	58
	06, July 2014	60
	06, August 2014	60
	Sub-total	236
Total		474

Effluent sampling was conducted at the Western Australian Meat Marketing Co-operative Limited (WAMMCO) abattoir (Katanning) daily over a 4 day period for 4 months (2 sample points in triplicate x 4 days = 24 samples /month (96 in total) (Table 11).

Table 11. Summary of effluent samples collected (WAMMCO) abattoir (Katanning) daily over a 4 day period for 4 months (2 sample points in triplicate x 4 days = 24 samples /month (96 in total).

Month	Sampling Date	Inlet	Outlet
Apr-14	11-Apr-14	3	3
	12-Apr-14	3	3
	13-Apr-14	3	3
	14-Apr-14	3	3
	Sub-total	12	12
May-14	09-May-14	3	3
	10-May-14	3	3
	11-May-14	3	3
	12-May-14	3	3
	Sub-total	12	12
Jun-14	09-Jun-14	3	3
	10-Jun-14	3	3
	11-Jun-14	3	3
	12-Jun-14	3	3
	Sub-total	12	12
Jul-14	08-Jul-14	3	3
	09-Jul-14	3	3
	10-Jul-14	3	3
	11-Jul-14	3	3
	Sub-total	12	12
Total		48	48

3.7 Development and validation of the *E. coli* O157:H7 qPCR (Milestone 6)

Primers and probes for *Escherichia coli* O157:H7 were designed using the software supplied by Biosearch Technologies. A 65 base pair (bp) product was amplified from a hypothetical protein in *E. coli* O157:H7 (GenBank accession CP008957, protein ID AIG71093) using the forward primer *E. coli* O157F 5'- TGC ATT GCA GCT CTG GTA A-3', the reverse primer *E. coli* O157R 5'- TGG CAG GCA GAT CGT TAG TTC -3' and the probe 5'- CAL-Fluor eRed-610 –CGC AGG TTT AAG CGT CTG TGC A-3' BHQ1.

An IAC consisting of a fragment of a coding region from Jembrana disease virus (JDV) cloned into a pGEM-T vector (Promega) and IAC primers were used as described under section 3.2.

The positive control used in the present study was *E. coli* O157:H7 broth (positive control) in the *E. coli* O157:H7 Latex Test kit (Thermo Scientific, Scoresby, Vic. Australia). DNA was extracted from 100 µL of the concentrated bacterial broth using a PowerSoil DNA extraction kit (Geneworks, Adelaide, Australia) according to the manufacturer's instructions.

The absolute numbers of *E. coli* O157:H7 (positive control) was determined using droplet digital PCR (ddPCR) on serial dilutions of bacterial DNA from 1:10 to 1:10,000 using the primers and probe described above. This data was used then used to calibrate the standard

curve for quantitative PCR (qPCR). ddPCR was conducted in triplicate using a QX100^{TM} droplet digital PCR system (BioRad, Gladesville, NSW, Australia) according to the manufacturer's instructions. Briefly, the ddPCR reaction mixture consisted of 12.5 µl of a 2 × ddPCR master mix (Bio-Rad), 2 µl of primer/probe mix (12.5mM each of the primer and probe), 1 µl of DNA (adjusted to 50 ng/µl) and 9.5 µl of H_2O to make a final volume of 25 µl. Droplets were generated using the Droplet Generator (DG) with 70 µL DG Oil per well with a DG8 cartridge and cartridge holder, 25 µL PCR reaction mix, and DG8 gasket. Droplets were dispensed into the 96-well PCR plate by aspirating 40 µL from the DG8 cartridge into each well. The PCR plate was then heat-sealed with a foil seal and the sealed plate was placed in the PCR thermocycler. Cycling consisted of 95°C for 10 min, followed by 45 cycles of 94°C for 30 s and 58°C for 45 s, 1 cycle of 98°C for 10 min with a 12°C hold. After the reaction, the droplets were read using the Droplet Reader, and QuantaSoft software converted the data into the number of template copies per µl of PCR mixture. The number of template copies in 1 µl of DNA solution was then calculated.

The analytical specificity of the qPCR assay was assessed by testing DNA from *Campylobacter jejuni, C. coli, Salmonella Typhimurium* (Koala)*, S. wandsbek II 21*:$_{z10:z6}$ (Koala), *S. bredeney* (Koala)*, S. muenchen* (Koala)*, S. Adelaide* (Koala)*, S. waycross* (Koala), *S. infantis* (Koala), *Chlamydia pecorum*, *Chlamydia abortus* (goat, Papua New Guinea), *Yersinia enterocolitica*, *Streptococcus bovis* (ATCC 33317), *Enterococcus durans* (ATCC 11576), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6633), *Serratia marcescens* (ATCC 14756 pigmented), *Citrobacter freundii* (NCTC 9750), *Enterobacter cloacae* (ATCC 13047), *Coxiella burnetti*, *Giardia duodenalis* assemblages A and E from sheep, *Cryptosporidium sp. (n=5)*, *Isospora* sp., *Tenebrio* sp., *Cyclospora* sp., *Toxoplasma gondii*, *Trichostrongylus colubriformis*, *Teladorsagia circumcincta*, *Haemonchus contortus* and *Eimeria* sp.*,* as well as human, sheep and cattle genomic DNA.

To determine the sensitivity of the assay, 10-fold serial dilutions of the bacterial DNA were prepared from 1,000,000 copies to 100 copies. These were then 'spiked' into faecal samples and the DNA was extracted and amplified as described above (final extracted DNA volume: 50 µL). Mean detection limits, the coefficient of determination - R squared (RSQ) values and % relative standard deviation (RSD) were calculated.

3.7.1 Specificity, sensitivity and efficiency

Evaluation of the specificity of the *E. coli* O157:H7 qPCR revealed no cross-reactions with other genera and no cross-reaction with other bacterial strains. Sensitivity analysis revealed that the mean minimum detection for *E. coli* O157:H7 was 5 copies /µL, which equates to ~1,250 organisms per gram (g^{-1}) of faeces. The mean RSQ value was 0.99. The % RSD was 3.2%. The frequency of PCR inhibition, as determined by IAC amplification, was ~2.5%. If inhibition was evident, then the sample was diluted and re-amplified. The mean efficiency was 98.5%.

4 Results

4.1 Faecal sample collection (Milestones 1-5)

The actual number of lambs sampled varied widely between farms and across states due to availability of lambs. We had anticipated sampling 3,840 faecal samples from 8 farms in 4 states (160 x 3 samplings x 8 farms), however the final number of faecal samples collected was 3,412 faecal samples collected directly from the rectum of 1,189 lambs (Table 12).

INF flocks were sampled in SA and NSW. We had initially planned to sample the INF flock in Katanning in WA but unfortunately due to mis-communication, INF staff did not inform us of the weaning sampling date until after lambs had been mustered and released. This necessitated using an additional non-INF flock in WA. We had also initially planned to sample in Trangie in NSW, but we were advised by David Hopkins that Trangie did not have the required number of XB lambs due to the lower conception rate. As a result of this, we sampled a third WA flock.

Problems were encountered with both data and sample collection, particularly from SA and NSW. In some cases, fewer samples arrived than was specified and in other cases, data on productivity measures were not included or were unavailable. Therefore not all samples that were analysed for pathogen prevalence were included in the final statistical analysis.

For example, for SA1 farm (Wirrega), a total of 160 post-weaning samples were collected on October 18th in 2011 and delivered to Murdoch on October 21st in 2011. However, the actual number of samples received was 159 (one sample container was empty) and five sample codes on the sample containers did not match the sample information on the Excel spread-sheet provided. Therefore only 154 samples were able to be analysed.

A total of 160 pre-slaughter samples were collected on the 8th of November in 2011 but upon delivery, one of the samples was missing and therefore only 159 samples were analysed for the pre-slaughter period. For the second SA farm analysed (Struan), although a total of 158 samples were collected on the 24th of December 2011, 2 were missing when received at Murdoch on the 24th of December, 2011. For pre-slaughter sampling, although 147 were sent to Murdoch in two batches (one on the15th of April, 2011 and one on the 20th of May, 2011, unfortunately 19 samples had no live weight and carcass weight data available. Therefore, only 128 samples were used for the final statistical analysis.

For the first Victorian farm (Lal Lal Estate), although 160 samples were received, only 133 samples had carcase weight data supplied and therefore only 133 samples could be included in the final statistical analysis. For the second Victorian farm (Millring), 178 samples were collected for the post-weaning sampling period, but only 173 samples were received by Murdoch, as five of the sample containers were empty. Only 128 samples were received from the pre-slaughter sampling.

For the NSW farm (Kirby), unfortunately the mob that was being analysed was slaughtered in three batches and the samples were sent to Murdoch over a period of 3 months. A total of 174 samples were received. On the 26th of May 2012, 101 samples were received, 51 samples were received on the 15th of July, 2012 and the final 22 samples were received on the 18th of August, 2012. Of these,19 samples did not appear to be from the same mob

sampled on the previous two occasions and due to the 3 month time lag in slaughtering, there were large differences in live weight and carcase data weights between the different batches of samples. Consequently, only 155 pre-slaughter samples were used in the final statistical analysis.

Table 12. Summary of numbers of sheep faecal samples collected from 8 farms in 4 states.

State		Farm name	INF/Private farm	Sampling conducted by	Sampling 1	Sampling 2	Sampling 3	Total/Farm
	Farm1	Pingelly	Non-INF	Murdoch	107	109	107	323
WA	Farm2	Arthur River	Non-INF	Murdoch	124	122	121	367
	Farm3	Fankland	Non-INF	Murdoch	101	101	100	302
								992
	Farm 1	Wirrega	Non-INF	SARDI	165	148	159	472
SA	Farm 2	Struan	INF	SARDI	169	156	147	472
								944
	Farm 1	Lal Lal Estate	Non-INF	U. of Melb	180	172	160	512
Vic	Farm 2	Millring	Non-INF	U. of Melb	176	173	128	477
								989
NSW	Farm1	Kirby	INF	INF staff	160	160	167	487
Sub-Total					1182	1141	1089	
Total								**3,412**

Sampling in WA was conducted by Murdoch Staff. Sampling in SA was conducted under the supervision of Dr. Ian Carmichael, SARDI. Sampling in Vic was conducted by Dr. Angus Campbell, University of Melbourne. Sampling in NSW was conducted by Justin Hoad.

4.2 Prevalence of bacteria, protozoa and worms across each state (Milestones 1-5)

The prevalence of each of the 10 pathogens on the eight study farm across NSW, SA, Vic and WA over the 3 sampling periods (weaning, post-weaning and pre-slaughter) is listed in Table 13. The overall prevalence for *Cryptosporidium, Giardia, Eimeria, Campylobacter, Yersinia, Salmonella, Chlamydia, Haemonchus, Teladorsagia* and *Trichostrongylous* was 16.9%, 20.2%, 18.1%, 13.3%, 21.5%, 5%, 30.1%, 14.8%, 43.3% and 49.4% (Table 13). For all pathogens there was no relationship between prevalence and the 3 sampling times ($p>0.05$), as the peak prevalence occurred at different sampling times across the farms tested.

4.2.1 Cryptosporidium

The prevalence of *Cryptosporidium* at WA2 was significantly higher than at the seven other farms ($p<0.05$). There were also significant differences between WA1 and SA1 and between NSW and SA1 ($p<0.05$). The prevalence of *Cryptosporidium* was highest in WA, which peaked at 43.9% and 37.1% at WA2 and WA1 respectively during weaning and at WA2 during pre-slaughter (36.4%). There were smaller peaks for *Cryptosporidium* at NSW (27.5% and 22.5% respectively during post-weaning and weaning respectively), at Vic 2 (21% at weaning), Vic1 (18.6% at post-weaning). In SA, the prevalence peaked at 19.2% at post-weaning at SA2. The overall prevalence in WA on the 3 farms was 25% (248/992). The prevalence in NSW was 20.7% (101/487), in Vic was 11.8% (117/989) and in SA, it was

11.3% (107/944), but these state-wide differences were not significant (p>0.05) (Fig 2). Only 4, 2 and 1 lambs from WA, Vic and NSW respectively were positive across all 3 samplings.

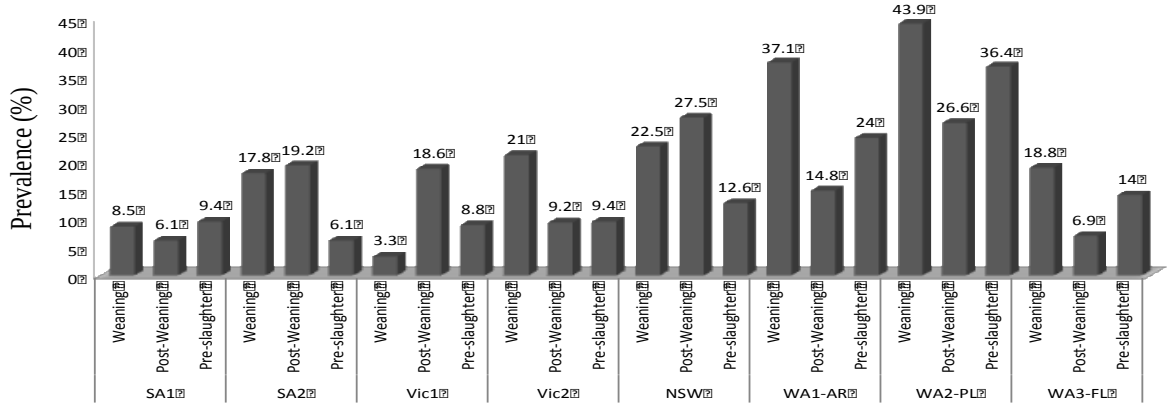

Figure 2. Prevalence (%) of *Cryptosporidium* in sheep faecal samples from 8 farms across 4 states (NSW, SA, Vic and WA) over 3 sampling times (weaning, post-weaning and pre-slaughter) as determined by qPCR.

4.2.2 Giardia

The prevalence of *Giardia* at WA1 was significantly higher than at the seven other farms (p<0.005) with the exception of WA2. The highest prevalences for *Giardia* were recorded at WA1, which peaked at 42.1% and 35.2% during pre-slaughter and post-weaning respectively. The lowest prevalence for WA farms was 7.9% (2.7-13) for WA3 at weaning (Table 13). At NSW, the peak prevalence was at pre-slaughter (34.7%). In Vic, peak prevalences were detected for Vic 2 (30.5%) at pre-slaughter and Vic1 (23.3%) at post-weaning. In SA, the prevalence peaked at 20.3% at post-weaning at SA1.

The overall prevalence in WA across the 3 farms was 25.4% (252/992). The prevalence in NSW was 23.2% (113/487), 16.9% (117/989) for Vic and 16.7% (158/944) for SA, but these state-wide prevalences were not significant (p=0.235)(Fig. 3).

A total of 24 lambs from WA (14 from WA1 and 10 from WA2) were positive across all 3 samplings. Only 4, 1 and 2 samples from SA, Vic and NSW respectively were positive across all 3 samplings.

Table 13. Prevalence of 10 pathogens in sheep faeces from 8 farms across 4 states (NSW, SA, Vic and WA) over 3 sampling times (weaning, post-weaning and pre-slaughter). 95% CI are given in parenthesis.

Farm	Sampling period	*Cryptosporidium*	*Giardia*	*Eimeria*	*Campylobacter*	*Yersinia*	*Salmonella*	*Chlamydia*	*Haemonchus*	*Teladorsagia*	*Trichostrongylus*
SA1	Weaning (n=165)	8.5 (4.2-12.7)	16.4 (10.7-22.0)	7.3 (3.3-11.2)	16.4 (10.7-22.0)	24.8 (18.3-31.4)	2.4 (0.1-4.8)	53.3 (45.7-60.9)	7.9 (3.8-12.0)	23.6 (17.2-30.1)	50.3 (42.7-57.9)
	Post-Weaning (n=148)	6.1 (2.2-9.9)	20.3 (13.8-26.7)	14.2 (8.6-19.8)	44.6 (36.6-52.6)	33.8 (26.2-41.4)	1.4 (0.0-3.2)	77.0 (70.2-83.8)	19.6 (13.2-26.0)	50.7 (41.9-58.1)	77.7 (70.2-83.8)
	Pre-slaughter (n=159)	9.4 (4.9-14.0)	11.9 (6.9-17.0)	20.8 (14.5-27.1)	27.7 (20.7-34.6)	26.4 (19.6-33.3)	1.3 (0.0-3.0)	16.4 (10.6-22.1)	15.1 (9.5-20.7)	42.8 (35.1-50.5)	71.7 (64.7-78.7)
SA2	Weaning (n=169)	17.8 (12.0-23.5)	14.8 (9.4-20.1)	7.7 (3.7-11.7)	15.4 (9.9-20.8)	27.2 (20.5-33.9)	1.9 (0.0-4.0)	52.7 (45.1-60.2)	5.9 (2.4-9.5)	31.4 (24.4-38.4)	53.3 (45.7-60.8)
	Post-Weaning (n=156)	19.2 (13.0-25.4)	19.9 (13.6-26.1)	16.0 (10.3-21.8)	59.0 (51.3-66.7)	44.2 (36.4-52.0)	9.6 (5.0-14.2)	94.2 (90.6-97.9)	9.0 (4.5-13.5)	67.9 (60.6-75.3)	93.6 (89.7-97.4)
	Pre-slaughter (n=147)	6.1 (2.2-10.0)	17.7 (11.5-23.9)	9.5 (4.8-14.3)	31.3 (23.8-38.8)	34.0 (26.4-41.7)	2.7 (0.1-5.4)	12.2 (6.9-17.5)	11.6 (6.4-16.7)	61.9 (54.1-69.8)	22.4 (15.7-29.2)
Vic1	Weaning (n=180)	3.3 (0.7-6.0)	7.2 (3.4-11.0)	10.6 (6.1-15.0)	0.6 (0.0-1.6)	20.0 (14.2-25.8)	2.2 (0.1-4.4)	18.9 (13.2-24.6)	10.0 (5.6-14.4)	33.3 (26.4-40.2)	50.6 (43.3-57.9)
	Post-Weaning (n=172)	18.6 (12.8-24.4)	23.3 (16.9-29.6)	13.4 (8.3-18.5)	2.3 (0.1-4.6)	34.9 (27.8-42.0)	2.3 (0.1-4.6)	17.4 (11.8-23.1)	7.0 (3.2-10.8)	27.3 (20.7-34.0)	39.0 (31.7-46.2)
	Pre-slaughter (n=160)	8.8 (4.4-13.1)	9.4 (4.9-13.9)	33.1 (25.8-40.4)	3.8 (0.8-6.7)	67.5 (60.2-74.8)	3.1 (0.4-5.8)	6.3 (2.5-10.0)	50.6 (42.9-58.4)	46.9 39.1(54.6-)	70.6 (63.6-77.7)
Vic2	Weaning (n=176)	21.0 (15.0-27.0)	13.6 (8.6-18.7)	16.5 (11.0-22.0)	26.7 (20.2-33.2)	9.1 (4.8-13.3)	5.7 (2.3-9.1)	1.1 (0.0-2.7)	13.1 (8.1-18.0)	25.6 (19.1-32.0)	35.2 (28.2-42.3)
	Post-Weaning (n=173)	9.2 (4.9-13.6)	20.8 (14.8-26.9)	10.4 (5.9-15.0)	2.3 (0.1-4.6)	35.8 (28.7-43.0)	5.2 (1.9-8.5)	19.1 (13.2-24.9)	2.9 (0.4-5.4)	31.8 (24.9-38.7)	32.9 (25.9-40.0)
	Pre-slaughter (n=128)	9.4 (4.3-14.4)	30.5 (22.5-28.4)	10.2 (4.9-15.4)	0.0 (0.0-0.0)	60.2 (51.7-68.6)	3.1 (0.1-6.1)	3.1 (0.1-6.1)	9.4 (4.3-14.4)	55.5 (46.1-63.3)	63.3 (54.9-71.6)
NSW	Weaning (n=160)	22.5 (16.0-29.0)	16.9 (11.1-22.7)	47.5 (39.8-55.2)	4.4 (1.2-7.5)	55.6 (47.9-63.3)	18.1 (12.2-24.1)	42.5 (34.8-50.2)	65.6 (58.3-73.0)	56.3 (48.6-63.9)	75.6 (69.0-82.3)
	Post-Weaning (n=160)	27.5 (20.6-43.3)	17.5 (11.6-23.4)	70.0 (62.9-77.1)	4.4 (1.2-7.5)	90.6 (86.1-95.1)	23.8 (17.2-30.3)	72.5 (65.6-79.4)	56.9 (49.2-64.5)	74.4 (67.6-81.1)	76.9 (70.3-83.4)
	Pre-slaughter (n=167)	12.5 (7.5-17.6)	34.7 (27.5-42.0)	12.0 (7.1-16.9)	1.2 (0.0-2.8)	61.7 (54.3-69.1)	3.6 (0.8-6.4)	80.8 (74.9-86.8)	7.8 (3.7-11.8)	70.7 (63.8-77.6)	82.0 (75.2-87.9)
WA1	Weaning (n=124)	37.1 (28.6-45.6)	27.4 (19.6-35.3)	8.1 (3.3-12.9)	10.5 (5.1-15.9)	7.3 (2.7-11.8)	4.0 (0.6-7.5)	7.3 (2.7-11.8)	2.4 (0.0-5.1)	61.3 (52.7-69.9)	32.3 (24.040.5-)
	Post-Weaning (n=122)	14.8 (8.5-21.0)	35.2 (26.8-43.7)	10.7 (5.2-16.1)	4.1 (0.6-7.6)	12.3 (6.5-18.1)	6.6 (2.2-10.9)	25.4 (17.7-33.1)	3.3 (0.1-6.4)	27.9 (19.9-35.8)	13.9 (7.8-20.1)
	Pre-slaughter (n=121)	24.0 (16.4-31.6)	42.1 (33.4-50.9)	5.8 (1.6-9.9)	5.8 (1.6-9.9)	8.3 (3.4-13.2)	10.7 (5.2-16.3)	10.7 (5.2-16.3)	0.8 (0.0-2.4)	39.7 (31.0-48.4)	16.5 (9.9-23.1)
WA2	Weaning (n=107)	43.9 (34.5-53.3)	28.0 (19.5-36.5)	12.1 (6.0-18.3)	12.1 (6.0-18.3)	21.5 (13.7-29.3)	4.7 (0.7-8.7)	16.8 (9.7-23.9)	5.6 (1.2-10.0)	29.0 (20.4-37.6)	18.7 (11.3-26.1)
	Post-Weaning (n=109)	26.6 (18.3-34.9)	25.7 (17.5-33.9)	13.8 (7.3-20.2)	4.6 (0.7-8.5)	5.5 (1.2-9.8)	2.8 (0.0-5.8)	16.5 (9.5-23.5)	0.9 (0.0-2.7)	73.4 (65.1-81.7)	75.2 (67.1-83.3)
	Pre-slaughter (n=107)	36.4 (27.3-45.6)	29.9 (21.1-38.6)	33.6 (24.7-42.6)	9.3 (3.8-14.9)	22.4 (14.5-30.3)	0.0 (0.0-0.0)	48.6 (39.1-58.1)	0.0 (0.0-0.0)	78.5 (70.7-86.3)	24.3 (16.2-32.4)
WA3	Weaning (n=101)	18.8 (11.2-26.4)	7.9 (2.7-13.2)	20.8 (12.9-28.7)	0.0 (0.0-0.0)	5.9 (1.3-10.6)	4.0 (0.2-7.8)	0.0 (0.0-0.0)	10.9 (4.8-17.0)	5.0 (0.7-9.2)	32.7 (23.5-41.8)
	Post-Weaning (n=101)	6.9 (2.0-11.9)	10.9 (4.8-17.0)	13.9 (7.8-20.6)	15.8 (8.7-23.0)	11.9 (5.6-18.2)	2.0 (0.0-4.7)	11.9 (5.6-18.2)	11.9 (5.6-18.2)	6.9 (2.0-11.9)	7.9 (2.7-13.2)
	Pre-slaughter (n=100)	14.0 (7.2-2-.8)	15.0 (8.0-22.1)	6.0 (1.3-10.7)	5.0 (0.7-9.3)	4.0 (0.2-7.8)	0.0 (0.0-0.0)	3.0 (0.0-6.3)	0.0 (0.0-0.0)	4.0 (0.2-7.8)	10.0 (4.1-15.9)
Total	**n=3,412**	**16.9 (15.6-18.1)**	**20.2 (18.9-21.6)**	**18.1 (16.8-19.3)**	**13.3 (12.1-14.4)**	**32.0 (30.5-33.6)**	**5.0 (4.3-5.7)**	**30.1 (28.6-31.6)**	**14.8 (13.6-16.0)**	**43.3 (41.7-45.0)**	**49.4 (47.7-51.1)**

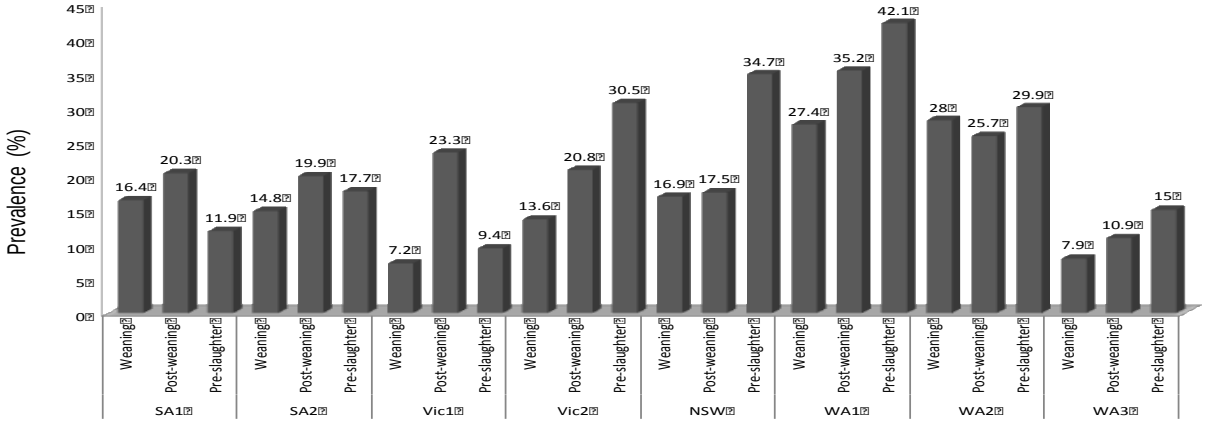

Figure 3. Prevalence (%) of *Giardia* in sheep faecal samples from 8 farms across 4 states (NSW, SA, Vic and WA) over 3 sampling times (weaning, post-weaning and pre-slaughter) as determined by qPCR.

4.2.3 Eimeria

The prevalence of *Eimeria* at the NSW farm was significantly higher than at the seven other farms ($p<0.05$) and peaked at 70% and 47.5% during the post-weaning and weaning periods respectively (Table 13 and Fig. 4). There were smaller peaks for *Eimeria* at WA2 (33.6%), Vic1 (33.1%) and SA1 (20.8%) (all at pre-slaughter) and at WA3 at weaning (20.8%). Only 3, 1, 2 and 1 lambs from NSW, Vic1, Vic2 and WA2 respectively were positive across all 3 samplings.

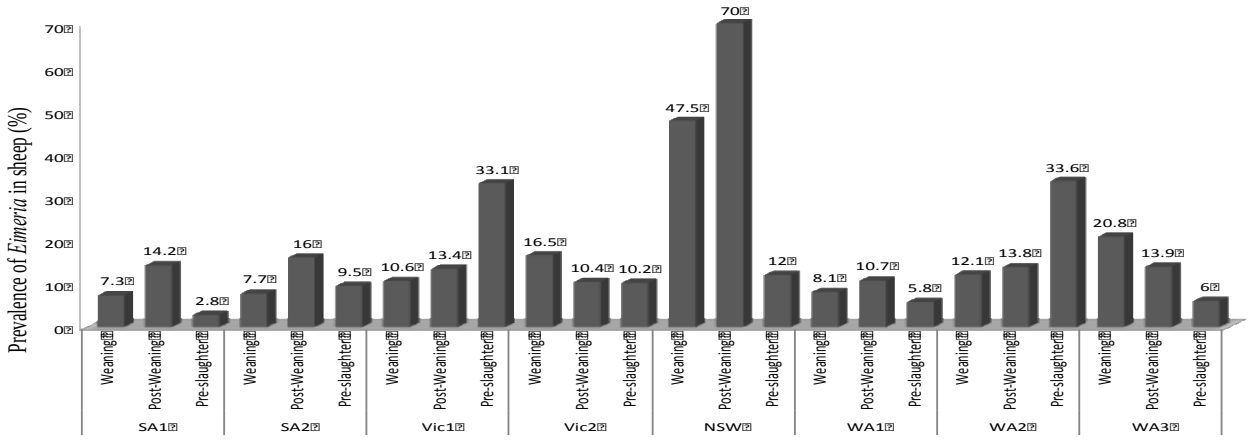

Figure 4. Prevalence (%) of *Eimeria* in sheep faecal samples from 8 farms across 4 states (NSW, SA, Vic and WA) over 3 sampling times (weaning, post-weaning and pre-slaughter) as determined by qPCR.

4.2.4 Campylobacter and Salmonella

The highest prevalence of *Campylobacter* spp. was in faecal samples from lambs on the two farms in SA (59% for SA2 and 44.6% for SA1, both from post-weaning samplings). Overall, there were significant differences between the prevalence of bacterial pathogens between

states ($P < 0.01$ for *Campylobacter* spp.; $P < 0.05$ for *S. enterica*). The highest prevalence of *S. enterica* in faecal samples from lambs was in NSW during the post-weaning period (23.8%), which was significantly higher than all three sampling times from the other farms and the pre-slaughter periods from the NSW farm ($P < 0.05$). In general, the prevalence of *S. enterica* was lower than *Campylobacter* spp. on all eight farms (Table 13 and Fig. 5).

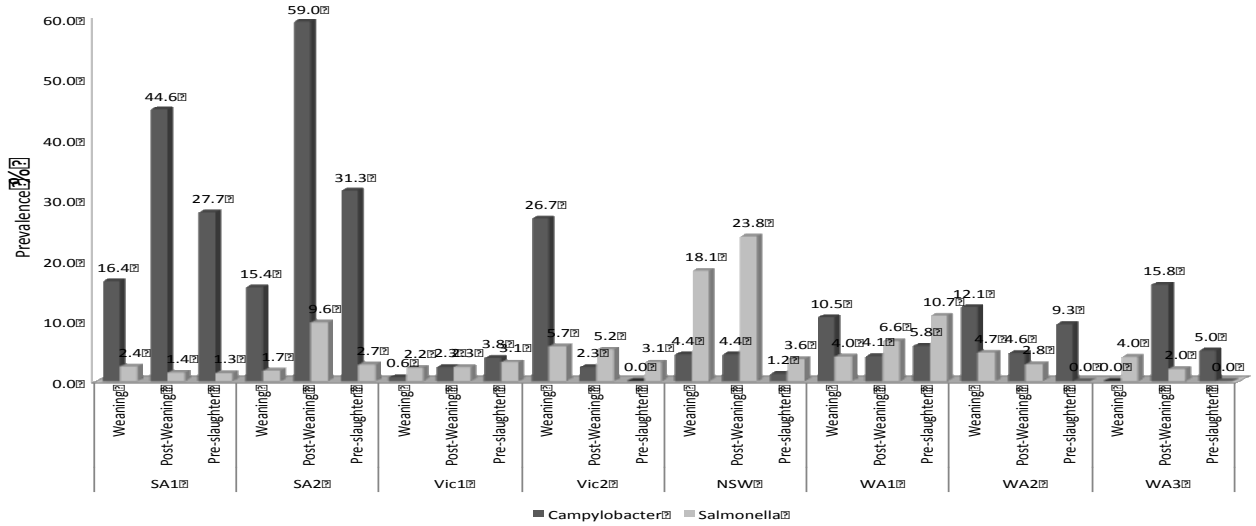

Figure 5. Prevalence (%) of *Salmonella* and *Campylobacter* in sheep faecal samples from eight farms across four states (NSW, SA, Vic and WA) over three sampling times (weaning, post-weaning and pre-slaughter) as determined by qPCR.

4.2.5 Yersinia

The overall prevalence of *Yersinia* spp. from eight farms over three sampling periods (weaning, post-weaning and pre-slaughter) was 32% (Table 13 and Fig. 6). Across each sampling time, the prevalence was 21.6%, 38.5% and 38.4%, respectively. Overall there were significant differences between the prevalence of *Yersinia* between states ($P < 0.001$). For example, the prevalence of *Yersinia* was much lower in WA compared to Eastern states (Fig. 6). *Yersinia* prevalence was highest at 90.6% at NSW during the post-weaning sampling, followed by 67.5% at Vic1 and 60.2% at Vic2 at pre-slaughter. Of the three WA flocks, the highest prevalence for *Yersinia* was 22.4% at pre-slaughter period for WA2.

A total of 65, 53, 2 and 0 individual lambs were positive for *Yersinia* spp. at all 3 samplings (weaning, post-weaning and pre-slaughter respectively) across the four states (SA, Vic, NSW and WA), respectively.

The overall prevalence of pathogenic *Y. enterocolitica* as determined by screening for the *yst* gene was 5.8% with the highest point prevalence in NSW during weaning and post-weaning at 48.8% and 31.3% respectively. There was no correlation between the overall prevalence of *Y. enterocolitica* and total *Yersinia* (Fig. 6).

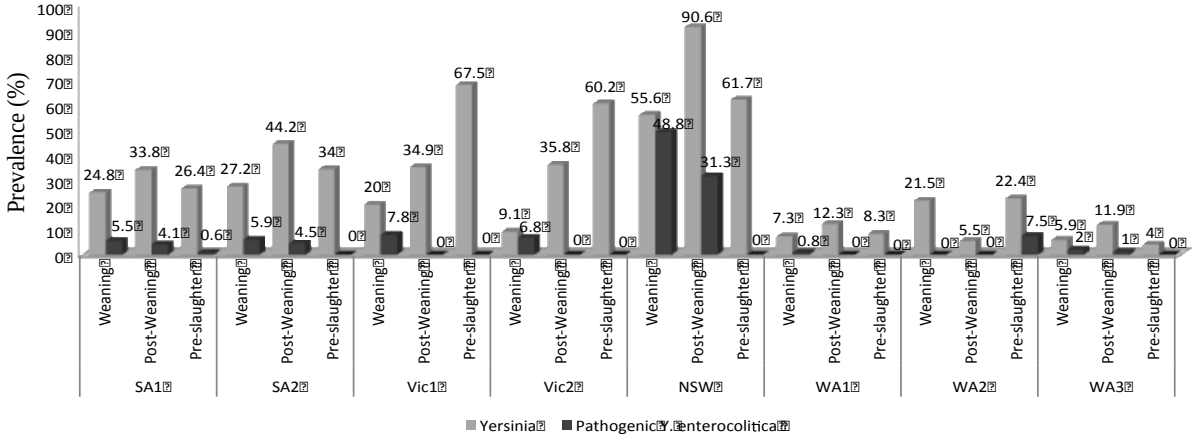

Figure 6. Overall prevalence (%) of *Yersinia* and prevalence (%) of pathogenic *Y. enterocolitica* in sheep faecal samples from 8 farms across 4 states (NSW, SA, Vic and WA) over 3 sampling times (weaning, post-weaning and pre-slaughter) as determined by qPCR of the *rpoB* and *yst* gene loci respectively.

4.2.6 Chlamydia

Chlamydia abortus was not detected in any samples. The overall prevalence of *C. pecorum* from eight farms across four states over three sampling times (weaning, post-weaning and pre-slaughter) was 30.1% (Fig. 7). Overall there were significant differences between the prevalence of *C. pecorum* between states ($P < 0.01$). For example, the prevalence of *C. pecorum* was much lower in WA than in the Eastern states (Fig. 7). The *C. pecorum* prevalence peaked at 94.2% in SA2 during the post-weaning period, followed by 80.8% in NSW during the pre-slaughter period and 77% in SA1 during the post-weaning period. The highest prevalence for *C. pecorum* in WA was 48.6% at WA2 during the pre-slaughter period. A total of 422, 114, 309 and 152 lambs were positive for *C. pecorum* across all three samplings at SA, Vic, NSW and WA, respectively.

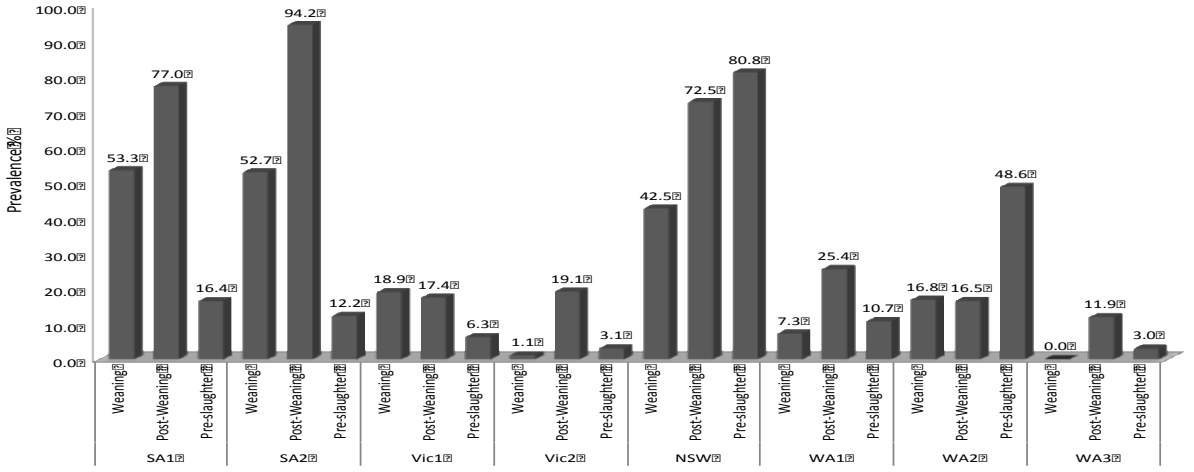

Figure 7. Prevalence (%) of *Chlamydia pecorum* in sheep faecal samples from eight farms across four states (NSW, SA, Vic and WA) over three sampling times (weaning, post-weaning and pre-slaughter) as determined by qPCR.

4.2.7 Haemonchus, Teladorsagia and Trichostrongylous

McMaster faecal worm egg counts for the eight flocks are shown in Table 24.

It should be noted that the WEC for these flocks were generally low and below the thresholds expected to be associated with clinical disease. This reflects the management of sheep, including anthelmintic treatments and grazing management, that was conducted to replicate typical management of slaughter lambs.

The overall prevalence of *Haemonchus, Teladorsagia* and *Trichostrongylus* from 8 farms across 4 states over 3 sampling periods (weaning, post-weaning and pre-slaughter) by qPCR was 14.8%, 43.3% and 49.4% respectively (Table 13 and Fig. 8).

Haemonchus prevalence was relatively low with the exception of peaks of 65.6% and 56.9% during the weaning and post-weaning periods respectively at NSW and a peak of 50.6% at Vic1 during the pre-slaughter period (Table 13). The prevalence of *Haemonchus* at weaning, post-weaning and pre-slaughter was 16.0%, 14.7% and 13.6% respectively. The highest prevalence was at NSW farm at 81.9%, followed by Vic1 (50.8%). The lowest prevalence was at WA2 (6.3%) and WA1 (6.5%). A total of 0 1, 4 and 0 individual lambs were positive for *Haemonchus* at all 3 samplings (weaning, post-weaning and pre-slaughter respectively) across the four states (SA, Vic, NSW and WA).

Teladorsagia peaked at 78.5% and 73.4% at WA2 during the pre-slaughter and post-weaning periods respectively and at 74.4% and 70.7% at NSW, during the post-weaning and pre-slaughter periods respectively (Table 13). There were also peaks in *Teladorsagia* prevalence at SA2 of 67.9% and 61.9% during the post-weaning and pre-slaughter periods respectively and a peak at SA1 during post-weaning (50.7%). The overall prevalence of *Teladorsagia* at weaning, post-weaning and pre-slaughter was 33.8%, 45.8% and 51.2% respectively. The highest prevalence was at the NSW farm at 95.0%, followed by SA2 (87.1%); the lowest prevalence was at WA3 (14. 9%). A total of 19, 7, 47 and 21 individual lambs were positive for *Teladorsagia* at all 3 samplings (weaning, post-weaning and pre-slaughter respectively) across the four states (SA, Vic, NSW and WA).

Trichostrongylus prevalence peaked at 93.6% in SA during the post-weaning period at SA2 and reached 77.7% at SA1 during post-weaning. At NSW, prevalences reached 82%, 76.9% and 76.5% during the pre-slaughter, post-weaning and weaning periods respectively (Table 13). The prevalence of all worms was lowest at WA3 across all sampling periods with the exception of *Trichostrongylus,* which peaked at 32.7% during the weaning period. The overall prevalence of *Trichostrongylus* at weaning, post-weaning and pre-slaughter was 45.8%, 53.6% and 49.0% respectively. The highest prevalence was at NSW farm at 98.1%, followed by SA1 (95.8%); the lowest prevalence was at WA1 (44.1%) and WA3 (44.3%). A total of 58, 22, 75 and 3 individual lambs were positive for *Trichostrongylus* at all 3 samplings (weaning, post-weaning and pre-slaughter respectively) across the four states (SA, Vic, NSW and WA).

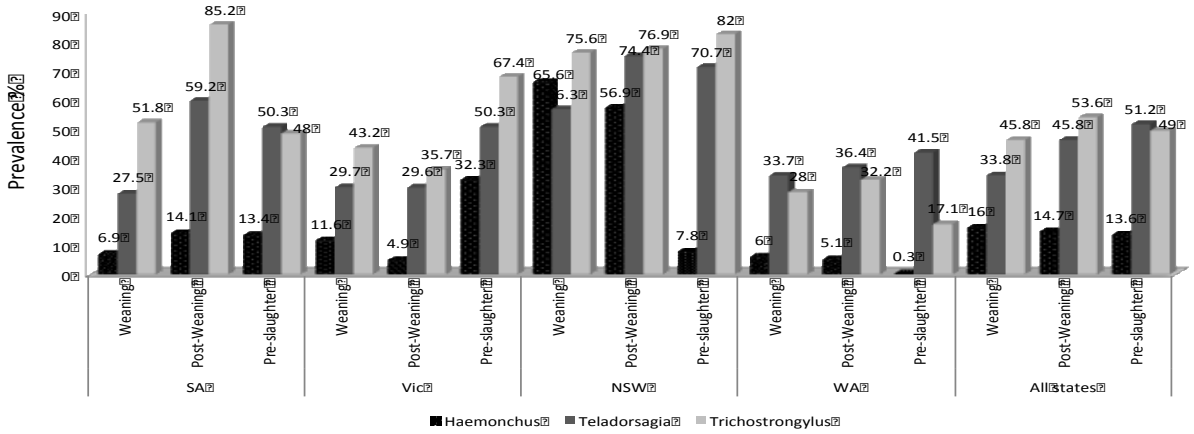

Figure 8. Prevalence (%) of *Haemonchus, Teladorsagia and Trichostrongylous* in sheep faecal samples across 4 states (NSW, SA, Vic and WA) over 3 sampling times (weaning, post-weaning and pre-slaughter) as determined by qPCR.

4.2.8 Comparison of qPCR prevalence data for strongyle worms with the traditional McMaster WEC method

Overall there was 81% agreement between qPCR data with the traditional McMaster WEC method for the prevalence of strongyle worms ($k = 0.363 \pm 0.017$ - $p \leq 0.001$), with a number of exceptions (Fig. 9 and Table 14). For the SA2, at the post-weaning sampling there was a very large difference between McMaster WEC and qPCR, as only 3.8% of samples were positive by McMaster WEC and 95.6% of samples were positive by qPCR. At Vic1, the prevalence of worms was 30.6% during the post-weaning using McMaster WEC but was 62.2% by qPCR. Similarly for NSW, the prevalence of strongyle worms at pre-slaughter was 39.7% by McMaster WEC, whereas the qPCR prevalence was 95.2%. At WA1 and WA2, the prevalence by McMaster WEC was higher than by qPCR (Fig. 9). The overall prevalence across all states by McMaster WEC was 55% whereas the overall prevalence by qPCR for all 3 strongyle parasites was 64.9%.

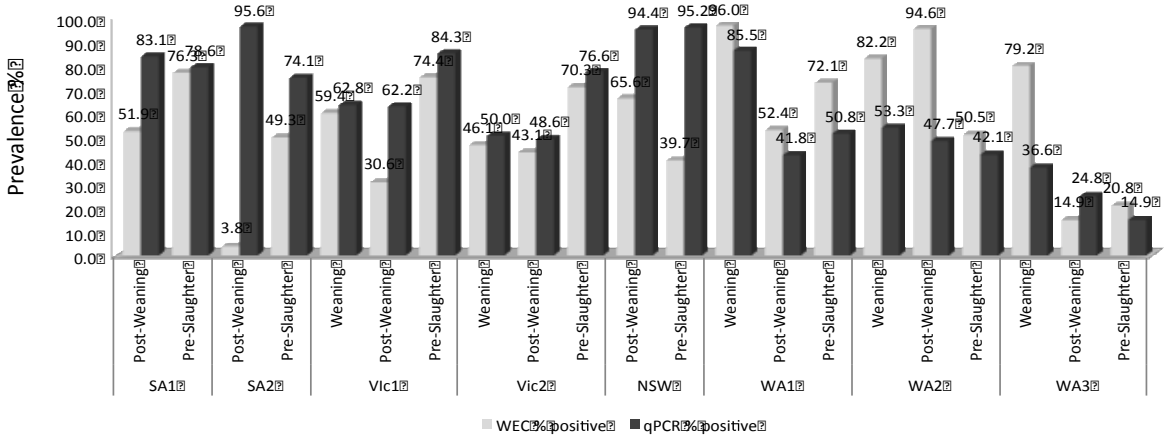

Figure 9. Comparison of qPCR and WEC for the detection of strongyle worms across 4 states.

Table 14. Comparison of the prevalence of strongyle worms on 8 farms across 4 states (NSW, SA, Vic and WA) and over 2 sampling times (post-weaning and pre-slaughter) as determined by WEC and qPCR. 95% CI are given in parenthesis. *Note that WEC's were only obtained for some farms during the weaning period as rectal swabs were used to limit discomfort to the lambs and therefore in some cases there was insufficient sample for WEC analysis.*

Farm	Sampling period	WEC % positive	qPCR % positive	*κ* statistic ± SE
SA1	Post-Weaning	51.9 (44.1-59.6)	83.1 (77.3-88.9)	0.153 ± 0.057 (p=0.07)
	Pre-Slaughter	76.3 (69.7-82.8)	78.6 (72.2-92.4)	0.603 ± 0.078 (p≤0.001)
	Two samplings together			0.328 ± 0.054 (p≤0.001)
SA2	Post-Weaning	3.8 (0.8-6.8)	95.6 (85.0-98.9)	0.021 ± 0.019 (p= 0.028)
	Pre-Slaughter	49.3 (41.1-57.5)	74.1 (67.1-81.2)	0.395 ± 0.069 (p≤0.001)
	Two samplings together			0.052 ± 0.027 (p=0.081)
Vic1	Weaning	59.4 (52.3-66.6)	62.8 (55.7-69.8)	0.817 ± 0.045 (p≤ 0.001)
	Post-Weaning	30.6 (23.5-37.8)	62.2 (55.0-69.5)	0.304 ± 0.054 (p≤ 0.001)
	Pre-Slaughter	74.4 (67.8-81.1)	90.6 (78.9-89.7)	0.429 ± 0.084 (p≤ 0.001)
	Three samplings together			0.554 ± 0.036 (p≤ 0.001)
Vic2	Weaning	46.1 (38.7-53.4)	50.0 (42.6-57.4)	0.886 ± 0.035 (p≤ 0.001)
	Post-Weaning	43.1 (35.7-50.5)	48.6 (41.1-56.0)	0.613 ± 0.060 (p≤ 0.001)
	Pre-Slaughter	70.3 (62.4-78.2)	76.6 (69.2-83.9)	0.708 ± 0.032 (p≤ 0.001)
	Three samplings together			0.494 ± 0.084 (p≤ 0.001)
NSW	Post-Weaning	65.6 (58.3-73.0)	94.4 (90.8-97.9)	0.129 ± 0.06 (p=0.011)
	Pre-Slaughter	39.7 (32.4-46.9)	95.2 (89.8-97.2)	0.083 ± 0.035 (p=0.029)
	Two samplings together			0.106 ± 0.032 (P=0.01)
WA1	Weaning	96.0 (92.5-99.4)	85.5 (79.3-91.7)	0.335 ± 0.094 (p≤ 0.001)
	Post-Weaning	52.4 (43.6-61.2)	41.8 (33.1-50.6)	0.063 ± 0.083 (p=0.478)
	Pre-Slaughter	72.1 (64.2-80.1)	50.8 (41,9-59.7)	0.129 ± 0.083 (p=0.121)
	Three samplings together			0.483 ± 0.052 (p≤0.001)
WA2	Weaning	82.2 (75.0-89.5)	53.3 (43.8-62.7)	0.355 ± 0.089 (p≤0.001)
	Post-Weaning	94.6 (90.4-98.8)	47.7 (38.3-57.1)	0.366 ± 0.161 (p≤0.001)
	Pre-Slaughter	50.5 (41.1-59.8)	42.1 (32.7-51.4)	0.102 ± 0.095 (p=0.016)
	Three samplings together			0.483 ± 0.056 (p≤0.001)
WA3	Weaning	79.2 (66.5-83.0)	36.6 (27.2-46.0)	0.072 ± 0.016 (p=0.267)
	Post-Weaning	14.9 (7.2-19.2)	24.8 (16.3-33.2)	0.632 ± 0.094 (p≤0.001)
	Pre-Slaughter	20.8 (11.6-26.2)	14.9 (7.9-21.8)	0.580 ± 0.106 (p≤0.001)
	Three samplings together			409± 0.053 (p≤0.001)
Total				**0.363 ± 0.017 (p≤ .001)**

4.2.9 Comparison of qPCR quantitation with WEC after log transformation

For log transformed data, overall there was a significant correlation between the qPCR quantitation and WEC for the eight farms (p=0.001). There were also significant correlations between individual farms, specifically NSW (p=0.001), SA2 (p=0.0327), Vic1 (p=0.001), Vic2 (p=0.0327) and WA1 (p=0.002). For non-log transformed data, there was only a significant correlation with WA1 (p=0.001) and no significant correlation overall for the eight farms (p=0.8989).

4.3 Prevalence of zoonotic pathogens in sheep at WA sale yards (Milestone 6)

With the exception of *Campylobacter,* the overall prevalence of pathogens was higher in lines of lambs from Muchea sale yards compared to Katanning sale yards (Table 15).

The prevalence of *Cryptosporidium* peaked at 8.3% in Katanning during the April collection and was ~5% for the other sampling times. *Cryptosporidium* prevalence peaked at 10.3% in Muchea during the May sampling and varied between 5-8.3% for the other sampling times. *Giardia* prevalence varied between 3.3% and 6.7% in Katanning and peaked at 20.7% in Muchea during the April collection. *Giardia* was not detected during the June sampling.

For *E. coli* O157:H7, the prevalence in Katanning, which peaked at 5% during the April collection and varied between 0%-3.4% for the other sampling times, was much lower than Muchea which peaked at 25.9%, during the April sampling and ranged between 6.7%-15% for the other sampling times. In contrast, the prevalence of *Campylobacte*r peaked at 12.1% at Katanning during the June sampling and varied between 3.3 and 11.7% for the other sampling times. The highest prevalence for *Campylobacter* at Muchea sale yard was 5.2% and varied between 1.7 and 5.0% for the other samplings. *Campylobacter* was not detected in the July sample at Muchea. *Salmonella* peaked at 6.7% in the July sample from Katanning but was not detected during the May sampling. At Muchea, the prevalence ranged between 1.7 and 5.2%.

Table 15. Prevalence (%) of zoonotic pathogens from lines of sheep selected at random at Muchea and Katanning saleyards over a 4 month period (95% confidence intervals are listed in parenthesis).

Saleyard	Time and sample numbers	*Cryptosporidium*	*Giardia*	*E. coli* O157:H7	*Campylobacter*	*Salmonella*
Katanning	April 2014 (n=60)	8.3 (1.3-15.3)	5 (0-10.5)	5 (0-10.5)	3.3 (0-7.9)	1.7 (0-4.9)
	May 2014 (n=60)	5 (0-10.5)	6.7 (0.4-13.0)	3.3 (0-7.9)	6.7 (0.4-13.0)	0
	June 2014 (n=58)	5.2 (0-10.9)	5.2 (0-10.9)	3.4 (0-8.1)	12.1 (3.7-20.5)	3.4 (0-8.1)
	July 2014 (n=60)	5 (0-10.5)	3.3 (0-7.9)	0	11.7 (3.5-19.8)	6.7 (0.4-13.0)
Sub-total	n=238	5.9 (2.9-8.9)	5.0 (2.3-7.8)	2.9 (0.8-5.1)	8.4 (4.9-11.9)	2.9 (0.8-5.1)
Muchea	April 2014 (n=58)	5.2 (0-10.9)	20.7 (10.3-31.1)	25.9 (14.6-37.1)	1.7 (0-5.1)	5.2 (0-10.9)
	May 2014 (n=58)	10.3 (2.5-18.2)	8.6 (1.4-15.8)	8.6 (1.4-15.8)	5.2 (0-10.9)	5.2 (0-10.9)
	June 2014 (n=60)	5 (0-10.5)	0	15.0 (6.0-24.0)	5.0 (0-10.5)	1.7 (0-4.9)
	July 2014 (n=60)	8.3 (1.3-15.3)	1.7 (0-4.9)	6.7 (0.4-13.0)	0	5.0 (0-10.5)
Sub-total	n=236	8.1 (4.6-11.5)	7.6 (4.2-11.0)	14.0 (9.6-18.4)	3.0 (0.8-5.1)	4.2 (1.7-6.8)
Overall	n=474	7.0 (4.7-9.3)	6.3 (4.1-8.5)	8.4 (5.9-10.9)	5.7 (3.6-7.8)	3.6 (1.9-5.3)

4.4 Pathogen prevalence in abattoir effluent at Katanning (Milestone 6)

Overall *Campylobacter* was the most prevalent pathogen (18.8%) and ranged in prevalence from 8.3% to 58.3% during the different sampling times (Table 16). *Cryptosporidium* was the second most prevalent pathogen in effluent (10.4%) and ranged in prevalence from 8.3% to 16.7% during the different sampling times. The overall prevalence of *Salmonella, Giardia* and *E. coli* O157:H7 in effluent was 6.3%, 5.2% and 5.2% respectively.

Table 16. Prevalence (%) of 5 zoonotic pathogens in effluent samples from Katanning abattoir over 4 sampling times (95% CI are given in parenthesis).

		Cryptosporidium		*Giardia*		*E. coli* O157:H7		*Campylobacter*		*Salmonella*	
Sampling time	**Location of sampling**	**No of positives**	**Prevalence (%)**	**No of positives**	**Prevalence (%)**	**No of positives**	**Prevalence (%)**	**No of positives**	**Prevalence (%)**	**No of positives**	**Prevalence (%)**
Apr-14	Inlet (n=12)	2	16.7 (0-37.8)	1	8.3 (0-24)	1	8.3 (0-24)	1	8.3 (0-24)	0	0
	Outlet (n=12)	1	8.3 (0-24)	2	16.7(0-37.8)	1	8.3 (0-24)	1	8.3 (0-24)	1	8.3 (0-24)
May-14	Inlet (n=12)	1	8.3 (0-24)	1	8.3 (0-24)	1	8.3 (0-24)	1	8.3 (0-24)	2	16.7 (0-37.8)
	Outlet (n=12)	1	8.3 (0-24)	0	0	1	8.3 (0-24)	0	0	1	8.3 (0-24)
Jun-14	Inlet (n=12)	1	8.3 (0-24)	1	8.3 (0-24)	0	0	4	33.3 (6.7-60.0)	0	0
	Outlet (n=12)	1	8.3 (0-24)	0	0	0	0	1	8.3 (0-24.0)	0	0
Jul-14	Inlet (n=12)	2	16.7 (0-37.8)	0	0	0	0	7	58.3 (30.4-86.2)	0	0
	Outlet (n=12)	1	8.3 (0-24)	0	0	1	8.3 (0-24)	3	25 (0.5-49.5)	2	16.7 (0-37.8)
Overall	Inlet (n=48)	6	12.5 (3.1-21.9)	3	6.3 (0-13.1)	2	4.2 (0-9.8)	13	27.1 (14.5-39.7)	2	4.2 (0-9.8)
	Outlet (n=48)	4	8.3 (0.5-16.2)	2	4.2 (0-9.8)	3	6.3 (0-13.1)	5	10.4 (1.8-19.4)	4	8.3 (0.5-16.2)
Total	**n=96**	**10**	**10.4 (4.3-16.5)**	**5**	**5.2 (0.8-9.7)**	**5**	**5.2 (0.8-9.7)**	**18**	**18.8 (10.9-26.6)**	**6**	**6.3 (1.4-11.1)**

4.5 Pathogen load (Milestone 1-5)

4.5.1 Cryptosporidium

The highest median concentration of *Cryptosporidium* oocysts were shed by lambs at WA2 (1.5×10^5 oocysts g^{-1}) (Table 17). Across the other farms, median *Cryptosporidium* oocysts concentration peaked during the pre-slaughter period at SA1, SA2, Vic 2 and WA3 (1.4×10^5, 8.3×10^4, 9.3×10^4 and 1.1×10^5 oocysts g^{-1} respectively). The median concentration of oocysts shed at Vic1 was low (1.6×10^3- 1.6×10^4), although individual sheep shed up to 3.7×10^9 oocysts g^{-1} during post-weaning. This corresponded with a peak prevalence of 18.6% at this time at Vic1. At NSW, the median concentration of oocysts shed was also low (6.3×10^3-1.7×10^4 oocysts g^{-1}) but individual sheep at NSW shed up to 2.1×10^8 and 1.4×10^7 oocysts during post-weaning and pre-slaughter respectively. Across both SA farms, the range of shedding at weaning was 375-7.9×10^6 and the median was 8.8×10^4. The range of oocyst shedding at weaning overall across all states was 63-7.9×10^6 and the median was 3.2×10^4. At pre-slaughter, the range was 260-4.8×10^7 and the median was 6.3×10^4. Of the 7 samples that were positive at all 3 samplings, no trends were seen in oocyst concentration; some were high at the weaning sampling (8.1×10^3-1.4×10^8) and continued

to decrease but others shed an increased concentration of oocysts at the pre-slaughter sampling.

4.5.2 Giardia

The highest median concentration of *Giardia* cysts were shed by lambs at SA1 during post-weaning (8.3 x 10^5 cysts g^{-1}) and WA3 during pre-slaughter (5.2 x 10^5 cysts g^{-1}) (Table 17). Across the other farms, median *Giardia* cyst concentration peaked during the pre-slaughter period at Vic 2, WA1 and WA2 (9.0 x 10^4, 7.5 x 10^4 and 5.2 x 10^4 cysts g^{-1} respectively). At SA2, the highest median concentration of cysts shed was 1.1 x 10^5 cysts g^{-1} at weaning and while the median concentration of cysts shed at post-weaning was low (9.8 x 10^3 cysts g^{-1}), individual lambs shed up to 2.1 x 10^9 cysts g^{-1} during this period. This corresponded with a peak prevalence of 19.9% at post-weaning at SA2. The median concentration of cysts shed at Vic1 was relatively low (1.2 x 10^4-4.2 x 10^4 cysts g^{-1}), although individual sheep shed up to 1.3 x 10^9 cysts g^{-1} during weaning and 1.0 x 10^9 cysts g^{-1} during post-weaning. This corresponded with a peak prevalence of 23.3% at post-weaning at Vic1. At NSW, the median concentration of cysts shed was also low (2.0 x10^3-4.3 x 0^3 cysts g^{-1}) as was the cyst shedding which ranged between 63- 4.5 x 10^5 cysts g^{-1}. The range of cyst shedding at weaning overall across all states was 63 – 1.3 x 10^9 and the median was 1.7 x 10^4. At post-weaning, the range was 63 - 1.1 x 10^9 and the median was 9.6 x 10^3. At pre-slaughter, the range was 63 – 4.7 x 10^9 and the median was 8.1 x 10^4.

4.5.3 Eimeria

The highest median concentration of *Eimeria* oocysts g^{-1} was shed by lambs at weaning in SA2 (1.8 x 10^5 oocysts g^{-1}) (Table 19). Across the other farms, median *Eimeria* oocyst concentration peaked during the weaning period at Vic1 and WA3 (8.3 x 10^3 and 6.5 x 10^4 oocysts g^{-1} respectively). Median *Eimeria* oocyst concentrations peaked during the post-weaning period at SA1 and NSW (5.5 x 10^3 and 7.1 x 10^3 oocysts g^{-1} respectively). Median *Eimeria* oocysts concentration peaked during the pre-slaughter period at WA1 (2.4 x10^3 oocysts g^{-1}). Median *Eimeria* oocyst concentrations in Vic2 were low at all samplings (290, 23 and 380 oocysts g^{-1} respectively). The range of oocyst shedding at weaning overall across all states was 23-2.1 x $10^7$and the median was 224. At post-weaning the range was 23-1.3 x10^7 and the median was 1.1x10^3. At pre-slaughter, the range was 23-2.1 x10^5 and the median was 1.1x10^3. Of the 7 samples that were positive at all 3 samplings, no trends were seen in oocyst concentration; some were low at the weaning sampling (89 to 774 oocysts g^{-1}) and continued to increase at the pre-slaughter sampling (6.5 x 10^3 to 3.5 x 10^4 oocysts g^{-1}) but others shed a decreased concentration of oocysts at the pre-slaughter sampling.

Table 17. Number of protozoan organisms per gram of faeces across 4 states (NSW, SA, Vic and WA) over 3 sampling times (weaning, post-weaning and pre-slaughter) as determined by qPCR.

		Cryptosporidium			*Giardia*			*Eimeria*		
Farm	**Sampling period**	**No. positive**	**Range**	**Median**	**No. positive**	**Range**	**Median**	**No. positive**	**Range**	**Median**
SA1	Weaning	14	938-1.8 x 10^6	4.8 x10^4	27	63-3.7 x10^5	3.9 x 10^3	12	22-344	37
	Post-weaning	9	3.9 x 10^3-9.8 x10^5	2.3 x10^4	30	390-1.9 x10^8	8.3 x 10^5	21	57-2.9 x10^5	4.6 x10^3
	Pre-slaughter	15	7.3 x 10^3-1.7 x 10^5	1.4 x10^5	19	1.4 x10^3-1.7 x10^5	1.5 x 10^5	33	38-375	319
SA2	Weaning	30	375-7.9 x 10^6	3.1 x 10^3	25	1.1 x10^3-1.2 x10^5	1.1 x 10^5	13	60-1.8 x10^7	1.6 x10^5
	Post-weaning	30	313-3.0 x 10^5	4.7 x10^3	31	188-2.1 x 10^9	9.8 x 10^3	25	20-2.1 x10^7	1.3 x10^4
	Pre-slaughter	9	4.7 x 10^3-1.7 x10^6	8.0 x 10^4	26	438-9.5 x 10^5	8.2 x10^4	14	19-550	57
Vic1	Weaning	6	125-8.7 x 10^5	1.6 x 10^4	13	390-1.3 x 10^9	2.3 x 10^4	19	19-6.5 x10^4	7.0 x10^3
	Post-weaning	32	390-3.7 x 10^9	5.1 x 10^3	40	63-1.0 x10^9	4.2 x 10^4	23	19-5.0 x10^4	850
	Pre-slaughter	14	1.6 x 10^3-7.8 x 10^4	1.6 x 10^3	15	1.6 x10^3-4.8 x10^5	1.2 x 10^4	53	19-450	57
Vic2	Weaning	37	313-4.8 x 10^5	7.8 x 10^3	24	63-1.8 x 10^5	9.8 x10^3	29	20-455	244
	Post-weaning	16	1.0 x 10^3-7.1 x 10^6	1.8 x 10^3	36	313-2.0 x 10^8	1.8 x 10^3	18	19-1.0 x10^5	19
	Pre-slaughter	12	937-6.0 x 10^6	9.0 x 10^4	39	125-4.7 x 10^5	9.0 x10^4	13	38-714	319
NSW	Weaning	36	313-1.1 x 10^6	6.1 x 10^3	27	63-4.5 x 10^5	2.0 x 10^3	76	19-8.0 x10^4	550
	Post-weaning	44	563-2.1 x 10^8	1.7 x 10^4	28	313-2.2 x 10^5	3.9 x 10^3	112	20-3.7 x10^5	6.0 x10^3
	Pre-slaughter	21	262-1.4 x 10^7	1.2 x 10^4	58	125-2.0 x 10^5	4.3 x 10^3	20	20-3.3 x10^5	5.0 x10^3
WA1	Weaning	46	125-2.6 x 10^6	1.6 x 10^4	34	313-5.0 x 10^6	2.6 x10^4	10	19-2.9 x10^4	19
	Post-weaning	18	313-1.1 x 10^5	4.5 x 10^3	43	63-1.2 x 10^5	7.6 x 10^3	13	20-1.5 x10^5	38
	Pre-slaughter	29	375-1.6 x 10^7	5.8 x 10^4	51	813-9.5 x 10^3	7.5 x10^4	7	38-4.2 x10^4	2.0x10^3
WA2	Weaning	47	63-5.3 x 10^3	400	30	63-6.9 x 10^5	4.2 x 10^3	13	305-1.9 x10^3	950
	Post-weaning	29	313-2.4 x 10^7	1.5 x 10^5	28	63-1.1 x 10^7	2.8 x 10^4	15	19-300	57
	Pre-slaughter	39	1.6 x10^3-2.9 x 10^7	2.0x10^4	32	125-2.4 x10^6	5.2 x 10^4	36	38-4.7 x10^5	3.3 x10^4
WA3	Weaning	19	313-4.7 x 10^5	2.3 x 10^4	8	1.9 x10^3-1.5 x10^6	3.7 x 10^5	21	19-1.5 x10^7	5.5 x10^4
	Post-weaning	7	313-3.7 x 10^6	5.9 x 10^3	11	63-7.4 x10^8	3.1 x 10^4	14	19-2.5 x10^4	19
	Pre-slaughter	14	2.0x10^3-4.8 x 10^7	1.0 x 10^5	15	63-4.7 x10^9	5.2 x 10^5	6	20-1.8 x10^5	94
	Total	**576**	**63 – 3.7 x 10^9**	**2.6 x 10^4**	**690**	**63-4.7 x 10^9**	**1.6 x 10^4**	**616**	**19-1.8 x 10^7**	**188**

4.5.4 Campylobacter

Across all states, the median concentration of *Campylobacter* spp. in lambs was 3.4 x 10^6 (range 250 to 3.3 x 10^{10}) organisms/g faeces at weaning, 1.1 x 10^5 (250 to 8.2 x 10^{10}) organisms/g faeces post-weaning and 1.5 x 10^5 (range 250 to 1.0 x 10^7) organisms/g faeces at pre-slaughter (Table 18). The highest median number of *Campylobacter* spp. (2.1 x 10^8 organisms/g faeces) was detected on WA3 post-weaning, which coincided with the peak prevalence on this farm. The highest number of *Campylobacter* spp. shed by an individual lamb was also on WA3 post-weaning (8.2 x 10^{10} organisms/g). In SA, high median concentrations of *Campylobacter* spp. were detected on SA1, SA2 and Vic2 during weaning (9.4 x 10^6, 1.6 x 10^9 and 2.9 x 10^6 organisms/g faeces, respectively); this coincided with the peak prevalence on Vic2 (26.7%), whereas the peak prevalence on SA1 and SA2 occurred post-weaning.

4.5.5 Salmonella

Across all states, the median concentration of *S. enterica* in lambs was 9.3 x 10^4 (range 250 to 5 x 10^8) organisms/g faeces at weaning, 7.5 x 10^4 (250 to 1.5 x 10^7) organisms/g faeces post-weaning and 1.8 x 10^5 (range 250 to 1.1 x 10^8) organisms/g faeces at pre-slaughter (Table 18). The highest median number of *S. enterica* (7.4 x 10^7 organisms/g faeces) was detected on SA2 at weaning (Table 18).

4.5.6 Yersinia

The largest median concentration of *Yersinia* spp. organisms/g detected was at Vic2 and SA2 during the post-weaning period (7.6 x 10^7 and 6.4 x 10^7 organisms/g respectively) and SA2 had the highest concentration of organisms shed by an individual during this period (5.5 x 10^{11} organisms/g) (Table 18). This coincided with the peak *Yersinia* spp. point prevalence of 44.2% at SA2 during post-weaning. The point prevalence at Vic2 during the same period (post-weaning) was 38.5% and peaked at 60.2% during pre-slaughter. At WA3 and WA1, the median number of organisms/g at pre-slaughter was 7.9 x 10^6 and 4.2 x 10^6 respectively. The highest concentration of *Yersinia* spp. organisms shed by individual lambs (1.8 x 10^{10}, 4.6 x 10^{10} and 2.1 x 10^{10} organisms/g) across the 3 sampling periods at NSW, which coincided with point prevalences of 55.6%, 90.6% and 61.7% for *Yersinia* spp. across the sampling times respectively. The range of *Yersinia* spp. shedding concentration at weaning overall across all states was 300 – 8.1 x 10^{10} organisms/g and the median was 1.1 x 10^6 organisms/g. At post-weaning, the range was 250 – 5.5 x 10^{11} and the median was 2.8 x 10^6. At pre-slaughter, the range was 221 – 2.1 x 10^{10} and the median was 5.6 x 10^5.

4.5.7 Chlamydia

The highest median number of *C. pecorum* organisms/g was detected at NSW at post-weaning, Vic1 at weaning and WA2 at pre-slaughter (3.1 x 10^9, 2.3 x 10^9 and 1.4 x 10^9 organisms/g, respectively), with a maximum pathogen load of 4.4 x 10^{12} organisms/g detected in one NSW sample at post-weaning (Table 18). This corresponded to *C. pecorum* prevalences of 72.5 % at NSW during post-weaning and 80.8% at pre-slaughter. There were also peaks in the median number of organisms for the weaning and post-weaning periods at SA2 (7.4 x 10^7 and 3.8 x 10^7 organisms/g, respectively) and the post-weaning period at Vic 2

(6.9 x 10^7 organisms/g). The range of *C. pecorum* shedding at weaning overall across all states was 250 to 3.8 x 10^{11} organisms/g and the median was 1.8 x 10^7 organisms/g. At post-weaning, the range was 250 to 4.4 x 10^{12} and the median was 1.2 x 10^7. At pre-slaughter, the range was 250 to 1.4 x 10^{11} and the median was 9.6 x 10^5.

Table 18. Number of bacterial organisms per gram of faeces across 4 states (NSW, SA, Vic and WA) over 3 sampling times (weaning, post-weaning and pre-slaughter) as determined by qPCR.

		Campylobacter			*Yersinia*			*Salmonella*			*Chlamydia*		
Farm	**Sampling period**	**No. positive**	**Range**	**Median**	**No. positive**	**Range**	**Median**	**No. positive**	**Range**	**Median**	**No. positive**	**Range**	**Median**
SA1	Weaning	27	$3.1x10^4$-$6.2x10^9$	$9.4x10^6$	41	$4.0x10^3$-$8.1x10^{10}$	$3.2x10^6$	4	$6.3x10^3$-$1.7x10^7$	$2.1x10^5$	32	500-9.0 $x10^9$	$1.9 x 10^4$
	Post-weaning	66	750-$9.4x10^7$	$1.6x10^5$	50	$3.5x10^3$-$4.1x10^7$	$6.1x10^5$	2	$4.5x10^3$-$2.5x10^6$	$8.8x10^3$	127	250-$6.6x10^7$	$1.3 x 10^5$
	Pre-slaughter	44	$3.8x10^3$-$2.4x10^6$	$1.7x10^5$	42	250 -$1.8x10^7$	$1.4x10^5$	2	$4.5x10^3$-$2.8x10^5$	$1.4x10^5$	25	250-$2.4x10^6$	$1.7 x 10^5$
SA2	Weaning	26	$3.0x10^3$-$1.6x10^9$	$6.5x10^6$	46	$1.3x10^3$-$8.1x10^9$	$6.3x10^6$	3	$6.3x10^3$-$1.7x10^7$	$7.4x10^7$	83	$1.7x10^4$-$1.6x10^{11}$	$7.4 x 10^7$
	Post-weaning	92	250-$1.1x10^8$	$1.9x10^4$	69	$7.8x10^3$-$5.5x10^{11}$	$6.4x10^7$	15	250-$1.5x10^7$	$4.4x10^4$	138	$1.3x10^3$-$3.0x10^{10}$	$3.8 x 10^7$
	Pre-slaughter	46	750-$1.0x10^7$	$2.1x10^5$	50	500-$3.9x10^7$	$2.4x10^5$	4	250-$6.9x10^4$	$2.1x10^3$	17	$4.5x10^3$-$1.0x10^6$	$8.4 x 10^4$
Vic1	Weaning	1	$1.3x10^3$-$1.3x10^3$	$1.3x10^3$	26	750-$2.9x10^8$	$1.8x10^6$	0	0	0	37	$3.0x10^3$-$2.3x10^{11}$	$2.3 x 10^9$
	Post-weaning	4	$8.1x10^5$-$4.3x10^8$	$1.3x10^6$	60	250-$3.0x10^6$	$3.1x10^4$	3	$1.3x10^3$-$6.3x10^3$	$6.3x10^3$	30	250-$1.6x10^8$	$1.7 x 10^5$
	Pre-slaughter	6	250-$8.1x10^4$	$1.4x10^4$	108	500-$2.4x10^7$	$1.4x10^5$	5	$3.8x10^3$-$3.7x10^5$	$2.4x10^4$	10	250-$2.8x10^5$	$1.9 x 10^4$
Vic2	Weaning	47	250-$3.4x10^{10}$	$2.9x10^6$	16	$3.8x10^3$-$4.2x10^9$	$5.1x10^6$	10	250-$7.8x10^5$	$1.3x10^4$	0	0	0
	Post-weaning	4	$6.9x10^4$-$6.3x10^6$	$2.5x10^5$	62	$2.3x10^3$-$7.2x10^9$	$7.6x10^7$	9	250-$7.5x10^6$	$1.9x10^4$	33	$7.9x10^5$-$8.2x10^{10}$	$6.9x10^7$
	Pre-slaughter	0	500-$4.1x10^6$	$6.9x10^5$	77	$1.1x10^4$-$3.3x10^9$	$1.4x10^7$	0	0	0	4	500-$6.3x10^3$	$1.3x10^3$
NSW	Weaning	7	$4.8x10^3$-$6.8x10^6$	$3.4x10^5$	89	750-$1.8x10^{10}$	$1.3x10^6$	28	$5.5x10^3$-$4.5x10^6$	$7.8x10^4$	68	250-$3.8x10^{11}$	$7.7x10^6$
	Post-weaning	7	$3.0x10^3$-$7.2x10^6$	$2.1x10^4$	145	750-$4.6x10^{10}$	$4.0x10^6$	38	250-$7.3x10^6$	$1.2x10^5$	116	$4.5x10^4$-$4.4x10^{12}$	$3.1x10^9$
	Pre-slaughter	2	931-$1.1x10^7$	$6.1x10^3$	103	222-$2.1x10^{10}$	$9.9x10^5$	6	$1.8x10^3$-$1.6x10^7$	$3.0x10^5$	0	250-$4.0x10^9$	$8.9x10^5$
WA1	Weaning	13	$2.0x10^3$-$5.7x10^8$	$7.1x10^5$	9	$1.3x10^3$-$9.6x10^7$	$1.2x10^6$	0	0	0	9	250-$1.8x10^9$	$3.5x10^6$
	Post-weaning	5	$6.3x10^3$-$1.4x10^5$	$3.2x10^4$	15	250-$1.3x10^8$	$6.3x10^3$	8	$1.4x10^4$-$9.2x10^5$	$2.3x10^5$	30	$1.3x10^3$-$1.7x10^9$	$9.3x10^5$
	Pre-slaughter	7	$4.0x10^3$-$5.0x10^6$	$1.7x10^5$	10	5.9 $x10^4$-$6.9x10^7$	$4.2x10^6$	13	$1.5x10^3$-$1.1x10^8$	$6.6x10^6$	13	$3.0x10^3$-$1.1x10^6$	$9.4x10^4$
WA2	Weaning	13	475-$2.9x10^5$	$5.4x10^4$	23	300-$2.7x10^7$	$3.8x10^5$	5	$1.8x10^5$-$5.0x10^8$	$7.4x10^6$	19	$1.5x10^3$-$2.9x10^7$	$1.5x10^6$
	Post-weaning	5	$1.3x10^3$-$7.5x10^7$	$1.7x10^5$	6	$3.5x10^3$-$3.2x10^7$	$9.3x10^5$	3	$5.6x10^4$-$7.1x10^5$	$1.3x10^5$	18	250-$1.7x10^8$	$1.0x10^6$
	Pre-slaughter	10	$4.0x10^3$-$5.0x10^6$	$1.7x10^5$	24	$8.0x10^3$-$6.5x10^7$	$7.8x10^5$	0	0	0	52	$1.1x10^6$-$1.4x10^{11}$	$1.4x10^9$
WA3	Weaning	0	0	0	6	$6.3x10^3$-$3.1x10^9$	$1.3x10^4$	4	$5.8x10^5$-$8.3x10^6$	$1.6x10^6$	0	0	0
	Post-weaning	16	$4.5x10^3$-$8.2x10^{10}$	$2.1x10^8$	12	$1.3x10^3$-$1.2x10^8$	$3.2x10^5$	8	$2.1x10^4$-$3.7x10^6$	$3.3x10^5$	8	$1.5x10^3$-$1.6x10^{11}$	$5.2x10^8$
	Pre-slaughter	5	750-$6.4x10^4$	$1.1x10^4$	4	500-$1.6x10^7$	$7.9x10^6$	0	0	0	3	250-$8.9x10^7$	$1.3x10^3$
	Total	**453**	**0-$8.2x10^{10}$**	**$3.9x10^5$**	**1093**	**250-$5.5x10^{11}$**	**$1.2x10^6$**	**170**	**0-$5.0x10^8$**	**$9.4x10^4$**	**1027**	**0-$4.4x10^{12}$**	**$8.1x10^5$**

4.5.9 Trichostrongylid worms

Overall the pathogen load of *Haemonchus* was low across most farms (Table 19) as was the prevalence. There was a large peak in the median *Haemonchus* epg detected at NSW during the pre-weaning sampling period (1.3 x 10^3 epg) and a smaller peak at weaning (294 epg). At SA1 and SA2, there were also two small peaks in the median *Haemonchus* epg during the weaning period (71 and 72 epg respectively). These peaks reflect the fact that *Haemonchus* is a summer rainfall dominant parasite.

Teladorsagia, displayed a very large peak in median epg at WA1 during the weaning sampling period (4.4 x 10^3 epg) with 8.7 x 10^3 *Teladorsagia* epg detected in one individual at the same sampling period at the same farm. At SA1, the median epg peaked at 1.4 x 10^3 in the weaning sampling period. There were also two medium peaks from SA2 during in the weaning sampling period (856 epg) and from NSW during the pre-slaughter period (550 epg).

For *Trichostrongylus*, the highest median epg detected was at NSW at pre-slaughter (5.9 x 10^3), followed by Vic2 at post-weaning (946 epg), SA2, NSW and SA1 at weaning (693, 650 and 625 epg respectively).

The range of worm egg concentration by qPCR at weaning overall across all states was 1-1.2 x 10^4, 1-5.4 x 10^4 and ~1-1.3 x 10^4 epg respectively for *Haemonchus, Teladorsagia* and *Trichostrongylus* and the medians were 191, 835 and 447 epg respectively. At post-weaning, the range was 1-3.3 x 10^4, 1-1.5 x 10^4 and 1-4.5 x 10^4 epg respectively for *Haemonchus, Teladorsagia* and *Trichostrongylus* and the medians were 705, 69 and 268 epg respectively. At pre-slaughter, the range was 1-1.4 x 10^3, 1-1.1 x 10^4 and 1-1.5 x 10^4 epg and the medians were 12, 38 and 1.2 x 10^3 epg.

Table 19. Number of trichostrongylid worms per gram of faeces across 4 states (NSW, SA, Vic and WA) over 3 sampling times (weaning, post-weaning and pre-slaughter) as determined by qPCR.

		Haemonchus			*Teladorsagia*			*Trichostrongylus*		
Farm	**Sampling period**	**No. positive**	**Range**	**Median**	**No. positive**	**Range**	**Median**	**No. positive**	**Range**	**Median**
SA1	Weaning	13	<1-588	71	39	<1-2.3x10^4	1.4x10^3	83	<1-1.3x10^4	625
	Post-weaning	29	<1-121	7	74	<1-1.4x10^3	29	113	<1-3.510^3	25
	Pre-slaughter	24	2.2-35	2.6	68	1.6-3.2x10^3	176	114	20-229	29
SA2	Weaning	10	<1-557	72	53	<1 -1.2x10^3	856	90	<1-1.2x10^3	693
	Post-weaning	14	<1-157	17	106	<1-2.4x10^3	165	146	<1-4.5x10^4	98
	Pre-slaughter	17	<1-<1	<1	91	<1-180	5	33	1.3-1.6x10^3	90
Vic1	Weaning	18	<1-47	6.2	60	<1-569	38	91	<1-5.6x10^3	112
	Post-weaning	12	<1-181	11	47	<1 -8.7x10^3	10	67	<1-7.8x10^3	148
	Pre-slaughter	81	10-240	10	75	2.2-164	31	113	<1-233	38
	Weaning	23	<1-480	67	45	10-5.4x10^4	277	62	<1-5.6x10^3	177
Vic2	Post-weaning	5	<1-<1	<1	55	10-1.5x10^4	140	56	<1 -1.1x10^4	946
	Pre-slaughter	12	<1-1.4x10^3	4	70	10-1.1x10^4	20	81	<1 -8.7x10^3	155
NSW	Weaning	105	<1-1.2x10^4	294	90	<1 -510	30	121	<1-2.0x10^3	650
	Post-weaning	91	<1-3.3x10^4	1.3 x10^3	119	<1-372	8	123	<1-2.3x10^3	490
	Pre-slaughter	13	<1-126	14	118	<1-2.0x10^3	550	137	<1-1.5x10^4	5.9x10^3
WA1	Weaning	3	0	0	76	<1-8.7x10^3	4.4 x10^3	40	<1-9.7x10^3	381
	Post-weaning	4	<1-<1	<1	34	<1-428	7	17	<1-465	15
	Pre-slaughter	1	5.0-5.0	5	48	<1-<1	<1	20	<1-124	7
WA2	Weaning	6	<1-45	5	31	<1-5.5x10^3	180	20	<1-3.9x10^3	40
	Post-weaning	1	<1-<1	<1	80	<1-428	6.9	82	<1-466	15
	Pre-slaughter	0	0	0	84	<1-577	13	26	<1-1.5x10^3	83
WA3	Weaning	11	<1-2	<1	5	<1-48	13	33	<1-9.9x10^3	526
	Post-weaning	12	<1-16	1.4	7	<1-9.2x10^3	13	8	1.2-120	10
	Pre-slaughter	0	0	0	4	<1-492	123	10	<1-474	48
	Total	**505**	**<1-3.3x10^4**	**12**	**1479**	**<1-5.4x10^4**	**230**	**1686**	**<1-4.5x10^4**	**260**

4.6 Pathogen load in sheep at WA sale yards and in abattoir effluent at Katanning (Milestone 6)

4.6.1 Pathogen load at WA sale yards

The highest median numbers of *Cryptosporidium* oocysts were shed by lambs at the Katanning sale yard during the May collection (1.9 x 10^5 oocysts g^{-1}) and ranged between 1.5 x 10^3 and 3.7 x 10^3 for the other sampling times (Table 20). The highest median numbers of *Cryptosporidium* oocysts shed at Muchea was 2.5 x 10^3 oocysts g^{-1} for the July sampling and ranged from 461-849 oocysts g^{-1} for the other sampling times. The highest median numbers of *Giardia* cysts were shed by lambs at the Katanning sale yard during the July collection (1.4 x 10^4 cysts g^{-1}) and ranged from 1.6 x 10^3 to 3.9 x 10^3 cysts g^{-1} for the other sampling times. At Muchea, the highest median numbers of *Giardia* cysts were shed by lambs during the August collection (7.5 x 10^5 cysts g^{-1}) and ranged from 1.9 x 10^3 to 3.2 x 10^3 cysts g^{-1} for the other sampling times, with the exception of the July sampling, where no cysts were detected.

Overall, *Campylobacter* had the highest median number of organisms shed (4.7 x 10^3 organisms g^{-1}), followed by *Salmonella* (4.5 x 10^3 organisms g^{-1}) and *E. coli* 0157:H7 (2.7 x 10^3 organisms g^{-1}). The highest median numbers of *E. coli* 0157:H7 were shed by lambs at Katanning during the April sampling (1.6 x 10^4 organisms g^{-1}) and ranged between 1.7 x 10^3 and 4.2 x 10^3 organisms g^{-1} for the other sampling times (Table 20). In Muchea, the highest median numbers of *E. coli* 0157:H7 were shed by lambs during the May sampling (5.4 x 10^3 organisms g^{-1}) and ranged between 1.6 x 10^3 and 2.2 x 10^3 for the other sampling times. The highest median numbers of *Campylobacter* organisms were shed by lambs at Katanning during the May sampling (5.4 x 10^5 organisms g^{-1}) and ranged between 2.3 x 10^3 and 3.2 x 10^4 organisms g^{-1} for the other sampling times. In Muchea, the highest median numbers of *Campylobacter* organisms were shed by lambs during the June sampling (2.2 x 10^4 organisms g^{-1}) and ranged between 2.6 x 10^3 and 3.5 x 10^3 for the other sampling times. The highest median numbers of *Salmonella* organisms were shed by lambs at Katanning, during the June sampling (4.5 x 10^3 organisms g^{-1}) and ranged between 2.3 x 10^3 and 4.0 x 10^3 for the other sampling times. In Muchea, the highest median numbers of *Salmonella* organisms were shed by lambs during the July sampling (7.9 x 10^3 organisms g^{-1}) and ranged between 2.7 x 10^3 and 5.6 x 10^3 for the other sampling times.

4.6.2 Pathogen load in abattoir effluent at Katanning

The median numbers of *Cryptosporidium* oocysts detected in effluent ranged from 587 to 1.5 x 10^4 oocysts g^{-1} (Table 21). Generally the numbers of oocysts detected were lower in the outlet compared to the inlet with the exception of the May sampling when the median number of oocysts detected was 1.2 x 10^3 oocysts g^{-1} in the inlet and 1.5 x 10^4 oocysts g^{-1} were detected in the outlet. For *Giardia*, the median numbers of cysts detected in effluent ranged from 759 to 8.1 x 10^3 cysts g^{-1}. No *Giardia* cysts were detected in the May and June outlet samples and in the inlet and outlet samples in July.

The highest median numbers of *E. coli* 0157:H7 in effluent (2.4 x 10^4 organisms g^{-1}) was detected in the outlet during the July sampling (Table 21) and ranged between 1.2 x 10^3 and 5.0 x 10^3 for the other sampling times (Table 21). For *Campylobacter*, the highest median

numbers of organisms in effluent (4.1×10^4 organisms g^{-1}) was detected in the inlet during the June sampling and ranged between 2.9×10^3 and 4.0×10^4 for the other sampling times. The highest median numbers of *Salmonella* in effluent (2.1×10^4 organisms g^{-1}) was detected in the inlet during the May sampling and ranged between 1.1×10^3 and 8.4×10^3 for the other sampling times.

Table 20. Number of protozoan and bacterial organisms per gram of faeces (range and median) from Katanning and Muchea sale yards as determined by qPCR.

		E. coli O157:H7			*Campylobacter*			*Salmonella*			*Cryptosporidium*			*Giardia*		
		No. positive	**Range**	**Median**	**No. positive**	**Range**	**Median**	**No. positive**	**Range**	**Median**	**No. positive**	**Range**	**Median**	**No. positive**	**Range**	**Median**
Katanning	April 2014 (n=60)	3	$4.2x10^3$-$2.1 x10^4$	$1.6x10^4$	2	$1.8x10^3$-$2.7x10^3$	$2.3x10^3$	1	$2.3x10^3$-$2.3x10^3$	$2.3x10^3$	5	175-$3.2x10^3$	$1.6x10^3$	3	331-$2.1x10^3$	$1.6x10^3$
	May 2014 (n=60)	2	260-$3.3x10^3$	$1.7x10^3$	4	430-2.0 $x10^6$	5.4 $x10^5$	0	0	0	3	265-$3.7x10^6$	$1.9x10^5$	4	143-9.7 $x10^3$	2.8 $x10^3$
	June 2014 (n=58)	2	$2.5x10^3$-$6.0x10^3$	$4.2x10^3$	7	430-$6.1x10^5$	$3.2x10^4$	2	$2.9x10^3$-$6.0x10^3$	$4.5x10^3$	3	250-$1.9x10^5$	$3.7x10^3$	3	$1.7x10^3$-$9.7x10^3$	$3.9x10^3$
	July 2014 (n=60)	0	0	0	7	355-$3.2x10^4$	$4.4x10^3$	4	296-$1.3x10^4$	$4.0x10^3$	3	963-$3.1x10^4$	$1.5x10^3$	2	246-$2.7x10^4$	$1.4x10^4$
	Sub-total (n=238)	7	260-$2.1x10^4$	$2.8x10^3$	20	355-$2.0x10^6$	$6.7x10^3$	7	296-$1.3x10^4$	$3.8x10^3$	14	175-$3.7x10^6$	$3.5x10^3$	12	143-$2.7x10^4$	$3.2x10^3$
Muchea	May 2014 (n=58)	15	660-$3.5x10^4$	$5.4x10^3$	1	2.6x103-$2.6x10^3$	$2.6x10^3$	3	$2.3x10^3$-$2.0x10^4$	$5.6x10^3$	3	233-825	461	12	600-$3.8x10^5$	$2.9x10^3$
	June 2014 (n=58)	5	910-$1.3x10^5$	$2.2x10^3$	3	$1.2x10^3$-$4.8x10^4$	$2.2x10^4$	3	$2.2x10^3$-$3.1x10^3$	$2.7x10^3$	6	65-$2.4x10^5$	849	5	$1.7x10^3$-$2.1x10^4$	$3.2x10^3$
	July 2014 (n=60)	9	146-$2.0x10^4$	$2.0x10^3$	3	$2.2x10^3$-$5.3x10^3$	$3.5x10^3$	1	$7.9x10^3$-$7.9x10^3$	$7.9x10^3$	3	32-$6.1x10^4$	$2.5x10^3$	0	0	0
	Aug 2014 (n=60)	4	$1.1x10^3$-$2.8x10^3$	$1.6x01^3$	0	0	0	3	$2.5x10^3$-$2.2x10^5$	$4.2x10^3$	5	260-$2.2x10^3$	590	1	$7.5x10^5$-$7.5x10^5$	$7.5x10^5$
	Sub-total (n=236)	33	146-$1.3x10^4$	$2.6x10^3$	7	$1.2x10^3$-$4.8x10^4$	$3.7x10^3$	10	$2.2x10^3$-$2.2x10^5$	$5.5x10^3$	17	32-$2.4x10^5$	849	18	600-$7.5x10^5$	$1.9x10^3$
Overall	**n=474**	**40**	**146-$2.1x10^4$**	**$2.7x10^3$**	**27**	**355-$2.0x10^6$**	**$4.7x10^3$**	**17**	**296-$2.2x10^5$**	**$4.5x10^3$**	**31**	**32-$3.7x10^6$**	**$1.7x10^3$**	**30**	**143-$7.5x10^5$**	**$2.5x10^3$**

Table 21. Number of protozoan and bacterial organisms per gram of effluent at Katanning as determined by qPCR.

		Ecoli O157:H7			*Campylobacter*			*Salmonella*			*Cryptosporidium*			*Giardia*		
Sampling period	**Inlet/Outlet**	**No. positive**	**Range**	**Median**	**No. positive**	**Range**	**Median**	**No. positive**	**Range**	**Median**	**No. positive**	**Range**	**Median**	**No. positive**	**Range**	**Median**
Apr-14	Inlet (n=12)	1	393-2.0x10^3	1.2x10^3	1	1.8x10^4-1.8x10^4	1.8x10^4	0	0	0	2	393-2.0x10^3	1.2x10^3	1	759-759	759
	Outlet (n=12)	1	2.6x10^3-2.6x10^3	2.6x10^3	1	6.7x10^3-6.7x10^3	6.7x10^3	1	5.4x10^3-5.4x10^3	5.4x10^3	1	587-587	587	2	1.5x10^3-4.8x10^3	3.2x10^3
May-14	Inlet (n=12)	1	5.0x10^3-5.0x10^3	5.0x10^3	1	2.9x10^3-2.9x10^3	2.9x10^3	2	3.5x10^3-4.0x10^3	2.1x10^4	1	1.2x10^3-1.2x10^3	1.2x10^3	1	8.1x10^3-8.1x10^3	8.1x10^3
	Outlet (n=12)	1	3.6x10^4-3.6x10^4	3.6x10^4	0			1	8.4x10^3-8.4x10^3	8.4x10^3	1	1.5x10^4-1.5x10^4	1.5x10^4	0	0	0
Jun-14	Inlet (n=12)	0	0	0	4	1.5x10^3-7.8x10^5	4.1x10^4	0	0	0	1	750-750	750	1	1.8x10^3-1.8x10^3	1.8x10^3
	Outlet (n=12)	0	0	0	1	3.2x10^4-3.2x10^4	3.2x10^4	0	0	0	1	894-894	894	0	0	0
Jul-14	Inlet (n=12)	0	0	0	7	1.8x10^3-4.5x10^5	4.7x10^3	0	0	0	2	1.9x10^3-7.5x10^3	2.5x10^3	0	0	0
	Outlet (n=12)	1	2.4x10^4-2.4x10^4	2.4x10^4	3	6.9x10^3-6.6x10^4	4.0x10^4	2	1.0x10^3-1.3x10^3	1.1x10^3	1	1.3x10^3-1.3x10^3	1.3x10^3	0	0	0
Total	**n=96**	4	293-3.6x10^4	7.8x10^3	18	1.5x10^3-7.8x10^5	3.2x10^4	6	1.0x10^3-8.4x10^3	2.5x10^3	**10**	587-1.5x10^4	1.3x10^3	5	759-4.8x10^3	587-1.5x10^4

Salmonella was not detected in the April inlet, June inlet and outlet samples and July inlet samples.

4.7 Genotyping (Milestones 1-5)

4.7.1 Cryptosporidium

The 576 positives detected at the actin locus were screened using *C. parvum/C. hominis* specific primers and the 18S primers and the positives sequenced. Of these, 500 were successfully genotyped. The following species/genotypes were identified; *C. xiaoi* (69% - 345/500), *C. ubiquitum* (17.6% - 88/500), *C. parvum* (9.8% - 49/500), *C. scrofarum* (previously pig genotype II) (0.8% - 4/500), mixed *C. parvum* and *C. xiaoi* (2.4% - 12/500), *C. andersoni* (0.2% -1/500) and sheep genotype 1 (0.2% -1/500).

Across the states, *C. xiaoi* was the most prevalent and peaked at 81.4% (57/70) of positive samples for NSW (Fig. 10). *Cryptosporidium ubiquitum* was the second most prevalent species in SA at 43.2% (43/88) positive samples and in Vic at 21.7% (20/92), whereas in NSW and WA, *C. parvum* was the second most prevalent species at 10% (7/70) and 10.4% (26/250) compared to 8.6% (6/72) and 9.6% (24/250) respectively for *C. ubiquitum*. *Cryptosporidium scrofarum* was only identified in Vic and mixed *C. parvum, C. xiaoi* infections as well as *C. andersoni* and sheep genotype I were only identified in WA.

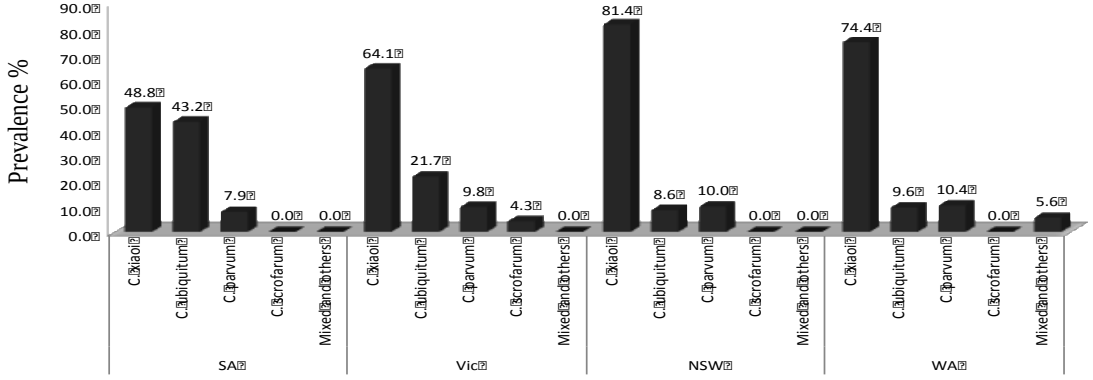

Figure 10. The prevalence (%) of *Cryptosporidium* species in sheep faecal samples from SA, Vic, WA and NSW.

Subtyping of *C. parvum* identified IIa and IId subtype families. All *C. ubiquitum* isolates typed (n=88) were XIIa. The following *C. parvum* subtypes were identified; IIaA15G2R1 (n=5), IIdA19G1 (n=10), IIdA18G1 (n=23). The *C. parvum* subtype IIaA15G2R1 was identified at Vic1 (n=3) and NSW (n=2). Subtype IIdA19G1 was identified in SA1 (n=3), SA2 (n=3), Vic1 (n=1) and Vic2 (n=3). Subtype IIdA18G1 was identified in SA1 (n=1), NSW (n=3), WA1 (n=2), WA2 (n= 9) and WA2 (n=8).

4.7.2 Giardia

The 690 positives detected at the *gdh* locus were screened using assemblage specific primers at the *tpi* locus. Of these, 473 were successfully genotyped; assemblage A was identified in 22.4% (106/473) of positive samples typed, assemblage E in 75.9% (359/473) and mixed A and E assemblages in 1.7% (8/473) of samples. Assemblage E was the most prevalent across all states and peaked at 20.1% for post-weaning in WA (Fig. 11). Assemblage A was most prevalent in NSW and peaked during the pre-slaughter sampling

(10.8%). Mixed A and E infections were only identified in WA and were confirmed by sequencing.

Two assemblage A and 2 assemblage E isolates from each of the 8 farms (n=32) were sequenced at both the *gdh* and *beta-giardin* loci. All the 16 assemblage A *gdh* sequences were identical to each other and were identified as sub-assemblage AII by aligning with reference AII *gdh* sequence AY178737. The 16 Assemblage E sequences at the *gdh* locus were also identical to each other and were confirmed as assemblage E by aligning with reference assemblage E *gdh* sequence AY178741. Similarly, at the *beta-giardin* locus, all 16 assemblage A sequences were identical to each other and were typed as sub-assemblage AII.

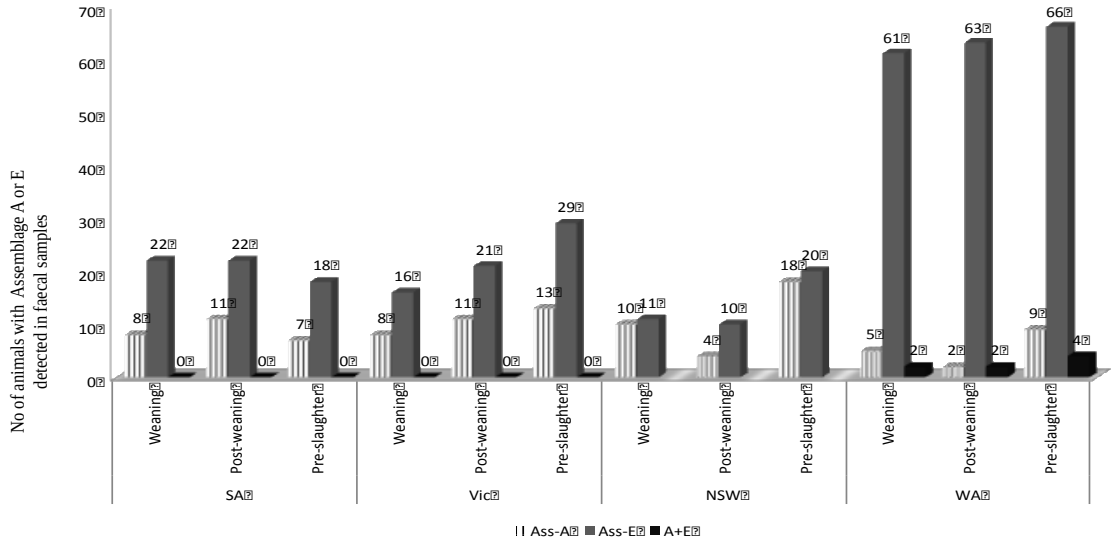

Figure 11. The number of animals with *G. duodenalis* assemblage A or E sequences detected in sheep faecal samples from SA, Vic, WA and NSW and across different sampling times.

4.7.3 Eimeria

Of the 616 positives, a subset of 5 positives from each sampling in each farm (n=120) were sequenced and of these, 118 sequences were successfully obtained (only 3 sequences were obtained from WA3 at post-weaning). The following species were identified; *E. crandallis* (48.3%, 57/118), *E. ahsata* (28%, 33/118)*, E. ovinoidalis* (10.1%, 12/118)*, E. weybridgensis* (10.1%, 12/118) and *E. cylindrica* (4.2%, 5/118). No mixed infections were detected.

Of the 118 isolates sequenced, *E. crandallis* was the most prevalent across all states and peaked at 53.3% (8/15) for NSW (Fig. 12). *Eimeria ahsata* was the second most prevalent species across all states and peaked at 33.3% (10/30) in Vic, with peaks of 25.6-26.7 across the other states. In WA and NSW, *E. weybridgensis* was the third most prevalent species, which peaked at 16.3% (5/38) in WA and 13.3% (2/15) in NSW. However, the prevalences for SA and Vic were lower at 6.7% (2/30) and 3.3% (1/30) respectively. *Eimeria ovinoidalis* was the fourth most prevalent with a 10% prevalence (3/30) for both SA and Vic. In WA and NSW, *E. ovinoidalis* prevalence was at 11.6% (5/43) and 6.7% (1/15) respectively. *Eimeria cylindrica* was detected in SA and Vic only at a prevalence of 6.7% (2/30) and 10.0% (3/30)

respectively. All *E. cylindrica* isolates sequenced (n=5) were 100% identical to each other and exhibited 2 single nucleotide polymorphisms (SNP's) from bovine-derived *E. cylindrica* (GenBank accession number AB769616).

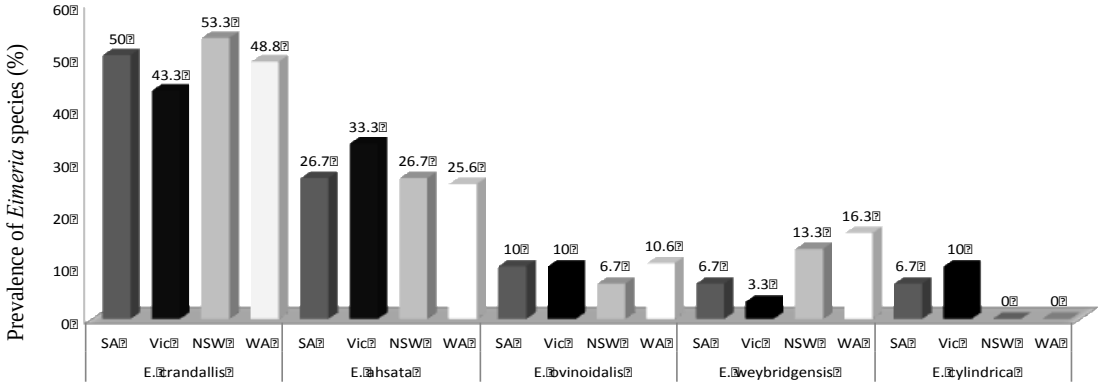

Figure 12. The prevalence (%) of *Eimeria* species from a subset of 118 sheep faecal samples from SA, Vic, WA and NSW.

4.7.4 Campylobacter and Salmonella

All *Campylobacter* spp. sequenced (*n* = 120) were identified as *C. jejuni* (16S rRNA sequence identical to GenBank CP001876) and all *S. enterica* sequenced (*n* = 120) were identified as *S. enterica* Typhimurium (*ompF* sequence identical to GenBank Z31594) (data not shown).

4.7.5 Chlamydia

A subset of 48 positives (two from each sampling in each farm; *n* = 48) were sequenced and confirmed the identity of *C. pecorum* in all positives analysed (data not shown).

4.7.6 Yersinia

A subset of up to five positives randomly chosen from each sampling in each farm, (*n* = 111) were sequenced. A total of four species were identified; *Y. enterocolitica* (*n* = 69), *Y. pseudotuberculosis* (*n* = 32)*, Y. intermedia* (*n* = 7) *and Y. frederiksenii* (*n* = 3). Of the subset of positive isolates analysed, *Y. enterocolitica* was the most common species identified across all farms ranging from 46.7% at WA1 to 86.7% in NSW (Fig. 13). *Yersinia pseudotuberculosis* was the second most common species identified in positive samples across all farms and ranged from 6.7% in NSW to 45.5% at WA3. *Yersinia intermedia* was only detected in SA2, Vic2, NSW, WA1 and WA2 ranging from 6.7-13.3% of positive samples. *Yersinia frederiksenii* was detected in Vic 2 and at WA1 and 2 and ranged from 6.7% at WA2 to 10% at Vic2 of positive samples (Fig. 13).

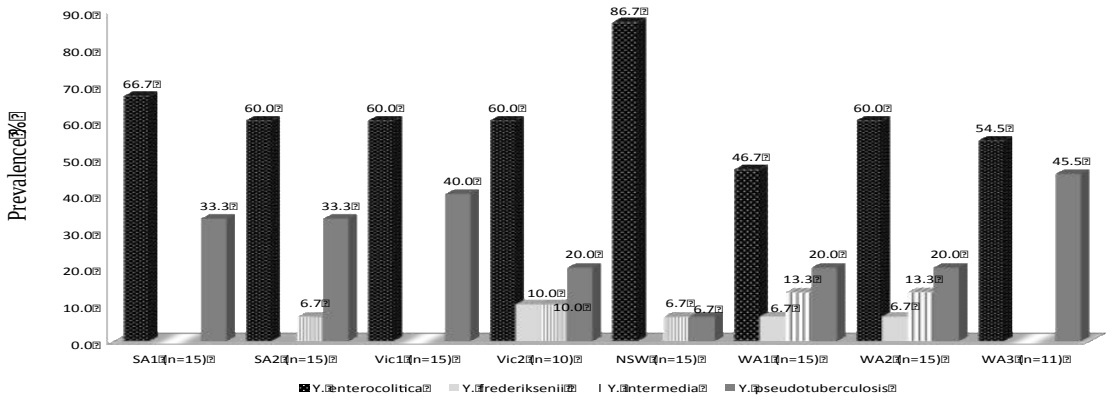

Figure 13. *Yersinia* species detected in a subset of isolates (n=111) from 8 farms across 4 states at 3 sampling periods.

4.8 Genotyping (Milestone 6)

Genotyping was only successful for *Cryptosporidium* and *Giardia* positives from the saleyard faecal samples as there was insufficient template and/or mixed chromatograms for the positives from abattoir effluent. For *Cryptosporidium*, twenty-four out of thirty-one qPCR positive isolates were successfully genotyped. Three *Cryptosporidium* species were identified; *C. parvum* (16.7%, 4/24), *C. ubiquitum* (37.5%, 9/24) and *C. xiaoi* (45.8%, 11/24) (data not shown). Subtyping of *C. parvum* and *C*. *ubiquitum* isolates at the gp60 locus, revealed that all four *C. parvum* isolates belonged to the IId subtype (IIdA18G1) and the nine *C*. *ubiquitum* isolates belonged to XIId subtype family. For *Giardia*, twenty-seven out of the thirty positives were successfully genotyped. Of these, eight were assemblage A (29.6%) and twenty-two were assemblage E (81.5%) (data not shown).

4.9 Association with Production Parameters (Milestones 1-5)

Statistical analysis revealed no association between WEC and production parameters. Only three pathogens (*Cryptosporidium, Giardia* and *Yersinia*) consistently showed significant effects.

4.9.1 Growth, carcase weight and DP

4.9.1.1 Cryptosporidium (all species), growth and carcase productivity

Cryptosporidium spp. shedding at pre-slaughter was associated with about 1.2% lower DP, within both high shedding (P<0.01) and low shedding (P<0.01) lambs compared to non-shedding lambs (Table 22). The impact of high and low shedding categories on DP did not differ from each other. In contrast there was no association between *Cryptosporidium* spp. shedding category and DP at either weaning or post-weaning. There was also no association between *Cryptosporidium* spp. shedding category with either HSCW (at any of the sampling time points) or liveweight.

4.9.1.2 Cryptosporidium parvum, growth and caracse productivity

Cryptosporidium parvum shedding at pre-slaughter was associated with between 2.2 – 2.6kg lower HSCW, in both high shedding (P<0.01) and low shedding lambs (P<0.01) compared to

non-shedding lambs (Table 22). The impact on HSCW did not differ between these two groups.

This HSCW effect was also reflected at the post-weaning time point, but was only evident in the high shedding group where high *C. parvum* shedding was associated with about 3.2kg lower HSCW (P<0.01) compared to non-shedding lambs (Table 22). At weaning there was no association between *C. parvum* shedding category and HSCW.

For DP, the only association evident with *C. parvum* shedding category was observed at post-weaning where the high shedding group had 2.7% lower DP (P<0.01) than the non-shedding lambs (Table 22).

Cryptosporidium parvum shedding category was associated with lower live weight (Table 22). Compared with non-shedding lambs, high shedding lambs were 2.78kg lighter at weaning (P<0.01), 4.52kg lighter at post-weaning (P<0.01) and 2.31kg lighter at slaughter (P<0.01; Table 22). Live weight in low shedding lambs were 2.78kg lighter at slaughter than non-shedding lambs at weaning (P=0.058).

4.9.1.3 Giardia, growth and caracse productivity

Giardia shedding at post-weaning was associated with lower HSCW, with HSCW reductions of 0.59kg in high shedding lambs relative to non-shedding lambs (P<0.05; Table 22). High *Giardia* shedding lambs had 0.66kg lower HSCW relative to low shedding lambs (P=0.053) at post-weaning. There were no associations between *Giardia* spp. shedding category and HSCW at weaning or pre-slaughter, nor between *Giardia* spp. shedding category and either DP (any time point) or live weight.

4.9.1.4 Associations of frequency of Cryptosporidium and Giardia detection with carcase weight and DP

Increased frequency of detection of *C. parvum* and *Giardia* were both associated with reductions in HSCW and DP (Table 23). Specifically, detection of *C. parvum* on three occasions was associated with 4.02kg lower HSCW (P<0.001) and 3.85% lower DP (P<0.05) compared with lambs in which *C. parvum* was never detected. Detection of *C. parvum* on two occasions was associated with 2.588kg lower HSCW (P<0.05) compared with detection on one occasion, and *C. parvum* detection on one occasion was associated with 1.427kg lower HSCW (P<0.001) compared with lambs in which *C. parvum* was never detected. A similar pattern (albeit with smaller magnitude of difference) was observed for *Giardia* spp. with detection on three occasions associated with 0.93kg lower HSCW (P=0.065) and 1.41% lower DP (P<0.05) compared with lambs in which *Giardia* was never detected, and detection on two occasions was associated with 0.82kg lower HSCW (P<0.01) and 0.63% lower DP (P=0.086) compared with lambs in which *Giardia* was never detected.

4.9.1.5 Association of mixed Giardia and Cryptosporidium infections with HSCW, DP and live weight

There was no significant main effect (P>0.100) of infection type (no infection, single *Giardi*a infection, single *Cryptosporidium* infection, mixed *Giardia* and *Cryptosporidium* infection) on live weight, HSCW (for any sampling occasion) or DP at weaning or post-weaning. There was an association between infection type at pre-slaughter and DP (P<0.01) whereby lambs with shedding *Cryptosporidium* only (44.79%) or mixed *Giardia* and *Cryptosporidium*

(44.29%) had lower DP than lambs with no infection (45.77%). Similarly, lambs shedding mixed *Giardia* and *Cryptosporidium* (44.29%) had lower DP than lambs shedding *Giardia* only (45.36%).

Table 22. Association of parasite shedding category with lamb carcase weight, DP and live weight with least square means ± standard error and F value for parasite main effect. F values significant <0.05 are shown in bold.

	Sampling occasion	Shedding category and main effect	Parasite		
			C. parvum	*Cryptosporidium* spp.	*Giardia* spp.
HSCW (kg) [#]	Weaning	High shedding	20.550±0.726	21.333±0.248	21.307±0.285
		Low shedding	20.164±0.800	21.573±0.250	21.159±0.288
		Not detected	21.614±0.079	21.621±0.090	21.663±0.086
		Parasite F value	2.61	0.58	1.92
	Post-weaning	High shedding	18.404±0.848[a]	21.361±0.284	21.068±0.221[a]
		Low shedding	21.619±1.204[b]	21.13±0.293	21.727±0.259[b]
		Not detected	21.622±0.079[b]	21.612±0.086	21.61±0.089[b]
		Parasite F value	**7.14****	0.45	**3.3***
	Pre-slaughter	High shedding	19.468±0.798[a]	21.488±0.284	21.557±0.219
		Low shedding	19.074±1.069[a]	21.324±0.308	21.345±0.228
		Not detected	21.676±0.077[b]	21.690±0.084	21.704±0.090
		Parasite F value	**6.69****	0.8	1.12
DP [#]	Weaning	High shedding	44.62±1.02	45.56±0.35	45.52±0.401
		Low shedding	43.76±1.12	45.26±0.35	45.07±0.406
		Not detected	45.52±0.11	45.51±0.13	45.52±0.121
		Parasite F value	1.56	0.25	0.56
	Post-weaning	High shedding	42.81±1.21[a]	45.14±0.41	45.28±0.316
		Low shedding	47.42±1.72[b]	45.90±0.42	45.43±0.369
		Not detected	45.52±0.11[b]	45.51±0.12	45.55±0.128
		Parasite F value	**3.12***	0.86	0.35
	Pre-slaughter	High shedding	44.35±1.14	44.43±0.40[a]	45.15±0.309
		Low shedding	44.66±1.52	44.47±0.43[a]	45.09±0.323
		Not detected	45.53±0.11	45.68±0.12[b]	45.65±0.127
		Parasite F value	0.7	**7.27****	2.02
Live weight (kg)[##]	Weaning	High shedding	28.00±0.95[a]	31.52±0.34	30.91±0.38
		Low shedding	30.78±1.13[ab]	31.02±0.35	32.19±0.39
		Not detected	31.58±0.14[b]	31.59±0.15	31.52±0.15
	Post-weaning	High shedding	35.31±1.16[a]	39.69±0.39	39.62±0.31
		Low shedding	38.20±1.51[ab]	39.27±0.39	39.62±0.36
		Not detected	39.84±0.14[b]	39.83±0.15	39.83±0.15
	Pre-slaughter	High shedding	44.72±1.13[a]	47.15±0.41	47.04±0.32
		Low shedding	46.02±1.38[ab]	47.79±0.44	47.21±0.34
		Not detected	47.03±0.15[b]	46.94±0.16	46.96±0.16
		Parasite F value	**15.39****	0.37	1.23

HSCW: hot standard carcase weight
[ab] values within time points and parasite with different superscripts are significantly different (P<0.05). Note – post hoc tests were performed for all parasites/sample occasions and values within parasites/sample occasions without superscripts are not significantly different (P>0.05)
** F value <0.01
* F value < 0.05
[#] General linear model (separate model for each parasite and timepoint)
[##] Linear mixed effects model (separate model for each parasite, each model includes all 3 timepoints)

Table 23. Association of frequency of parasite detection with lamb carcase weight and DP with least square means ± standard error, and F value for parasite main effect. F values significant <0.05 are shown in bold.

	Frequency of detection and main effect	Pathogen		
		C. parvum	***Cryptosporidium* spp.**	***Giardia* spp.**
HSCW (kg)	0 positive occasions	21.652±0.081[a]	21.629±0.105	21.699±0.109[a]
	1 positive occasions	20.225±0.389[b]	21.579±0.144	21.686±0.143[a]
	2 positive occasions	17.637±1.195[c]	21.401±0.291	20.882±0.237[b]
	3 positive occasions	n/a	20.694±0.589	20.771±0.489[ab]
	Frequency F value	**11.72****	0.9	**4.23****
DP	0 positive occasions	45.59±0.12[a]	45.59±0.15[a]	45.60±0.15[a]
	1 positive occasions	44.86±0.55[a]	45.55±0.20[a]	45.62±0.20[a]
	2 positive occasions	41.68±1.70[b]	45.06±0.41[ab]	44.97±0.33[ab]
	3 positive occasions	n/a	43.58±0.83[b]	44.19±0.69[bc]
	Frequency F value	**3.2***	2.2	**2.2***

HSCW: hot standard carcase weight
[ab] values with different superscripts are significantly different (p<0.05)
** F value <0.01
* F value < 0.05
n/a – insufficient numbers

4.9.1.6 Association between WEC and Haemonchus status with HSCW, DP and live weight

Faecal WEC and prevalence of *H. contortus* (by qPCR) are shown in Table 24. The mean WEC for all farms and timepoints were below 1000 eggs per gram, suggesting that whilst *H. contortus* was identified by qPCR on all 8 farms included in the study, clinical Haemonchosis was unlikely at a flock level. Whilst *Teladorsagia* and *Trichostrongylus* are more likely to be associated with reduced growth, the presence of *Haemonchus* was expected to increase WEC in individual sheep due to increased fecundity, therefore Haemonchus status was included as a fixed factor in analyses to determine if this was a determinant on realationship between WEC and productivity measures (growth, caracse weight, DP).
No statistically significant main effects for either WEC (P>0.05) or *Haemonchus* status (P>0.05) were identified for any of the sheep meat productivity measures (liveweight, HSCW and DP). Trends were observed towards a negative association between WEC and liveweight (P=0.086), WEC at weaning and DP (P=0.069) and *Haemonchus* status at slaughter and DP (no *Haemonchus* 45.4% vs *Haemonchus* present 46.1%; P=0.057). This suggests that at the level of worm challenge present in the eight flocks, the magnitude of WEC (after accounting for presence of *Haemonchus*) was not associated with effects on liveweight, HSCW and DP.

Table 24. Concentration of Trichostrongylid DNA or worm eggs (eggs per gram) by qPCR and McMaster WEC in faecal samples collected on three occasions from lambs on eight Australian farms

Farm	Sampling occasion	McMaster WEC (epg)		qPCR *H. contortus*	
		mean	range	Valid samples (n)	Prevalence % (95% CI)
SA1	Weaning	-	-	165	7.9 (4.5, 12.7)
	Post-weaning	282 ± 35	0 - 2600	154	19.5 (13.8, 26.3)
	Pre-slaughter	372 ± 37	0 - 2500	158	14.6 (9.7, 20.7)
SA2	Weaning	-	-	158	8.2 (4.7, 13.3)
	Post-weaning	1 ± 1	0 – 1500	151	8.6 (4.9, 13.9)
	Pre-slaughter	210 – 21	0 - 1350	123	13.0 (7.9, 19.8)
Vic1	Weaning	853 ± 117	0 – 12 000	176	9.7 (6.0, 14.7)
	Post-weaning	164 ± 24	0 - 3100	170	7.1 (3.9, 11.6)
	Pre-slaughter	315 62	0 - 6500	159	50.3 (42.6, 58.0)
Vic2	Weaning	124 ± 20	0 - 1500	175	13.1 (8.8, 18.7)
	Post-weaning	198 ± 32	0 - 2500	175	2.9 (1.1, 6.1)
	Pre-slaughter	219 ± 33	0 - 3000	127	2.4 (0.7, 6.2)
NSW	Weaning	-	-	146	63.7 (55.7, 71.2)
	Post-weaning	625 ± 125	0 – 15 000	146	56.8 (48.7, 64.7)
	Pre-slaughter	0 ± 0	0 - 50	131	7.6 (4.0, 13.1)
WA1	Weaning	239 ± 17	0 - 800	123	0 (0, 2.0)
	Post-weaning	50 ± 7	0 - 400	122	0.8 (0.1, 3.8)
	Pre-slaughter	205 ± 40	0 - 3200	121	0.8 (0.1, 3.8)
WA2	Weaning	183 ± 44	0 - 3150	107	5.6 (2.4, 11.2)
	Post-weaning	133 ± 46	0 - 2500	109	0.9 (0.1, 4.2)
	Pre-slaughter	575 ± 87	50 - 3800	107	(0, 2.3)
WA3	Weaning	172 ± 20	0 - 1200	101	10.9 (5.9, 18.1)
	Post-weaning	41 ± 11	0 - 650	101	2.0 (0.4, 6.2)
	Pre-slaughter	41 ± 11	0 - 650	100	0 (0. 2.5)

4.9.1.7 Association between Yersinia and liveweight, carcase weight and DP

Associations between *Yersinia* and pathogenic *Yersinia* shedding categories with liveweight are shown in Table 25.

There was no significant association between *Yersinia* shedding category and live weight at any of the three sampling occasions.

There was a significant main effect of pathogenic *Y. enterocolitica* shedding category and liveweight (P<0.001). Lambs categorized as high shedding for pathogenic *Yersinia* were 1.407kg (p=0.004) lighter at weaning, 4.958kg (p<0.001) lighter at post-weaning and 3.881kg (p=0.013) lighter at pre-slaughter than lambs in which pathogenic *Yersinia* were not detected. There was no difference in liveweight between lambs that were categorized as low shedding for pathogenic *Yersinia* at ay of the sampling occasions. This is the first report of

reduced growth of lambs in lambs with higher faecal shedding of pathogenic *Y. enterocolitica* relative to non-infected counterparts.

Table 25: Association of *Yersinia* shedding category with live weight with least square means and F value for parasite main effect. F values significant <0.05 are shown in bold.

Sampling occasion	Shedding category and main effect	Liveweight (kg)	
		Yersinia	**Pathogenic *Yersinia*.**
Weaning	High shedding	31.69	30.18^{A}
	Low shedding	31.89	31.85^{B}
	Not detected	31.45	31.59^{B}
Post-weaning	High shedding	39.80	34.97^{A}
	Low shedding	39.67	39.48^{B}
	Not detected	39.82	39.92^{B}
Pre-slaughter	High shedding	46.82	43.15^{A}
	Low shedding	47.35	46.04^{AB}
	Not detected	46.96	47.03^{B}
	Pathogen F value	0.67	**16.36**
	Pathogen P value	0.512	**<0.001**

^{AB} values within time points and pathogen with different superscripts are significantly different (P<0.05). Note – post hoc tests were performed for all pathogens/sample occasions. Values within pathogen/sample occasions without superscripts are not significantly different (P>0.05)

4.10 Associations between pathogens with FCS and dag

4.10.1 Association between WEC and FCS

Correlations between WEC and FCS are shown in Table 26. Significant correlations between WEC and FCS were noted only for two (WA2 and WA3) of the eight flocks, and for these flocks only at one (WA3) or two (WA2) sampling occasions.

Table 26: Faecal consistency scores (FCS) observed over three sampling occasions with bivariate correlation between faecal worm egg count (WEC) and faecal consistency score (FCS). Significant (two-tailed P<0.05) correlations are shown in bold.

Flock	Sampling occasion	FCS		WEC and FCS association	
		mean	range	Pearson correlation	P value (2 tailed)
SA1	Weaning	na	na	na	na
	Post weaning	3.25 ±.0.46	2 - 5	0.120	0.139
	Pre slaughter	3.07±0.05	1 - 5	0.059	0.463
SA2	Weaning	na	na	na	na
	Post weaning	2.91 ± 0.04	1 - 5	0.014	0.863
	Pre slaughter	2.46 ± 0.09	1 - 5	0.064	0.487
VIC1	Weaning	1.99 ± 0.08	1 - 5	-0.012	0.876
	Post weaning	1.12 ± 0.03	1 - 4	-0.010	0.897
	Pre slaughter	1.16 ± 0.03	1 - 3	0.080	0.321
VIC2	Weaning	1.27 ± 0.05	1 - 4	0.013	0.866
	Post weaning	3.90 ± 0.02	3 - 5	0.092	0.231
	Pre slaughter	2.52 ± 0.07	1 - 4	0.092	0.315
NSW	Weaning	na	na	na	na
	Post weaning	1.82 ± 0.08	1 - 5	0.098	0.248
	Pre slaughter	1.13 ± 0.04	1 - 4	na	na
WA1	Weaning	1.97 ± 0.09	1 - 5	**0.367**	**0.000**
	Post weaning	1.47 ± 0.06	1 - 4	**0.187**	**0.039**
	Pre slaughter	2.80 ± 0.07	1 - 5	0.091	0.327
WA2	Weaning	1.75 ± 0.08	1 - 5	0.062	0.529
	Post weaning	2.46 ± 0.09	1 - 5	0.005	0.961
	Pre slaughter	2.10 ± 0.11	1 - 5	0.125	0.228
WA3	Weaning	2.98 ± 0.05	1 - 4	0.053	0.597
	Post weaning	2.59 ± 0.09	1 - 5	0.133	0.186
	Pre slaughter	2.91 ± 0.06	1 - 5	**0.200**	**0.046**

na: insufficient faecal material available to determine FCS

4.10.2 Associations between pathogen presence and FCS

Associations between pathogen presence and FCS are shown in Table 27. FCS was higher for lambs in which *Cryptosporidium* (all species combined) were detected at both weaning and post-weaning, and a trend (p=0.09) in the same direction was observed at pre-slaughter. There were no significant associations (pathogen main effect) with FCS for any of the other pathogens, with the exception of a trend (P=0.087) to lower FCS in sheep in which pathogenic *Yersinia* were detected.

Table 27: Association between pathogen detection with faecal consistency score (FCS) with least square means and F value for parasite main effect. F values significant <0.10 are shown in bold.

		Pathogen						
Sampling occasion	**Pathogen category and main effect**	***Cryptosporidium* (all species)**	***C. parvum***	***Giardia***	***Salmonella***	***Campylobacter***	**Pathogenic *Yersinia***	***C. percorum***
Weaning	Detected	2.12[A]	2.04	2.00	2.08	1.85	2.09	2.13
	Not detected	1.94[B]	1.98	1.98	1.98	2.00	1.98	1.97
	Pathogen F value	**4.36**	0.06	0.07	0.23	1.4	0.38	1.78
	Pathogen P value	**0.037**	0.812	0.791	0.634	0.236	0.536	0.182
Post-weaning	Detected	2.71[A]	2.65	2.47	2.40	2.52	2.56	2.39
	Not detected	2.39[B]	2.44	2.43	2.44	2.42	2.44	2.48
	Pathogen F value	**26.04**	1.22	0.48	0.26	1.93	1.11	2.17
	Pathogen P value	**<0.001**	0.270	0.486	0.611	0.165	0.293	0.141
Pre-slaughter	Detected	2.36	2.55	2.23	2.40	2.37	1.83	2.31
	Not detected	2.25	2.26	2.28	2.25	2.25	2.27	2.25
	Pathogen F value	**2.86**	2.19	0.78	2.54	2.67	**2.93**	0.65
	Pathogen P value	**0.091**	0.139	0.377	0.111	0.102	**0.087**	0.420

[AB] values within time points and pathogen with different superscripts are significantly different (P<0.05). Note – post hoc tests were performed for all pathogens/sample occasions. Values within pathogen/sample occasions without superscripts are not significantly different (P>0.05)

4.10.3 Associations for pathogen intensity category with FCS and dag

Associations for pathogen shedding intensity category with FCS and dag are shown in Table 28. Least square means for significant pathogen shedding intensity category main effects (P<0.100) are shown in Table 29. Farm was a significant main effect for both FCS and dag score at all three timepoints. Pathogen shedding intensity category was not consistently associated with FCS or dag score over the three sampling occasions. High shedding of *C. parvum* was associated with the greatest increase in FCS (relative to lambs in which *C. parvum* was not detected) and the only pathogen with FCS over 3 (soft unformed faeces), but only at post-weaning sampling. WEC was significant as a covariate only for dag score at pre-slaughter sampling

Table 28: Significance of main effects (F value probability) for associations between faecal consistency score (FCS) or dag score with pathogen faecal shedding intensity category (high, low or none). P values <0.100 are shown in bold.

	FCS			Dag		
	Weaning	Post-weaning	Pre-slaughter	Weaning	Post-weaning	Pre-slaughter
Farm	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Kill group	-	-	**0.007**	-	-	0.623
C. parvum	0.708	**<0.001**	0.490	0.697	0.302	0.107
Cryptosporidium	**0.057**	**<0.001**	0.218	0.868	0.231	**0.028**
Giardia	0.968	0.960	0.535	**0.091**	0.876	0.246
Eimeria	0.619	**0.019**	**0.031**	0.269	0.845	0.196
Salmonella	0.448	0.105	0.611	0.974	0.996	0.757
Pathogenic *Yersinia*	0.133	0.318	**0.094**	**0.065**	0.132	0.982
Yersinia	0.288	0.434	0.669	0.441	0.443	0.758
Campylobacter	0.837	0.692	0.452	0.682	**<0.001**	0.125
WEC	0.671	0.232	0.625	0.558	0.890	**0.021**

Table 29: Least square means for significant main effects (p<0.100) for associations between faecal consistency score (FCS) or dag score with pathogen faecal shedding intensity category (high, low or not detected).

	Shedding intensity category				
	High	Low	Not detected	F value	P value
FCS					
Weaning					
Cryptosporidium	2.28 ± 0.34	2.42 ± 0.34	2.12 ± 0.33	2.87	0.057
Post-weaning					
C. parvum	3.49 ± 0.29	1.57 ± 0.33	2.55 ± 0.13	11.2	<0.001
Cryptosporidium	2.63 ± 0.19	2.67 ± 0.19	2.31 ± 0.19	12.31	0.000
Eimeria	2.45 ± 0.19	2.50 ± 0.19	2.66 ± 0.18	3.98	0.019
Pre-slaughter					
Eimeria	1.99 ± 0.26	2.29 ± 0.26	2.21 ± 0.25	3.49	0.031
Pathogenic *Yersinia*	2.23 ± 0.39	1.73 ± 0.42	2.53 ± 0.18	2.37	0.094
Dag					
Weaning					
Giardia	2.24 ± 0.29	1.97 ± 0.30	1.96 ± 0.27	2.4	0.091
Pathogenic *Yersinia*	2.41 ± 0.37	1.98 ± 0.34	1.77 ± 0.26	2.75	0.065
Post-weaning					
Campylobacter	2.19 ± 0.22	2.10 ± 0.22	1.77 ± 0.20	8.8	0.000
Pre-slaughter					
Cryptosporidium	2.11 ± 0.34	1.72 ± 0.35	1.92 ± 0.34	3.61	0.028

5 Discussion

5.1 Prevalence and pathogen load (Milestones 1-5)

Objective 1 of this project was to develop a high throughput qPCR multiplex assay, for identifying and quantitating *Salmonella, Campylobacter, Yersinia, Chlamydia*, C*ryptosporidium, Eimeria, Giardia*, *Haemonchus, Teladorsagia and Trichostrongylus* in sheep faeces.

This objective has been met as multiplex assays were successfully developed to detect and quantitate 10 different pathogens (*Salmonella, Campylobacter, Yersinia, Chlamydia, Eimeria, Cryptosporidium, Giardia, Haemonchus, Teladorsagia and Trichostrongylus)* across 8 farms over 3 different time points (weaning, postweaning and pre-slaughter) in 4 states (NSW, SA, Vic and WA).

All assays were shown to be very specific and sensitive with detection limits of <1 to 10 organisms (see section 3.2 and Table 6). Data on the overall prevalence and pathogen load of the 10 different pathogens are discussed in the following section.

Whilst numerous studies have conducted single point prevalence analysis by sampling a random selection of lambs or sheep within a flock at a specific time, few studies have conducted longitudinal analysis. This is important as the determination of prevalence at one

sampling, may not provide a true indication of the overall prevalence in flocks over an extended period of time. Reported sporadic excretion of protozoa and bacteria requires multiple faecal samples to be collected for an accurate diagnosis, as a negative specimen would indicate that an animal was not infected when there might actually be a pattern of intermittent oocyst excretion (Skirnisson and Hansson, 2006; Skirnisson, 2007, Xiao, 2010; Feng and Xiao, 2011).

Differences in prevalence were reported for the different pathogens and also between farms. These differences could be related to a wide range of factors, including environment, stocking density and potential for contamination of feed/water. The sites chosen represented a range of different environments in which sheep are farmed throughout Australia. The WA farms were all located in southern Western Australia and experienced a Mediterranean environment (hot, dry summers and cool, wet winters) with a predominantly winter rainfall pattern and had similar average annual rainfall, ranging between 450 and 550 mm. The SA farms also experienced a Mediterranean climate with average rainfall ranging between 430 and 550 mm. The Vic farms experienced higher rainfall in winter with (620-750 mm/year) but milder summers whereas the NSW farm experienced relatively cold winters with warm wet summers (495 mm/year). However, given the lack of detailed weather information available for each site, it is difficult to link these prevalences with obvious environmental effects.

5.1.1 Cryptosporidium

The overall prevalence of *Cryptosporidium* from 8 farms with a history of scouring across 4 states over 3 sampling periods (weaning, post-weaning and pre-slaughter) was 16.9% (576/3412). There was no relationship between prevalence and lamb age, which ranged from ~12 weeks (weaning) to ~29 weeks (pre-slaughter). Previous studies in cattle have shown that the highest prevalence occurs in very young animals (~ 2 weeks of age) (Santín *et al.,* 2008). In sheep, it has been shown that ewes (2-6 years in age) had a much higher prevalence of *Cryptosporidium* than lambs aged 7-21 days in age (Santín *et al.,* 2007). However previous studies in WA have reported prevalences by PCR of 26% for slaughter age lambs in WA (Ryan *et al.,* 2005) and 24.5% for pre-weaned lambs (aged 1-8 weeks) in WA (Yang *et al*., 2009). A recent study reported that the prevalence in 3-4 week-old and 15-16 week-old lambs was 18.4% and 26.7% respectively (Ye *et al.,* 2013). Another study reported that the prevalence in 5-6 week old lambs increased from 15% to 25% in 6-10 week old lambs (Robertson *et al.,* 2010). Further longitudinal research is required to better understand the relationship between the prevalence of *Cryptosporidium* and lamb age.

The average range of *Cryptosporidium* oocyst shedding concentration at weaning overall (across all states) was 63 - 7.9 $x10^6$ and the median was 3.2 x 10^4 g^{-1}. At pre-slaughter, the average range was 260-4.8 x 10^7 and the median was 6.3x10^4 g^{-1}. These shedding rates are higher than the previous study and highlights the advantages of using a method that does not require purification of oocysts and utilises a PCR-based detection method, which has been shown to be much more sensitive than microscopy (Ryan *et al.,* 2005). The data shows that although the prevalence in SA was lower than WA, oocyst shedding concentrations were higher in SA.

5.1.2 Giardia

The overall prevalence of *Giardia* from 8 farms with a history of scouring across 4 states over 3 sampling periods (weaning, post-weaning and pre-slaughter) was 20.2% (690/3412) and ranged from 7.9% to 42.1% (WA). Previous studies have reported prevalences by PCR of 4% - 89.2% in lambs (Ryan et al., 2005; Santín *et al.,* 2007; Geurden *et al.,* 2008a; 2008b; Gómez-Muñoz *et al.,* 2009; Yang *et al.,* 2009; Nolan *et al.,* 2010; Robertson *et al.,* 2010; Sweeny *et al.,* 2011a; Gómez-Muñoz *et al.,* 2012). In Australia, previous studies in WA have reported prevalences of 11.1% and 44% in pre and post-weaned lambs (Yang *et al.,* 2009), while in Vic, a prevalence of 15.1% was reported in lambs (<7 weeks) (Nolan *et al.,* 2010).

The data showed that although the prevalence for WA3 was the lowest for all farms sampled (7.9-15%), the median *Giardia* cyst shedding concentration was relatively high and peaked at 5.2 x10^5 cysts g^{-1} during pre-slaughter. SA1 had a relatively low prevalence (11.9-20.3%) but had the highest median cyst shedding which peaked at 8.3 x 10^5 cysts g^{-1} during post-weaning. There are only limited reports on the concentration and environmental loading of *Giardia* cysts as a result of faecal contamination by sheep. One study reported that the range of cyst shedding for adult sheep in Sydney catchments was 0 – 504 cysts g^{-1} with a median of 26 cysts g^{-1} (Cox *et al.,* 2005).

5.1.3 Eimeria

Previous prevalence studies on *Eimeria* in sheep, conducted in Australia, have been based on microscopy. One study conducted in South Australia detected *Eimeria* oocysts in 80% of sheep examined (n=136) (O'Callaghan *et al*., 1987). The most recent study, reported overall prevalences of 58.9% and 71.4% for *Eimeria* in two West Australian farms (Sweeny *et al.,* 2011a). In Papua New Guinea, a prevalence of 17% was reported for *Eimeria* in sheep by microscopy (Koinari *et al.,* 2012).

The overall median *Eimeria* oocyst shedding (across the eight farms with a history of scouring in the four states) peaked during post-weaning and pre-slaughter. This is in contrast to previous studies that have reported that peak oocyst shedding was reached around the period of weaning (Chartier and Paraud, 2012). For example, in Bangladesh, it was reported that younger sheep passed higher numbers of *Eimeria* oocysts than older animals with a peak at around 4-6 weeks of age (7.9 x10^5 oocysts/g of faeces) (Karim *et al.,* 1990), with a similar pattern seen in UK sheep (Gregory *et al.,* 1983). Another study reported that *Eimeria* shedding in sheep in Iceland ranged from 300 to 4.9 x 10^4 g^{-1}, peaking just after weaning (Skirnisson and Hansson, 2006; Skirnisson, 2007). Other studies have reported a mean of ~2,838 g^{-1} for sheep in Sudan (Abakar *et al.,* 2013), while a study in Kenya reported peaks of 1.1 x 10^4 in sheep with a mean of 1.1 x 10^3 in sheep < 1 year of age (Kanyari, 1993). One study suggested a genetic component in the determination of oocyst ouput (Yvore *et al.,* 1992). The reasons for the higher oocyst output during post-weaning and pre-slaughter (compared to weaning) in the present study are unknown, but may be due to stress or to polyparasitism reducing host immunity as these sheep were known to be co-infected with *Giardia*, *Cryptosporidium*, bacteria and strongyle worms (Yang *et al*., 2014a; 2014b; 2014c; 2014d; 2014e).

5.1.4 Campylobacter and Salmonella

The overall prevalence of *Campylobacter* spp. and *S. enterica* on the eight farms with a history of scouring from four states was 13.3% and 5.0%, respectively. However, the prevalence varied widely among states and at different sampling points. As an example, the prevalence of *Campylobacter* spp. was 15.4% during the weaning period in SA2 but was 59% during the post-weaning period. Similarly, *S. enterica* prevalence peaked at 23.8% in NSW during the post-weaning period but was only 3.6% during the pre-slaughter period. In a study of slaughter age lambs in NSW and Queensland, 14/19 (73.7%) of all flocks tested were positive for *C. jejuni* by culture and the prevalence in individual lambs was 8% (Bailey *et al.,* 2003). In Scotland, the prevalence of *Campylobacter* spp. by culture in ovine faeces was 14-49% (Garcia *et al.,* 2010a; Sproston *et al.,* 2011).

In a national baseline microbiological survey of Australian sheep carcasses and frozen boneless sheep meat conducted in 2004, *S. enterica* was isolated from 0/1117 carcases and from 3/560 (5.4%) samples of boneless product. *Campylobacter* spp. were isolated from 4/1117 (0.4%) carcases and from 1/560 (0.2%) boneless samples (Phillips *et al.,* 2006). However, it is important to note that low or no recovery rates of these organisms in faeces and carcasses during slaughter may be due to the limited sensitivity of culture methods. For example, a recent study, which compared qPCR and culture methods for the detection of *S. enterica* in faeces, reported that 45 samples were positive using the PCR assay which were not positive by bacteriological culture methods (Maciel *et al.,* 2011).

The prevalence of *S. enterica* in the faeces of healthy slaughtered sheep appears to vary widely. In a recent study of 486 samples from 164 sheep and lambs at two abattoirs in Australia, *S. enterica* was isolated from 20% of faeces, 13% of fleeces and 1.3% of pre-chill carcases (Duffy *et al.,* 2010). Previous studies have reported prevalences ranging from 0.1% in the UK (Davies *et al.,* 2004) to 42% in Australia (Samuel *et al.,* 1981).

For *S. enterica*, the pathogen load was relatively low across all farms, with the exception of SA2 at weaning, where, although the shedding was high, the prevalence was low (1.7%). The highest median number of *Campylobacter* spp. detected was at WA3 during post-weaning (2.1×10^8 organisms/g faeces), although individuals at SA2 shed up to 1.6×10^9 organisms/g faeces. Little is known about bacterial shedding of *Campylobacter* spp. in sheep and the reasons for the high *Campylobacter* spp. output are unknown, but may be due to stress or to multiple infections reducing host immunity, as these sheep were co-infected with *Giardia* spp., *Cryptosporidium* spp., *Eimeria* spp.*, Chlamydia pecorum, Yersinia* spp. and strongyles (Yang *et al.,* 2014a, 2014b, 2014c, 2014d; 2014e).

5.1.5 Yersinia

The overall prevalence of *Yersinia* spp. in ovine faecal samples from 8 farms with a history of scouring across four states over three sampling periods was 32% and as with the other pathogens, the prevalence varied widely between states and at different sampling occasions. For example, the prevalence of *Yersinia* spp. peaked at 60.2% in Vic2 during the pre-slaughter period but was only 9.1% during the weaning period. Differences in prevalence could be related to a wide range of factors, including environmental conditions, stocking density, potential for contamination of feed/water and acquisition of host immunity.

Yersinia spp. were identified an all eight flocks included in this study. The three highest *Yersinia* spp. point prevalences identified were at NSW (90.6% at post-weaning) and Vic1 (67.5% at pre-slaughter) and Vic 2 (60.2% at pre-slaughter). The lowest point prevalences were in WA (0-25.4% across the 3 WA farms). A previous study in NSW recovered 53 isolates of *Yersinia* spp. by culture from 45 sheep in 37 flocks from sheep in southern NSW from 1981 to 1989 (Philbey *et al.,* 1991). Another study in Vic reported that *Y. enterocolitica* was isolated by culture from one or more sheep in 78 (17%) of 449 flocks and that *Y. enterocolitica* infection was most common in sheep less than 1 year old (Slee and Skilbeck, 1992). A more recent study of 19 flocks of slaughter-age lambs in NSW and Queensland failed to identify *Y. enterocolitica* by culture (Bailey *et al.,* 2003). It has been suggested that culture methods may underestimate the prevalence of *Yersinia* spp. (Zheng *et al.,* 2006; 2007) and this may, in part, explain the differences in prevalences observed between studies utilising culture or molecular methodologies. On the other hand, the prevalence of infection detected by the sensitive qPCR assay may also be overstated due to the detection of non-viable bacteria.

The estimated overall prevalence of pathogenic *Y. enterocolitica* as determined by screening for the *yst* gene was 5.8% with the highest prevalence in NSW (up to 48.8% of positive samples). The chromosomal *yst* gene encodes a low-molecular-weight, heat-stable enterotoxin which belongs to a family of structurally and functionally related enterotoxins produced by several species of diarrheagenic bacteria (Robins-Browne *et al.,* 1979; Delor *et al.,* 1990). Although *yst* is confined to pathogenic bioserotypes of *Y. enterocolitica* and hence is a useful marker of potential virulence, a homologous gene is found in some isolates of *Y. intermedia* and *Y. kristensenii* (Kechagia *et al.,* 2008; Ibrahim *et al.,* 1997). However, the primers used in the present study were designed to be specific to the *yst* gene in *Y. enterocolitica* (Ibrahim *et al.,* 1997).

Yersinia spp. concentration in faeces were determined using qPCR. The largest median concentration of *Yersinia* spp. organisms was detected at Vic2 and SA2 during the post-weaning period. For other farms, concentration of *Yersinia* spp. shedding were higher at weaning (SA1, Vic1, WA3). *Yersinia* spp. shedding concentration for NSW was high across all 3 sampling periods. Virtually nothing is known about bacterial shedding of *Yersinia* spp. in sheep and the reasons for the high *Yersinia* spp. output are unknown but may be due to stress or to mixed infections affecting host immunity as these sheep were known to be co-infected with other potentially pathogenic organisms including *Giardia*, *Cryptosporidium*, *Eimeria*, other bacteria and strongyle worms (Yang *et al*., 2014a; 2014b; 2014c; 2014d; 2014e and unpublished).

5.1.6 Chlamydia

Chlamydia pecorum was the only *Chlamydia* spp. identified in Australian sheep in the present study and a recently published serological survey of 891 sheep from 109 properties across southern Australia supports this (McCauley *et al.,* 2010). An obvious limitation of the present study is that vaginal swabs were not screened and therefore, conclusive evidence for the absence of *C. abortus* in Australian sheep is lacking. However, *C. abortus* has been detected in faeces as well as the genital tract (Tsakos *et al.,* 2001; Longbottom *et al.,* 2002; Longbottom and Coulter, 2003; Jee *et al.,* 2004; Lenzko *et al.,* 2011; Talafha *et al.,* 2012).

Across the eight farms with a history of scouring from the four states, *C. pecorum* had median bacterial concentrations at weaning, post-weaning and pre-slaughter of 1.8×10^7, 1.2×10^7 and 9.6×10^5 per gram, respectively. However individual sheep shed up to 4.4×10^{12} per gram (NSW-post-weaning). Virtually no information is available on the numbers of *C. pecorum* in sheep faeces. Previous studies on koalas have reported intensity levels of 1-979,081 copies of rRNA target per microlitre of DNA extract (Wan *et al.,* 2011). The reasons for the higher bacterial shedding during weaning and post-weaning (compared to pre-slaughter) in the present study are unknown, but may be due to stress or to co-infection with the other pathogens in this study, reducing host immunity.

5.2 Association between the prevalence of bacteria, protozoa and WEC with production parameters

Objectives 2 and 3 were as follows: (2) Use qPCR and WECs to monitor flocks of sheep on four farms to determine if there is an association between the prevalence of bacteria, protozoans and WEC with scouring; (3) Develop molecular tools to examine if scouring and production loss is associated with particular protozoan or bacterial species/genotypes and with particular management systems under Australian conditions.

These objectives have been met. Firstly molecular tools were developed to type the various protozoan and bacterial species. This was done in order to identify the prevalence of zoonotic species and genotypes as this has implications for carcase contamination at abattoirs and secondly to identify if particular species and subtypes were associated with production loss.

Sections 5.2.1 – 5.2.4 discusses the protozoan and bacterial species identified and any zoonotic implications. Section 5.2.5 discusses the production consequences.

5.2.1 Cryptosporidium and Giardia

A total of 6 *Cryptosporidium* species/genotypes were identified including *C. xiaoi*, *C. ubiquitum*, *C. parvum*, *C. scrofarum, C. andersoni* and sheep genotype 1, with *C. xiaoi* and *C. ubiquitum* responsible for 86.6% of infections typed compared to 12.2% for *C. parvum* (includes the mixed *C. parvum, C. xiaoi* isolates). *Cryptosporidium ubiquitum* is a common human pathogen (Xiao, 2010). In Australia, *C. ubiquitum* has not been identified in the limited typing of Australian human *Cryptosporidium* isolates that has been conducted to date (Ryan and Power, 2012), however *C. ubiquitum* has been identified in source water in Australia (unpublished) and should be considered a zoonotic species. *Cryptosporidium xiaoi* has only been reported once in two HIV-positive individuals in Ethiopia (Adamu *et al.,* 2013). *Cryptosporidium scrofarum* was detected in 4 lambs from Vic and not in any other samples. It is primarily a porcine parasite (Kváč *et al.,* 2013), but has previously been identified in sheep and cattle in WA (Ryan *et al.,* 2005; Ng *et al.,* 2011a and 2011b) and has been reported in an immunocompetent human (Kváč *et al*., 2009). Sheep genotype I was identified in one sheep at WA1. This genotype has not been identified in humans and is genetically distinct at both the 18S and actin loci but most closely related to *C. ubiquitum* (Sweeny *et al.,* 2011a). *Cryptosporidium andersoni* was also identified in one isolate from WA. This is primarily a bovine parasite but has previously been identified in sheep in WA (Ryan *et al.,* 2005) and a human in NSW (Waldron *et al.,* 2011a). Therefore 30.8% (154/500) of the positive samples identified were potentially zoonotic. This is the first report of ovine

genotypes from NSW, Vic and SA. Previous studies have also reported that *C. xiaoi* and *C. ubiquitum* are the dominant species infecting sheep (Yang *et al.,* 2009; Robertson *et al.,* 2010; Wang *et al.,* 2010; Fiuza *et al.,* 2011), although other studies have reported that *C. parvum* (Ryan *et al.,* 2005; Mueller-Doblies *et al.,* 2008; Cacciò *et al.,* 2013; Imre *et al.,* 2013) and even *C. hominis* were more dominant than *C. ubiquitum* in sheep (Connelly *et al.,* 2013).

At the *gp60* locus, two subtype families were identified (IIa and IId). At least 12 *C. parvum* subtype families (IIa-IIl) have been identified at this locus, but only IId and especially the most common subtype family, IIa, appear to be zoonotic (Xiao, 2010). Prior to the present study, ovine-derived *C. parvum* isolates from Australia had not been subtyped at the *gp60* locus. The *C. parvum* subtype IIaA15G2R1 was identified in lambs in Vic and NSW. This is a dominant subtype in ruminants and has been reported in humans and calves in Australia (O'Brien *et al.,* 2008; Waldron *et al.,* 2011b) and worldwide (Xiao, 2010). This is the first report of IIaA15G2R1 in lambs in Australia. This subtype was also previously seen in three lambs linked to a human infection in the United Kingdom (Chalmers *et al.,* 2005).

The *C. parvum* IId subtype family is less common and has been reported mainly from sheep and goats but has also been reported in humans and cattle overseas (Xiao, 2010). The IId subtype family has not been reported in cattle in Australia (as previous studies have only identified IIa subtypes in cattle), but has been reported in humans (Waldron *et al.,* 2009; Ng *et al.,* 2010a; 2010b). In the present study, subtype IIdA19G1 was identified in lambs from SA and Vic and subtype IIdA18G1 was identified in SA, NSW and WA. Subtype IIdA18G1 was previously identified in lambs in Spain and subtype IIdA19G1 was identified in both lambs and goats in the same study (Quilez *et al.,* 2008a; 2008b). Both subtypes are rare and have not been reported in humans in Australia. Previous studies have identified IIdA15G1 (Ng *et al.,* 2010) and IIdA24G1 (Waldron *et al.,* 2009) in individual human patients. In Spain, where both IIa and IId have been identified, IIa subtypes appear to preferentially infect calves, whereas IId subtypes preferentially infect lambs and goat kids (Quilez *et al.,* 2008a; 2008b). Of the 38 *C. parvum* subtypes identified in the present study, the IId subtype family accounted for 87% (33/38) of the subtypes identified. This data along with evidence from studies overseas suggest that subtype family IId is adapted to lambs (and goat kids), and may therefore be to be one of the most important reservoirs for this zoonotic group of *C. parvum* isolates (Quilez *et al.,* 2008a, 2008b; Imre *et al.,* 2013).

All *C. ubiquitum* isolates analysed at the *gp60* locus were typed as subtype XIIa. To date six subtype families (XIIa to XIIf) have been identified in *C. ubiquitum* (Li *et al.,* 2013). XIIa has been found in ruminants world-wide, XIIb to XIId in rodents in the United States, XIIe and XIIf in rodents in the Slovak Republic. XIIa, XIIb, XIIc, and XIId have been found in humans, therefore XIIa is a potentially zoonotic subtype (Li *et al.,* 2013).

For *Giardia*, the non-zoonotic assemblage E was responsible for the majority (75.9%) of positive isolates typed, whereas the potentially zoonotic Assemblage A was identified in 22.4% of positive isolates typed with mixed A and E infections in 1.7% of samples. Previous studies have also reported that assemblages E and A are the dominant assemblages infecting sheep and although assemblage E is usually more prevalent (Feng and Xiao, 2011; Caccio and Ryan, 2013), one Australian study in Victoria reported that assemblage A was more prevalent than E in sheep (Nolan *et al.,* 2010) and in Italy, in one study, only assemblage A was found in sheep (Giangaspero *et al.,* 2005).

Subtyping at the *gdh* and *beta-giardin* loci identified sub-assemblage AII. Within assemblage A, three main sub-assemblages have been identified; AI, AII and AIII. AI and AII have been reported in both humans and animals, while AIII is associated mostly with wild hoofed animals (Caccio et al., 2008; Feng and Xiao, 2011; Caccio and Ryan, 2008). Relatively few studies have subtyped assemblage A isolates in sheep but both AI and AII have been reported (Feng and Xiao, 2011; Caccio and Ryan, 2008). Sprong et al., (2009) reported that 78% of the assemblage A sequences obtained from sheep and goats were sub-assemblage AI. This sub-assemblage was also frequently found in other studies carried out in sheep (Giangaspero *et al.,* 2005, Lebbad *et al.,* 2010 and Gómez-Muñoz *et al.,* 2009; Sweeny *et al.,* 2011a; Gómez-Muñoz *et al.,* 2012; Zhang *et al.,* 2012). In the present study sub-assemblage AII was identified on all 8 farms and was identical to AII sub-assemblages, which have been identified in humans in Australia (Yang *et al.,* 2010), which indicates that sheep are a potential zoonotic reservoir for human *Giardia* infections.

5.2.2 Eimeria

Of the subset of 118 isolates sequenced in the present study, a total of 5 species were identified; *E. crandallis, E. ahsata, E. ovinoidalis, E. weybridgensis and E. cylindrica.* Of these, *E. crandallis* and *E. ovinoidalis,* the most pathogenic species in sheep were responsible for 58.5% of infections typed. This is the first report of ovine species of *Eimeria* from NSW, Vic and WA. A previous study in South Australia identified the following *Eimeria* species in sheep; *E. crandallis/E. weybridgensis* (76%), *E. ovina* (55%), *E. ovinoidalis* (54%), *E. granulosa* (49%), *E. parva/E. pallida* (44%), *E. intricata* (37%), *E. ahsata* (31%), *E. faurei* (24%), and *E. punctata* (1%). (O'Callaghan *et al.*, 1987). In the present study, only 5 species of *Eimeria* were detected, but this may be due to the fact that only a small subset of positives (118/616) were typed. Individual sheep faecal samples usually contain several species of *Eimeria* (O'Callaghan *et al.,* 1987; Saratsis *et al.,* 2011), however in the present study, no mixed infections were detected. This is likely due to the most abundant species being amplified by PCR. This could be overcome by the use of species-specific primers or by cloning each amplicon and sequencing multiple clones or via deep sequencing using next generation sequencing. However, this was beyond the scope of the present study. Around the world, it has been reported that in temperate areas like western Europe, the most prevalent *Eimeria* species are *Eimeria ovinoidalis* followed by *Eimeria weybridgensis/crandallis* in sheep (Reeg *et al.,* 2005). In lambs in Germany, faecal samples with 3, 4 or 5 species of *Eimeria* are frequent with a predominance of *E. ovinoidalis* in high oocyst count samples (Dittmar *et al.,* 2010; Reeg *et al.,* 2005). In dry tropical areas such as Senegal, the main species of *Eimeria* in sheep are *E. ovinoidalis* (76%), *E. crandallis* (62%), *Eimeria ahsata* (28%) and *E. parva* (25%) (Vercruysse, 1982).

In the present study, *E. cylindrica* was detected in two out of 15 sheep in SA2 and 3 out of 15 sheep in Vic2. This species is a bovine *Eimeria* species (Duszynski *et al.,* 2000) and has not been reported previously in sheep as *Eimeria* species are thought to be host-specific (Chartier and Paraud, 2012). Both SA2 and Vic2 properties also had cattle and thus it is possible that this was mechanical transmission due to ingestion of infected cattle faeces. Oocyst shedding numbers in these animals ranged from 19 to 3.2×10^5 g^{-1}. It may also be possible that ruminant *Eimeria* species are not as rigidly host-specific as previously thought as has been shown to be the case with *Cryptosporidium* (Xiao, 2010). However, it also be noted that only a single locus was used in the present study and no microscopic evidence of

E. cylindrica in these samples was obtained. Further studies are required to determine if the identification of *E. cylindrica* in these sheep was due to an actual infection or mechanical transmission.

5.2.3 Campylobacter and Salmonella

Campylobacter jejuni and *S. enterica* serovar Typhimurium were the only species identified from a subset of 120 positive samples for each bacteria. However since only a subset of positive samples were sequenced, other species and serovars may also be present. A previous study of 55 *S. enterica* isolates from Australian sheep at slaughter identified 11 different serovars (Duffy *et al.,* 2010). In Scotland, *C. jejuni* accounted for 75% of all the positive samples from sheep sent for slaughter, followed by *C. coli* (16%), *Campylobacter upsaliensis* (2.52%) and *Campylobacter fetus* (1.26%) (Garcia *et al.,* 2010a).

5.2.4 Yersinia

Of the subset of 111 positives sequences across all farms, *Y. enterocolitica* was most commonly identified (62% of infections typed), followed by *Y. pseudotuberculosis* (29% typed), *Y. intermedia* (6% typed) and *Y. frederiksenii (*3% typed). In a NSW study by Philbey *et al.,* (1991), 53 isolates that were cultured from 37 sheep flocks identified as *Y. pseudotuberculosis* (49%), *Y. enterocolitica* (38%), *Y. intermedia* (9%) and *Y. frederiksenii* (4%). The 20 *Y. enterocolitica* isolates were categorised biochemically as biotype 5 strains and, of 6 isolates serotyped, all belonged to serogroup 2, 3 (Philbey *et al.,* 1991).

5.2.5 Production consequences for *Crytosporidium* and *Giardia* on liveweight and carcase productivity

The key finding of this study was that associations between protozoan infections and reduced caracse productivity were identified on widely distributed farms representing a range of sheep production environments. Despite evidence that the protozoan parasites *Cryptosporidium* and *Giardia* are common and widespread in livestock, the effects of infection on livestock productivity are surprisingly poorly understood. This is the first report of associations for intensity and frequency of faecal protozoan shedding with growth and meat (carcase) productivity in a ruminant species, specifically sheep. High *C. parvum* shedding was associated with reduced live weight, HSCW and DP. High *Cryptosporidium* (all species) shedding was associated with reduced DP and high *Giardia* shedding was associated with reduced HSCW. Relationships between protozoan shedding intensity and productivity were observed at specific time points that varied between the parasites. Importantly, high shedding at post-weaning and pre-slaughter were associated with reduced productivity, suggesting that *Cryptosporidium* and *Giardia* infections have sub-clinical consequences for ruminants beyond the neonatal period and challenges the notion that *Cryptosporidium* and *Giardia* are only of relevance in the neonatal period as a cause of neonatal diarrhoea. Apart from intensity of protozoan shedding, repeated detection of *C. parvum* and *Giardia* shedding in a specific animal was associated with reduced HSCW and DP, again suggesting that infection beyond the neonatal period has consequences for sheep meat productivity (HSCW, DP and live weight).

Sheep flocks included in this study were distributed across a wide geographical area and represented a range of sheep production environments. For example, the distance between WA3 (Frankland, Western Australia) and NSW (Armidale, New South Wales) was approximately 3300km with considerable variation in rainfall pattern (Mediterranean in WA farms versus Summer rainfall in NSW). This was an observational study with natural (not experimental) parasite infections and sheep were managed under normal commercial extensive grazing conditions. Both carcase weight and DP are important profit drivers for the sheep meat industry, and reductions in HSCW and DP reduce efficiency and increase costs of processing. *Cryptosporidium* (including *C. parvum)* and *Giardia* were identified in all eight flocks included in the present study, and have been widely reported in sheep in Australia (Ryan et al., 2005; Yang et al., 2009; Sweeny et al., 2011; Sweeny et al., 2012a; Yang et al., 2014a; Yang et al., 2014b) and worldwide (Xiao, 2010). Therefore, the observations of reduced HSCW and DP associated with these organisms have important implications for sheep meat industries worldwide.

Sweeny et al. (2012b) and Sweeny (2012) reported reduced HSCW and DP associated with detection of *Cryptosporidium* and *Giardia* by PCR on at least one occasion at the three Western Australian farms (WA1, WA2 and WA3). The present study has extended findings from the previous reports by examining protozoan shedding intensity (determined by qPCR) rather than simply presence of organism (by PCR). In addition, the present study included a much larger number of sheep representing wider genetic diversity and located over a much wider geographical area (across Australia) to confirm the relationship between shedding and reduced carcase productivity. This study was also able to identify differences at specific sampling timepoints as well as identify effects related specifically to C*. parvum*. The previously reported associations between reduced HSCW and DP with identification of *Cryptosporidium* or *Giardia* (parasite detected by PCR on at least one occasion) were largely consistent with the present study, although the present study identified that relationships between shedding and HSCW existed only for *C. parvum* (but not all *Cryptosporidium* spp.). Other studies have also reported reductions in DP associated with nematode infections in sheep (Liu et al., 2005; Jacobson et al., 2009).

The productivity consequences of greatest magnitude were for lambs categorized as high shedding for *C. parvum* at post-weaning (3.2kg lower HSCW than non-shedding lambs) and pre-slaughter (2.2kg lower HSCW). *Cryptosporidium parvum* was identified on all eight farms at either post-weaning or pre-slaughter, albeit at low prevalences (below 6%). A recent review by Ryan et al. (2014) reported geographic differences in prevalence and predominant *Cryptosporidium* species and genotypes in sheep, with *C. parvum* apparently the dominant species in Europe based on available epidemiological studies. It is likely that host age influences distribution of *Cryptosporidium* species, but further evidence derived from longitudinal studies is required to confirm this.

Lambs shedding *Cryptosporidium* pre-slaughter had lower DP than non-shedding lambs, but no differences in HSCW or live weight were observed. DP is the ratio of HSCW divided by live weight, and differences in DP may be reflecting variation in either HSCW or the visceral (and non-carcase tissue) of the animal. Although not significant, the HSCW was 0.3kg lighter in lambs shedding *Cryptosporidium* pre-slaughter, whilst their visceral tissue (live weight - HSCW) was 0.8kg heavier, implying that impact on visceral (non-carcase) tissue weight was the key driver of the reduced DP response observed for these lambs. This observation was consistent with previous studies showing DP (but not necessarily live weight) was reduced in

sheep infected with nematodes (Liu et al., 2005; Jacobson et al., 2009) and protozoan parasites (Sweeny 2012; Sweeny et al., 2012b), and supports the suggestion that live weight may underestimate carcase productivity losses in sheep associated with parasitism.

Increased frequency of detection of both *C. parvum* and *Giardia* was associated with reduced carcase production and processing efficiency, specifically greater magnitude of reductions in HSCW and DP. Whether the repeated observations of shedding were due to ongoing (chronic) infection, separate infections (different genotype) or re-infection (same genotype) could not be determined. Both *Cryptosporidium* and *Giardia* infections are thought to be mostly self-limiting although chronic infection and reinfections have been reported at least in humans (Cama et al., 2008; Halliez and Buret, 2013). Cama et al. (2008) reported that reinfections with both the same and different genotypes were common. Little is known about the situation in sheep.

Observations in this study highlighted the importance of longitudinal sampling to identify relationships between protozoan shedding and outcomes for livestock productivity. Importantly, the findings suggest that *Cryptosporidium* and *Giardia* infections have consequences for ruminant productivity beyond the neonatal period. Furthermore, the results suggest that that both intensity and timing of shedding relative to slaughter impact productivity outcomes. Shedding of *C. parvum* or *Giardia* post weaning were associated with reduced HSCW at slaughter, approximately 10 weeks later. Importantly, the reductions in carcase weight in high shedding sheep at post-weaning sampling were both statistically significant and of a magnitude relevant for commercial sheep meat production. Relationships between protozoan shedding and subsequent carcase weight and DP were not significant at the weaning sampling, possibly due to the larger time period between sampling and slaughter. Testing animals pre-slaughter was likely to underestimate impacts on carcase productivity in the flock. For example, *Giardia* shedding at the post-weaning (but not pre-slaughter) sampling was associated with reduced HSCW and *C. parvum* shedding at the post-weaning sampling was associated with reductions in both HSCW and DP. This suggests that protozoan infections have chronic impacts on carcase productivity that may not be identified if samples are collected only at the point of slaughter.

Thresholds for “high”, or pathological oocyst shedding concentration have not been established for either *Cryptosporidium* or *Giardia*. In this study, we nominally used the median oocyst shedding concentration in positive animals for each parasite/farm/timepoint as the cutoff for categorising shedding animals as low or high shedding, and significant effects were observed using this methodology despite the range in median shedding intensity observed across farms and timepoints. This is the first study to our knowledge that has used longitudinal sampling and quantitative molecular measures of shedding to determine impacts on growth and carcase productivity in sheep, so there was little evidence available to support or refute the approach using median values when determining appropriate methods for analyses. Larger data sets that in turn include a larger number of positive (shedding) animals, may be able to identify thresholds for shedding concentration where future impacts on carcase productivity are more likely to be observed. This would be of clinical relevance to production animals where treatment and management decisions are likely to incorporate cost-benefit analyses that incorporate production consequences as well as zoonotic potential and animal welfare considerations. The pathogenesis of infection and subsequent effects on growth and caracase productivity are likely to reflect a combination of parasite and host factors (Geurden et al., 2010b), so interpretation of shedding intensity

around a given threshold for decision making would likely need to include a range of factors impacting resilience to infection such as sheep age, expected time to slaughter, host nutrition, parasite genotype and so on.

The mechanisms by which protozoan infections may reduce growth, or in the case of this study HSCW and DP, are not well understood (O'Handley and Olson, 2006), but are likely related to alterations in gut epithelial structure and function (Buret, 2007). Olson et al. (1995), attributed reduced carcase weight in lambs to reduced feed conversion efficiency in specific pathogen-free barn raised lambs subsequent to surgical infection with *Giardia* (in contrast to the present study with paddock-raised lambs and naturally acquired infections). Ralston et al. (2003) observed associations between reduced feed intake and *Giardia* in steers, but a review by Geurden et al. (2010b) concluded that there was no experimental data "to conclusively indicate an economical impact" of *Giardia* on production in calves. Similarly, whilst *Cryptosporidium andersoni* has been associated with reduced weight gain (Anderson 1987; Ralston et al., 2010) and reduced feed efficiency (Ralston et al., 2003) in feedlot cattle, and reduced milk production in dairy cows shedding *C. muris* (Esteban et al., 1995), reviews by O'Handley and Olsen (2006) and Santin (2013) concluded that there is a lack of evidence to indicates that intestinal cryptosporidiosis results in any long-term production effects in ruminants. All lambs in the present study had access to grazing and feed intake was not recorded, so it was not possible to determine whether differences in HSCW were associated with reduced appetite (feed intake) and/or feed conversion efficiency. It should be noted that associations between infection and reduced growth (*C. parvum*) or carcase size (*C. parvum* and *Giardia* spp.) in this observational study could not be confirmed as causative (i.e. parasitism causing reduced growth or carcase size). It may be the case that sheep that otherwise had restricted growth were more susceptible to infection (shedding) and high shedding intensity.

Interactions between protozoan parasites and a range of host factors determining disease outcomes have been reported in sheep, although these are much less well studied than nematode parasite-host factor interactions. Repeatability of *Eimeria* shedding has been reported as suggestive of a genetic component (Yvore et al., 1992), and Reeg et al. (2005) showed *Eimeria* shedding in sheep older than 60 days of age had high heritability in German Merino lambs. Other factors including stress, litter size, stocking intensity, poor hygiene and concurrent infections have been implicated as increasing risk of clinical coccidiosis and cryptosporidiosis in young lambs (de Graaf et al., 1999; Taylor, 2008; Chartier and Paraud, 2012). In the present study, mixed infections were not associated with reduced live weight, HSCW or DP, except at pre-slaughter where lambs with mixed infections had lower DP. The role of host factors in determining resilience of sheep to *Cryptosporidium* and *Giardia* infection are poorly understood and further evidence is required to draw conclusions about the role of host genetics, concurrent infections, nutrition and management on subsequent productivity losses.

The study was conducted using flocks of sheep being raised for meat production under commercial farming conditions. The potential for biases due to factors that impact growth/weight such as date of birth (lambing periods extended up to 8 weeks), sex and parity and litter size exists and whilst the size of the data set in this study are likely to have addressed some potential biases, assumptions of similarity of factors (such as distribution of litter size across each flock) between flocks was made. Future studies conducted under controlled conditions could be used to better define associations between infection and

growth in sheep accounting for wider range of factors that have potential to impact growth rate within flocks such as estimated breeding values, sex, parity, litter size and the types of anthelmintic treatments that may impact protozoan parasite shedding.

This study was not designed to investigate the effect of nematodes on live weight or carcase productivity, so farmers employed their normal strategic anthelmintic treatments and grazing management. Treatment with benzimidazoles (BZ) may impact shedding intensity (Xiao et al., 1996; O'Handley et al., 1997; O'Handley et al., 2000; Geurden et al., 2006a; 2006b). Records showed that BZ treatments were not used for WA1, WA2, WA3, SA1 or VIC1. Records for SA2, VIC2 and NSW were incomplete, and it is possible that a anthelmintic treatments included a BZ as part of a combination treatment. The BZ dosage required to reduce cyst excretion in sheep is not known, but a review by Geurden et al. (2010b) suggests that for calves the total dosage (5-20mg/kg fenbendazole or albendazole daily for 3 consecutive days) is higher compared with helminth treatment. No sheep in this study were treated with 3 consecutive doses of any anthelmintic. Furthermore, studies in calves have shown that cyst suppression after 3 days of BZ treatment was either not complete or short acting in field conditions, and it has been proposed that this may reflect rapid re-infection in contaminated environments (O'Handley et al., 2000; Guerden et al., 2006; Geurden et al., 2010b). Therefore, any BZ treatments used in the present study were likely sub-therapeutic, and reinfection rapid. The results reported for this study therefore reflect production losses in sheep with oocyst excretion observed under field conditions (ie contaminated environment and reinfection likely) and with anthelmintic treatment protocols typical for lambs being raised for slaughter. Further studies are required to determine the effect of BZ on cyst excretion by sheep at doses recommended for nematode control and under field conditions where reinfection is likely.

No significant main effects for WEC or *Haemonchus* status for live weight, HSCW or DP were observed in this study. Resilience to trichostrongylid parasites by prime lambs with nutrition adequate to support growth rates of 200g/d has been reported in the same regions included in this study, specifically southern Australia in prime lambs up to slaughter (Carmichael et al., 2011; Carmichael et al., 2013) and northern NSW in prime lambs up to weaning (Dever et al., 2016). Furthermore, the effect of infection is likely to have been complicated by variations in resistance both between and within flocks which may impact on lamb growth during the acquisition of immunity (Greer, 2008). **It should also be noted that lambs in the present study were being raised for slaughter using management typical for each property, including anthelmintic treatments and grazing management. It is possible that the nematode infection pressure and range of WEC observed in these flocks was not sufficient for effects on live weight, HSCW and DP to be observed.**

5.2.6 Production consequences for *Yersinia* on liveweight

High shedding of pathogenic *Y. enterocolitica* shedding category was associated with lower liveweight compared with lambs in which pathogenic Yersinia was not detected. The greatest impact was observed at post-weaning (4.958kg), but was still evident pre-slaughter (3.881kg). This is the first report of reduced growth of lambs in lambs with higher faecal shedding of pathogenic *Y. enterocolitica* relative to non-infected counterparts. It was not possible to determine causation with this longitudinal observational study, specifically it was not clear whether pathogenic *Yersinia* was responsible for lower liveweight or if smaller

lambs that were otherwise compromised, were more likely to be shedding higher concentration of *Yersinia* bacteria in faeces.

Ovine yersiniosis was first recognised in the 1930's, but since the early 1990's it has emerged as a cause of enterocolitis in south-eastern Australia. Clinical signs of yersiniosis include scouring, depression, dehydration, ill thrift and deaths. The diarrhoea varies from green and fluid to black and mucoid and may be foul smelling or blood tinged. Diarrhoea is due to hypersecretion caused by enterotoxin and the inflammatory response that results in mucosal erosion and severe exudation of water, electrolytes and protein into the intestinal lumen (Glastonbury 1990; Radostits *et al.* 1994; West *et al.* 2002). In the present study, the overall prevalence of pathogenic *Y. enterocolitica* as determined by screening for the *yst* gene was 5.8% with the highest point prevalence in NSW during weaning and post-weaning at 48.8% and 31.3% respectively.

5.2.7 Associations between pathogens, FCS and dag

Significant correlations between WEC and FCS were noted only for two (WA2 and WA3) of the eight flocks, and for these flocks only at one (WA3) or two (WA2) sampling occasions. Of the pathogens identified by qPCR (specifically *Cryptosporidium* spp., *C. parvum*, *Giardia* spp, *Salmonella* spp., *Campylobacter*. spp., pathogenic *Yersinia* and *C. percorum),* significant associations between pathogen presence and FCS were identified only for *Cryptosporidium* (all species). Lambs shedding *Cryptosporidium* (all species) at weaning and post-weaning had higher FCS, meaning shedding was associated with looser faecal consistency. A complex relationship between pathogen presence and shedding intensity category with FCS and dag score was identified. Analysis identified that farm was a significant main effect for both FCS and dag score at all three sampling timepoints. Pathogen shedding intensity category was not consistently associated with FCS or dag score over the three sampling occasions. High shedding of *C. parvum* was associated with the greatest increase in FCS (relative to lambs in which *C. parvum* was not detected) and the only pathogen with FCS over 3 (soft unformed faeces), but only at post-weaning sampling. WEC was significant as a covariate only for dag score at pre-slaughter sampling. Analysis is ongoing looking at FCS and dag as categorical outcomes and using logistic regression to account for potential confounders.

5.3 Prevalence and pathogen load of zoonotic pathogens in sheep at WA sale yards and in abattoir effluent at Katanning (Milestone 6)

Objective 4 (and milestone 6) of this project was to develop a qPCR assay for *Escherichia coli* O157:H7 and determine the prevalence and pathogen load of zoonotic pathogens (*Salmonella, Campylobacter, Cryptosporidium, Giardia* and *Escherichia coli* O157:H7 in sheep faeces collected from WA sale yards (Muchea and Katanning) and in abattoir effluent (Katanning) (inlet and outlet).

This objective has been met as a quantitative multiplex PCR (qPCR) for detecting and enumerating *Salmonella enterica, Campylobacter* and *E. coli* O157:H7 in one assay in sheep faeces was successfully developed. The multiplex qPCR assay was specific for *Campylobacter*, *S. enterica* and *E. coli* O157:H7, as only the relevant organisms were detected and the detection limit was 5 organisms/µL faecal DNA extract for each pathogen.

This multiplex qPCR assay and a previously developed multiplex qPCR assay for *Cryptosporidium* and *Giardia* (Milestones 1-5) were used to determine the prevalence and concentration of *Campylobacter* spp., *S. enterica, E. coli* O157:H7, *Cryptosporidium* and *Giardia* in 474 sheep faecal samples from WA saleyards (Katanning and Muchea) and in 96 effluent samples from Katanning abattoir.

Shedding of bacterial and protozoan pathogens has implications beyond the farm gate. It can result in contamination of meat carcasses with potentially zoonotic pathogens, impacting on public health. The median concentrations of both *Campylobacter* spp. (4.7 $x10^3$ organisms/g faeces) and *S. enterica* (4.7 $x10^3$ organisms/g faeces) in faeces, was lower than that observed in Australian lambs on farms across Australia (3.9 x 10^5 and 9.4 $x10^4$ organisms/g faeces respectively) in the longitudinal study (Milestones 1-5). The range of *Cryptosporidium* oocyst shedding at sale yards overall was 32 - 3.7 $\times10^6$ and the median was 1.7 × 10^3 oocysts g^{-1}. In abattoir effluent, the range was 587 - 1.5 $\times10^4$ and the median was 1.3 × 10^3 oocysts g^{-1}. This is similar to oocyst shedding concentrations in lambs in the previous milestone where the range of oocyst shedding at pre-slaughter overall (across all states) was 260 - 4.8 × 10^7 and the median was 6.3 × 10^4 g^{-1} (Yang *et al.,* 2014a). The range of *Giardia* cyst shedding at sale yards overall was 32 - 3.7 × 10^6 and the median was 1.7 × 10^3 oocysts g^{-1}. In abattoir effluent, the range was 250 – 4.8 × 10^3 and the median was 1.3 × 10^3 cysts g^{-1}. This was lower than the cyst concentrations in pre-slaughter lambs overall (across four states in Australia) in the previous milestone which was 63-4.7 × 10^9 and the median was 8.1 × 10^4 g^{-1} (Yang *et al.,* 2014b).

An important difference to the previous longitudinal study (milestones 1-5) was that in the present study (Milestone 6), sheep were selected at random and age was not known. The previous milestone was a longitudinal study of lambs with faecal samples collected on 3 occasions between weaning (approximately 12 weeks old) and the period pre-slaughter (approximately 29 weeks of age).

The factors that determine shedding (both prevalence of animals shedding and concentration in faeces) in sheep are not well described, but age is likely to have a significant impact as younger animals are more susceptible to infection. The role of common stressors in sheep meat enterprises (for example, transport, curfew-lairage time where feed and water is withheld, mixing of groups of animals, seasonal factors and management practices such as shearing), may affect the shedding of bacterial pathogens in faeces. However, the impact of these factors on shedding has not been well described. None-the-less there is evidence that protracted fasting may cause protozoan and bacterial load to be more hazardous (Pointon *et al.,* 2012). The time spent off feed prior to sampling was not known for lines sampled in the present study. The prevalence and median shedding concentration of organisms in faeces would be expected to increase where sheep are consigned to slaughter directly from saleyards and therefore subjected to protracted fasting times (>24 hours) and additional stressors related to mixing of animals and transport between sale yards and abattoirs.

5.3.1 Salmonella enterica, Campylobacter and E. coli O157:H7

The overall prevalence of *E. coli* O157:H7 in sheep at sale yards was 8.4% with the organism identified in faeces at 7 of the 8 sampling occasions, and point prevalence ranging from 0% (Katanning, July sampling) to 26% (Muchea, April sampling). This was comparable

with a previous study in which *E. coli* O157:H7 was isolated from 5% of sheep (faeces), 3% of fleeces and 0.6% of pre-chill carcases at two Australian abattoirs (Duffy et al., 2010), but higher than that observed in a study that observed *E. coli* O157:H7 in 2.5% in sheep faeces and 2.9% on hides at a Saudi Arabian abattoir (Bosilevac et al., 2015). Other studies have reported prevalences of 0% in sheep from North Wales (Alhelfi *et al.,* 2013), 3.9% in sheep from Iran (Gencay, 2014) and 18% in sheep from Turkey (Tahamtan and Namavari, 2014).

The overall prevalence of *S. enterica* in sheep at saleyards was 3.6%, with the organism identified in faeces at 7 of the 8 sampling occasions and point prevalence's ranging from 0% (Katanning, May sampling) to 6.4% (Katanning, July sampling). This was comparable with the longitudinal study (Milestone 1-5) that observed *S. enterica* point prevalence ranging from 0-24% in lambs on farms across Australia, but lower than that observed in studies that identified *Salmonella* in 20% of faeces, 13% of fleeces and 1.3% of pre-chilled carcases at two Australian abattoirs (Duffy et al., 2010), and 23.2% of faeces and 67.6% of hides at an abattoir in Saudi Arabia (Bosilevac et al., 2015). The overall prevalence of *Campylobacter* spp. in sheep at saleyards was 5.7% with the organism identified at 7 of the 8 sampling occasions and point prevalence's ranging from 0% (Muchea, July sampling) to 12.1% Katanning, June sampling).

Apart from potential contamination of carcasses and meat products, *Campylobacter* spp. was identified in abattoir effluent (inlet or outlet) at all 4 sampling occasions and both *S. enterica* and *E. coli* O157:H7 were identified on 3 out of 4 sampling occasions. This has implications for the management of effluent waste-water from abattoirs, particularly where this water may come into contact with humans.

5.3.2 Cryptosporidium and Giardia

The overall prevalence of *Cryptosporidium* and *Giardia* in sheep faeces from sale yards in Western Australia was 6.5% and 6.3% respectively, which is similar to the previous milestone where on farm pre-slaughter prevalences ranging from 6.1- 36.4% and 9.4%-42.1% for *Cryptosporidium* and *Giardia* respectively were identified. Few studies have examined the prevalence of *Cryptosporidium* and *Giardia* in sheep sale yards and none have examined abattoir effluent. One study examined lines of sheep sent for slaughter at the Fletcher International abattoir at Narrikup, on the south coast of Western Australia (Ryan *et al.,* 2005). In that study, the PCR prevalence was 44% and 26% for *Giardia* and *Cryptosporidium* respectively. The differences in prevalence between these two studies may be due to the fact that a more sensitive nested PCR approach was used for screening for both parasites in the 2005 study, whereas a qPCR assay was used in the present study. An important advantage of using a qPCR approach however, is that it allows much higher throughput screening and allows numbers of organisms per gram of faeces to be determined. Differences in prevalence may also be due to sheep breeds and levels of stress and nutrition. Other studies have reported microscopy prevalences of 13.2% and 0.7% for *Cryptosporidium* in faeces from slaughtered sheep at slaughterhouses in Turkey and Nigeria respectively (Faleke *et al*., 2006; Ciçek *et al.,* 2008). However as these studies were based on microscopy, they are likely to underestimate the prevalence.

Apart from potential for contamination of carcasses and meat products, *Cryptosporidium* was identified in abattoir effluent (inlet or outlet) at all 4 sampling occasions (overall prevalence of

10.4%) and *Giardia* were identified on 3 out of 4 sampling occasions (overall prevalence of 5.2%). This has implications for the management of effluent waste-water from abattoirs.

The extent to which protozoa find their way from faeces to fleeces/caracases and whether protozoa remain viable on/in meat products is as yet largely unknown, although data from bacteria suggests it is likely where pathogens are present in faeces (Pointon *et al*., 2012). Similarly, the factors that drive pathogen output are largely unknown, although there is evidence that protracted fasting may cause bacterial load to be more hazardous (Pointon *et al*., 2012). There is also very little known about the effect of diet and stress on patterns of protozoa shedding (i.e. whether changes in genotype, prevalence or concentration are driven by stress or diet factors). This is clearly an area that needs to be investigated (see section 6.2.4).

5.4 Extension of recommendations for management strategies to minimise the risk of bacterial and protozoal scouring in prime lambs to sheep producers

Objective 5 of this project was to publish the data in a series of manuscripts and to provide recommendations for management strategies to minimise the risk of bacterial and protozoal scouring in prime lambs to sheep producers.

5.4.1 Publications arising from this project

The following are papers that have already been published from this study and papers that are currently being written (Table 28 - see also appendix).

Table 28. Manuscripts arising from this study.

No.	Authors and Title	Status
1	Yang, R., Jacobson, C., Gardner, G., Carmichael, I., Campbell, A.J.D., Ng-Hublin, J., Ryan, U., 2014. Longitudinal prevalence, oocyst shedding and molecular characterisation of *Cryptosporidium* species in sheep across four states in Australia.	Published. 2014 Veterinary Parasitology 200(1-2), 50-8.
2	Yang, R., Jacobson, C., Gardner, G., Carmichael, I., Campbell, A.J.D., Ryan, U., 2014. Development of a quantitative PCR (qPCR) for *Giardia* and analysis of the prevalence, cyst shedding and genotypes of *Giardia* present in sheep across four states in Australia.	Published. 2014 Experimental Parasitology. 137, 46-52.
3	Yang, R., Jacobson, C., Gardner, G., Carmichael, I., Campbell, A.J.D., Ryan, U., 2014. Longitudinal prevalence, oocyst shedding and molecular characterisation of *Eimeria* in sheep across four states in Australia. Experimental Parasitology 145, 14-21.	Published. 2014 Experimental Parasitology 145, 14-21.
4	Yang, R., Jacobson, C., Gardner, G., Carmichael, I., Campbell, A.J.D., Ryan, U., 2014. Longitudinal prevalence and faecal shedding of *Chlamydia pecorum* in sheep.	Published. 2014 The Veterinary Journal. 201(3), 322-326.
5	Yang, R., Jacobson, C., Gardner, G., Carmichael, I., Campbell, A.J.D., Ryan, U., 2014. Longitudinal prevalence, faecal shedding and molecular characterisation of *Campylobacter* spp. and *Salmonella enterica* in sheep.	Published. 2014. The Veterinary Journal. 202(2), 250-254
6	Yang, R., Ryan, U., Gardner, G., Carmichael, I., Campbell, A.J.D., Jacobson, C., 2015. Longitudinal prevalence, faecal shedding and molecular characterisation of *Yersinia* spp. in sheep across four states in Australia	Published 2016 Australian Veterinary Journal 94(5), 129-137.

No.	Authors and Title	Status
7	Yang, R., Ryan, U., Gardner, G., Carmichael, I., Campbell, A.J.D., Ng-Hublin, J., Jacobson, C., 2015. Prevalence of *Haemonchus, Teladorsagia* and *Trichostrongylus* in sheep across four states in Australia and comparison of traditional McMaster faecal worm egg counts (WEC) with qPCR for detection of strongylid worms.	In Preparation
8	Yang, R., Gardner, G., Ryan, U., Jacobson, C., 2015. Prevalence and pathogen load of *Campylobacter* spp., *Salmonella enterica* and *E. coli* O157:H7 in sheep faeces collected from sale yards and in abattoir effluent in Western Australia.	Published 2016 Australian Veterinary Journal. In press
9	Yang, R., Gardner, G., Ryan, U., Jacobson, C., 2015. Prevalence and pathogen load of *Cryptosporidium* and *Giardia* in sheep faeces collected from sale yards and in abattoir effluent in Western Australia.	Published 2015 Small Ruminant Research. 130, 216–220
10	Jacobson, C., Williams, A., Yang, R., Ryan, U., Carmichael, I., Campbell, A.J.D., Gardner, G. Greater intensity and frequency of *Cryptosporidium* and *Giardia* oocyst shedding beyond the neonatal period is associated with reductions in growth, carcase weight and dressing efficiency in sheep.	Published 2016 Veterinary Parasitology 228: 42 – 51
11	Jacobson, C., Williams, A., Yang, R., Ryan, U., Carmichael, I., Campbell, A.J.D., Gardner, G. Impacts of *Yersinia* on carcase production and growth in lambs.	Will be submitted to Veterinary Parasitology

As can be seen from Table 28, a total of 11 manuscripts have either been published or will be published in international journals from this project and therefore this aspect of the objective has clearly been met.

5.4.2 Recommendations for management strategies to minimise the risk of bacterial and protozoal scouring in prime lambs to sheep producers

This project was the first study to detect and quantitate 10 different pathogens in sheep faecal samples from 8 farms across 4 different states. The multiplex qPCR diagnostic developed can be used to ensure that producers have a full knowledge of the suite of disease problems likely to be encountered in a property-specific manner.

A key finding of the present study was that statistical analysis revealed no association between WEC and production parameters. However, three pathogens (*Cryptosporidium, Giardia* and *Yersinia*), consistently showed significant production effects.

For *Cryptosporidium* and *Giardia,* a variety of chemotherapeutic agents have shown promise in sheep, goats and cattle, with examples including benzimidazole chemicals (O'Handley *et al.*, 2000; O'Handley *et al.*, 2001; Thompson *et al.*, 2008), paromomycin (Griffiths *et al.*, 1998; Viu *et al.*, 2000; Rossignol, 2010), halofuginone lactate (Giadinis *et al.*, 2007; Giadinis *et al.*, 2008; De Waele *et al.*, 2010), nitazoxanide (St Jean *et al.*, 1987; Xiao *et al.*, 1993; Plutzer and Karanis, 2009; Schnyder *et al.*, 2009; Rossignol, 2010) (Schnyder *et al.* 2009; Rossignol 2010), cyclodextrin (Castro-Hermida *et al.*, 2001; Castro-Hermida *et al.*, 2004) and lasalocid (Castro-Hermida *et al.*, 2000).

The benzimidazoles, commonly found in many strongylid nematode anthelmintics used in Australia, require consecutive days of administration (3–5 days) for the reliable treatment of

giardiasis. However this is not a sustainable option, with trichostrongylid worm resistance already proving a major challenge for sheep enterprises in southern Australia.

Nitroimidazoles are considered an alternative chemotherapeutic treatment for cryptosporidiosis and giardiasis, although here too there are concerns of resistance developing (Rossignol, 2010) and treatment failures have been reported (Schnyder *et al.,* 2009).

Treatment of *Cryptosporidium* and *Giardia* infections in ruminants is also problematic due to the expense and a high risk of re-infection when treated animals are released back to extensive grazing of pastures (Olson, 2000).

In the absence of effective treatment, knowledge of the transmission dynamics of *Cryptosporidium* and *Giardia* is crucial. The infective stages of both *Cryptosporidium* and *Giardia*, are encysted when released in faeces and are capable of prolonged survival (3 months or more) in a variety of different environments (Robertson *et al.*, 1992; Fayer *et al.*, 1996; Olson *et al.*, 1999; Carey *et al.*, 2004). It is thought that sheep become infected with *Cryptosporidium* and *Giardia* through ingesting pasture or supplementary feed contaminated with oocysts (Taylor *et al.*, 1993; Fayer *et al.*, 1996; Olson *et al.*, 1997; O'Handley and Olson, 2006). It is also common for livestock to become infected with *Cryptosporidium* and *Giardia* via the consumption of contaminated water (Fayer *et al.*, 1996; Olson *et al.*, 2004; O'Handley and Olson, 2006; Thompson *et al.*, 2008).

Different species of flies have been reported to be capable of carrying and enhancing the transmission of both *Cryptosporidium* and *Giardia* between livestock*,* particularly those intensively managed within confined barns, stalls, feedlots or during blowfly strike outbreaks (Graczyk *et al.*, 1999; Graczyk *et al.*, 2005; Fetene *et al.*, 2011).

For extensively managed sheep on pastures, livestock management including time of lambing, husbandry practices, paddock rotations, livestock water source and stocking rate all have the potential to influence the spread of both *Giardia* and *Cryptosporidium*. This presents an opportunity for the development of livestock control programs to minimise both the risk of transmission and also the impact of protozoan parasites (Geurden *et al.*, 2006). For example, cleaning of the lambing area has been identified as a major factor associated with reduced risk of infection with *Cryptosporidium* and *Giardia* (Causapé *et al.,* 2002).

Yersinia infections in sheep are likely to come from contaminated pastures (Sunderland *et al.,* 2009) and from contaminated water as *Yersinia* survives well in spring, river, or groundwater (Guan and Holley, 2003), and is often isolated from water (Ford, 1999). *Yersinia* infections in sheep are rarely treated. Doxycycline in combination with an aminoglycoside is sometimes used in severe infections. Other antibiotics active against *Y. enterocolitica* include trimethoprim-sulfamethoxasole, ceftriaxone, and chloramphenicol (Bottone, 1997). Chloramphenicol is banned for use in food producing animals in most countries, including Australia. *Yersinia enterocolitica* is usually resistant to penicillin G, ampicillin, and cephalotin due to beta-lactamase production (Bottone, 1997).

Further studies are required to identify potential risk factors for *Cryptosporidium, Giardia* and *Yersinia* infections in Australian extensively grazing lambs across different states to more effectively limit transmission of these pathogens.

6 Conclusions/recommendations

6.1 Conclusions

The focus of this project was to examine the epidemiology and potential pathogenesis of protozoan and bacterial parasites in extensively grazing lambs and also in lambs in sale yards and abattoir effluent.

The project identified that the protozoan parasites *Cryptosporidium, Giardia* and *Eimeria* were present in lambs on eight sheep properties with a history of scouring across Australia and that lambs are capable of harboring *Cryptosporidium* and *Giardia* species that are known to be zoonotic as well as those that appear to be host-specific.

Zoonotic *C. parvum* and *C. ubiquitum* were identified in 29.8% of positive samples typed. For *Giardia*, the potentially zoonotic Assemblage A was identified in 22.4% of positive isolates typed. Of the 118 *Eimeria* isolates typed, pathogenic *Eimeria* species were found in 58.5% of samples.

Campylobacter spp. and, to a lesser extent, *S. enterica* were prevalent in lambs on eight farms with a history of scouring in this study, and bacterial shedding in these animals was high. *Chlamydia abortus* was not detected in sheep faecal samples, but further screening of vaginal swabs are required to confirm the absence of *C. abortus* in sheep in Australia. This is the first study of *C. pecorum* of such magnitude in sheep in Australia and high prevalences were identified.

Statistical analysis revealed no association between WEC and production parameters and only three pathogens (*Cryptosporidium, Giardia* and *Yersinia*) consistently showed significant production effects.

Cryptosporidium shedding was associated with a reduction in DP (1.21-1.25%) and *Giardia* was associated with a reduction in HSCW (0.594 kg). More specifically, this study identified that shedding of the zoonotic *Cryptosporidium parvum* was associated with reductions in liveweight (2.411-3.457 kg), HSCW (2.602-2.208 kg) and DP (4.6%). Importantly, this was a longitudinal study, therefore relationships between protozoan shedding at different time points and productivity measures at different time points could be identified. For example, *Giardia* shedding at the post-weaning (but not pre-slaughter) sampling was associated with reduced HSCW (0.594 kg), and *C. parvum* shedding at the post-weaning sampling was associated with reductions in both HSCW and DP suggesting that protozoan infections can have chronic impacts on carcase productivity.

Significant correlations between WEC and FCS were noted only for two (WA2 and WA3) of the eight flocks, and for these flocks only at one (WA3) or two (WA2) sampling occasions. Of the pathogens identified by qPCR (specifically *Cryptosporidium* spp., *C. parvum*, *Giardia* spp, *Salmonella* spp., *Campylobacter*. spp., pathogenic *Yersinia* and *C. percorum),* significant associations between pathogen presence and FCS were identified only for *Cryptosporidium* (all species). Lambs shedding *Cryptosporidium* (all species) at weaning and postweaning had higher FCS, meaning shedding was associated with looser faecal consistency.

Analysis also identified a complex relationship between pathogen shedding intensity category and FCS or dag score, as it was not consistently associated with FCS or dag score over the three sampling occasions. High shedding of *C. parvum* was associated with the greatest increase in FCS (relative to lambs in which *C. parvum* was not detected) and the only pathogen with FCS over 3 (soft unformed faeces), but only at post-weaning sampling. WEC was significant as a covariate only for dag score at pre-slaughter sampling.

Yersinia *enterocolitica* shedding was associated with reductions in liveweight (3.4 kg). Furthermore, there was a significant interaction between sampling period (time) and level of shedding category on liveweight, with reductions in liveweight evident at the post-weaning (4.9kg, post hoc P) and pre-slaughter (3.9kg post hoc P) sampling occasions in the lambs classified as high shedding compared to non-shedding lambs. This is the first report of reduced growth of lambs in lambs with higher faecal shedding of pathogenic *Y. enterocolitica* relative to non-infected counterparts.

Campylobacter spp., *S. enterica* and *E. coli* O157:H7 were identified in sheep at saleyards in Western Australia. Overall prevalences were generally low (<6%), but point prevalences ranged considerably in healthy sheep (up to 26% for *E. coli* O157:H7) suggesting that further work to determine risk factors for shedding of bacterial organisms in meat sheep in the pre-slaughter period (on-farm, sale yards and lairage at abattoirs) could further reduce the risk of contamination of meat products.

Cryptosporidium and *Giardia* are prevalent in pre-slaughter lambs in saleyards in Western Australia and in abattoir effluent. This has the potential to contaminate lamb carcases, which has important implications for public health.

6.2 Recommendations

6.2.1 Facilitate validation of a uniform diagnostic qPCR assay across laboratories

The qPCR assay developed as part of the present study was shown in our hands to be both specific and sensitive for the detection of 10 pathogens. The livestock industries will benefit from a wider adoption of this qPCR-based diagnostic approach to animal health. MLA should consider funding efforts to validate and optimise the diagnostic qPCR platform developed as part of this study across different laboratories to maximise time efficiency, accuracy and reliability and ensure that quality diagnostic services are provided for all Australian livestock producers. An investigation into the cost-benefits and practical implications for diagnostic laboratories of the multiplex qPCR diagnostic should be conducted, combined importantly with a forecasted uptake and probable demand by livestock enterprises. It is anticipated that the adoption of this multiplex qPCR assay across both commercial and government laboratories for both commercial on-farm testing and state or federally-funded disease out-break testing will provide economies of scale for all parts of the veterinary diagnostics complex.

6.2.2 More in depth analysis of the production impacts of *Cryptosporidium, Giardia* and *Yersinia* on sheep

A key finding of the present study was that *Cryptosporidium, Giardia* and *Yersinia* had production impacts on sheep. However, as these studies were conducted on-farm, many uncontrollable variables may have influenced the data, particularly live weight measurements. Some of these uncontrollable variables include feed intake, gut fill and time off feed (Thompson *et al.*, 1987; Warriss *et al.*, 1989) and lamb birth type (single or twin) (Kenyon *et al.*, 2004; Hatcher *et al.*, 2009).

Body Condition Score (BCS) was not measured in the present study, however it should be included in future studies. Assessment of BCS does not require sophisticated equipment and has been shown to be an effective measure of a sheep's "nutritional wellbeing" and body reserves across a range of genotypes and environments (van Burgel *et al.*, 2011). Body condition score is considered a more accurate measure of body reserves than live weight because unlike live weight, BCS is not confounded by factors such as gastrointestinal tract contents, sheep frame size, pregnancy and fleece weight (Russel *et al.*, 1969; Warriss *et al.*, 1987; Teixeira *et al.*, 1989; Sanson *et al.*, 1993; Oregui *et al.*, 1997). Consequently, BCS is potentially a more accurate and reliable indicator of the consequences of internal parasitism on productivity compared to live weight.

While acknowledging that internal parasitism has significant consequences upon livestock production profits (Sackett *et al.*, 2006), it is important to emphasise that other factors (such as genetics and nutrition) will have a major impact on lamb productivity and resilience to parasite challenge (Abbott *et al.*, 1986; Kahn *et al.*, 2003; Liu *et al.*, 2005; Louvandini *et al.*, 2006; Houdijk, 2008). Further more controlled studies including controlled feed intake are necessary to provide more accurate information regarding the observed production impacts. The study could also include ascertaining whether specific new broad spectrum anti-protozoal and anti-bacterial chemotherapeutic agents (e.g. inosine 5'-monophosphate dehydrogenase inhibitors – Mandapati et al., 2014) and or re-profiling of approved drugs such as auranofin (Debnath et al., 2013) are cost-effective against *Cryptosporidium, Giardia* and *Yersinia* infections. It would be important that such a trial examines the possible impacts that single or mixed (*Cryptosporidium, Giardia* and *Yersinia)* infections have on on-farm and off-farm profitable productivity; including feed consumption, carcase attributes (HSCW, DP and GR knife fat depth), production performance attributes (live weight, growth rate and body condition score) and faecal attributes (FCS, FDM% and breech fleece soiling score).

6.2.3 More in depth analysis of the pathogen presence, shedding intensity with FCS or dag score

A complex relationship between pathogen presence and shedding intensity category with FCS and dag score was identified and clearly further analysis is required. Cross-sectional studies described below should be designed to more thoroughly examine the relationship between pathogen presence and shedding intensity category with FCS and dag score.

6.2.4 Identification of potential risk factors for *Cryptosporidium, Giardia* and *Yersinia* infections in sheep

Little is known about the risk factors for *Cryptosporidium, Giardia* and *Yersinia* infections in sheep. A cross-sectional study should be carried out to identify management factors that may be contributing to disease. This would include a questionnaire being designed and administered to farmers to collect data on demographic, management and health factors hypothesized to be associated with the risk of infection with these pathogens in sheep.

6.2.5 Measure faecal output and pathogen load in lambs over time throughout curfew and lairage

Stress and shedding of potentially zoonotic pathogens (*Giardia, Cryptosporidium, Salmonella,* etc) has implications beyond the farm gate, particularly for the processing and retail sector, and these have not been well explored to date.

The present study measured prevalence and pathogen load of five pathogens (*Campylobacter* spp., *S. enterica*, *E. coli* O157:H7, *Cryptosporidium* and *Giardia)* in lambs at saleyards and in abattoir effluent. A more in depth study that measures faecal output and pathogen load in lambs over time throughout curfew and lairage is required. This will provide more meaningful information on risk factors for shedding of zoonotic pathogens at abattoirs with relevance to both carcase contamination and effluent management at the abattoir. These important factors have not been studied in detail and need to be managed/reduced in meat enterprises. The proposed study would use the multiplex qPCR tools developed by this project to provide baseline information from which recommendations can be made about management of lambs prior to slaughter in order to manage zoonotic pathogen risk. Specifically, a project should be designed to answer the following questions:

- Does pre-slaughter stress (mustering, curfew, transport, mixing, lairage) alter faecal pathogen shedding?
- Does pathogen shedding change the longer the lambs are off feed?
- Does the pattern of pathogen shedding change with diet?

If these actions are taken now, they are likely to enable effective future delivery of improved options in animal health management for Australian livestock producers. Acting now will provide opportunities to minimise production loss and animal health input costs. This will place Australian producers in a better position to respond to future market pressures regarding animal health and welfare issues.

A delay in responding could mean that Australia's producers could be denied the opportunity to adopt new technologies embraced by overseas competitors. Failure to act is likely to result in continuing declines in the provision of commercial tier veterinary diagnostic services for livestock producers.

If the benefits of qPCR veterinary diagnostic assays can be realised, each producer should be able to control disease more precisely, and have a full knowledge of the suite of disease problems likely to be encountered in a property-specific manner. By combining the results of repeated diagnostic testing with the livestock producer's experience, production data, market

information and weather data, it should be both feasible and profitable to implement a precise on-farm animal health management plan.

7 Key messages

- The protozoan parasites *Cryptosporidium, Giardia* and *Eimeria* were prevalent in lambs on eight farms with a history of scouring in four states across Australia.
- *Campylobacter* spp. and, to a lesser extent, *S. enterica* were prevalent in lambs on the eight studyfarms, and bacterial shedding in these animals was high.
- *Chlamydia abortus* was not detected in sheep faecal samples, but further screening of vaginal swabs are required to confirm the absence of *C. abortus* in sheep in Australia.
- High prevalences of *Chlamydia pecorum* in sheep were identified on the eight study farms.
- No significant main effects for WEC or Haemonchus status for live weight, HSCW or DP were observed in this study.
- Significant correlations between WEC and FCS were noted only for two (WA2 and WA3) of the eight flocks, and for these flocks only at one (WA3) or two (WA2) sampling occasions.
- Three pathogens (*Cryptosporidium, Giardia* and *Yersinia*) consistently showed significant production effects.
- *Cryptosporidium* shedding was associated with a reduction in DP (1.21-1.25%) and *Giardia* was associated with a reduction in carcase weight (0.594 kg).
- Shedding of the zoonotic *Cryptosporidium parvum* was associated with reductions in liveweight (2.411-3.457 kg), HSCW (2.602-2.208 kg) and DP (4.6%).
- Protozoan infections can have chronic impacts on carcase productivity.
- Yersinia *enterocolitica* shedding was associated with reductions in liveweight (3.4 kg).
- Lambs shedding *Cryptosporidium* (all species) at weaning and postweaning had higher FCS, meaning shedding was associated with looser FCS
- A complex relationship between pathogen shedding intensity category was identified. Pathogen shedding intensity was not consistently associated with FCS or dag score over the three sampling occasions.
- High shedding of *C. parvum* was associated with the greatest increase in FCS (relative to lambs in which *C. parvum* was not detected) and the only pathogen with FCS over 3 (soft unformed faeces), but only at post-weaning sampling.
- WEC was significant as a covariate only for dag score at pre-slaughter sampling.
- Overall prevalences of *Campylobacter* spp., *S. enterica* and *E. coli* O157:H7 in sheep at saleyards in Western Australia were generally low (<6%), but point prevalences ranged considerably in healthy sheep (up to 26% for *E. coli* O157:H7).
- Further work to determine risk factors for shedding of bacterial organisms in meat sheep in the pre-slaughter period (on-farm, sale yards and lairage at abattoirs) could further reduce the risk of contamination of meat products.

- *Cryptosporidium* and *Giardia* are prevalent in pre-slaughter lambs in saleyards in Western Australia and in abattoir effluent. This has the potential to contaminate lamb carcases, which has important implications for public health.

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