



Department of
Agriculture and Food



final report

Project Code: AHW.019
Prepared by: R.B.Besier
Department of Agriculture Western Australia
Date published: March 2003

PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Aetiology and epidemiology of scouring in sheep at abattoirs - the role of nematode and protozoalparasites

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

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Aetiology and epidemiology of scouring in sheep at abattoirs

*- the role of nematode and protozoal
parasites*

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Report prepared for MLA by:

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ISBN 1 74036 482 1

March 2003

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Animal Health and Welfare





ABSTRACT

A survey of sheep at an abattoir in Western Australia from September 2002 to February 2003 indicated unexpectedly high worm burdens in many lines of prime lambs. Mean worm egg counts exceeded 1500 eggs per gram, with counts of over 1000 eggs per gram in 40% of lines, suggesting that drenching would be beneficial. Higher counts than usually considered acceptable were also found in many adult lines. The protozoal parasites *Giardia* and *Cryptosporidium* were commonly detected, including some isolates not previously recorded in sheep or in Australia. The scouring observed in some lines was not clearly related to parasitic infections, though this remains the most likely cause. The production significance of the results requires investigation, as it appears that producers are generally not aware of the potential for loss due to parasites in sheep sent for slaughter.

EXECUTIVE SUMMARY

A survey of parasites in sheep sent for slaughter at the Fletcher International abattoir at Narrikup, on the south coast of Western Australia was conducted from September 2002 to January 2003. Faecal samples were taken each day from 6 lines of sheep selected at random, except that preference for sampling was given to lines showing evidence of scouring (diarrhoea). A total of approximately 4400 sheep from 367 lines from locations throughout WA were sampled (244 adult sheep, 10 hogget and 133 lamb), with scouring in 10% of lines. A mail questionnaire of production factors sent to the sheep vendors attracted a high response rate. The study results are considered generally applicable to the WA sheep meat industry.

The results indicate that sheep worms are an unrecognised burden on prime lamb production in WA, and that most producers had not taken effective worm control measures in lambs. Mean worm egg counts in lambs exceeded 1500 eggs per gram (epg), and in over 40% of lines, counts were above levels usually considered warranting drenching. Even allowing for a faecal concentration effect, these results suggest substantial losses in productivity. In adult sheep, counts were lower (mean 486 epg), but in 13%, counts were greater than 1000 epg.

The factors associated with scouring were more difficult to elucidate, largely because the number of cases was relatively low. However, although not statistically significant, high worm burdens remain the most likely cause in lambs. In adult sheep, scouring is also considered due to worms through the "larval hypersensitivity" syndrome. The association of *Giardia* and *Cryptosporidium* with scouring in adult sheep may be of relevance.

The worm burdens in slaughter sheep was surprising given the level of control typically used in wool enterprises, especially during the spring and summer period. Effective worm control in prime lambs should not prove difficult and would include worm egg count monitoring, pasture management and drench treatments. The mail survey indicated significantly lower worm burdens in lambs drenched in the 2 months prior to consignment. Pasture movements also aided worm control.

The survey also produced new information regarding protozoal infections in sheep at slaughter, and illustrated the value of molecular techniques in maximising sensitivity. Using PCR detection, *Giardia* infections in lambs were found in 45% of lines, and *Cryptosporidium* in 26%. Infection rates in adult lines were only one-third those of lambs, but were associated with scouring, whereas there was no such relationship in lambs. A number of *Giardia* genotypes were detected, including a livestock genotype and the potentially zoonotic group A (although no implications for Australian abattoir products are evident). The most common *Cryptosporidium* genotypes identified were the cervid type and the novel bovine B genotype, although little is known of their prevalence or zoonotic potential. The identification of *C.andersoni* is the first report of this species in Australia and the first report in sheep.

These findings indicate the need to communicate to sheep meat producers the importance of effective nematode control practices. Should these results also prove to apply to the industry outside WA, an extension campaign would provide a significant national productivity benefit.

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DURATION:

September 2002 – June 2003

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1. INTRODUCTION

Scouring in sheep delivered to abattoirs is considered a significant problem to the meat industry, but there is little objective information regarding the causes.

However, as nematode parasitism is a common cause of scouring, especially in lambs (and adult sheep in some situations), it was considered that an investigation should first quantify worm burdens in sheep sent for slaughter. As helminthiasis is a significant cause of poor growth rates, reduced body condition in sheep and a major cause of scouring (Cole 1986), it is likely that the existence of scouring may indicate an underlying effect on sheep production.

A survey of sheep at abattoir also provides the opportunity to investigate the role of protozoan organisms such as *Giardia* and *Cryptosporidia* as a cause of scouring in sheep, and as a potential zoonosis. There is little objective information on their clinical significance in sheep, and no prevalence data from Australia. The recent availability of sophisticated molecular tools to identify genotypes offers the opportunity for a preliminary survey of the presence and identity of potentially significant protozoan infections.

The survey information is expected to indicate the relative significance of parasitism in slaughter sheep, and if considered necessary, to provide the basis for communication to growers of strategies to manage the problems.

2. EXPERIMENTAL OBJECTIVES

1. To investigate the prevalence of helminth and protozoan parasites in prime lambs and adult sheep delivered to abattoirs
2. To relate scouring in slaughter sheep to parasite species and numbers, and to basic epidemiological factors
3. To document the prevalence of lines of sheep in which scouring occurs *
4. To assess the potential public health significance of organisms such as protozoa
5. To provide the basis for an economic assessment of the cost of scouring to the sheepmeat industry.*

* Objectives 3 and 5 did not prove possible as the necessary information was not available, but the survey results will facilitate an assessment of the industry significance (See "Outcomes", p 19).

3. EXPERIMENTAL METHODS

Lines of sheep were sampled whilst in lairage at the Fletcher International abattoir in Narrikup, 25 km north of Albany, Western Australia. The sampling period ran from September 2002 to January 2003, with a break in sampling during the month of December. This is the peak annual period of prime lamb turn-off as well as a period when surplus ewes are sold. During this time, 367 lines of sheep were sampled consisting of 113 lamb lines, 10 carry-over lamb or hogget lines and 244 mutton lines. For analysis of nematode samples, sheep were defined as lambs (less than 12 months of age), hoggets (12 to 24 months), and adults. For protozoal analyses, lambs were considered less, and adults older, than 12 months.

A line of sheep was defined as a group of 50 or more sheep consigned from an identified source. Lines were classified as either "scouring" (at least 10 animals showing evidence of active or recent scouring) or "non-scouring". From all lines, faecal samples were taken from 10 individual non-scouring animals, and in scouring lines, an additional 10 scouring sheep were sampled. On each day a maximum of 6 lines were sampled, with priority given to scouring lines when present.

3.1. Laboratory diagnosis

3.1.1. Nematode counts

Faecal samples were processed at the Albany Animal Health Laboratory, using the modified McMaster technique for worm egg counts. Larval differentiations were performed on the bulked samples from lines with worm egg counts above 100 eggs per gram (epg), and used as the basis for distinguishing the "scour worm" component of the mean line egg count. "Scour worms" were defined as strongyle genera other than *Haemonchus* and *Nematodirus* (i.e., chiefly *Ostertagia* and *Trichostrongylus*). Lines with a high proportion of *H. contortus* (a larval differentiation of more than 70%) were not used in the analysis.

3.1.2. Protozoal methods

Sub-samples for protozoal detection were sent to the parasitology laboratory at Murdoch University. A total of 1647 samples were screened, pooled within lines in lots of 5 samples. Microscopy was performed using malachite green negative staining for *Cryptosporidium* (Elliot et al. 1999), and salt floatation for the detection of *Giardia* (Hopkins et al. 1997) for the presence of *Cryptosporidium* and *Giardia*. A total of 240 samples and 147 samples were screened for *Cryptosporidium* and *Giardia*, respectively, at the 18S locus as previously described (Hopkins et al. 1997, Ryan et al. 2003a). Sequencing and phylogenetic analysis was performed as previously described (Ryan et al. 2003a).

Briefly, DNA was purified using a QiAmp stool kit (Qiagen, Hilden Germany). A two-step nested PCR protocol was used to amplify the *Cryptosporidium* 18S rDNA gene. For the primary PCR, a PCR product of 763 bp was amplified using the forward primer 18SiCF2 (5'-GAC ATA TCA TTC AAG TTT CTG ACC-3') (bp position 292) and the reverse primer 18SiCR2 (5'-CTG AAG GAG TAA GGA ACA ACC -3') (bp position 1007). The PCR reaction consisted of 200 μ M each of dNTP, 1 x PCR buffer (Fisher Biotech, Perth, Australia), 1.5 mM MgCl₂, 0.5 units of Taq polymerase (Fisher Biotech, Perth, Australia), and 12.5 pmoles of forward and reverse primers in a total of 25 μ l reaction. Forty-five PCR cycles (94°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec) were carried out in a Perkin Elmer Gene Amp PCR 2400 thermocycler with an initial hot start (94°C for 5 min) and a final extension (72°C for 10 min). For the secondary PCR, a fragment of ~587 bp was amplified using 1 μ l of primary PCR product and nested forward 18SiCF1 (5'-CCT ATC AGC TTT AGA CGG TAG G-3') (bp position 289) and nested reverse 18SiCR1 (5'-TCT AAG AAT TTC ACC TCT GAC TG-3') (bp position 851) primers. The PCR condition for the secondary PCR was identical to the primary PCR. Secondary PCR products were sequenced directly in both directions. Each isolate was sequenced at least twice. TAQ Extender™ (Stratagene, La Jolla, CA) was included in all reactions to minimise PCR error.

The primers, and their sequences, used to amplify a 292 bp region of the 5' end of the *Giardia* 18S rDNA gene were RH 11, forward primer (1-18), 5' CATCCGGTCGATCCTGCC 3' and RH 4, reverse primer (268-292), 5' AGTCGAACCCTGATTCTCCGCCAGG 3'. PCR amplification was performed in 25 μ l volumes using 12.5 pmol of each primer, 1 unit Tth plus DNA polymerase (Biotech International, Perth, Australia), 200 μ M of each dNTP and 2 mM MgCl₂. DMSO was added to a final concentration of 5%. Reactions were heated to 96°C for 2 min followed by 35 cycles of 96°C for 20 sec, 59°C for 20 sec and

72°C for 30 sec and 1 cycle of 72°C for 7 min using a GeneAmp PCR System 2400 thermocycler (Perkin-Elmer, Foster City, California).

PCR products were purified using Qiagen spin columns (Qiagen, Hilden, Germany) and sequenced using an ABI Prism™ Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA). Sequences were analysed using SeqEd v1.0.3. (Applied Biosystems, Foster City, CA). Phylogenetic analysis was performed using Treecon version 1.3b (<http://www.psb.rug.ac.be/bioinformatics/psb/Userman/treeconw.html>), based on evolutionary distances calculated with the Kimura 2-parameter model. The confidence of grouping was accessed by bootstrapping, using 1,000 replicates.

3.2. Mail survey

A survey (Appendix 1) was sent to the named consigner of all lines sampled, with a separate survey form for lambs and adult sheep. Questions included sheep age or birth month, nutritional regimen (pasture, supplemented or feedlot), whether lambs were weaned, paddock changes at weaning, drenching history and products used, signs of scouring, and whether crutched or shorn. A reply paid envelope was included with the survey. Of the 244 and 111 mutton and lamb survey forms delivered, 160 and 76 respectively were returned, a response rate of 66% for mutton and 68% for lambs.

3.3. Statistical analysis

Worm egg count (WEC) data was analysed using log transformed geometric mean of WEC+25 (half the smallest detectable unit of 50 epg). Non-parametric tests (Mann-Whitney U, Kruskal-Wallis and Wilcoxon signed rank tests) were used to compare means due to unequal variances and the skewed and non-normally distributed nature of the data. For protozoal analyses, Chi-square, risk analysis and non-parametric tests were performed. All statistical analysis was conducted using SPSS 11.0 (Statistical Package for the Social Sciences) for Macintosh OS X (SPSS inc. Chicago, USA).

4. STAFF EMPLOYED

The following staffs were employed for varying time periods using MLA-provided funds:

Mr Garnett O'Connell, Technical Officer for abattoir sampling (casual employment)

Ms Wendy Nekele, Technical Officer for abattoir sampling (casual employment)

Ms Heide Guetelich, Laboratory Technical Officer, Albany Animal Health Laboratories (permanent Department of Agriculture officer, approximately 10% time on survey)

Mr Ramin Nikravan, Laboratory Technical Officer, Albany Animal Health Laboratories (permanent Department of Agriculture officer, approximately 20% time on survey)

Ms Esther Spence, Laboratory Technical Officer, Albany Animal Health Laboratories (casual)

Ms Aileen Elliott, Technical Officer, Parasitology Laboratory Murdoch University.

5. RESULTS

5.1. Sheep sampled: number and origin

5.1.1. Sheep sampled

A total of over 4400 sheep were faecal sampled, with a preponderance of adult lines as there were fewer lines of lambs, especially when the survey began. Relatively few lines were hoggets. Most lines were described as Merinos, but this is likely to be more accurate for the adult sheep than the lambs.

Table 1. Number and breeds of sheep sampled in lairage

Lines/sheep	Lambs	Hoggets	Adults	Total
No. Lines sampled	113	10	244	367
No. Sheep sampled	1350	160	2920	4430
Breeds (judgements by sample collectors, not information from vendors)				
Merino	70*	8	236	324
Cross-breed or British Breed	38	1	8	46
Mixed or unspecified	5	2	0	2

* Lambs listed as “prime lambs” were Merinos by appearance, but some incorrect specifications are likely

5.1.2. Seasonal sampling pattern

The usual seasonal lamb turn-off pattern was evident, with few lines in September.

There was no perceptible seasonal turn-off trend for adult sheep, with ewes being sought for slaughter when lambs were scarce.

Table 2. Lines of sheep sampled in each month

Month	Number of Lines	
	Lambs	Adult sheep
September	5	83
October	50	68
November	34	27
January	24	66
TOTAL	113	244

5.1.3. Lamb samples by location

Although weighted towards the southern coastal region, most lambs came from the “typical” Western Australian sheep zones with hot dry summers (even in the southern coastal regions, few sheep derive from the temperate coastal fringe). The sample is a reasonable cross-section of the lamb industry.

Table 3. Regions of origin of lambs sampled

Month	No. Lines	Mean worm egg count	No. lines scouring
South West	0	0	
Southern coastal	44	872	8
Great Southern	20	1373	2
South Eastern	4	211	1
Midlands	18	1559	2
Eastern Wheatbelt	9	601	0
Northern Wheatbelt	16	1057	0
Unspecified	2		0

5.1.4. Adult sheep samples by location

A good distribution of sheep origin by population in different zones was achieved, as the Great Southern region contains more than other areas.

Table 4. Regions of origin of adult sheep sampled

Month	No. Lines	Mean worm egg count	No. lines scouring
South West	7	384	0
Southern coastal	13	764	4
Great Southern	72	300	5
South Eastern	31	466	3
Midlands	29	497	5
Eastern Wheatbelt	50	302	7
Northern Wheatbelt	35	566	0
Unspecified	7		2

5.2. Nematode egg counts and scouring

5.2.1. Worm egg counts and proportions scouring (see also Figures 1& 2, p. 37)

Mean counts were very high in the lamb lines, and low to moderate in the adult sheep. The proportions of lamb lines in excess of 1000 and 2000 eggs per gram indicate that worm control practices were ineffective or absent on many properties. A number of lines contained *H. contortus*, but as the mean count was low the association with disease is likely to reflect scour worm activity. The mean difference between total (ie, including *H. contortus*) and scour worm lines was 375 eggs per gram, but this was due chiefly to a small number of high count lines. Hogget counts were also high, closer to the lamb than adult sheep pattern.

There was visual evidence of scouring in an overall 10% of lines, although many of these were dry dags rather than active scouring, and the relationship with worm egg counts may not be clear in these cases. It is of interest that the prevalence of scouring was identical in adults and lambs, despite the vastly different worm egg counts.

Table 5. Faecal worm egg counts in sheep sampled in lairage

	Lambs	Hoggets	Adults	Total
Mean worm egg count: Total*	1525	1159	486	825
Mean worm egg count: Scour worms**	1150	1013	364	625
% lines > 1000 epg	42.5%	40%	13.1%	22.9%
% lines > 2000 epg	22.1%	30%	6.1%	12%
% lines scouring	9.5%	30%#	9.3%	10%
% line with high <i>H.contortus</i>	7%	0	3.5%	4.3%
No. lines sampled	113	10	244	367

* Strongyle counts: all nematodes except *Nematodirus*

** Scour worm counts: strongyle genera excluding *Haemonchus*

Small number of lines, probably not representative

5.2.2. Worm egg counts in scouring and non-scouring sheep (Table 6, p. 13)

There was no significant difference between the mean WEC in scouring and non-scouring individual sheep in any age group, although there was a trend to a difference in hoggets (higher in scouring sheep) and adults (lower in scouring sheep). Both within and between scouring lines, there was no difference in the strongyle or scour worm FEC in the scouring and non-scouring individuals in any age category (scour worm results only shown).

5.2.3. Nematode genera (Table 7, p. 14)

The genera encountered were typical of the catchment area, with *Ostertagia* and *Trichostrongylus* dominant and accounting for over 80% of worm eggs. *H.contortus* occurred in relatively few lines (33% of lambs, 17% adults) and in small proportions where present (although indications of clinical haemonchosis were present in a few lines). As expected, the less abundant genera, *Chabertia*, *Oesophagostomum* and *Cooperia* accounted for less than 10% of worm eggs.

Table 6. Faecal egg counts (eggs per gram) in relation to scouring

	Lambs	Hoggets	Adults
Individual sheep (all worms)			
Scouring	1501	1616	437
Non-scouring	1238	1269	462
P value	0.963	0.122	0.065
Within lines (scour worms only*)			
Scouring	1767	2021	412
Non-scouring	1097	1061	365
P value	0.571	0.285	0.167
Between lines (scour worms only*)			
Scouring	1185	1527	391
Non-scouring	958	1686	356
P value	0.575	0.909	0.356

* Non-*H.contortus* strongyles

Table 7. Genus or species of worms shown by larval differentiations on each line (not calculated for hoggets due to small number of lines)

Genus	LAMBS		ADULT SHEEP	
	Mean % Non-scouring	Mean % Scouring		Mean % Scouring
<i>Haemonchus contortus</i>	13	17	12	9
<i>Ostertagia circumcincta</i>	46	33	32	24
<i>Trichostrongylus</i> spp	32	30	49	65
<i>Chabertia ovina</i> & <i>Oesophagostomum</i> spp	7	14	7	2
<i>Cooperia</i> spp	2	6	0	0

5.2.4. Seasonal pattern of worm egg counts, scouring and genera present: lambs

The trend to reducing worm egg count from spring to summer is expected given the rapid onset of dry conditions and the common use of drenches. No seasonal effect on scouring is evident.

Table 8. Lamb worm egg counts by month, evidence of scouring and worm identity

Month	Mean WEC	Mean scour WEC	N. lines scouring	% Ost.	% Trich.	% Haem.
September	2268	5038	1	28	47	25
October	1896	2265	4	51	31	11
November	1254	3175	5	48	32	7
January	933	253	2	36	34	25

5.2.5. Seasonal pattern of worm egg counts, scouring and genera present: adults

The increase in worm egg counts in summer may indicate that farmers did not drench sheep intended for sale, as most of these would have received a “summer drench” had they remained on the farm. In contrast to the lambs, a strong seasonal trend on scouring was observed.

Table 9. Adult sheep worm egg counts by month, evidence of scouring and worm identity

Month	Mean WEC	Mean scour WEC	N lines scouring	% Ost.	% Trich.	% Haem.
September	348	430	17	21	63	9
October	267	298	7	39	48	5
November	462	-	0	33	49	13
January	884	-	0	37	37	19

5.3. Protozoa

5.3.1. Detection of protozoa: microscopy

A total of 1647 samples were screened, pooled within lines in lots of 5 samples. Lambs were far more likely to be infected than adult sheep for both *Giardia* and *Cryptosporidium*.

For *Cryptosporidium*, lines of lambs were 3.7 times more likely to be positive for than adult lines (odds ratio 95% confidence interval (CI): 1.5 - 9.3), and 7.0 times more likely to be positive for *Giardia* than adult sheep (95% CI: 4.1 - 11.9).

Table 10. Number of isolates positive for *Giardia* and *Cryptosporidium*

	Lambs*	Adults*
<i>Cryptosporidium</i>	12/112 (10.7%)	8/225 (3.1%)
<i>Giardia</i>	54/112 (42.8%)	30/225 (13.3%)

* Lambs, < 12months; adults, > 12 months of age

Relationship with scouring/survey factors (Appendices 7, 8)

Although protozoa were more likely to be detected in lambs, lines of adult sheep positive for *Cryptosporidium* were 9.7 times more likely to be scouring than negative lines (odds ratio 95% CI: 2.3 - 41.6), and lines positive for *Giardia* were 3.1 times more likely to be scouring than lines in which *Giardia* was not detected (95% CI: 1.2 - 8.2).

There was no significant relationship between scouring and *Cryptosporidium* or *Giardia* in lambs (figures in Appendices 7 and 8).

Genotype analysis (PCR)

Sequence analysis of 36 *Cryptosporidium* positive samples identified 7 distinct species/genotypes: the cervid genotype, 16; the novel bovine B genotype, 8; the marsupial genotype, 5; the pig genotype II, 4; *C. andersoni*, 1; *C. hominis*, 1; unknown genotype, 1.

Sequence analysis of 30 *Giardia* isolates identified 4 distinct genotypes; the *G. duodenalis* Livestock genotype, 12; the *G. duodenalis* – assemblage A, 16; and 2 unidentified genotypes.

Table 11. PCR and genotyping results for protozoans

Parasite	Microscopy (% positive)	PCR (% positive)	Species/genotypes (N)
Giardia	8.7% (144/1647)	44% (65/147)	<i>G. duodenalis</i> Livestock genotype (12) <i>G. duodenalis</i> – assemblage A (16) Unknown genotypes (2)
<i>Cryptosporidium</i>	2.6% (43/1647)	26.25 (63/240)	<i>Cryptosporidium andersoni</i> (1) <i>Cryptosporidium hominis</i> (1) Cervid genotype (16) New Bovine B genotype (8) Pig genotype II (4) Marsupial genotype (5) Unknown genotype (1)

5.4. Survey results: lambs

(NB: See Appendix 1 for Survey questionnaire, Appendix 3 & 4 for data and statistics)

5.4.1. Survey

A total of 78 replies were received from 123 questionnaires mailed, a very good response rate (63.4%). However, not all replies were useable for all questions. Associations are reported only for nematodes, as there was no relationship between *Cryptosporidium* and *Giardia* in adults or lambs and any survey factors.

5.4.2. Month of lamb birth

There was no association between month of birth and the likelihood of scouring. However, the prevalence of scouring is more likely to be related to age at turn off and month of turn-off, than month of birth. There is no apparent relationship of month of age and WEC.

Table 12: Month of birth of lambs sampled (July, August: too few samples for analysis)

Month	No.	%	Not scouring	Scouring	Strongyles non scouring	Scour worms non scouring
March	7	9.1	7	0	801	612
April	15	19.4	13	2	2052	1123
May	31	40.3	26	5	978	772
June	20	26	17	3	1635	1082
p-value, scouring/not scouring			0.981		N/A	N/A

5.4.3. Age at slaughter

The great majority of prime lambs (i.e., born in the 12 months prior to slaughter) were 5 to 7 months of age. Although there may appear to be more scouring and higher worm egg counts in younger lambs, any trend is biased by the low numbers in older age groups, preventing objective comparison. However, there was no association of age with either the presence of scouring or the worm egg count.

Table 13. Age of lambs sampled

Age (months)	Percent of total	Mean WEC (scour worms)	N scouring/N total
3	3.1		
4	20.0	1633	1/6
5	35.4	885	3/27
6	13.8	1114	1/21
7	9.2	683	2/7
8	4.6	710	0/4
9	6.2	750	0/3
10	3.1	339	0/4
14	1.5	430	0/1
15	1.5	Hoggets	Hoggets
17	1.5	Hoggets	Hoggets

5.4.4. Feeding system

Worm egg counts were significantly lower in feedlot lambs for all strongyles 279 epg in feedlot lambs v. 950 epg in lambs on pasture ($p = 0.034$).

5.4.5. Weaned or nor weaned prior to consignment

There was no association of weaning status with scouring ($p = 0.738$), but an association with worm egg count was apparent ($p = 0.091$); 535 epg if weaned, against 1075 epg for scour worms.

5.4.6. Grazing in the lambing paddock

There was no association with scouring or with worm egg count category.

5.4.7. Pre-lamb drench to ewes

There was no significant difference in the strongyle WEC in the lambs born to ewes that received a pre-lambing drench (905epg) and the lambs born to ewes not drenched prior to lambing (1172 epg) ($P = 0.336$).

There was increased scouring in the lambs born to ewes which received a pre-lambing drench with these lambs 10.2 times more likely to be scouring than lambs born to ewes that did not receive a pre-lambing drench (odds ratio, 95% confidence interval 1.1 – 93.3).

5.4.8. Post lambing drench to ewes

There was no association with a post-lambing drench given to ewes and scouring or worm egg count category, although worm egg counts were lower in drenched flocks (984 and 755 epg) than non-drenched flocks (1509 and 1036 epg, strongyles and scour worm counts, respectively).

5.4.9. Lamb drenching

5.4.9.1. Effect on scouring and worm egg counts

Scouring

Drenching lambs within 2 months of slaughter had no apparent effect on the likelihood of scouring, although this analysis was of low sensitivity due to low numbers.

Worm egg counts

Drenching prior to consignment appeared to reduce lamb worm egg counts (lower counts in lambs drenched up to 2 months earlier: scour worms, 644 v. 1306 epg, $p < 0.001$).

Table 14: Lamb drenching in relation to age and time of consignment

Birth Month	N replies	Median month of slaughter	Mean days birth to slaughter	% lambs drenched	Mean days from drench to slaughter
March	7	October	239	41	83
April	15	October	239	29	59
May	31	October	177	74	75
June	20	November	161	65	90
Total	73		191	63	78

Of 5 lines finished in a feedlot, 3 had been drenched, 2 of them apparently prior to entry. None of the anthelmintics was administered within the withholding period for the particular product, though 2 were within 3 weeks of consignment.

5.4.9.2. Anthelmintics used

Table 15: Anthelmintics used to treat lambs in the months prior to sampling

Anthelmintic	Number where anthelmintic specified (%)
Benzimidazole	3 (7)
Levamisole	2 (4.5)
Combination benzimidazole-levamisole	2 (4.5)
Ivermectin	19 (43)
Abamectin	6 (13.5)
Moxidectin	12 (27)
Not specified	3

5.4.10. Shearing, crutching prior to consignment

Most lambs were not shorn prior to consignment (~ 70%). Most were either crutched (~ 40%), or dirty lambs were crutched (18%). Most lambs (~ 70%) were reported as not being dirty prior to consignment, but some 30% of lines contained some dirty lambs.

5.5. Survey results: adult sheep

(NB: See Appendix 1 for Survey questionnaire, Appendix 5 & 6 for data and statistics)

5.5.1. Replies to questionnaire

A total of 151 replies were received (66% return), although not all were useable for all questions. Associations are reported only for nematodes, as there was no relationship between *Cryptosporidium* and *Giardia* in adults or lambs and any survey factors.

5.5.2. Feeding system

There was no association with scouring or with worm egg count category.

5.5.3. Lamb in 2002

There was no association with scouring or with worm egg count category.

5.5.4. Drench in the 2 months prior to lambing

There was no effect of drenching: mean worm egg counts for all worms and scour worms were 618 and 256 epg for drenched and 324 and 274epg for non-drenched ewes. Scouring occurred 11 of 132 non-drenched lines compared with none of 8 drenched lines, suggesting an association, but the numbers are too low for analysis.

6. SURVEY OUTCOMES

Objectives 1 and 2, of assessing the parasite status of sheep sent to the Fletcher International abattoir, and relationships with scouring and production practices were achieved for both nematode and protozoan parasites. The results were surprising and of significance to the industry, and potentially have implications on a national basis. They form the basis for both the communication of better practices and further investigation.

Objective 4, regarding the public health significance, was also achieved in that the molecular techniques employed enabled the identification of protozoan genotypes, and hence inferences of their importance. The significance in practice would require research on meat products, and is a separate issue from the present.

Objectives 3 and 5, the prevalence and economic significance of scouring, were not investigated, and as the project proceeded it became obvious that this requires a separate study. The available staff resources were required for sampling, laboratory preparation and testing, and the mail survey, and more specialist skills are necessary. Investigations will require contact with several abattoirs, and the development of an economic structure. This work should also include estimates of the production loss due to parasite infections, and treatment costs. It is suggested that this is considered as an additional project, though with the information from the present study, it would not be a difficult undertaking for an appropriately skilled investigating team.

7. DISCUSSION

7.1. Sheep sampled

The sheep sampled appear to be a good representation of the present sheep meat industry in Western Australia, and the results are hence likely to be generally applicable. As expected, almost all adult sheep were ewes, as wethers are typically sent for overseas export. Although identifications of breeds could not be made accurately, sheep were listed as Merinos or crossbreds except where identification features were present. Almost all were apparently Merinos, but a far higher proportion of lambs were crossbreds. This is of interest as it would be expected that the management practices used for Merinos flocks would also be applied to the sheep destined for slaughter, although the survey results suggest that this was not the case.

Lambs were aged from 4 to 8 months of age, and all but 7 lines were consigned directly from pasture. This reflects the nature of the Western Australian sheep meat industry, which is largely a by-product of wool enterprises, with lambs from Merino ewes mated to British Breed rams, or full-blood Merinos. The strong seasonality is due to the preference to finish lambs on pasture rather than incur feedlot costs, and hence there is often an end-of-year glut. However, due to strong prime lamb prices in recent years, there is now a strong move towards more specialist lamb production, with more ewes mated to a terminal sire.

Sheep originated from throughout the state, with most from inland zones (100 km from the coast), which is most typical of the Western Australian sheep areas.

7.2. Inferences from the data

The demographic basis of the sample taken is considered a sound reflection of the Western Australian sheep meat industry, and findings are not likely to differ from the situation in sheep sent to other abattoirs in WA. Whether this is representative of sheep meat production practices in other states cannot be predicted, and results may vary according to the number of specialist prime lamb enterprises.

As a prevalence survey, the worm egg count data for is probably a good guide to the usual pattern, as lines were sampled at random, unless scouring lines were present. However, as there were relatively few of the latter (10%), the large sample base is likely to be representative. However, the figures are not a prevalence survey for scouring, as the proportions of non-scouring lines were not recorded.

7.3. Nematode results

7.3.1. Worm egg counts

The worm egg counts of lambs and hoggets were surprisingly high, and clearly indicate damagingly high worm burdens in many lines. Evidently, parasites are not recognised as a concern by many sheep owners, as neither effective treatment regimes or worm monitoring procedures were in place. This is especially concerning, as late spring is the time of year well recognised as a potential danger period for worm problems.

Interpretation may be complicated by the likelihood that some concentration of faeces occurred during transport when the sheep did not have access to feed. Where this occurred, mean counts would be over-estimated, requiring some weighting when applying the diagnostic values used for sheep on pasture. However, it is unlikely that counts would more than double, and in many cases would be inflated to a considerably lower degree. Placing this in context, a flock mean worm egg count exceeding 500 eggs per gram of the scour worm genera is usually considered to indicate some production loss, sufficiently significant in a proportion of the flock to warrant drenching.

Hence, the mean of 1525 eggs per gram (epg) in lambs, and counts exceeding 1000 epg in over 40% of lines, and 2000 epg in approximately 25%, is of concern. Most worms were of the "scour worm" genera, chiefly *Ostertagia* and *Trichostrongylus*, which are associated with production loss, and with scouring where burdens are very high. Allowing for the concentration factor when interpreting the worm egg counts, it is likely that in at least one-third of cases, a drench was indicated. (See Figure 2 for graphical illustration.)

As expected, worm egg counts in adult sheep lines were far lower, with the mean count of 486 epg unlikely to indicate significant parasitism, especially if the values are overestimated due to faecal concentration. However, the unpredictable nature of the development of worm burdens was indicated by the proportion (13%) of mature sheep lines with counts exceeding 1000 epg. A significant number of adult sheep flocks would also be expected to benefit from drenching some time before consignment. (Figure 3 shows adult sheep worm egg counts.)

Also surprising was the extreme counts, mostly associated with *H. contortus*. Counts exceeding 10,000 epg, and individual sheep, 20,000, occurred in 6 lines. On the basis of larval differentiations and egg counts, clinical haemonchosis was suspected in 8 lines, some of which were visibly anaemic or weak when sampled.

7.3.2. Potential production significance

Significantly compromised growth performance must be expected in sheep with parasite burdens of the size detected in this study, especially in prime lambs which are at their most susceptible to infection. Detailed pen studies indicate that nematodes significantly reduce growth rate in lambs, and that the development and expression of resistance and resilience to nematode infection is compromised through inadequate nutrition (Coop and Kyriazakis 1999, van Houtert and Sykes 1996, Steel 2003). As there is considerable potential for pasture nutrition to fall below levels necessary for both immune development and maximal growth rate, significant effects must occur frequently.

Pen study results are supported by evidence from field studies, in which reduced growth rates and parasitic disease were routine in Merino lamb flocks exposed to high worm challenge (Barger 1983). Reduced weight gains of over 10% are typically associated with poor parasite control, and mortalities due to worms are common (Besier *et al.* 1996). Effects on a similar scale would also be expected in lambs raised for slaughter, hence reducing carcass weights, increasing the time required to reach target weights, and also increasing the proportion of a flock which fails to reach turn-off weights. This is particularly likely in lambs maintained on pasture, where nematode infection occurs continually, and pasture nutritive value is often below the optimal.

In adult sheep, reduced production performance due to worm infections is also common (for example, Morris *et al.* 1977), which in sheep sent for slaughter may be reflected either in lower returns on sheep sold to a weight specification, or fewer sheep being accepted by buyers.

The evidence that poor worm control is routine, and the demonstrated effects on sheep performance, suggest that this is an unrecognised problem of major proportion. It is the more surprising given that the strategic summer drenching program is used routinely in weaner sheep kept for wool purposes, as similar burdens would surely be expected in sheep of the same age but destined for slaughter.

7.3.3. Scouring

Worm egg counts were considerably higher in scouring individuals in prime lamb lines, and although not statistically significant, helminthiasis remains the most likely cause. The small number of scouring lines reduces the statistical power, and hence the association with worm egg counts may not have been adequately tested. Further, in a number of lines classified as "scouring", the faecal soiling had dried, suggesting that the problem had resolved or the sheep had been treated shortly prior to consignment. It is hence likely that some lines with low counts counted "scouring" were in fact mis-classified, but would bias downward the mean count of that group.

There are few other causes, as other common infectious cause are rare in lambs on pasture in this age group (coccidiosis, Salmonellosis), or absent from Western Australia (Yersiniosis, Campylobacterial scours). Some of the protozoa recorded in this survey may have a role in gut disease in sheep under some circumstances, but are not likely to be the major explanation (see below). Non-infectious causes also appear unlikely explanations in this environment (Bath 2003), although whether transport stress can activate latent infections such as with *Salmonella* is not clear.

In adult sheep, the absence of an association of scouring with worm egg counts is not unexpected, as low counts are typical of sheep exhibiting a hypersensitivity to ingested nematode larvae (Larsen *et al.* 1995).

The association of scouring with *Giardia* and *Cryptosporidium* in adult sheep, but not lambs, is a new finding, and requires further study.

The scale of the scouring problem was not established in this study, and the survey does not provide prevalence data, as lines in which scouring occurred were sampled preferentially. However, on most sampling days, scouring was not apparent in any lines, only minority of the total lines sampled (a mean of 10%) contained scouring sheep, and the proportion of affected sheep within lines was always low. It hence appears that scouring is not highly prevalent, which is expected as abattoir operators actively discourage producers from consigning sheep with these signs. Nevertheless, abattoir operators consider it a significant issue where processing must be interrupted to trim affected carcasses, or badly scouring lines must be kept aside until the condition resolves. It will be worth quantifying the financial and operational significance to processors.

7.4. Association with management factors – nematodes

The mailed survey attracted an excellent response rate (nearly 70%), and this as well as comments by owners contacted indicated keen producer interest.

Several sheep management practices with implications for parasite infection were associated with worm egg counts. Trends were most clear regarding worm egg counts, as the number of cases of scouring were low and usually precluded clear associations with most factors.

7.4.1. Feeding system

Most lambs were finished from pasture (93%), mostly without supplementation. Of the 6 lines in the survey which originated from feedlots, most were fed for only 21 days or less. Lambs from pasture were more likely to have high worm burdens, compared to those from feedlots, presumably because 5 of the 6 lines were drenched (probably on entry to the lot).

7.4.2. Weaning status prior to consignment

This was considered a potentially significant worm-risk factor, as unweaned lambs are exposed to larval pick-up from pasture contamination by the ewes. Most lambs (63%) were not weaned, reflecting the typical age of turn-off. However, weaned lambs had worm egg counts of one-half the non weaned lambs, which relates to weaning drenches (22 of 26 weaned lines were drenched), as well as the higher pasture contamination levels of paddocks also occupied by ewes.

7.4.3. Whether grazed in lambing paddock

Similarly to the above factor, grazing in the paddock where the lambs were born is considered a potential risk due to worm larval pasture contamination by the ewes. Marginally more lambs (56%) remained in the lambing paddock until sending for slaughter. However, there was no effect on scouring or worm egg count, possibly because on most farms, at the time of year most lambs were turned off, almost all pastures would be contaminated with worm larvae. No effect of drenching was apparent as in both cases two-thirds of the lambs had been drenched.

7.4.4. Pre-lambing drench to ewes

There was a significant association of pre-lamb drenches to ewes with scouring in lambs, although there was a trend towards lower WEC in these lambs. This indicates that pre-lambing treatments may be of questionable value, especially where the lambing paddock is already contaminated with worm larvae, as a temporary suppression of worm egg counts in ewes probably has little effect. However, the general principle regarding the desirability of lambing on “clean” pastures is sound, and communication to producers on how this may be achieved would be worthwhile.

7.4.5. Post lambing drench to ewes

There was no significant association with worm egg counts or scouring, but worm egg counts were 50% higher (although not significantly) where no post-lamb treatment was given to ewes. Presumably, pastures would be contaminated by the time drenches were given, as this would usually be some weeks after lambing started.

7.4.6. Lamb drenching

Two-thirds of lamb flocks had received a drench, on an average of 77 days prior to turn off. This was, as expected, highly effective in reducing worm egg counts, with counts of both strongyle and scour worms approximately one-half of the non-drenched lambs. However, drenching did not prevent scouring, as there were many more flocks scouring if drenched (5/40) as if not (1/23). Furthermore, of the drenched flocks, worm egg counts were similar in the scouring (894 scour worm eggs per gram) and non-scouring flocks (603 epg). This apparently contradictory result suggests that drenching was too far away from turn off to affect scouring, although the difference in worm egg counts between drenched and non-drenched flocks does suggest that treatment would probably have enhanced production.

No association of the effect of drenching on scouring could be made as no scouring lines had been drenched within 2 months of consignment. Likewise, no realistic assessment of the effect of drenching on worm egg count could be made, as only 9 lines had been treated.

Of interest, drenches expected to be highly effective were used in almost all cases: 83% were a macrocyclic lactone product, and while ivermectin resistance is present in Western Australia (Palmer *et al.* 2000), even where this exists it is rarely severe.

7.4.7. Shearing, crutching prior to consignment

Over 20% of lamb lines had been shorn prior to consignment. Surprisingly, some 80% had been crutched, although only 30% were reported as having ever been "dirty" (scouring or daggy). No significant association with worm egg counts or scouring was evident.

7.5. Protozoa

7.5.1. Detection methods

In the present study, PCR detection was much more sensitive than microscopic detection of protozoans. *Giardia* was identified in 8.7% (144/1647) of sheep samples by microscopy and 45.5% (67/147) of sheep by PCR. *Cryptosporidium* was identified in 2.6% (43/1647) of sheep by microscopy and 26.25% (63/240) by PCR. Shedding of *Giardia* and *Cryptosporidium* can be sporadic and in low numbers, which can make microscopy difficult (Elliot *et al.* 1999).

7.5.2. *Cryptosporidium*

Sequence analysis of 36 *Cryptosporidium* positive samples identified 7 distinct species/genotypes: the cervid genotype, 16; the novel bovine B genotype, 8; the marsupial genotype, 5; the pig genotype II, 4; *C. andersoni*, 1; *C. hominis*, 1; unknown genotype, 1.

Cryptosporidium andersoni

This is the first report of *C. andersoni* in Australia and also the first report of this species in sheep as prior this, *C. andersoni* has only been reported in cattle, camel, marmots and a European wisnet (Lindsay *et al.* 2000; Ryan *et al.* 2003b). This species is not zoonotic but it is associated with long-term chronic infections and reduced weight gain. Therefore, its finding in Australian sheep is significant and warrants further investigation.

Cervid genotype

The cervid genotype, identified in 16 isolates, is genetically very distinct from all genotypes and species of *Cryptosporidium* but like the *C. parvum* "cattle" genotype, it has a wide host range, including humans and could possibly emerge as an important human pathogen with increasing contact between humans and wildlife (Ong *et al.* 2002; Ryan *et al.* 2003b; Xiao *et al.* 2002).

Cryptosporidium hominis

C. hominis (1 sample) is a newly described species, previously referred to as the *C. parvum* 'human' genotype/genotype 1 (Morgan-Ryan *et al.* 2002). *Cryptosporidium hominis* primarily infects humans although experimental infections have been produced in gnotobiotic pigs (Widmer *et al.* 2000) and a lamb

(Giles et al. 2001) and there has been one report of a natural infection in a Dugong (*Dugong dugon*) (Morgan et al. 2000) and one report of a natural infection in a lamb (Giles et al. 2004).

Pig genotype II

The pig genotype II, found in 4 isolates, was first identified in a pig-derived *Cryptosporidium* isolate from Switzerland in 1997 (Ryan *et al.* unpublished). It has subsequently been identified in pigs in Western Australia during a three-year survey (Ryan *et al.* 2003b) and in secondary sewage effluent (Ryan *et al.* 2003c) suggesting that it may be zoonotic. Further studies are required to confirm this.

Marsupial genotype

This genotype (found in 5 isolates) was first identified in a koala (*Phascolarctos cinereus*) from South Australia (Morgan *et al.* 1998) and has subsequently been identified in kangaroos from Western Australia and in Eastern Grey Kangaroos (*Macropus giganteus*) in New South Wales (Power, 2003). This genotype is not zoonotic and this is the first report of this genotype in a non-marsupial host.

Novel bovine B genotype

Detected in 8 isolates, this genotype was first identified in cattle in the USA in 2002 (Xiao *et al.* 2002). It is genetically very distinct and little is known of its prevalence, distribution or zoonotic potential. This is the first report of this genotype in sheep.

An unknown genotype was identified in one sample. This genotype is genetically very distinct and may represent a new species of *Cryptosporidium*. Further studies are required to confirm this.

7.5.2. Giardia

Sequence analysis of 30 *Giardia* isolates identified 4 distinct genotypes; the *G. duodenalis* Livestock genotype (12), the *G. duodenalis* – assemblage A (16) and 2 unidentified genotypes, one of which was 100% identical to a *Giardia* isolate recovered from water in Italy and the second genotype was 99% identical to a novel *Giardia* isolate from a goat. Genotype A is geographically the most widespread genotype and as it has been identified in both livestock and humans, it is thought to be zoonotic (Thompson *et al.* 2000). The livestock genotype is not considered to be zoonotic and the zoonotic status of the 2 unidentified genotypes remains unknown.

7.6. Significance of protozoa

Two species/genotypes of *Cryptosporidium* and one genotype of *Giardia* identified in sheep as part of this study have known zoonotic potential (*C. hominis*, the *Cryptosporidium* cervid genotype and the *Giardia* genotype A respectively). However the potential for zoonotic transmission of *Cryptosporidium* and *Giardia* in abattoirs is probably low. Human infection is more likely to happen in situations where sheep are slaughtered outside abattoirs or where poor hygiene is employed by sheep handlers.

Of concern, however, is the potential role that these parasites may play as pathogens in sheep as there was a significant association between lines of adult sheep that were positive for *Cryptosporidium* and *Giardia* and scouring. Previous studies have shown that the presence of these parasites in sheep may result in significant economic losses. For example, a study in Canada reported that *Giardia* infection in sheep was associated with a decrease in rate of weight gain and impairment in feed efficiency. In addition, time to reach slaughter weight was extended in infected lambs, and the carcass weight of *Giardia*-infected lambs was lower than that of control lambs (Olson *et al.* 1995). The authors concluded that “giardiasis in domestic ruminants is an economically important disease, thus necessitating control or elimination of the infection.” Further studies are required to determine (1) the prevalence of *Cryptosporidium* and *Giardia* in sheep in Western Australia (2) the extent of economic losses associated with these parasites.

8. CONCLUSIONS

8.1. Implications of worm egg counts

This study has produced strong evidence that worm egg counts, and hence presumably worm burdens, in prime lambs are frequently far higher than is compatible with efficient sheep production. More than 40% of lines had counts which would usually indicate that treatment is required, and overt or impending clinical disease was evident in many. As a result, reductions in carcass weights and increased periods necessary to attain target weights in the WA prime lamb industry almost certainly occur commonly due to significant worm burdens.

It appears that in general, prime lamb producers do not consider worm control to be an important consideration. This may reflect the generally rapid growth rates of prime lambs, but this may obscure a production limitation of major significance. The failure to institute management procedures for worm control is surprising, as routine "summer drenches" would have been given to wool-enterprise sheep on the same farms, and monitoring worm egg counts in lambs is now common practice on many farms. Where scouring occurred, it is also surprising that this well known indication of parasitism in lambs did not suggest a worm problem.

In adult sheep, also, worm egg counts in some lines were far higher than expected, with counts likely to indicate significant production loss in at least 13%, and counts at levels associated with parasitic disease in a smaller proportion. There is less incentive to ensure maximum carcass weights in sheep sent for mutton slaughter, but lighter lines are not attractive to buyers, and prices for the entire draft may be reduced. As for lambs, drenches are routinely used around the time of the study, and it may be that producers considered there was little point in treating cull animals. In some lines sampled, it is certain that worm burdens would have had substantial effects on the sheep weights.

Investigations are needed to determine whether similar nematode infections occur in the meat sheep industries in other regions, as there is the potential for a significant unappreciated impact on a national scale.

8.2. Occurrence and causes of scouring

Although scouring was not clearly related to worm burdens in prime lambs, this remains the most likely cause. The study design does not permit a clear examination of this relationship, as lambs in which treatment may have been given could not always be identified. However, most scouring lines had counts above values considered pathogenic, or appeared to have been recently treated.

In adult sheep, the complete lack of association of scouring with worm egg counts was expected, as in this age group, the most common cause of scouring in winter rainfall region is hypersensitivity to nematode larvae.

Research to more clearly associate scouring with causal and epidemiological factors is required.

8.3. Recommendations to producers

The strong association of drenching lambs, or ewes before and after lambing, indicates that a single treatment typically has a major effect. Although it is recommended that chemical use is minimised in sheep intended for slaughter in the short term, treatment some weeks before consignment is likely to have sufficient effect to reduce the adverse effect of worm burdens on production. The use of slow release anthelmintic capsules in ewes is common in some states, but poses a risk of drench resistance, and any control approach based chiefly on anthelmintic use prevents access to chemical-free markets.

A preferred alternative is to minimise pasture infectivity by either anthelmintic treatment of ewes, or pasture management for ewes and/or lambs. Monitoring worm egg counts is a simple procedure to ensure that worm burdens do not impeded productivity. The interest shown by producers through the high return rate of the mail survey provide encouragement for adoption of recommendations for improved worm control.

8.4. Protozoal infections

The high detection level of *Giardia* and *Cryptosporidium* is a new finding in Australia sheep at slaughter. Identification of the genotypes is a clear illustration of the power of molecular tools, as there are significant differences in the potential effect on host animals, and the zoonotic risk, between isolates.

The potential role of both genera in scouring in adult sheep, especially, was highlighted by the prevalence in survey samples and the association with scouring. The lack of association with survey factors was not unexpected, as there has been little previous indication of a major problem, but both sub-clinical effects and unrecognised disease outbreaks are likely in some circumstances. Whether these infections have an unrecognised but occasionally costly sheep production impact in Australia is not known at this stage.

The potential public health significance is also indicated, as some genotypes of both *Giardia* and *Cryptosporidium* are considered zoonotic, or possibly so. While no increased risk to consumers of meat products from commercial Australian abattoirs is suggested, risks may exist where animals are slaughtered for home consumption, in less hygienic circumstances.

The survey findings clearly indicate the need for investigations of the prevalence (on a national scale), clinical significance, potential zoonotic significance, and associations with sheep management routines, of protozoal infections.

9. RECOMMENDATIONS AND FUTURE RESEARCH

1. Sheep producers in Western Australia should be acquainted with the demonstrated potential for losses due to inadequate worm control, and recommendations for improved control provided. Given the interest shown in this field through the high response rate to the mail questionnaire, a significant impact from an extension campaign can be expected. However, a communication campaign should include a survey of industry attitudes and information gaps, to explain the failure of many producers to apply routine worm control practices, and hence indicate where extension messages should be targeted.
2. The application of these results in other Australia states should be investigated, as it is unlikely that only producers in Western Australia would be unaware of the need for vigilance regarding worm burdens in lambs.
3. The production significance of worm burdens in prime lambs requires examination, especially in relation to different nutritional regimens, to indicate the levels of worm control appropriate in different production systems.
4. Factors related to scouring require further research, including on-farm investigation, in terms of bot causes and control. The potential role of protozoal infections in scouring warrants investigation.
5. Worm control programs for different situations should be developed, especially programs based on no-chemical approaches. The potential for nutritional supplements to offset parasitic loss where continued larval intake is unavoidable should be pursued, as this may be more effective and sustainable than anthelmintic treatments.
6. The prevalence, and pathogenic significance and production effects of protozoal infections in sheep (and other livestock) have received comparatively little attention in Australia, and their association with different management systems and environments should be elucidated. The power of molecular techniques in distinguishing genotypes of varying significance has been demonstrated in this study.
7. The potential zoonotic role of different genotypes *Giardia* and *Cryptosporidium*, and the relative prevalence of different genotypes, requires investigation. Although there appears no indications of risks associated with Australian abattoir products, meat slaughtered on farms and for home consumption may pose risks.

10. COMMUNICATIONS

A communication campaign to inform the industry of the need for better parasite control will be planned in conjunction with MLA, and may depend on further investigations of the extent and significance of the findings.

All press releases and technical articles have been (and will be) subject to approval by MLA.

Release/in press to July 2004

- "Feedback" (October 2002)
- "Prograzier" (December 2003)
- Australian Sheep Veterinary Society conference (May 2004)
- Australian Sheep Veterinary Society Proceedings 2004 (Vol. 14, pp 146-150)
- Sheep Updates seminar (WA Dept. Agric consultant forum, July 28, 2004)
- Protozoology conference (Barcelona, Spain, July 2004)

Planned

- Prime Time for lamb seminars
- Wormwise newsletter (consultant/veterinarian publication, WA Dept Agric.)
- Australian Veterinary Journal article
- Farming Ahead article (invited)
- Specialist parasitology journals

11. ACKNOWLEDGEMENTS

The management and staff of Fletcher International, WA, provided willing cooperation for the sampling operations. Garnet O'Connell and Wendy Nekele were efficient sample collectors, and Aileen Elliot, Jill Lyon, Heide Gutelich, Ramin Nikravan, Jonno Prosser and Esther Spence provided expert laboratory assistance. The assistance of Ian Robertson with statistical advice is also gratefully acknowledged.

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Appendix 1

Questionnaire introduction



Department of Agriculture
Government of **Western Australia**

Division of Veterinary and Biomedical Studies
Murdoch University
MURDOCH WA 6150

<date>

<NAME
ADDRESS
ADDRESS/ etc >

Dear <Mr/Mrs >

SHEEP PARASITE STUDY 2002/03

We are carrying out a study on to investigate parasite burdens in sheep at slaughter, with the support of Meat and Livestock Australia.

Samples for parasites were taken from lines of sheep consigned to **Fletcher's International** at Narrikup during 2002/03. Some of the sheep sampled came from a line from your property.

Some brief background information on the sheep will allow us to interpret our findings and develop recommendations for producers and processors.

Where dates are asked for, a month or approximate time relative to lambing is sufficient. There is some space at the end of the questionnaire for any comments that you may like to make.

If you have any questions about this study, please call any of the researchers named below.

A reply paid envelope has been included for the return of the questionnaire. Alternatively, questionnaires can be faxed to (08) 9360 2235.

Results are strictly confidential and will be used for research purposes only. No information will be given to anyone not involved with this research project.

Yours sincerely

Dr Brown Besier (Veterinary Parasitologist, Dept. Agriculture, Albany; 9892 8470)
Dr Una Ryan (Parasitology research scientist, Murdoch University; 9360 2482)
Caroline Bath (Post-graduate student, Murdoch University; 9360 2235)

Questionnaire: Lambs

Name under which the sheep were consigned:

Date of consignment:

1. Were the lambs in the consignment carry over lambs (2001 drop – white tag) or 2002 drop (orange tag)?
 - 2001 drop (white tag)
 - 2002 drop (orange tag)

2. In which month did lambing commence? _____

3. How were the lambs fed prior to consignment?
 - Straight off pasture
 - Pasture with supplementary feeding
 - FeedlotHow long were the lambs in the feedlot? _____

4. Were the lambs grazing/feeding with their mothers in the 4 weeks prior to consignment?
 - No
 - Yes

5. Were the lambs grazing in the paddock(s) in which they lambed prior to consignment (or up to a month before this)?
 - No
 - Yes

6. Were the lambs drenched at any time before sending for slaughter?
 - No
 - YesApproximate date and product used: _____

7. Did the ewes (mothers of the lambs) receive a drench in the month prior to lambing?
 - No
 - YesApproximate date and product used: _____

8. Did the ewes (mothers of the lambs) receive a drench after lambing?
 - No
 - YesApproximate date and product used: _____

9. Were the lambs shorn prior to consignment?
 - No
 - YesApproximate date of shearing: _____

10. Were the lambs crutched prior to consignment?
 - No
 - YesApproximate date of crutching: _____

11. Were the lambs “dirty” prior to consignment?

- No
- Yes

Approximate percentage (%) dirty: _____

Comments:

I WOULD LIKE TO RECEIVE A COPY OF THE FINAL REPORT

(tick if "yes")

Questionnaire: Mutton

Name under which the sheep were consigned:

Date of consignment:

1. Were the sheep in the consignment:

- Ewes
- Wethers
- Mixed

2. What was the age or tag colour (or colours) of the sheep in the consignment?

3. How were the sheep fed prior to consignment?

- Straight off pasture
- Pasture with supplementary feeding
- Feedlot

How long were the sheep in the feedlot? _____

4. If ewes were included in the consignment, did they have a lamb in 2002?

- No
- Yes

5. Did the sheep receive a drench in the 2 months before consignment?

- No
- Yes

Approximate date and product used of the most recent drench:

6. When were the sheep last shorn? _____

7. Did the sheep need crutching in the 2 weeks prior to consignment?

- No
- Yes

Approximate percentage (%) dirty: _____

Comments:

I WOULD YOU LIKE TO RECEIVE A COPY OF THE FINAL REPORT

(tick if "yes")

Appendix 2

Worm Egg Counts, Lambs

Figure 1. Worm egg counts, all lines of lambs

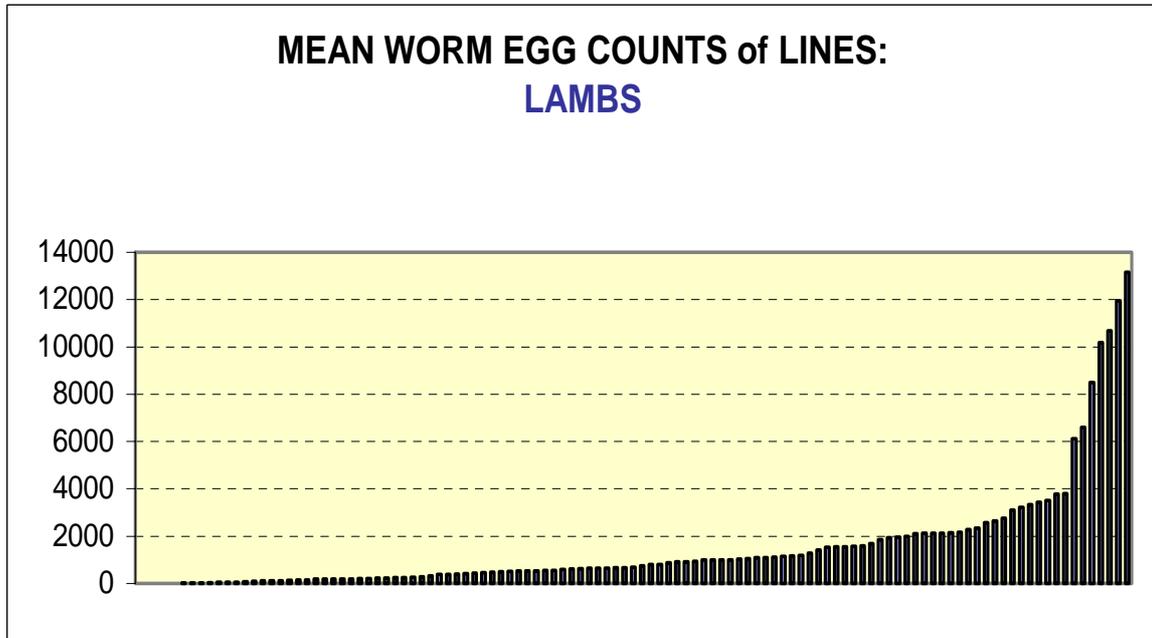
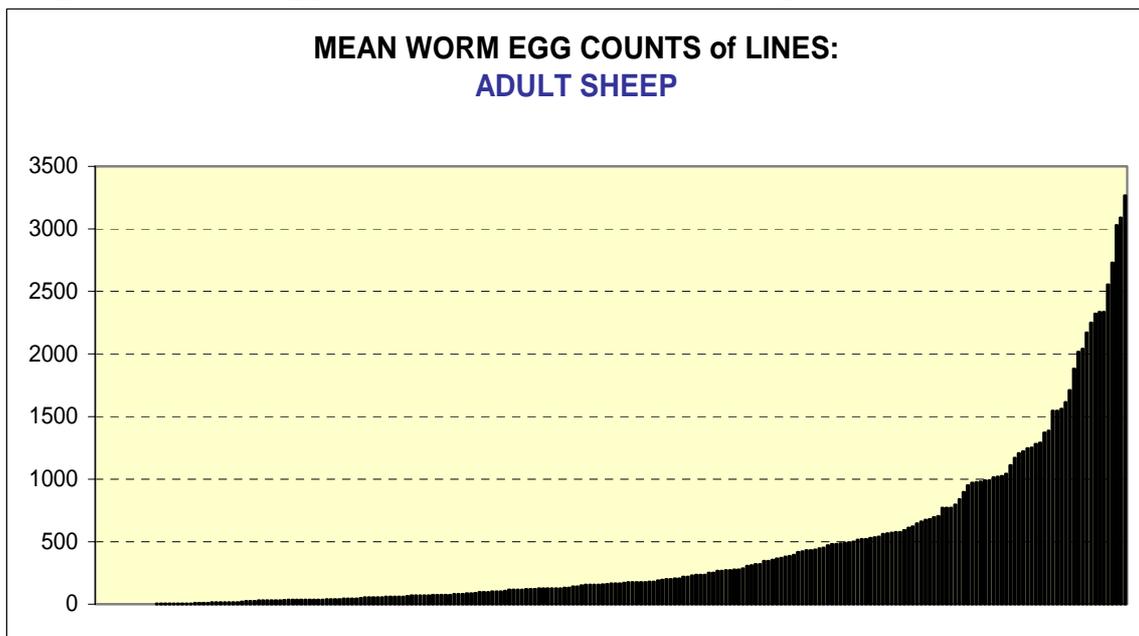


Figure 2. Worm egg counts, all lines of adult sheep



Appendix 3: Association of survey factors with lamb worm egg counts

Table A3.1: Associations: Lambs, all strongyles

	Strongyle* worm egg counts				Mean counts
	<1000epg	>1000epg	<2000epg	>2000epg	
Pasture	39	25	53	11	1310
Feedlot	5	1	6	0	279
P value	0.401		0.580		
Pasture only	33	24	47	10	1310
Supplemented	11	2	12	1	1994
P value	0.111		0.676		
Not drenched	11	13	19	5	1894
Drenched	33	12	40	5	997
P value	0.024		0.275		
Pre-lambing drench	17	9	22	4	1517
No pre-lambing drench	27	17	37	7	996
P value	0.737		1.000		
Post-lamb drench for ewe	16	9	22	3	1509
No post-lamb drench for ewe	27	17	36	8	984
P value	0.828		0.734		
Weaned	17	5	20	2	1181
Grazing with ewe	27	21	39	9	1390
P value	0.091		0.483		
Grazing lambing paddock	25	18	37	6	1574
Not lambing paddock	18	8	21	5	1165
P value	0.357		0.562		
Scouring	5	5	9	1	2447
Not scouring	60	35	77	18	1405
P value	0.415		0.685		

*Strongyles: all nematodes except *Nematodirus*

Table A3.2: Associations: Lambs, scour worms only

	Scour worms*				Mean WEC
	Scour*worms <1000 epg	Scour worms >1000 epg	Scour worms <2000epg	Scour worms >2000ep	
Pasture	145	7	53	11	1018
Feedlot	1	0	6	0	279
P value	1.000		1.000		
Pasture only	35	22	47	10	1018
Supplemented	11	2	12	1	490
P value	0.194		0.676		
Not drenched	12	12	19	5	1306
Drenched	34	11	40	5	644
P value	0.032		0.275		<0.001
Pre-lambing drench	17	9	37	7	984
No pre-lambing drench	29	15	22	4	713
P value	0.964		1.000		
Post-lamb drench for ewe	18	7	36	8	1036
No post-lamb drench for ewe	27	17	22	3	755
P value	0.438		0.734		
Weaned	17	5	20	2	535
Grazing with ewe	29	19	39	9	1075
P value	0.168		0.483		
Grazing lambing paddock	26	17	21	5	845
Not grazing lambing paddock	19	7	37	6	938
P value	0.286		0.736		
Scouring	6	4	9	1	1220
Not scouring	63	32	78	17	1105
P value	0.733		0.203		

*Scour worms: strongyles except *H.contortus*

Appendix 4: Association of survey factors with scouring in lambs

Table A4: Associations, scouring in lambs

	Not Scouring	Scouring
Pasture	158	6
Feedlot	6	0
P value	1.000	
Pasture only	51	6
Supplemented	3	0
P value	0.585	
Not drenched	23	1
Drenched	40	5
P value	0.657	
Pre-lambing drench	43	1
No pre-lambing drench	21	5
P value	0.024	
Post-lamb drench for ewe	22	3
No post-lamb drench for ewe	41	3
P value	0.660	
Weaned	21	1
Grazing with ewe	43	5
P value	0.657	
Grazing lambing paddock	25	1
Not grazing lambing paddock	38	5
P value	0.398	
Strongyle <1000	60	5
Strongyle >1000	35	5
P value	0.415	
Strongyle <2000	77	9
Strongyle >2000	18	1
P value	0.685	
Scour worm <1000	63	6
Scour worm >1000	32	4
P value	0.733	
Scour worm <2000	78	9
Scour worm >2000	17	1
P value	1.000	

Appendix 5: Association of survey factors with adult sheep worm egg counts

Table A5.1: Associations: Adult sheep, all worms

	Strongyles*				Mean counts
	<1000epg	>1000epg	<2000epg	>2000epg	
Pasture	142	10	147	5	407
Feedlot	1	0	1	0	342
P value	1.000		1.000		
Pasture only	131	9	135	5	407
Supplemented	12	1	13	0	N/A
P value	0.600		1.000		
Not drenched	134	9	139	4	324
Drenched	7	1	7	1	581
P value	0.430		0.241		
Lamb in 2002	122	9	18	1	379
No lamb in 2002	18	1	127	4	331
P value	1.000		0.497		
Scouring	19	3	20	2	497
Not scouring	187	26	202	11	381
P value	0.741		0.348		

*Strongyles: all nematodes except *Nematodirus*

Table A5.2: Associations: Adult sheep, scour worms only

	Scour worms*				Mean
	<1000epg	>1000epg	<2000epg	>2000epg	
Pasture	41	23	150	2	392
Feedlot	5	1	1	0	267
P value	0.656		0.580		
Pasture only	134	6	138	2	329
Supplemented	12	1	13	0	N/A
P value	0.470		0.837		274
Not drenched	137	6	141	2	251
Drenched	7	1	8	0	
P value	0.322		1.000		
Lamb in 02	19	0	129	2	251
No lamb in 02	124	7	19	0	271
P value	0.596		1.000		
Scouring	19	3	20	2	364
Not scouring	192	20	205	7	332
P value	0.462		1.000		

*Scour worms: strongyles except *H.contortus*

Appendix 6: Association of survey factors with scouring in adult sheep

Table A6: Associations with scouring: adult sheep

	Not Scouring	Scouring
Pasture	141	11
Feedlot	1	0
P value	1.000	
Pasture only	129	11
Supplemented	13	0
P value	0.600	
Not drenched	132	11
Drenched	8	0
P value	1.000	
No lamb in 02	18	1
Lamb in 02	123	8
P value	1.000	
Strongyle <1000	187	19
Strongyle >1000	26	3
P value	0.741	
Strongyle <2000	202	202
Strongyle >2000	11	11
P value	0.348	
Scour worm <1000	192	19
Scour worm >1000	20	3
P value	0.462	
Scour worm <2000	205	20
Scour worm >2000	7	2
P value	0.203	

Appendix 7: Association of survey factors with *Cryptosporidium*

Table A7: Associations with *Cryptosporidium*

	Cryptosporidium positive	Cryptosporidium negative
Adults	8	247
Lambs	12	100
P value	0.005	
Non-scouring adults	4	224
Scouring adults	4	23
P value	0.005	
Non-scouring lambs	9	90
Scouring lambs	3	10
P value	0.145	
Lamb: Paddock	64	6
Lamb: Feedlot	4	2
P value	0.118	
Adult: paddock	2	159
Adult: feedlot	0	1
P value	1.0	
Lamb: weaned	3	23
Lamb: not weaned	5	45
P value	1.000	
Lamb: not in lambing paddock	3	28
Lamb: in lambing paddock	5	39
P value	1.000	
Adult: Lamb in 02	1	22
Adult: No lamb in 02	0	133
P value	0.147	

Appendix 8: Association of survey factors with *Giardia*

Table A8: Associations with *Giardia*

	Giardia positive	Giardia negative
Adults	30	225
Lambs	54	58
P value	<0.0001	
Non-scouring adults	23	205
Scouring adults	7	20
P value	0.025	
Non-scouring lambs	47	52
Scouring lambs	7	6
P value	0.445	
Lamb: Paddock	36	34
Lamb: Feedlot	5	1
P value	0.209	
Adult: paddock	20	141
Adult: feedlot	0	1
P value	1.000	
Lamb: weaned	11	15
Lamb: not weaned	24	26
P value	0.809	
Lamb: not in lambing paddock	13	18
Lamb: in lambing paddock	22	22
P value	0.639	
Adult: Lamb in 02	4	19
Adult: No lamb in 02	14	119
P value	0.309	