DETERMINING THE IMPACT OF PROTOZOAN AND STRONGYLID PARASITES ON MEAT LAMB PRODUCTIVITY

UTILISING MOLECULAR DIAGNOSTIC METHODS FOR THE DETECTION OF INTERNAL PARASITES IN LAMBS

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ABSTRACT

Internal parasites (strongylid gastrointestinal helminths) have been reported to decrease lamb productivity in extensive grazing sheep enterprises. Increased interest into intestinal, protozoan parasites; *Cryptosporidium* and *Giardia*, has arisen due to their potential public health risks. Little research has examined their prevalence and impact on productivity in extensively managed livestock. Despite molecular diagnostic techniques having the capability to facilitate rapid identification, improve control and enhance prevention strategies for disease pathogens, little investigation has been conducted to compare molecular tests with traditional diagnostic methods.

Longitudinal studies observed that 47–81% of lambs sampled, tested positive for *Cryptosporidium* or *Giardia* at least once in their lives over five sampling occasions. *Cryptosporidium xiaoi* and *G. duodenalis* assemblage E were the most common species/genotypes isolated from Pingelly (Farm A) and Arthur River (Farm B). Zoonotic species/genotypes were also isolated but in low numbers. *Cryptosporidium xiaoi* was isolated on two occasions from dam water on Arthur River, while *C. ubiquitum* and *G. duodenalis* assemblage E were detected in dam water from Frankland. A novel, possibly new genotype (sheep genotype I) was identified in six *Cryptosporidium* isolates from Arthur River. *Cryptosporidium parvum* and *C. ubiquitum* were the most common species detected in Boyup Brook and Kojonup flocks.

Statistical analyses revealed lambs positive for *Cryptosporidium* on at least one sampling occasion had lighter HCWs and lower dressing percentages when compared to lambs never positive for *Cryptosporidium* for Farms A and B, respectively. On Farm B,

lambs positive for *Giardia* on at least one occasion had lighter HCWs and lower dressing percentages when compared to lambs never positive for *Giardia*. *Cryptosporidium*-positive lambs at the second sampling were 3.84–4.72 times more likely to have non-pelleted faeces (faecal consistency score [FCS] ≥ 3), when compared to *Cryptosporidium*-negative lambs for Farms A and B. Lambs on Boyup Brook and Kojonup farms that were positive for *Cryptosporidium*, *Giardia* or both, were 2.4–14.0 times more likely to have non-pelleted faeces. Furthermore, a higher number of internal parasites detected per lamb was associated with lower body condition score (BCS) and higher FCS on the Boyup Brook and Kojonup farms. *Cryptosporidium*-positive lambs were 3.36–2.96 times more likely to have moderate to severe breech fleece faecal soiling scores (3 – 5), when compared to *Cryptosporidium*-negative lambs at the second sampling for Farms A and B. Live weight, growth rate and BCS were inconsistently associated with protozoa detection across different samplings and farms.

A further study compared the performances of two lamb flocks exposed to different natural strongylid larval challenges. A new innovative, molecular approach was developed to recover strongylid larvae from pasture, which had a strong, negative correlation (r^2=0.91–0.95) with pasture larval counts used to detect and quantify strongylid larvae species on pasture. Flock L (exposed to a low larval challenge) had greater dressing percentages greater than Flock S (exposed to a higher larvae challenge). Within flock analyses of the Frankland flocks found lambs positive for *Giardia* at least once had lighter HCWs and lower dressing percentages, when compared to lambs never positive for *Giardia*.

A written questionnaire which surveyed 139 (41.4%) meat lamb enterprise owners/managers in southern Western Australia, found evidence of diarrhoea was reported on 64.8% of farms. A binary logistic regression analysis revealed that the source of livestock drinking water was associated with the incidence of diarrhoea. Lamb flocks that sourced water from a dam, were 117 times more likely to have active or recent evidence of diarrhoea. Overall, 10.1% and 14.4% of respondents were aware of *Cryptosporidium* and *Giardia*, respectively.

Comparison between a molecular diagnostic technique (identifying strongylid species by screening genomic DNA extracted directly from faeces) and the traditional McMaster WEC method, found high levels of agreement (kappa statistic ≥0.93) between the test results for detecting patent strongylid infections in two separate epidemiological studies. The findings that some lambs tested negative for strongylid infections while grazing pastures known to be infested with larvae, together with the strong correlations between WEC and the number of strongylid species detected per lamb, both suggest that strongylid eggs are the likely main source of strongylid DNA.

The findings of this thesis suggest that molecular identification of internal parasites is potentially negatively associated with phenotypic performance traits of lambs. Protozoa-positive lambs had reduced production performances (lighter carcase weights and reduced dressing percentage), when compared to protozoa-negative lambs. For such molecular techniques as that were employed in this research to be introduced into routine veterinary diagnostics, they need to: (1) quantify the magnitude of infections, (2) provide cost-benefits to sheep producers, (3) display consistent associations/correlations with phenotypic performance traits of livestock and (4) be cost-beneficial for diagnostic laboratories to

conduct (sales volume and equipment costs). The future development of multiplex, real-time, quantitative PCR (qPCR) assays capable of detecting and quantifying multiple pathogen infections (parasites and bacteria) in a single assay, would facilitate the uptake of such tests for both veterinary and human diagnostics

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DECLARATION

I declare this thesis is my own account of my research and contains as its main content, work which has not been previously submitted for any degree and is not currently being submitted for any other degree or qualification. I declare that I have conducted the research described except where otherwise acknowledged.

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Signature

Joshua Paul Alexander Sweeny

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SYMBOLS

~:	approximately
β:	beta
^{o}C:	degrees Celsius
=:	equals
>:	greater than
≥:	greater than or equal to
κ:	kappa statistic
<:	less than
≤:	less than or equal to
µ:	micro
ï:	naive

ABBREVIATIONS

ABARE:	Australian Bureau of Agricultural and Resource Economics
ad libitum:	freely available (Latin)
ANOVA:	Analysis of variance
AQIS:	Australian Quarantine and Inspective Services
AUD:	Australian dollars
BZ:	benzimidazole
C. jejuni:	*Campylobacter jejuni*
C. ovina:	*Chabertia ovina*
CI:	confidence interval
CMI:	cell mediated immunity
Cp. pecorum:	*Chlamydophila pecorum*
C_q:	cycle number at which the fluorescence threshold was exceeded
DM:	dry matter
DM%:	dry matter percentage
DMI:	dry matter intake
DNA:	deoxyribonucleic acid

dNTP:	Deoxyribonucleotide triphosphate
DSE:	dry sheep equivalent
E. coli:	*Escherichia coli*
ELISA:	enzyme-linked immunosorbent assay
epg:	eggs per gram
et al.	and others (Latin *et alii*)
FCS:	faecal consistency score
FDM%:	faecal dry matter percentage
FECRT:	faecal (worm) egg count reduction test
FOO:	feed on offer
g:	gram
g:	unit
H. contortus:	*Haemonchus contortus*
HCW:	hot carcase weight
hr:	hour(s)
kg:	kilogram
km:	kilometres
L:	litres

L_1:	first stage larvae
L_2:	second stage larvae
$L_{3:}$	third stage larvae
$L_{4:}$	fourth stage larvae
$L_{5:}$	fifth stage adult larvae
LV:	levamisole
mRNA:	mitochondrial ribonucleic acid
mg:	milligram
$MgCl_2$:	Magnesium Chloride
min:	minute(s)
mL:	millilitre
ML:	marcocyclic lactone
MLA:	Meat and Livestock Australia
mm:	millimetres
mM:	milli molar
n/a:	not applicable
n/s:	not significant
OP:	organophosphate

OJD:	Ovine Johne's disease
pg:	picogram
pH:	negative log of hydrogen ion concentration
PETA:	People for the Ethical Treatment of Animals
PCR:	polymerase chain reaction
PPRI:	peri-parturient relaxation of immunity
qPCR:	quantitative real-time PCR
rDNA:	ribosomal deoxyribonucleic acid
r^2:	linear regression correlation coefficient
RNA:	Ribonucleic acid
rRNA:	ribosomal ribonucleic acid
s:	second(s)
S.E.M.:	standard error of the mean
S.E.D.	standard error of the difference
swt:	shipped weight
spp.:	species
T. circumcincta:	*Teladorsagia circumcincta*
U:	units

UK:	United Kingdom
µM:	micromolar
USA:	United States of America
WA:	Western Australia
WEC:	faecal worm egg count

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