

NZSP 47

NEW ZEALAND SOCIETY FOR PARASITOLOGY

ANNUAL MEETING NO. 47

23-24 OCTOBER 2019

THE DUNEDIN CENTRE

1 HARROP STREET

DUNEDIN



CONFERENCE PROGRAMME

The Society would like to thank the following
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PROGRAMME

Wednesday 23 October

	08:30	Registration	
	09:00	Welcome	
Chair			
1	09:05	Ian Scott	Hookworms in New Zealand Dogs
2	09:25	Seer Ikurior	Evaluating on-animal sensors to assess gastrointestinal nematode effects on sheep behaviour
3	09:45	Ian Scott	Comparison of the egg reappearance times for ivermectin and moxidectin in horses
4	10:05	David Heath	Large-scale field application of eg95 vaccine contributes to blocking the transmission of hydatidosis from dog to sheep and goat
	10:35	Morning tea - Sponsored by PGG Wrightsons	
5	11:00	Matt Playford	Drench resistance in Livestock a. Update on the Australian status for cattle and sheep including recent trials b. Measures to manage and prevent drench resistance c. Update on diagnosing drench resistance
6	11:45	Kathryn McRae	25 years of WormFEC
7	12:05	Dovile Murauskaite	Restriction enzyme reduced representational sequencing (RE-RRS) of pooled samples to investigate genetic diversity in Irish and New Zealand <i>Teladorsagia circumcincta</i>
	12:30	Lunch	Sponsored by Elanco
8	13:30	Matt Playford	New active ingredients and formulations a. Introduction of new actives into a worm control program b. Management of long acting formulations c. Wormboss guidelines – take home messages
9	14:15	Richard Sides	Pragmatism in parasite management: what can we expect from controlled release capsules?
10	14:45	Tony Rhodes	Drenching ewes with ML's at lambing - accounting for the unintended consequences
	15:05	Afternoon tea	Sponsored by Alleva

Chair

11	15:30	Paul Brett	The seasonal pattern of the primary strike flies of sheep (<i>Lucilia cuprina</i> , <i>Lucilia sericata</i> and <i>Calliphora stygia</i>) in New Zealand together with a preliminary study of baits to be used fly traps
12	15:50	Paul Hughes	Ongoing investigations into Trichostrongyloid drench resistance in sheep in the Taihape district.
	16:10	NZSP AGM	Sponsored by Techion
	18:30	Pre-dinner drinks	In White Room above Vault 21 Sponsored by Elanco
	19:15	Dinner	Vault 21 Sponsored by Alleva

Thursday 24 October

13	09:00	Rakesh Sehgal	Genetic Characterization of DARC (Duffy Antigen /Receptor for chemokines) gene & DBP (Duffy Binding Protein) in complicated and uncomplicated <i>Plasmodium vivax</i> infected patients.
14	09:20	Sarah Coker	Morphological description of coccidia in Haast tokoeka (<i>Apteryx australis</i> "Haast").
15	09:40	Jerusha Bennett	Parasites of little blue penguins
	10:00	Morning tea	Sponsored by Elanco
16	10:30	Matt Playford	Current parasitology topics a. Theileriosis in cattle and other tick-borne diseases b. Lice control in sheep and cattle
17	11:15	Felix Guerrero	The <i>de novo</i> assembled and annotation genome of the New Zealand cattle tick, <i>Haemaphysalis longicornis</i> Neumann, 1901
18	11:35	Scott Ferguson	Methagens and animal only antibiotic research

12:00 **Lunch** **Sponsored by Alleva**

19	13:00	Marin Milotic	Parasites Lost: A Meta-analysis of Parasite Infections in Captive vs. Wild Hosts
20	13:20	Antoine Filion	Validating a non-invasive method for detecting malaria in wild bird populations.
21	13:40	Brandon Ruehle	Risky business: influence of eye-flukes on use of “risky” microhabitats and conspicuousness of a fish host
22	14:00	Bronwen Presswell	Completing the cycle of trematode ‘supermodels’.
23	14:20	Eunji Park	Uncovering hidden diversity: Microsporidians from New Zealand freshwater amphipods
24	14:40	Jeff Doherty	Contrasting effects of host specificity and host mobility on the reproductive strategies of parasites

Presentation Abstracts

1 Hookworms in New Zealand Dogs

Ian Scott, Barb Adlington, Anne Tunnicliffe

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Two species of hookworm have been identified in dogs in New Zealand, *Ancylostoma caninum* and *Uncinaria stenocephala*, with the latter generally assumed to be the most common in the general dog population. In the early 70s, in a post mortem survey of 51 dogs from the Palmerston North area, 31% were infected with *U. stenocephala*, and no *A. caninum* were recovered, and similar findings were found in a later survey of 55 dogs from different areas of NZ (7 North Island sites, 4 South Island).

Nevertheless, *A. caninum* has been reported in this country on several occasions, with the first report in a greyhound puppy in 1975. In the next few years there were several additional reports of ancylostomosis, all from the North Island. An unpublished 1975 survey conducted by the Fielding and Districts Veterinary Club examined the faeces of 202 dogs. Based on egg size, *U. stenocephala* was found in 36%, and *A. caninum* in 3.5%. Of the 7, *A. caninum* infected dogs, 5 were greyhounds. The most recent finding of *A. caninum* was in 2018 in another greyhound, one that collapsed after racing.

Based on more recent surveys, hookworm is arguably still the most prevalent nematode found in dogs, especially dogs held in council pounds and other shelters, and also in working farm dogs, at least in the North Island, however no recent surveys have distinguished which hookworm species was present.

For our study we used egg size to identify which hookworm spp. was present. 343 faecal samples were collected from dogs held in City Council Pounds (Palmerston North, Wanganui, Christchurch, and the Henderson and Silverdale suburbs of Auckland) and SPCA shelters (Invercargill, New Plymouth, Nelson, Palmerston North). In addition, 113 samples were obtained from owned pet dogs and 76 samples from racing greyhounds, all from the Manawatu area. A centrifugal floatation test was used initially to detect hookworm eggs and if present, the length and width of typically 50 hookworm eggs per sample were measured using a calibrated eyepiece micrometer.

In total, 76 hookworm positive dogs were identified (16 greyhounds and 60 other dogs), with the prevalence of infection being 21%, 16.6% and 2.7% in greyhounds, pound/shelter dogs and owned pet dogs respectively. Based on egg size, all the hookworm positive greyhounds were infected with *A. caninum* (mean egg length + SD, $61.9\mu + 1.5$), whereas all other dogs appeared to be carrying *U. stenocephala* ($81.0\mu + 2.6$). In the general dog population, hookworm infections slightly outnumbered those with the next most common nematode, *Toxocara canis* (60 vs. 56 dogs (out of 456)), but as might be expected, *T. canis* was more common in young puppies < 6months old (26.3% vs. 21.2%). In dogs over one year of age, hookworm was however about twice as common (7.7% vs. 3.6%). Hookworm infection was much more prevalent in the pound/shelter dogs than in the owned pet dogs (16.6% vs. 2.7%).

These findings have important implications for the control of hookworms in dogs in New Zealand since not all licensed canine anthelmintics have a label claim for efficacy against *U. stenocephala*. The results should also be of concern to the racing greyhound industry since *A. caninum* is considered a voracious blood-feeder and can therefore impact severely on racing performance.

2... Evaluating on-animal sensors to assess gastrointestinal nematode effects on sheep behaviour

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The ability to objectively measure behavioural changes has clear application for assessing health and welfare. Behavioural changes, such as reduced activity, may indicate poor wellbeing or disease. Gastrointestinal nematodes (GIN) can alter the grazing behaviour of ruminants marked by a voluntary reduction in feed intake. Tri-axial accelerometers afford a sensitive platform for measuring this effect. In this study we validated the ActiGraph wGT3X-BT[®] tri-axial accelerometer for its ability to identify periods of grazing, standing, lying and walking for sheep. Three experiments were conducted involving six ewe-lambs fitted with an accelerometer mounted on a neck collar. Acceleration data was collected at 30Hz. Lambs were in a grazing paddock (Experiment One), a holding pen (Experiment Two) or walked through a lane way (Experiment Three). All six lambs were filmed during these experiments and a behaviour profile was created from videos by annotating activity of each animal at five seconds intervals. Accelerometer data were aggregated to five seconds intervals and merged with the annotated behaviour data. By using random forests for classifying behavioural patterns of lambs on the created dataset of annotated and accelerometry data, an out-of-bag "cross-validation" model returned an overall model accuracy of 89.5% (overall estimate of error rate of 10.5%). Higher levels of accuracy in distinguishing between activity were achieved when combining lying and standing behaviours. Thus, the final model accurately predicted grazing at 94%, standing combined with lying at 88% and walking at 78%. A further "sensitivity analysis" of prediction was performed by removing the six ewe-lambs one after another for training the model and then testing for accuracy of prediction for each animal. This resulted in overall model accuracies of 89%, 87%, 88%, 88%, 92% and 87% for each respective round of sensitivity analysis. These results demonstrate the ability of the ActiGraph wGT3X-BT[®] AM to distinguish different activities in ewe-lambs. Applying this algorithm, the movement and activity patterns of twenty-two lambs naturally infected with gastrointestinal nematodes were monitored from weaning to approximately 11 months of age using the ActiGraph wGT3X-BT[®] attached to GPS neck collars. These lambs were allocated to two treatment groups on a six-weekly drenching cycle, drenched at the same frequency but staggered by half a drenching cycle from each other. Each lamb was weighed and faecal sampled on a weekly basis. Parasite burdens measured by faecal egg counts were moderate (mean = 300 eggs/g). The activity "walking" was significantly different from week to week the longer lambs were from a drench ($p=0.012$). The activities "grazing" ($p=0.754$) and being "stationary" (standing and lying; $p=0.225$) were non-significantly different from week to week. Overall, these preliminary results indicate that the activity "walking" responds to anthelmintic treatment, whilst the activities grazing and being stationary did not show anything under these experimental conditions.

3... Comparison of the egg reappearance times for ivermectin and moxidectin in horses

Ian Scott, Erica Gee, Chris Rogers, Bill Pomroy, Mike Reilly, Barb Adlington, Kylie Legg

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Shortened egg reappearance periods (ERP) following macrocyclic lactone treatments of horses are seen as indicative of developing rather than overt anthelmintic resistance. The egg laying adult parasites are still being killed, but later larval stages are being left behind, which subsequently mature and commence laying eggs. Of all the ML anthelmintics, moxidectin was considered to have a much longer ERP originally, in the order of at least 15 weeks (double that for ivermectin), but much shorter ERP have been observed; as little as 5 weeks in one instance on a stud in the Waikato, with similar reports overseas. Massey University maintains a herd of horses for teaching purposes. Management of the horses for parasite control for a long time involved the off-label use of an injectable moxidectin preparation given orally. This was eventually found to be inefficacious in 2014, likely due to sub-optimal pharmacokinetics associated with the use of an injectable product given by the wrong route, and there was no evidence of overt ML resistance at that time. Nevertheless, given the history of the likely sustained 'underdosing' of the animals on this property with a persistent anthelmintic, it seemed timely to assess the current efficacy and ERP for moxidectin in the teaching herd and to compare them to those for ivermectin.

On day 0, 23 horses were weighed, faecal sampled and treated with moxidectin (n=11) or ivermectin (12), and the horses were then faecal sampled at weekly intervals for 7 weeks. Egg counts were performed using a Modified McMaster method using 3g of faeces (rather than 2) and counting two standard, two chambered slides per animal rather than one. Thus each egg seen represented a count of 17 egg rather than the more typical 50. The ERP was judged to have been reached when the average egg count of a group reached or exceeded 10% of the pre-treatment average.

For all 23 horses no eggs were observed for the first 4 weeks after treatment. For the ivermectin treated animals, the egg counts were at 6.5, 13.0 and 31.9% of the pre-treatment counts in weeks 5, 6 and 7 respectively, thus the ERP for ivermectin could be determined as 6 weeks. For those treated with moxidectin, the same figures were 1.1, 9.8 and 16.3%.

Although technically the ERP for moxidectin could be considered to be 1 week longer than ivermectin, there was no significant difference between the egg counts of the two-groups over this period. This may indicate that the reported greater efficacy of moxidectin against L4 stages of the major parasites of horses (the cyathostomines) has largely been lost.

After many years of use of the ML class in horses, it is somewhat remarkable that there is still full (100%) efficacy against the adults. If efficacy versus L4 worms has declined, then whether the greater efficacy of moxidectin against L3, particularly those that are inhibited, is still intact remains unknown, but this could have important implications for the recommendations for the use of moxidectin to reduce the burdens of inhibited worms as a means of preventing the syndrome of larval cyathostominosis.

4. Large-scale field application of EG95 vaccine contributes to blocking the transmission of hydatidosis from dog to sheep and goat

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Cystic Echinococcosis (CE) also known as Hydatidosis caused by the larvae of *Echinococcus granulosus* is endemic and an important zoonosis in the pastoral and semi-pastoral area of Western China. Since 2010, the central government has allocated special funds to local governments for carrying out control measures using praziquantel in dogs in the key endemic areas, which has significantly reduced the transmission of the disease to intermediate hosts, but the epidemic situation remains serious. From 2011 to 2015, pilot field trials of the recombinant hydatid subunit vaccine (EG95) against sheep and goat were conducted in Xinjiang, Sichuan, Inner Mongolia and other provinces, and the results were very satisfactory. Since 2016, the central government has included animal hydatidosis in the list of compulsory immunization diseases, and launched comprehensive compulsory vaccination of sheep and goats with EG95 vaccine in seven provinces of Xinjiang, Sichuan, Qinghai, Gansu, Tibet, Ningxia and Inner Mongolia. Newborn lambs received two vaccination beginning at 4-month old and with a 28 days interval, then followed one booster injection every year. To date, 40.91 million vaccine doses, 47.76 million, 38.92 million and 40 million doses of EG95 vaccine were used in 2016, 2017, 2018 and 2019 respectively. The number of vaccinated animals was between 61.20 % and 94.90 %, and the positive rate of anti-EG95 antibodies after the third vaccination was between 60.09 % and 89.20 %. Slaughter examination and necropsy of sheep and goats in the seven provinces revealed that the prevalence has been decreased from 5.34%-44.72% prior compulsory immunization (2014/2015) to 1.23%-20.16% post compulsory immunization (2017/2018). In dogs, the positive rate for *Echinococcus granulosus* using arecoline purgation decreased from 3.12 % - 29.63 % to 1.04 % - 13.80 % in the seven provinces while the positive rate using copro-ELISA decreased from 12.88 % to 2.04 % in Ningxia. After nearly four years of comprehensive immunization with the EG95 vaccine in these difficult and remote environments, the prevalence and incidence in sheep/goats and dogs has decreased significantly, indicating that vaccination of sheep and goats with EG95 vaccine has effectively blocked the dog-sheep/goat transmission chain, which provides an economical, efficient and practical tool for controlling hydatidosis.

5. Drench resistance in Livestock

- a. Update on the Australian status for cattle and sheep including recent trials**
- b. Measures to manage and prevent drench resistance**
- c. Update on diagnosing drench resistance**

Matt Playford

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6. 25 years of WormFEC

Kathryn M McRae, Sheryl-Anne N Newman, Gordon J Greer, Neville C Amyes and John C McEwan

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Infection with gastrointestinal nematodes, resulting in clinical disease and loss of productivity, is one of the major constraints of ruminant production worldwide. Current control strategies in ruminants rely heavily on the use of anthelmintic treatment; the increasing prevalence of anthelmintic resistance in sheep nematodes throughout the world suggests that total reliance on chemotherapy is unsustainable. Selective breeding for host resistance is an alternative, sustainable option for nematode control. The most commonly used indicator of host resistance is faecal egg count (FEC), which is moderately heritable ($h^2 \sim 0.2-0.3$), with a wide variability between individuals. Rapid genetic progress has been demonstrated in selective breeding programmes using both experimental and commercial flocks. In New Zealand, WormFEC commenced in late 1994, providing sheep breeders with a service enabling selection for host resistance to gastrointestinal nematodes establishment. While nematode species composition may vary markedly between flocks, it has been shown that WormFEC breeding values are largely independent of flock, sex, or year in which they are measured, indicating a limited genotype-by-environment interaction. FEC is often thought to be strongly genetically associated with dagginess, however, a recent study utilising a whole industry data set consisting of more than 90,000 DAG measurements, and more than 100,000 FEC measurements showed low to zero genetic correlations between the two traits in New Zealand sheep. Using the New Zealand Maternal Worth + WormFEC selection index, animals can be selected that are both high producing and resistant to establishment of internal nematode parasites. In recent years genomic breeding values for the trait have been available via Beef + Lamb Genetics in most maternal and composite breeds.

7. Restriction enzyme reduced representational sequencing (RE-RRS) of pooled samples to investigate genetic diversity in Irish and New Zealand *Teladorsagia circumcincta*

D Murauskaite, O Keane, D Leathwick, R Poulin, T van Stijn, T Waghorn, K Hamilton, A McCulloch, R Brauning, M Hess, K Dodds, J McEwan

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Teladorsagia circumcincta is an important parasite of sheep in temperate climates, because it leads to millions of dollars in economic losses due to reduced production of meat, wool and milk and the high costs of treatment. Since 1960, five classes of anthelmintic compounds have been brought to the market. Unfortunately, the intensive use of these drugs has led to the development of anthelmintic resistant *T. circumcincta*. Simultaneous resistance to multiple drugs has now been documented in Ireland, New Zealand and other countries. The genetics of resistance is still poorly understood in nematodes. There are a

number of possible molecular mechanisms by which resistance to a new drug could rapidly evolve in multi-drug resistant *T. circumcincta*, in particular: there is a common (shared) resistance “path” - locus/loci. Another possibility is that multi-drug resistant worms are more genetically adaptable as they are more genetically diverse due to a higher mutation, or recombination rate especially as it relates to gene duplication.

In order to investigate genetic mechanisms of resistance we first need to know the background genetic diversity of these nematodes. For that purpose, we sampled farms in New Zealand and Ireland pre and post anthelmintic treatment. Faecal samples of sheep from 45 farms across New Zealand and 14 across Ireland were collected to see how polymorphic *T. circumcincta* is across different latitudes and geographical locations. Using a low-temperature method, pure *T. circumcincta* larvae cultures have been cultivated from eggs obtained in faeces. DNA was extracted, pooled and genotyped by RE-RRS. DNA reads were filtered bioinformatically against multiple references to select *T. circumcincta* sequence. The relationship (similarity) among the samples were compared by MDS plots to determine that there is significant population structure among *T. circumcincta* from different farms in New Zealand and Ireland. Weather data from both countries was utilized and correlation with population structure will be presented in the talk.

8. New active ingredients and formulations

- a. Introduction of new actives into a worm control program**
- b. Management of long acting formulations**
- c. Wormboss guidelines – take home messages**

Matt Playford

Dawbuts Pty Ltd, 9 Mitchell St, Cambden NSW 2570, Australia

9. Pragmatism in parasite management: what can we expect from controlled release capsules?

Richard Sides

Boehringer-Ingelheim, 31b SargoodDrive, Wanaka

Objectives:

- 1. To describe some field-trial data from around NZ, and a case study in the application of clinical reasoning to parasite management in a commercial sheep farm with evidence of triple-drench resistance.**

2. To briefly review current evidence (trial-work and underlying scientific understanding) for when and how long-acting parasite control can result in sheep-farm productivity gains; and when and how this can be a sustainable practice.

The use of controlled release anthelmintic capsules (CRCs) in ewes around lambing is a current topic of interest, especially with regard to anthelmintic-resistant parasite populations.

There are several trials and reports in the literature demonstrating the potential productivity outcomes of CRC use. It appears clear that productivity gains will depend on the level of parasitic challenge to parasite-susceptible sheep (heavily pregnant ewes especially of younger age) by anthelmintic-susceptible parasites, as well as other factors such as nutrition and sheep genetics. There is consistent scientific work over many years on the over-arching factors that lead to selection for drug resistance in biological populations. There is clarity in the literature around the conditions in which anthelmintics (particularly combinations) may either delay resistance or provide some clinical efficacy in the presence of resistant parasites.

Despite this body of scientific understanding there is still plenty of debate around the expected performance and consequences from CRC usage. This positioning, from both the proponents and the critics of CRCs, appears to reduce the consideration of the role of other anthelmintic tools (and in fact non-anthelmintic factors), in both resistance development and in productivity.

There are a number of veterinary advisors and farmers around New Zealand interested in gathering farm-level data, with outcome measures based on the current monitoring tools of faecal egg counts and larval cultures. There are some interesting data sets appearing from the 2019 lambing season, showing what expectations farmers might have for the use of CRCs versus not using them, and also the potential consequences of using primer drenching (concurrent short-acting oral drench at time of administration of CRC). The overall outcomes are consistent with the scientific understanding mentioned above, and can be demonstrated by a description of a commercial sheep farm case through the 2018-2019 season. This case and the other data clearly demonstrate the critical need to balance short term productivity gains with longer term sustainability requirements when using CRCs.

10. Drenching ewes with ML's at lambing - accounting for the unintended consequences

AP Rhodes

PGG Wrightson Consulting, PO Box 42, Dannevirke 4942, New Zealand

A series of on-farm trials were undertaken to test the hypothesis that pre-lambing administration of long-acting macrocyclic lactone anthelmintics to ewes would impact the survival of lambs via the transfer of actives across the placenta and in colostrum and milk.

On each of ten farms, 133 mixed-age twin-bearing ewes were allocated to each of three treatment mobs; an untreated control, a combination-controlled release capsule or a long-acting moxidectin injection. Treatment groups were grazed together and were observed daily throughout the lambing period. In year one lambs were tagged and allocated to a birth mother. In year two, DNA samples were collected from ewes at treatment and from lambs at weaning. Lambs were subsequently allocated to dam and treatment group on this basis. Dead lambs were recovered and identified daily, and frozen for subsequent autopsy.

For each treatment group, the number of lambs born dead or dying post birth was compared and expressed as a difference in lamb survival relative to the control group. Lamb survival figures varied considerably between farms (range 67-97%) and between treatments relative to the untreated (-3.9 to +9.3% for the CRC and -6.9 to +6.1% for moxidectin injection). While there was no consistent positive or negative effect of treatment on lamb survival, considerable unexplained between farm variation was evident.

An evaluation of the impact of response to anthelmintic treatment and of variation in lamb survival data indicates that, as in previous studies, variation in lamb survival is likely to be a more significant driver of financial return than anthelmintic treatment. Farmers would be better advised to invest in increasing lamb survival than in treating ewes with anthelmintic.

11. The seasonal pattern of the primary strike flies of sheep (*Lucilia cuprina*, *Lucilia sericata* and *Calliphora stygia*) in New Zealand together with a preliminary study of baits to be used fly traps

P.T.J.Brett¹, K.E. Lawrence¹, P.R. Kenyon², W.E. Pomroy¹

1. School of Veterinary Science, Massey University, Palmerston North, New Zealand.
2. School of Agriculture and Environment, Massey University, Palmerston North, New Zealand

Flystrike continues to be an important problem for sheep farmers throughout New Zealand. The main species of fly involved are *Lucilia cuprina*, *Lucilia sericata* and *Calliphora stygia* which have a seasonal distribution that varies across the country. The principle aim of Study 1 was to establish the seasonality of these species by monitoring their occurrence on eight sheep farms spread around New Zealand. Flies were collected on five sheep farms in the North Island (Waikato, King Country, Hawkes Bay, Wairarapa, Manawatu) and three sheep

farms in the South Island (Canterbury). The flies were captured using three commercial fly traps ("LuciTrap") installed at various locations on each farm. A "sticky" flytrap was attached to the lid of the LuciTrap to enhance the capture of flies. These traps were emptied on a weekly basis. A weather station (Davis instruments Vantage Pro 2 6322C) together with a 10 cm soil temperature probe (HOBO MX2201) were installed on each farm. Trapping was commenced in early September 2018 and continued until three successive weeks were observed with no target species recovered. The aim of Study 2 was to investigate more practical options for baits in flytraps that could be used on-farm in New Zealand. A comparison was made of the commercially available LuciTraps using chemical lures, a modified Western Australian trap design using either the same chemical lures as in the LuciTrap, squid or sheep liver +10% Na₂S. These were set up on a Latin Square design on a sheep property in the Manawatu over a six week period from late January until March 2019.

Results for Study 1 obtained to date indicate variation between locations for all three species. In the North Island, *L. cuprina* first appeared in the second week of October in Waikato and King Country but not until the first or second week of November for Hawkes Bay, Wairarapa and Manawatu. In the South Island, the first appearance of *L. cuprina* varied from the second week in October until the first week in November. Numbers peaked in mid-January to mid-February 2019. The first appearance of *L. sericata* was later than for *L. cuprina*. Further results will be presented. A further study will be conducted on the same farms in 2019/2020 to examine the variation in seasonality across each of these sites. The results of both of these studies will form the basis of the prediction model. Results for Study 2 indicate that the most successful trap was the Western Australia trap using sheep liver with Na₂S.

12. Ongoing investigations into Trichostrongyloid drench resistance in sheep in the Taihape district.

P L Hughes, A W R Oswald, H R Bowsher

Taihape Veterinary Services, 2 Kotare Street, Taihape 4720

An update from previous reports. In 2004 a report detailed the resistance of *Teladorsagia circumcincta* to moxidectin, in 2006 a report discussed management factors from a survey of 82 farms associated with the development of resistance to Ivermectin and in 2018 a report of triple drench resistance in *Trichostrongylus colubriformis*.

The sudden appearance of *Trichostrongylus* spp resistance in the district is a serious development as it is by far the most pathogenic parasite. This resistance was not detected 3 years prior on the property or in annual monitoring of other properties and, as well, a follow-up slaughter study in 2007 failed to detect any *Trichostrongylus* spp resistant to Ivermectin.

This 2019 update details the current state of triple drench resistance and situation regarding the effectiveness of Monepantel/Abamectin (Zolvix Plus) and Derquantel/Abamectin (Startect). It also suggests an approach to mitigate, at least theoretically, the development of further resistance to these newer actives.

The drench effectiveness tests reported here were part of routine surveillance, none were initiated because of concern about clinical disease due to drench resistance and all cases had larval cultures undertaken.

Using our previously described criteria for faecal egg count reduction tests (FECRTs) 22 farms were tested (2017-2019) using triple active drenches with 12 returning resistant results (55% prevalence). All of the resistance cases involved *Trichostrongylus* spp with some also having *Teladorsagia* spp present. Interestingly 6 of the 10 negative properties used long acting drenches regularly in their ewes with 9 out of the 12 positive also doing so. Drench checks where follow-up faecal egg counts (FEC) are carried out 10-14 days post drenching and the pre-treatment counts are unknown have also been used. Of 31 farms that carried out drench checks using triple actives 12 had detectable strongyle eggs (@100 epg) and a further 8 cases had larvae detected that returned a zero egg count.

Trichostrongylus spp dominated the resistant profile in 17 cases and *Teladorsagia* spp in 3 cases.

FECRT's and drench checks were performed in 2018 and 2019 for Zolvix plus and Startect. Both drenches have to date had very little use in the district.

Of the 8 FECRT's for Startect, one returned a 54.7% reduction for *Trichostrongylus* spp, one only just exceeded the 95% threshold and a further 6 had larvae cultured. The resistant profile was evenly split between *Trichostrongylus* spp and *Teladorsagia* spp. The one case of a drench check for Startect returned a zero FEC but *Trichostrongylus* spp larvae were cultured.

Of the 8 FECRT's for Zolvix Plus, one returned a 62% reduction for *Teladorsagia* spp and one other had an egg count detected and a further 4 had larvae cultured. Zolvix Plus was tested in 8 drench checks with one returning a positive egg count and 3 further cases returning a positive larval culture. *Trichostrongylus* spp tended to dominate the resistant profile over *Teladorsagia* spp.

Acknowledging the small number of farms tested these results are very concerning for both of the newer actives with Startect performing appreciably worse. As a consequence of these findings we are recommending that these two drenches are used in combination with another drench administered at the same time. Zolvix Plus with Ivermatrix (Minidose), and Startect with Trimox. To date 3 FECRT's carried out on properties with larvae cultured after a Zolvix Plus drench have all returned clear results after the Zolvix Plus/Ivermatrix double drenches. Unfortunately this is not the case for the Startect/Trimox double drench with 2 out of 5 cases returning positive larval cultures (both *Trichostrongylus* spp and *Teladorsagia* spp identified).

We have developed a double drench gun to allow the separate administration of two drenches with a single pass. This will be illustrated.

In the future more careful monitoring of all drench effectiveness will be required. Property specific programmes based on resistance profiles will be required to avert clinical parasitism.

13. Genetic Characterization of DARC (Duffy Antigen /Receptor for chemokines) gene & DBP (Duffy Binding Protein) in complicated and uncomplicated *Plasmodium vivax* infected patients.

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BACKGROUND:

Human malaria parasite *Plasmodium vivax* infects red blood cells through a key pathway that requires interaction between Duffy Binding Protein II (DBPII) and its receptor on reticulocytes, the Duffy antigen/receptor for chemokines (DARC). A high proportion of *P. vivax*-exposed individuals fail to develop antibodies that inhibit DBPII-DARC interaction, and genetic factors that modulate this humoral immune response are poorly characterized. This study was aimed to determine the extent of genetic polymorphisms and positive natural selection at domain II of the *PvDBP* gene and DARC gene among *P. vivax* isolates.

METHODS:

A total of 112 blood samples (40 from malaria patients and 72 from healthy controls) were collected after informed consent at the Outpatient Department, Postgraduate Institute of Medical Education and Research, Advanced Pediatrics Centre, PGIMER, Chandigarh as well as from Government Medical College and Hospital, Sector-32 Chandigarh during period 2017-2018. The confirmation of *P. vivax* infection was by microscopy and Nested PCR targeting 18S RNA region. Conventional PCR targeting DARC gene was done in positive samples and PCR product were used in sequencing for polymorphism and region II flanking *pvdbpII* gene was also PCR amplified and sequenced. Genetic diversity of *pvdbpII* were done using Clustal W, Laser gene software and MEGA ver 5.0 programs.

RESULTS:

All *P. vivax* positive patients as well as control samples were found to be Duffy positive with the presence of T at 33rd nucleotide position in the promoter region. The exon 2 region of *P. vivax* positive patient and control samples had either G/A or both at 125th nucleotide position. The presence of only G or A at 125th position suggested samples as homozygote (FyA/FyA and FyB/FyB) and the presence of both the peaks (G and A) at 125th position considered them heterozygotes. About half of the individuals were heterozygotes (FyA/FyB) in both complicated (11/21) and uncomplicated (10/19) group of *P. vivax* patients and also in control group (38/72). The homozygote FyA/FyA was found to be the second highest in all three groups of individuals with the frequency of (8/21), (8/19) and (25/72) in complicated, uncomplicated and control group respectively.

Using Sal I as the reference strain, all the isolates showed the monomorphic mutation in DBP gene. The highest frequency of polymorphism was found in five variant amino acids positions (residues D384G, R390H, L424I, W437R, I503K) as reported earlier by Gosi et al., 2008.

14. Morphological description of coccidia in Haast tokoeka (*Apteryx australis* “Haast”).

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Coccidia (*Eimeria* spp.) causes severe disease and mortality in kiwi (*Apteryx* spp.). The purpose of this research was to morphologically describe coccidia in Haast tokoeka (*Apteryx australis* “Haast”). Faecal samples from kiwi from Haast (n=2), Rona Island (n=3), and Frans Joseph (n=1) were collected in 2017/2018 and incubated at room temperature in 2.5% potassium dichromate. Sporulated oocysts were rinsed, isolated in MgSO₄ (SG 1.28) in a cavity slide under a coverslip, imaged under oil immersion, and measured using ImageJ. We describe four novel morphotypes of *Eimeria* that share similarities to eimeriid species described in brown kiwi (*Apteryx mantelli*, Morgan et al. in Parasitol Res 116(5): 1433-1441). Morphotype “A” (14.74 × 13.42µm, n=102) was circular to elliptic with one polar granule and a striated wall, similar to *Eimeria kiwii*. Morphotype “B” (14.27 × 13.41µm, n=107) was identical to Morphotype “A” but with a smooth wall. Morphotype “C” (19.09 × 12.65µm, n=46) was ovate to elliptic with no visible micropyle, usually 1-2 polar granules, and a smooth wall, similar to *Eimeria mantellii*. Lastly, Morphotype “D” (21.81 × 13.17µm, n=107) was ovate to elliptic and, like *Eimeria apteryxii*, had a micropyle present, usually 1-2 polar granules, and a smooth wall. While similar to those previously described, we are considering these morphotypes to be novel species, as they were recovered from geographically separated host species. However, the parallels in characteristics between brown kiwi and Haast tokoeka coccidia morphotypes indicate that molecular characterisations are required to confirm *Eimeria* species distinctions.

15. Parasites of little blue penguins

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Our knowledge of the helminths of New Zealand birds is limited, even in the case of penguins which have large cultural, economic and ecological significance within New Zealand. Penguins are important components of marine systems, and tracking their helminth communities through time can be indicative of ecosystem health. Information on helminth communities can also provide insights into the health, feeding habits, behaviour

and evolution of their hosts. We report unpublished results from our lab that constitute the first quantitative study on the gastrointestinal helminth assemblage of little blue penguins from Otago, New Zealand, using recently dead birds from Dunedin Wildlife Hospital, and older specimens from the Otago Museum. We used integrative taxonomy to resolve the identities of each helminth species infecting little blue penguins which consisted of two tapeworms (*Tetrabothrius lutzi* and *Tetrabothrius pukui* n. sp.), three nematodes (*Contraecaecum eudyptulae*, *Capillaria* sp. and *Stegophorus macronectes*), two acanthocephalans (*Andracantha sigma* and *Bolbosoma balaenae*) and one trematode (*Galactosomum otepotiense*). The most prevalent were *T. lutzi* and *C. eudyptulae*. These results will set a baseline for comparison in future studies on penguin population, prey availability and ecosystem change, including global climate change.

16. Current parasitology topics

- a. Theileriosis in cattle and other tick-borne diseases
- b. Lice control in sheep and cattle

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17. The *de novo* assembled and annotation genome of the New Zealand cattle tick, *Haemaphysalis longicornis* Neumann, 1901

Felix D. Guerrero, Joseph Guhlin, Kristene R. Gedye, Kevin E. Lawrence, Peter K. Dearden, Thomas W. R. Harrop, Allen C. G. Heath, Patrick J. Biggs, William E. Pomroy, Stanley E. Bellgard, Daniel M. Tompkins

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The longhorned tick, *Haemaphysalis longicornis*, feeds upon a wide range of mammalian hosts, including cattle, deer, sheep, goats, humans, and horses. This tick can transmit a number of tick-borne diseases, and was the cause of a recent serious outbreak of theileriosis in New Zealand. A New Zealand-USA consortium was established to sequence, assemble, and annotate the genome of this tick, using ticks obtained from New Zealand's North Island. In New Zealand, the tick is considered exclusively parthenogenetic and this trait was deemed useful for genome assembly. Very high molecular weight genomic DNA was sequenced on the long-read Pac Bio Sequel platform, utilizing 28 SMRT cells that produced 21.3 million reads. These reads were assembled with Canu on a reserved supercomputer node with access to 12 TB of RAM, running continuously for over 24 days. The final assembly dataset consisted of 34,211 contigs with an average contig length of 215,205 bp. The tick's genome size was estimated from the assembly to be ~ 7 Gbp, conflicting with flow cytometric analysis that has estimated this tick's genome size to be

~4.5 Gbp. The quality of the annotated genome was assessed by BUSCO analysis, an approach that provides quantitative measures for the quality of an assembled genome. Over 95% of the BUSCO gene set was found in the assembled genome. Only 48 of the 1066 BUSCO genes were missing and only 9 were present in a fragmented condition. The raw sequencing reads and the assembled contigs/scaffolds are archived at the National Center for Biotechnology Information.

18. Methagens and animal only antibiotic research

Scott Ferguson

University of Otago

19. Parasites Lost: A Meta-analysis of Parasite Infections in Captive vs. Wild Hosts

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In addition to their significant contribution to biodiversity, parasites are important drivers of ecosystem functions and play a key role in the maintenance of ecosystem health. However, they may be quietly going extinct, as they are under threat from host extinction and host conservation. The aim of our study was to examine how captivity affects parasites and to determine which parasite life-history traits (modes of transmission) are the strongest predictors of their decline. We compared the odds of infection by parasites in host populations from captivity and the wild and found that captive populations had 1.80 times lower odds of infection compared to wild host populations. In addition, the modes of transmission that predicted lower odds of infection in captivity were vector transmission, complex life cycles, and environmental transmission, having 3.42, 2.55 and 1.43 lower odds, respectively. As a group, helminths were under the greatest threat, with 2.12 lower odds of infection in captivity. Our results suggests that certain modes of transmission may be predictors for parasite losses in captivity with likely causes being the use of anti parasitic drugs, and the biotic and abiotic differences between captivity and the wild. Due to their vulnerability, we suggest that these parasite types should be targeted for conservation in the future, as a world without parasites may not necessarily be a better one.

20. Validating a non-invasive method for detecting malaria in wild bird populations.

Antoine Filion, Stephanie Godfrey and Robert Poulin

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Emerging infectious diseases are a serious threat to naïve individuals that have not evolved with them. For instance, avian malaria occurrence has been shown to be on the rise in New Zealand avifauna, potentially threatening indigenous bird species that are considered to be the most extinction-prone in the world. However, detecting avian malaria in the wild can be difficult: birds in acute phase of infection are less active, therefore less likely to be sampled with traditional techniques. So far, no solution has been found to improve the detection of infected individuals. To this end, we test the potential for a novel, non-invasive tool, namely infrared thermal imaging, to accurately detect early signs of infections in wild populations. Using a multi-species approach, we provide general guidelines for conservation biologists wanting to use this method for rapid assessment of disease status in wild individuals.

21. Risky business: influence of eye-flukes on use of “risky” microhabitats and conspicuousness of a fish host

Brandon Ruehle & Robert Poulin

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A prerequisite for a parasitic manipulation to be considered adaptive is that it confers a fitness benefit to the parasite, such as increased transmission to another host. These manipulations can involve alterations to a wide range of host phenotypic traits, including microhabitat choice. Eye-flukes of the trematode family Diplostomidae use fish as intermediate hosts, and must be transmitted by predation to a piscivorous bird. In New Zealand, the diplostomid *Tyloodelphys darbyi* infects the eyes of a widespread endemic freshwater fish, the common bully *Gobiomorphus cotidianus*. Within the eye, *T. darbyi* metacercariae achieve large sizes and move freely about the aqueous and vitreous humors of the eye. We hypothesized that higher intensities of *T. darbyi* would (i) cause bullies to show increased activity and spend more time moving about in open space (i.e. more conspicuous, risky microhabitat), and (ii) reduce their ability to compete for shelter with fish harboring lower infection levels. Our experiments showed that heavily-infected fish were more active and spent more time in the open, although the effect was age-dependent, with immature fish displaying decreases in activity and time spent in the open with increasing intensities of infection. We also demonstrated that heavily-infected female bullies have a lower probability of using shelter, but that males show the opposite pattern. It is possible that using more risky microhabitats increases the likelihood of the fish being eaten by the parasite's predatory avian definitive hosts. However, our findings indicate that age- and sex-dependent effects call for a more nuanced interpretation.

22. Completing the cycle of trematode ‘supermodels’.

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Over a period of many years, parasite species within certain ecological systems have become favoured as models for study by the Otago parasitology group, some having been so much written about that perhaps they qualify for the title ‘supermodels’. However, these species are often only known from larval stages and their adults have never been described or named. Two new species, found in birds donated by the Dunedin Wildlife Hospital, are examples of these ‘supermodels’. *Philophthalmus attenuatus* is famous for being a well-studied example of a eusocial trematode at the larval stage. The asexually reproducing stage is found as two different morphs, the usual reproductive one and a tiny, soldier morph that defends the colony from secondary infections by other parasites. Their two-host lifecycle is unusual as well as their site in the definitive host; the membranes of the eye in gulls. *Galactosomum otepotiense* has featured in a number of studies as larval stages. Members of this genus migrate in their fish second intermediate hosts to the optic lobe of the brain, where they cause behavioural changes that make the fish more noticeable to seabird predators. We found the adults in four different host bird species and, although molecularly identical, specimens from each of these birds differed morphologically in a case of host-induced variability.

23. Uncovering hidden diversity: Microsporidians from New Zealand freshwater amphipods

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Freshwater amphipods are an important component of freshwater ecosystems and host diverse parasites. Microsporidians are obligatory intracellular parasites and have been reported from almost all animal phyla with more than 1,500 described species. Considering their wide distribution and diversity, very little is known about them in New Zealand. Here, we aimed at understanding distribution, diversity and abundance of microsporidians from freshwater amphipod species. We screened more than 2,600 individuals of pooled samples of 12 freshwater amphipod species from 79 locations collected throughout South and North Islands, by amplifying a partial SSU rRNA region. In particular, the most abundant freshwater amphipod species, *Paracalliope* species complex, was collected from 60 populations and 46 of them were positive for microsporidian infections with low to high prevalence. In addition, microsporidians were detected from 7 other amphipod species. Among all the microsporidians detected from this study, the genus *Dictyocoela* was the most common, which is the dominant microsporidian taxon in Europe as well. Our Bayesian phylogenetic tree shows that most of the Dictyocoelan species discovered in New Zealand formed a

distinct clade, sister to the clade of *D. mulleri* and *D. duebenum*, and several minor lineages were closely related to *D. cavimanum*. Also, two unique lineages were discovered from Corophiidae and Talitridae amphipods. In conclusion, microsporidians are abundant, but previously not studied, microparasites in New Zealand freshwater amphipods, and targeted screening effort would no doubt uncover a greater hidden diversity of parasites.

24. Contrasting effects of host specificity and host mobility on the reproductive strategies of parasites

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Reproductive strategies of living organisms vary greatly across taxa and are shaped by an evolutionary history resulting from the complex interactions between individuals and their environment. Therefore, uncovering the dominant factors that impact species reproductive strategies requires both an ecological and evolutionary point of view. One of the main ways to compare these different strategies is through the use of r/K selection theory derived from population dynamics. Organisms that are r -selected are generally found in unstable environments and have higher fecundity rates, whereas K -selected organisms tend to produce less offspring in more stable environments. This theoretical framework has helped ecologists understand the forces shaping reproductive strategies in free-living organisms, but little data is available for parasitic ones. Hence, we ask whether the reproductive strategies of parasites are determined by ecological and evolutionary pressures such as host specificity and host mobility. To answer this question, we scoured the literature to determine the host specificity of 150 species of parasitic copepod that infect either fish or corals (i.e., mobile vs immobile hosts). Using clutch size and egg size as proxies of reproductive strategy for each copepod species, we found that female size had strong positive effects on both clutch size and egg size, albeit for copepods only infecting fish. Interestingly, host specificity only had a weak positive effect on egg size in copepods infecting fish, but it had a strong negative impact on clutch size in copepods infecting corals, meaning that the reproductive strategies differ not only between copepods with different host specificities, but also the type of host they infect. These differences may reflect the evolutionary history they share with their immediate environment, in other words the hosts that they prey upon.

POSTER ABSTRACTS

1 Investigating the molecular basis of water seeking behaviour in earwigs induced by nematode infection.

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Abstract:

Parasites can have profound effects on their hosts, affecting their survival, distribution, and evolution. Host behaviour manipulation has evolved as one of the primary tools through which parasites increase their survival and reproduction, and is observable in all major parasitic lineages. One example of such manipulation is the water-seeking behaviour that some nematodes and nematomorphs induce in their arthropod hosts, sacrificing their host so that the worm can exit in water to continue its life cycle. In this project, we use the Earwig (*Forficula auricularia*) and its parasitic nematode (*Mermis nigrescens*) to study the mechanisms through which the parasite alters host behaviour. The main hypotheses we will test include the idea that the parasite secretes compounds (RNAs or proteins) that mimic components of the host signalling systems, or hijacks the host systems through novel factors that interfere with these processes. To explore these possibilities, the nuclear genome of both the host and the parasite is being sequenced using long, short, and linked reads. Different genome assemblers and polishing pipelines are being tested to determine which generate better assemblies. Our primary long-read sequencing data from Oxford minion for *M. nigrescens* has thus far yielded 512,545 reads with a median read length of 11,743 bp. These long reads were assembled and polished to produce 9374 contigs with an N50 of 105,290 bp. The best genome assembly has many intact genes represented: when evaluated with Quast, 73.93% of the BUSCO (Benchmarking Universal Single-Copy Orthologs) gene set were complete, with a further 12.21% present as partial sequences. Annotation of the nematode genome is planned using denovo repeat libraries and RNA-seq data. Gene duplication, gene losses and horizontal gene transfer between the parasite and host will be evaluated through comparative genomic frameworks. Comparative transcriptome analysis of *M. nigrescens* will also be conducted for stages before, during, and after manipulation. Differential gene expression analysis and gene set enrichment analysis will be carried out. With these genomic and transcriptomic approaches, we aim to uncover the underlying genetic mechanism *M. nigrescens* employs to trigger the water seeking behaviour in its host, and explain the genetic association and genetic exchanges between these host and parasite during coevolution.

2 Infection intensity and disease progression of *Bonamia* parasites in New Zealand flat oysters (*Ostrea chilensis*)

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Bonamia exitiosa and *Bonamia ostreae* are protistan parasites responsible for Bonamiosis (microcell disease or haemocyte disease) of flat oysters. *Bonamia* spp. have been found within the haemocytes and have also caused significant mortalities of flat oysters (*Ostrea chilensis*) in New Zealand. A detailed histopathological examination was undertaken on flat oysters collected from the Marlborough Sounds, New Zealand after the first known incursion of *Bonamia ostreae* to NZ in 2015. In this study, the overall prevalence of *Bonamia* spp. infection was 36% in oyster samples. The intensity of *Bonamia* spp. infection for the whole oyster and different tissues including gills, mantle, connective tissue around digestive tubules and haemocytes surrounding the gut were evaluated semi-quantitatively by a modified intensity grading scale modified from Hine (1991). *Bonamia* microcells were observed within haemocytes in blood spaces of gills and sinuses of oysters. Diapedesis (the passes of haemocytes with microcells through the unruptured walls of the capillaries) was also observed in different oyster tissues (mantle, gills and gut). The intracellular parasites multiplied (number increased) in haemocytes and the intensity of infection seemed to be associated with haemocyte destruction and diapedesis. This study focused on the assessment of the intensity of *Bonamia* spp. infection and on identifying specific infection patterns relating to disease progression. Thus, this study provides important information helping to understand host-parasite interactions, transmission mechanisms, and disease progression in *Ostrea chilensis*.

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