NZSP 46

NEW ZEALAND SOCIETY FOR PARASITOLOGY

ANNUAL MEETING No. 46

25-26 OCTOBER 2018

COACHMAN HOTEL

PALMERSTON NORTH

CONFERENCE PROGRAMME

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PROGRAMME

Thursday 25 October

	08:30	**Registration desk open**	
	09:00	**Welcome**	
Chair			
Tania Waghorn		**Horses**	
1	09:05	**Martin Nielsen**	**Equine parasite control – where are we heading?**
2	09:50	**Lee Morris**	Anthelmintic efficacy of single active and combination products against commonly occurring parasites in foals
3	10:10	**Martin Nielsen**	New take on an old concept: automated fecal egg counts
	10:30	**Morning tea**	
Paul Mason		**Sheep**	
4	11:00	**Dave Leathwick**	Nematode parasites are not a major cause of illthrift in adult ewes in New Zealand
5	11:15	**Alex Chambers**	Wild worms: gastrointestinal nematodes in feral sheep
6	11:30	**Anthony Oswald**	Triple Resistant *T.columbriformis*
7	11:45	**Tania Waghorn**	Host effects on *Haemonchus contortus* larval traits
8	12:00	**Seer Ikurior**	Using Remote Sensing Technologies for Detection of Parasitism in Sheep
9	12:15	**Mark Vickers**	Ewes behaving badly: An ongoing investigation into continued high egg output in ewes on a Southland sheep farm, it effect on production, and methods to control this.
	12:30	**Lunch**	
Cathryn Christie		**Sheep and cattle**	
10	13:30	**Anne Ridler**	Use of long-acting anthelmintic capsules in ewe hoggets pre-lambing
11	13:45	**Mark Vickers**	Field studies in sheep demonstrating the impact of host immunity on the observed anthelmintic treatment effect and measurement of resistance, and its potential use in managing worm resistance
12	14:00	**Dave Leathwick**	Combination capsules will ACCELERATE rather than SLOW the development of multiple drench resistance
13	14:15	**Christian Sauermann**	A climate-driven model for the free-living stages of Cooperia oncophora
14	14:30	**Paul Mckee**	Emergence of triple resistance in two worm genera of cattle without using a triple combination drench
15	14:45	**Colin Mckay**	A nasty case of anthelmintic resistance – what can we learn?

	15:00	**Afternoon tea**	
Christian Sauermann		**Invertebrates**	
16	15:30	**Stan Bellgard**	Vaccinology in the age of genomics
17	15:50	**Dallas Bishop**	Two cases of human myiasis in New Zealand
18	16:05	**Jeff Doherty**	Host-parasite interactions of insects and hairworms
19	16:20	**Christian Selbach**	Parasite transmission in changing ecosystems
	16:35	**AGM**	
	18:30	**Pre dinner drinks**	At The Chalet on Centennial Lagoon
	19:00	**Dinner**	https://www.boatshedcatering.co.nz/

Friday 26 October

	08:30	**Registration desk open**	
Laryssa Howe		**Host-Parasite interactions**	
20	09:00	**Vanessa Ezenwa**	**Exploring the consequences of helminth coinfection**
21	09:45	**Robert Poulin**	The geography of parasite discovery versus host diversity, across taxa and over time
22	10:00	**Eleanor Hay**	Do hosts drive parasite diversification?
23	10:15	**Bronwen Presswell**	Preserving parasite diversity
	10:30	**morning tea**	
Robert Poulin		**Wildlife**	
24	11:00	**Brandon Ruehle**	Effects of an Eye-Dwelling Parasite on the Behaviour of Common Bully
25	11:15	**Anusha Beer**	Role of ecology and phylogeny in determining tapeworm assemblages in skates (Rajiformes)
26	11:30	**Jerusha Bennett**	Parasites that travel along feeding links
27	11:45	**Marin Milotic**	Exposure to a cyanobacterial toxin increases larval amphibian susceptibility to parasitism
28	12:00	**Adrienne French**	*Toxocara cati* is one cause for nematode *larva migrans* in kiwi (*Apteryx* spp.)
29	12:15	**Sarah Coker**	Comparing the accuracy of mini-FLOTAC and centrifugal faecal flotation for the detection of coccidia (*Eimeria* spp.) in kiwi (*Apteryx mantelli*).

	12:30	**Lunch**	
Dallas Bishop		**General Parasitology**	
30	13:30	**Kiliana Bekelaar**	Abomasal nematode species differ in their response to exsheathment triggers
31	13:45	**Richard Shaw**	Relationships between CarLA IgA, productivity and parasites in deer
32	14:00	**Nik Palevich**	Identifying the genetic mechanisms that evoke parasitic exsheathment.
33	14:15	**Andrew Dowling**	Liver fluke serum ELISA and milk production analysis in nine herds supplying Westland Milk Products
	14:30	**Afternoon tea**	
	15:00	**Close**	

Keynote Speakers

Dr. Martin Nielsen, DVM, Ph.D., DipEVPC

University of Kentucky, USA

Dr. Nielsen graduated with his DVM degree from the Royal Veterinary and Agricultural University, Denmark in 2001. He then spent three years in equine veterinary practice before joining graduate school in 2004. He received his Ph.D. in equine parasitology at University of Copenhagen in 2007, and served as assistant professor there until 2011. He then joined the M.H. Gluck Equine Research Center at University of Kentucky where he currently serves as associate professor in equine parasitology and holds the title of Schlaikjer Professor in Equine Infectious Disease.

He is board certified in veterinary parasitology with the European Veterinary Parasitology College (EVPC) and with the American College of Veterinary Microbiologists (ACVM). Dr Nielsen is chair of the AAEP Equine Parasite Control Subcommittee with published its guidelines in 2013. He currently serves as co-editor in chief for the scientific journal *Veterinary Parasitology*, and he is a member of the WAAVP Anthelmintic Resistance Guideline Committee.

Dr. Nielsen's research has resulted in more than 90 peer-reviewed publications in scientific journals, and over 150 peer-reviewed abstracts for presentations at conferences and meetings. He is co-author of "Handbook of Equine Parasite Control" which was recently published in its second edition. He has published over 50 popular and tutorial articles, and is the author of five book chapters.

Dr. Vanessa Ezenwa, PhD

University of Georgia, USA

Dr. Vanessa Ezenwa is a Professor at the University of Georgia, USA, where she holds joint appointments in the Odum School of Ecology and College of Veterinary Medicine. She received a BA in Biology from Rice University, and PhD in Ecology and Evolutionary Biology from Princeton University. Her research centers on the ecology of infectious diseases in wild animal populations, with a specific focus on linking processes across scales of biological organization. Work in Dr. Ezenwa's lab

The Ezenwa Lab at the University of Georgia webpage:
http://ezenwalab.uga.edu/index.html

PRESENTATION ABSTRACTS

1. Equine parasite control – where are we heading?

Martin K. Nielsen

M.H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, USA.

In recent decades, levels of anthelmintic resistance in important equine nematode parasites have been reported to be increasing across the world, and no new anthelmintic classes with new modes of action have introduced during this time period. This complicates approaches for equine parasite control and forces the industry to revise the strategies employed. This presentation will outline some of the more pressing research questions emerging from the efforts to devise more sustainable parasite control strategies and will summarize recent research findings relevant to these questions. Anthelmintic resistance is widely reported in cyathostomins and ascarids across the world, but generally at least one anthelmintic class appears to still be working against each of these. Combination products can be constructed to ensure activity against both cyathostomins and ascarids, but very few will contain more than one active ingredient with full activity against any one of them. Recent work performed by our group illustrated a quickly declining efficacy of a pyrantel pamoate/ oxibendazole combination against a population of cyathostomins with resistance already developed to both active ingredients. Parasite biology is widely different between ascarid and cyathostomin parasites, and computer simulations have proven useful for identifying knowledge gaps and developing less intensive treatment strategies and we are currently evaluating some of these in field. Although fecal egg counts are widely recommended in equine parasite control programs, recent questionnaire surveys illustrate that horse owners and farm managers are generally very reluctant to do so, unless mandated by national legislation. Reasons could be many, but cost, a cumbersome technology, and data variability all seem to play a role. More refined diagnostic methods with improved accuracy and precision may help address this challenge. Even with novel anthelmintic classes eventually

2. Anthelmintic efficacy of single active and combination products against commonly occurring parasites in foals

Lee H. Morris, Sally Colgan, Dave M. Leathwick, Martin K. Nielsen

EquiBreed NZ Ltd, 399 Parklands Rd, RD 1, Te Awamutu, 3879, New Zealand

Parasite control in foals is complicated due to the concurrent presence of biologically different parasites and developing levels of anthelmintic resistance within these. Several combination anthelmintic products are available for use in equines, but information on the efficacy of these against important equine parasites is scarce. This trial evaluated the efficacy of two combination anthelmintic products and three single-active macrocyclic lactone (ML) products in reducing common gastrointestinal parasites in foals. A total of 110 foals, located on three farms with substantially different anthelmintic treatment histories, were allocated to one of six anthelmintic treatment groups, i) oxfendazole/pyrantel embonate (N = 20), ii) pyrantel

embonate/ivermectin/praziquantel (N = 20), iii) ivermectin/praziquantel (N = 17), iv) abamectin/praziquantel (N = 17), v) moxidectin/praziquantel (N = 13), and vi) placebo-treated control (N = 23). During the three month pre-treatment period, foals were monitored monthly with determination of *Parascaris* spp., strongylid, and *Strongyloides westeri* faecal egg counts (FEC). Foals were systematically allocated to treatment group and treated accordingly following the first appearance of *Parascaris* spp. eggs in the faeces. FECs were then determined at two, four and eight weeks post treatment. None of the three macrocyclic lactones achieved >95% reduction of ascarid FECs, whereas only the pyrantel embonate/oxfendazole combination demonstrated full efficacy. This contrasts the anti-strongylid efficacies determined, where the same combination failed to reduce FECs on one farm, whereas the macrocyclic lactones generally had good efficacy. Only 22 foals were *S. westeri*-positive pre-treatment, but results suggested >95% faecal egg count reduction (FECR) for all tested products. However, the placebo-treated control group had a mean FECR of 90%, which complicates the interpretation of these results. Taken together, this study illustrates the complexity of parasite control in foals, where effective treatment of important parasites remains challenging despite the availability of several anthelmintic products.

3. New take on an old concept: automated fecal egg counts

Martin K. Nielsen

M.H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, USA.

Parasite fecal egg counts remain important tools for surveillance and diagnosis of patent parasite infections and evaluation of anthelmintic treatment efficacy. Recent surveys have indicated limited routine usage of fecal egg counts in equine parasite control programs, and this may, in part, be due to the cumbersome nature of fecal egg count procedures and the inherent variability associated with the data generated. We sought to develop a user-friendly technology capable of determining ascarid and strongylid fecal egg counts without the need of a traditional microscope. We fluorescently stain nematode eggs within a sample and use image analysis to identify and count the eggs. Over the past years, we have evaluated different camera systems and improved and optimized sample preparation and staining, algorithm refinement, and camera setting. Our current prototype is capable of identifying and counting ascarid and strongylid egg types, and sample processing times is about five minutes per sample. A recent study determined coefficient of variation (CV) between repeated counts performed on the same equine fecal samples naturally infected with strongylid parasites and found the system to have lower CV (21.2) compared to McMaster (23.9) and Wisconsin counts (36.3). The main reason for this improved method precision is likely the elimination of the operator-dependency from the reading of samples. We observed statistically significant linear correlations between egg counts determined with all three methods ($p<0.0001$), and the magnitude of egg counts determined was similar between McMaster and our technology, whereas the Wisconsin method returned markedly lower counts. Beta versions of our technology are currently under evaluation by equine veterinarians and a final commercially available product is underway.

4. Nematode parasites are not a major cause of illthrift in adult ewes in New Zealand

Dave Leathwick, Siva Ganesh, Chris Miller

AgResearch Grasslands, Private Bag 11008, Palmerston North

Illthrift, defined as low body condition score (BCS), is a problem in adult ewes on many farms in New Zealand, and nematode parasites are often considered a significant contributing factor. Here we test the hypothesis that, over the lambing-lactation period, parasites are a significant causal factor in ewe illthrift, and that anthelmintic administration to low BCS ewes results in a greater improvement in condition than treatment of other ewes. Faecal nematode egg count (FEC) prior to lambing and ewe liveweight change over the study period were poorly correlated, and both were independent of pre-lambing BCS, as was ewe response to anthelmintic treatment. Some ewes lost, or gained, considerable amounts of weight independent of their BCS, the number and size of lambs reared, and anthelmintic treatment. We find no evidence that ewes of low BCS have higher FECs, or a larger response to anthelmintic treatment than ewes in better condition.

5. Wild worms: gastrointestinal nematodes in feral sheep

Alex Chambers, Fiona Kenyon, Russell Avramenko, Dan Nussey, John Gilleard, Elizabeth Redman and Neil Sargison

Host fitness is influenced by the presence of co-infecting parasites, and understanding the population dynamics is important in gaining an insight into parasite transmission and host health. Researching the nematode population structure in the absence of control measures is required to recognise the impact of management decisions on sustainable control. The unmanaged, feral population of Soay sheep on St Kilda (Scotland, UK) provides an ideal study population.

Faecal samples were collected over 8 sampling months, from 7 different sex-age groups. Individual faecal egg counts were conducted by a cuvette salt floatation method, the faeces were then pooled into composite cultures (per test group/season) and incubated to grow third -stage larvae (L3). Pools of 500 L3 were speciated and quantified by next generation sequencing (Illumina MiSeq). This allows for a high-throughput and fine scale partitioning of different species, which cannot be achieved with traditional methods of nematode speciation. The development of a deep sequencing assay of the ITS-2 region of the rDNA cistron has enabled the accurate identification and quantification of clade V parasites in mixed-species pools.

This is the first field application of this method, and has been validated for the species present on St Kilda. Validation corrects potential species-specific biases during sequencing, in addition to testing amplicon repeatability and species detection threshold. *Bunostomum trigonocephalum*, *Teladorsagia circumcincta*, *Trichostrongylus axei*, *Trichostrongylus vitrinus* and *Chabertia ovina* were identified, with seasonal, age and sex differences in species

composition. Trends appear to correspond with the sheep's dynamic life-history in addition to the life-cycle of the parasite; including a spring peri-parturient rise in pregnant females and high winter mortality. L3 speciation provides a means of identifying sources of contamination of pastures, not as a tool to determine levels of infection (i.e. worm burden). This study offers a preliminary parasitological snapshot of what is going on in the Soay sheep.

6. Triple Resistant *T.columbriformis*

Anthony Oswald

Taihape Veterinary Services, Kotare St, Taihape 4720

As part of ongoing anthelmintic resistance monitoring a third FECRT in sheep was performed on a commercial sheep and beef farm in the Taihape area. The results indicated that triple combination anthelmintics had poor efficacy against the *Trichostrongylus* genera. In 2018 a worm count study was performed on this property revelling *T.columbriformis* as the resistant species to the triple combination. This study showed that the triple combination was only 50% effective against *T.columbriformis.* Triple resistant *Trichostrongylus* was confirmed on three other targeted properties after FECRTs in 2018. As a follow on from this drench checks were performed on 36 farms following triple combination anthelmintic treatment in the autumn to assess the extent of triple resistant *Trichostrongylus* on farms within the Taihape area. 50% of these drench checks had eggs present, and the quantitative larvae cultures done on all the drench check samples showed that *Trichostrongylus* was the dominant genera of nematode present.

7. Host effects on *Haemonchus contortus* larval traits

Christian Sauermann, Paul Candy, Tania Waghorn, Kiliana Bekelaar, Dave Leathwick

AgResearch Grasslands Research Center, Tennent Drive, Private Bag 11-008, Palmerston North 4442

Nematode parasite control is currently based largely around the use of anthelmintics however, alternative approaches including the breeding of animals for resistance to nematode infection is based on the capacity of grazing ruminants to mount a strong immune response against nematode infection. It has been shown that resistant animals are able to influence the percentage establishment of ingested larvae, the survival rate of adult worms and the fecundity of adult females. In this work we investigate the influence of host animal on a range of life-history variables associated with the free-living stages of *H. contortus*.

A group of 5 lambs was infected with the same batch of *Haemonchus contortus* and after patency individual faecal samples were collected, separately cultured and a series of experiments assessing different larval traits were undertaken: the length of the larvae, larval survival, response to ivermectin in a migration assay, the ability of the larvae to exsheath in vitro, and establish and develop to the adult stage in young lambs.

The results for all traits indicate a significant difference between the host animals, with larvae from specific hosts following a consistent pattern with the highest or lowest trait results.

Compared to larvae from Host 1 the larvae from Host 5 were, had a longer median survival, were less susceptible to ivermectin, exsheathed to a lesser degree, but showed a higher establishment rate in the consecutive host.

Regarding the survival time, anthelmintic susceptibility and establishment rate as indicators for fitness, the parasites populating Host 5 produced progeny of greater fitness. The findings indicate that the host animal of the parental parasite generation has a significant effect on the parasite progeny.

8. Using Remote Sensing Technologies for Detection of Parasitism in Sheep

Seer Ikurior[1], Bill Pomroy[1], Ian Scott[1], Rene Corner-Thomas[1, 2], Stephan Leu[3]

[1]School of Veterinary Science, Massey University, Tennent Drive, Palmerston North, New Zealand 4442, [2]School of Agriculture and Environment, Massey University, New Zealand, [3]Department of Biological Sciences, Macquarie University, NSW, Australia

Animals suffering from parasitism typically display altered grazing behaviour and a voluntary reduction in feed intake. These changes are potentially important as indicators of disease. Recent advances in GPS and accelerometer technologies provide the opportunity to objectively measure animal behaviour while on pasture. GPS tracking collars are well suited to monitor and detect changes in movement patterns. Triaxial accelerometers measure body movement in terms of acceleration, which can then be used to estimate physical activity over time. Two preliminary studies using these technologies are presented. In study one, the accuracy and performance of seven commercially sourced GPS receivers were assessed. A motion test was used to assess the receivers' prediction of distance travelled against the inner lane of an athletics track. Stationary tests assessed how dispersed location estimates from the receivers were from a "true" location landmark. Six of seven receivers returned <5 m errors in location estimates. Implications on logging frequency, error rates and usability to measure distance travelled by grazing lambs is discussed. In study two, the total activity levels of two groups of crossbred Romney and Suffolk ram lambs were monitored over a period of four days using triaxial accelerometer sensors after 42-days exposure to treatment. On day zero, all lambs were given anthelmintics. Thereafter, a Suppressive Treatment Group (S) (n=12) was treated with anthelmintics every two weeks. An Untreated Group (U) (n=12) did not receive anthelmintics. Total activity levels were monitored from days' 42 – 46. Activity level was calculated as vectorial dynamic body acceleration (VeDBA). Parasite burdens measured by faecal egg counts were low (μS = 50 eggs/g; μU = 500 eggs/g). Activity levels in untreated lambs were significantly less than in the Suppressive Treatment group (P=0.024). This small but significant difference in total activity indicates usefulness of this approach in detecting subclinical parasitism.

9. Ewes behaving badly: An ongoing investigation into continued high egg output in ewes on a Southland sheep farm, it effect on production, and methods to control this.

Mark Vickers, Julie Wagner

201 Dominion Road, Tuakau 2121

Investigation of suspected triple drench failure on a Southland sheep farm in February 2018, found the worm population was surprisingly susceptible. Reductions for oxfendazole, levamisole or abamectin were 97% or greater, with complete reductions (100%) with two dual, and three different triple active formulations in lambs. The same triples Trio Sheep, Matrix Hi-Min and Triplemax oral were also fully effective in ewes. The high egg count of untreated ewes of 390epg (100-1050epg) in this study was of more concern, triggering a second weight gain and egg count study.

In this second study 60 ewes were individually ear tagged, weighed and then allocated to two groups by weight. Forty ewes allocated to Group 1 remained untreated, while 20 ewes allocated to Group 2 were treated with a Centramax capsule. In lambs allocation was identical, but the 40 lambs in Group 1 were treated with a triple oral (Triplemax) at 28 day intervals, while the 20 in Group 2 were treated with a Centramax capsule.

In lamb and ewe groups with capsules, no eggs were found to Day 112, and no larvae except *Trichostrongylus* in lambs at Day 112. In untreated ewes the mean egg count declined slowly to be 100 epg (0-200epg) at Day 112. In triple oral treated lambs egg counts were zero or low positive 28 days after treatment.

There was no weigh gain difference in lambs, with a 4.25kg gain for the triple and 4.15kg for the capsule over the 85 days.

In contrast the capsule treated ewes gained 6.15 kg compared to a 3.31kg gain in the untreated controls.

The high egg output in these ewes and possible causes including sheep genetics or a loss of normal ewe immunity is discussed. Current pastoral sheep farming and anthelmintic practice relies on the ewe's ability to control egg output.

10. Use of long-acting anthelmintic capsules in ewe hoggets pre-lambing .

Anne Ridler, Dave Leathwick, Chris Miller, Siva Ganesh, Chris Garland, Hannah Bruce

School of Veterinary Science, Massey University, Private Bag 11-222, Palmerston North

Anecdotally, there is a perception that a greater and/or more consistent benefit will accrue when long-acting anthelmintic capsules are administered to young ewes (hoggets) compared with MA-adult ewes. Production trials were undertaken on two commercial farms to investigate the responses associated with anthelmintic capsule administration to ewe

hoggets prior to lambing. An abamectin/albendazole capsule was administered to half of the 737 pregnant ewe hoggets while the other half remained untreated. The weaning weight and subsequent pre-mating weight of the ewe hoggets, their lamb weaning weight and lamb survival were evaluated. In both flocks the treated ewes had a higher weaning weight and their lambs were heavier at weaning but there was variation in the magnitude of the response between the flocks. Lamb survival was not different between treated and untreated ewes. A cost-benefit analysis showed that in one flock there was an approximately $5.40/ewe benefit whereas for the other flock the production gains did not quite cover the cost of treatment (cost-benefit approximately -$0.10/ewe). Any potential production benefits also need to be weighed up against the risk of selection for anthelmintic resistance. In summary, using long-acting anthelmintic capsules in ewe hoggets prior to lambing produced benefits similar to those seen in adult ewes and will not always be economically worthwhile.

11. Field studies in sheep demonstrating the impact of host immunity on the observed anthelmintic treatment effect and measurement of resistance, and its potential use in managing worm resistance.

Mark Vickers, Gary Harrison, Jon Petherick

201 Dominion Road, Tuakau 2121

In 2016, a Suffolk sheep stud farmer in Northland reported that a triple drench was not working in his sheep. A Nexan sponsored on farm investigation of the ill thrifty and scouring sheep from this property confirmed very severe triple resistance, predominantly *Trichostrongylus* species, with approximately 50 % reductions in egg counts with triple oral drenches (Matrix or Triplemax) 14 days after treatment in 6-8 month old lambs. The faecal egg output of sheep drenched with Bionic capsules was also high, and did not reduce when dosed with a triple drench. Ewes also were found to have high egg counts, and they also failed to respond to triple drenches or capsules treatments, with reductions similar to lambs. Small numbers of *Trichostrongylus* larvae were also recovered from monepantel (Zolvix) treated lambs, including with Bionic capsules running.

Twenty clinically affected 6-8 month old lambs with high egg counts were then transferred from this farm to a South Auckland farm and grazed on previous hay paddocks and remained undrenched for approximately 2 months until the paddocks were heavily contaminated. These 8-10 month old lambs were then drenched with a triple oral drench or Bionic capsule. On this occasion both the triple oral and Bionic capsules appeared fully effective (100%) but small numbers of *Trichostrongylus* spp were recovered in larval cultures post treatment.

Changes were made to the Northland farm, including better pasture and parasite management with higher levels of nutrition, cross grazing, and a small number of untreated ewes for refugia. In 2018 the lambs were still noted as scouring and illthrifty and both ewes and lambs were treated with capsules and or triple drenches. At this time ewes responded fully to treatment, but in lambs the reductions were similar to the earlier trials, with approximately 50 % reductions. An interesting finding was that in the 8-10 month old lambs

on the South Auckland farm in summer months, and also the ewes on the Northland farm in 2018, very few *Trichostrongylus* were found in culture. The importance of immunity both in contributing to the observed treatment effect and quantification of resistance, as well as its role in the future management of resistant worms of sheep and domestic animals is discussed.

12. Combination capsules will ACCELERATE rather than SLOW the development of multiple drench resistance

Dave Leathwick

AgResearch Grasslands, Private Bag 11008, Palmerston North

Statements claiming that combination capsules will slow the development of anthelmintic resistance have persisted in advertising, the popular press and vet newsletters for many years. However, the evidence disputes this claim.

Short-acting combination anthelmintics kill more resistant genotype worms than single actives, creating a greater degree of dilution when these survivors mate with susceptible worms which establish soon after treatment. Establishment of susceptible worm genotypes is therefore essential for this process, and in its' absence the combination has no benefit.

A capsule which contains two or more actives does not allow establishment of susceptible genotypes and so does not allow for the single most important factor in combination therapy. Use of these products in adult ewes over lactation is even worse because the immune competence of the ewe returns quite early after lambing and the host becomes highly refractory to establishment of new infection. Because of this resistant worms which establish while the capsule is active will remain largely undiluted for weeks after it expires. A combination capsule administered to ewes pre-lambing will pass only eggs resistant to both drugs for at least 150 days after treatment.

The low daily release rate of capsules has been shown insufficient to attain high efficacy against resistant worm genotypes, at least for albendazole and ivermectin. Hence, the survival of worms heterozygous for resistance is likely in the presence of the capsule when they don't survive following treatment with an oral combination containing the same actives.

Long-acting products have also been shown to alter worm dynamics in favour of resistant survivors i.e. the adult worms will be bigger, produce more eggs and live longer, increasing the contribution of these multi-resistant genotypes to future generations.

Therefore rather than slowing the development of resistance, combination capsules are highly likely to accelerate the development of multi-drug resistance.

13. A climate-driven model for the free-living stages of *Cooperia oncophora*

Christian W Sauermann, Dave M Leathwick

AgResearch Grasslands, Private Bag 11008, Palmerston North, 4442, New Zealand

A climate-driven model to simulate the development, survival and herbage translocation of *Cooperia oncophora* free-living stages was developed using experimental results and published literature data. The model uses daily maximum and minimum temperatures to estimate hourly development and survival rates of the eggs to infective third stage larvae (L3) and daily survival rates of L3 in the faecal pat and in the herbage. Daily rainfall data were used to calculate the translocation rate of the L3 from the faecal pat into the herbage. The model outputs for the development and survival of the free-living stages were comparable to previous observations. The model estimates of developed L3 indicate an optimal temperature of 25.6 °C, with low estimates of developed L3 with temperatures below 6 °C or above 35 °C. Further, the model predicted that the developed L3 would be able to translocate from the faecal pat into the herbage in case of sufficient rainfall. When validating model output for the herbage contamination with *C. oncophora* infective stage larvae against results of a two year field experiment, the comparison indicated that the model was able to reproduce the observed contamination pattern. In addition, detailed examination of different model components helped to identify possible factors causing the decay of larval herbage contamination during winter-spring as occurred in the field experiment and the widespread prevalence of anthelmintic resistance in this species.

14. Emergence of triple resistance in two worm genera of cattle without using a triple combination drench.

Paul McKee, Julie Wagner, Mark Vickers

The emergence of triple resistant Ostertagia spp and Cooperia spp in cattle in the southern North Island over a 10 year period is demonstrated in two Ravensdown studies, on a farm with no previous triple drench use. The farm is a bull beef farm, administering drenches at approximately 4 week intervals using primarily dual benzimidazole+levamisole orals with one or two macrocyclic lactone (abamectin or moxidectin) pour ons per year.

In 2008 the faecal egg count reduction (FECR) 12 days after treatment demonstrated a reduction for oxfendazole + levamisole of 90% with only Ostertagia larvae found post treatment, and a FECR of 87% for abamectin injection, with mainly Cooperia, Trichostrongylus larvae, and very occasionally Ostertagia isolated in culture.

In 2018 the FECR 12 days after treatment for oxfendazole+levamisole 42% with Ostertagia, Trichostrongylus and Cooperia all isolated in larval culture. For abamectin injection the reduction was 28%, with Cooperia, Trichstrongylus and some Ostertagia present. A triple oral of oxfendazole+levamisole+abamectin gave 100% reduction in egg count, but quite large numbers of Ostertagia and Cooperia were found in the larval culture post treatment. These findings suggest triple resistance has emerged in these two worm genera while Trichostrongylus remained sensitive to oxfendazole+levamisole. The effect of repeat treatment on weight gain was also measured in 2018 and is presented.

15. A nasty case of anthelmintic resistance – what can we learn?

Colin Mckay

Elanco, Level 1, 123 Ormiston Road, Botany Junction, Auckland 2016

This paper details a case of confirmed resistance to a Zolvix Plus (monepantel plus abamectin) oral sheep drench on a small sheep farm in the North Island. The farm involved was diagnosed as having triple drench resistant parasites in 2013 and had been relying on either Zolvix (monepantel) or Zolvix Plus (monepantel plus abamectin) treatments as cornerstones to their worm control program for several years. Unfortunately, the use pattern followed proved unsustainable with significant consequences for the property owners.

Zolvix Plus is recommended to be used as part of a worm control programme as the fifth drench in a lamb preventative drenching program (knockout), as part of a farm protection (quarantine) control programme, or as an "exit" drench from long acting products. Zolvix Plus should also be used on farms with known resistance issues, however on properties with triple resistant parasites a total review of roundworm management practices is required to best implement the use of Zolvix Plus. Simply subjecting Zolvix Plus to the same resistance selection pressure that resulted in the emergence of triple drench resistant parasites will result, as in this unfortunate case, in parasites resistant to Zolvix Plus.

These recommendations were developed in conjunction with key opinion leaders and are currently part of Wormwise™ guidelines for anthelmintic use. In addition, they were developed to ensure responsible incorporation of new drenches, balancing the benefits to farmers of using a highly effective drench now while also ensuring that new anthelmintics weren't used inappropriately, potentially leading to faster development of resistance.

This case of resistance does not change the recommendations, and indeed supports the early incorporation of Zolvix Plus into sustainable worm control strategies.

16. Vaccinology in the age of genomics

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Tick-borne diseases of livestock cause large impacts to dairy herds. For example, acute outbreak costs of the disease 'oriental theileriosis', first reported in NZ in 2012, have been estimated as high as $1M on one large farm, with climate change likely to increase impacts. NZ is also threatened by a range of other tick-borne diseases that we do not yet have in the country, with greater trade levels increasing the risk of their introduction. There is currently no satisfactory approach to prevent tick-borne diseases of livestock in NZ. However, we are fortunate in that there is only a single tick in the country that infests livestock, the brown cattle tick. This provides an elegant and efficient solution to both current and potential future

disease impacts manage the tick, rather than specific diseases. Almost all existing vaccines have been developed based on traditional vaccinology methods, which rely on empirical screening of a few candidates at a time. However, the ability to sequence a tick's genome provides access to its entire antigenic repertoire. As such, genomics has catalysed a shift in vaccine development towards sequence-based 'Reverse Vaccinology' approaches, which use high-throughput in silico screening of the genome of a tick's mouth-parts to identify genes that encode proteins with the attributes of good vaccine targets. Vaccine candidates identified from a tick's genome or proteome can then be expressed as recombinant proteins and tested in appropriate in vitro or in vivo models to assess immunogenicity and protection. We have commenced a work plan to sequence the genome of the brown cattle tick, and use 'reverse vaccinology' to develop a livestock vaccine against it. This approach has proven highly successful overseas, with a vaccine against the brown cattle tick being a step-change in the approach to preventative animal health protection.

17. Two cases of human myiasis in New Zealand

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Following a 2010 review of human cases of myiasis in New Zealand, two further cases were recorded during 2017. One case occurred in Kaiwaka, Northland in February 2017 involved the third instar larvae of the blowfly *Lucilia cuprina* (Diptera: Calliphoridae). This was the first case of naturally-acquired myiasis in New Zealand recorded for this fly. The infestation was associated with an open facial wound.

The second case in October 2017 was an Aucklander recently returned from South America. Larvae were extracted from lumps on the right arm and submitted for identification. The larvae were identified as third instar *Dermatobia hominis,* the human botfly (Diptera: Oestridae). Previous imported cases attributed to this fly have been recorded in New Zealand, from residents and tourists, who spent time in South America. Two of these occurrences from Peru and Bolivia have been published (1996 & 2009).

18. Host-parasite interactions of insects and hairworms

Jeff Doherty & Robert Poulin

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Hairworms (phylum Nematomorpha) are a relatively obscure group of parasites with a complex life cycle including multiple invertebrate hosts, an aquatic and terrestrial phase, and five recognised life stages. The final aquatic life stage is a dormant cyst found within the tissues of several macroinvertebrate hosts inhabiting streams and rivers. Some of these mature into terrestrial adults, transporting cysts to dry land. These intermediate hosts

eventually die and are thus consumed by a number of scavenger and/or predatory insects, which act as definitive hosts. Hairworms grow inside the body cavity of their definitive host for several weeks or months. Once mature, they must exit the host in order to mate in water and lay their eggs. This coincides with remarkable behavioural changes in the host: they suddenly approach water and jump into it, usually to their death. It has been suggested that hairworms adaptively manipulate their insect hosts to commit suicide, but the few studies on this phenomenon have only looked at natural infections, which do not fully support the manipulation hypothesis. Several questions on this unique life cycle remain unanswered. Therefore, my goal is to study the hidden interactions between hairworms and both their aquatic and terrestrial hosts by combining field data and experimental infections. In this talk, preliminary data on a species of caddisfly larvae (*Olinga* sp.) showing high prevalence of hairworm cyst infection is discussed. The internal defence reaction of this intermediate host may hinder the life cycle of hairworms, since 52.3% of cysts were melanised to varying degrees. However, some encysted hairworm larvae are capable of exiting partially melanised cysts, raising doubts about the true lethality of this internal defence reaction. Furthermore, I will also discuss a non-invasive technique that I have developed for detecting hairworm infections, currently being tested on naturally infected cave weta.

19. Parasite transmission in changing ecosystems

Christian Selbach & Robert Poulin

University of Otago, Department of Zoology, 340 Great King Street, Dunedin 9016

The transmission from one host to another constitutes a challenging event in the life cycle of parasites and is a key determinant of their fitness. Due to their complex life histories, the free-living dispersal stages of trematodes, the cercariae, show a huge diversity in morphology and behaviour. Abiotic and biotic factors, such as temperature and predation, have been shown to have regulating effects on cercarial infection dynamics. Yet, on a finer scale, we still have a limited understanding of how and to what degree these factors affect the transmission of individual parasite species in an ecosystem. In the present study we assessed how (i) changes in temperature and (ii) predation affect the transmission abilities of cercariae from the New Zealand mudsnail *Potamopyrgus antipodarum* and the marine snail *Zeacumantus subcarinatus*. Our results show that increases in temperature positively affect the transmission dynamics of cercariae, whereas predation leads to a significant reduction of dispersal stages from the environment. However, the impacts of both temperature and predation varied considerably between the transmission stages of different trematode species, depending on their morphology and behaviour. This varying susceptibility to biotic and abiotic factors is likely to have far-reaching implications for the disease dynamics in changing ecosystems, since increases in temperature or changes in predator abundance can shift parasite community structure. Understanding these species-specific parasite transmission traits therefore remains a fundamental requirement to predict parasite dynamics under current global change, e.g., climate change, scenarios.

20. Exploring the consequences of helminth coinfection.

Vanessa Ezenwa

University of Georgia, USA

Co-infection with helminths can affect the host response to microbial infections (e.g. viral and bacterial infections), and an increasing number of studies are investigating the consequences of helminth-microbe coinfection in laboratory settings. To better understand the dynamics of co-infection in natural systems, we used a wild population of African buffalo to explore the consequences of gastrointestinal nematode infection for bovine tuberculosis (TB; causative agent: *Mycobacterium bovis*). Using a combination of experimental, longitudinal, and theoretical studies, we show that anthelmintic treatment has profound effects on the outcome of TB infection at both the individual and population levels. We also show that an individual's constitutive response to nematode infection has non-intuitive consequences for TB disease. Overall, our work is revealing that both active infection with nematodes, and host resistance to nematodes, can have profound implications for the epidemiology of microbial diseases.

21. The geography of parasite discovery versus host diversity, across taxa and over time.

Robert Poulin & Fátima Jorge

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Mapping global parasite diversity is crucial to identify geographic hotspots of emerging disease, guide public health and conservation efforts, and also complete our inventory of global parasite biodiversity. Assuming a bottom-up coupling between the diversity of resources and consumers, the geographical distribution of parasite diversity should match that of global host diversity. Using a large and representative dataset on the geographical coordinates where nearly 5000 helminth species were first discovered across the globe over the last few decades, we found only very weak spatial covariation (across 2x2° grid cells) between vertebrate species richness and the number of parasite species discovered. The deficit in parasite discovery is greatest in areas corresponding to hotspots of host diversity, where disproportionately fewer new parasites are discovered than expected based on local host richness. Our data also show (i) that parasite discovery rates are not spatially congruent across higher helminth taxa, i.e. areas where many trematodes are found are not also areas where many nematodes or cestodes are discovered; and (ii) parasite discovery rates are temporally inconsistent, i.e. the number of new parasite species discovered per decade in a particular region increases or decreases at random over time. Our findings suggest that the global parasite discovery effort is inefficient, spatially biased and subject to idiosyncrasies, problems for which we propose solutions.

22. Do hosts drive parasite diversification?

Eleanor Hay, Robert Poulin & Fatima Jorge

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Despite decades of research linking different factors to parasite diversification (result of the balance between speciation and extinction), we still do not understand why some parasite lineages speciate quickly and frequently switch host species, while others do not. The expectation that hosts may influence parasite diversification comes from the nature of their association, with the parasite depending on the host for survival, and consequently from their co-evolutionary interactions. The aim of this study is to test the hypothesis that a host-switch event drives parasite diversification, i.e. we predicted it would lead to an increase in parasite diversification observable across a variety of parasite taxa. To test this hypothesis, we compiled data on 15 host-parasite datasets (parasite time-calibrated phylogenies and respective host-parasite associations records) for a wide range of parasite groups. Using two different macroevolutionary approaches we estimated differences in diversification rate across parasite phylogenies. We first identified distinct branching patterns within trees using a model-free approach (RPANDA). We then used a Bayesian hypothesis-testing approach (BayesRate) to test various diversification regimes across different branches of parasite evolutionary trees (e.g. clades of parasites found to result from host switches versus those that are not). Focusing on just two examples from our dataset, avian body lice and avian malaria, this talk will highlight the complexities of testing diversification patterns within and between different parasite groups. It is of the utmost importance to elucidate the factors that drive parasite diversification, not only for pure scientific reasons, but also because their intimate associations with hosts have important ecological, environmental and health implications. It is our hope that this study provides further insight into what drives parasite diversification.

23. Preserving parasite diversity

Bronwen Presswell & Jerusha Bennett

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Species are going extinct at a rate higher than the planet has ever experienced, and there is pressure to document biodiversity. The diversity of life cannot be understood and measured without the collection and scientific description of specimens. But, parasites….?? Why bother to preserve (or *conserve*) them? It's estimated that around 50% of biodiversity consists of parasites. That is a lot to be ignorant about! And we are learning more about the important roles that parasites play in ecosystems: in host population regulation, in food webs, as bioaccumulators of pollutants, as drivers of evolution and maintainers of diversity. In this project we aim to collect, identify, curate and submit to Otago Museum, all helminth parasite species found in the last ten years during our taxonomic work. These are mainly from native birds, but also from fish, elasmobranchs and invertebrates. In addition, the Wildlife Hospital in Dunedin has given us access to all birds which die or are euthanased in their care. The material, either slide or ethanol-fixed specimens, will be submitted to the Museum along with

all relevant data, including our identifications, collection details, drawings, photographs, scanning electron micrographs and DNA barcode sequence numbers. The purpose is to gather as much data as possible to place on record for researchers to access in the future. The helminth parasite fauna of New Zealand vertebrates is so little known that every record and specimen is a valuable addition to our database. As a result of our work we have already found several species new to science that will be described and formally named after the initial phase has taken place.

24. Effects of an Eye-Dwelling Parasite on the Behaviour of Common Bully

Brandon Ruehle & Robert Poulin

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The trematode family Diplostomidae contains many species that inhabit the eyes of fishes as metacercariae. Of these, members of the genus *Diplostomum* are often studied as potential manipulators that cause their fish host to be predated at a higher rate. Recently, the first diplostomid described in New Zealand, *Tylodelphys* spp., was found in the eyes of a native freshwater fish, the common bully *Gobiomorphus cotidianus*. *Tylodelphys* spp., as other congeners, resides in the humors of the eyes of its fish host until it is eaten by the definitive host, a piscivorous bird. Common bullies are the most widespread of New Zealand's native fish and are important prey items for predators (e.g. birds and eels). Our study investigates how infection by *Tylodelphys* spp. affects fish behaviors that rely on vision (e.g. predator avoidance, microhabitat selection, foraging). Fish were tested in aquarium conditions for their response to predators (using a computer animation simulating a shadow moving over the fish) and their preference for microhabitats (e.g. light vs dark). Prevalence of infection in our study site, Lake Hayes (South Island), is ~98% with a mean abundance of 8.7 parasites per host. Our results show that infection has no impact on reaction to a passing visual stimulus, but that higher infection levels are associated with increased activity and time spend in the light in immature fish (gonads not well developed).

25. Role of ecology and phylogeny in determining tapeworm assemblages is skates (Rajiformes)

Anusha Beer, Travis Ingram and Haseeb S Randhawa

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An understanding of the mechanisms that determine host and parasite relationships is a central aim in parasitology. Association of a parasite species with a host species may be influenced primarily by phylogenetic constraints that cause parasite species to co-speciate with their host species, or predominantly by ecological parameters that influence all other co-evolutionary scenarios. This study aimed to investigate the role of co-speciation as well as other co-evolutionary scenarios in influencing the assemblages of tapeworm parasites (marine cestodes) in skate hosts (Rajiformes) using a modification of the PACo (Procrustean

Approach to Cophylogeny) method. The study found that phylogeny and host ecology are both significant predictors of skate–tapeworm relationships, implying that co-speciation as well as other co-evolutionary scenarios are shaping these associations. The study also investigated the key ecological. The study also investigated the key ecological parameters influencing host-switching and found that host diet, distribution depth, average body size and geographical location have a combined effect. Given the importance of parasites in ensuring healthy and stable marine ecosystems, the findings of this study have implications for conservation management worldwide.

26. Parasites that travel along feeding links

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Parasites are important components of natural systems and among their various roles, they may strongly influence the flow of energy between and within food webs. Over 1000 tapeworm species are known to parasitise elasmobranchs (sharks, skates and rays), although full life cycles are resolved for fewer than 10 of them. This lack of resolution stems from our inability to distinguish larval from adult stages using morphology alone. Molecular elucidation of transmission pathways is the next step in understanding the role of hosts and parasites within food webs. We investigated the parasite assemblage of New Zealand's rough skate, *Zearaja nasuta*. Skates and their prey items (obtained from the skates' stomachs) were dissected for the recovery of adult and larval tapeworms, respectively. A fragment of the 28S rDNA region was amplified for all worm specimens with the aim of confirming species identity of parasites within rough skates and to uncover trophic transmission pathways that follow the predation links between rough skates and their prey. We identified seven species of tapeworms from the skates, belonging to four tapeworm orders. By matching gene sequences between larval tapeworms in prey and adult worms in skates, four trophic transmission pathways were resolved: three involving prey items from skate stomachs, and one involving a larval tapeworm sequence from a New Zealand sole. These findings contribute to our limited understanding of cestode life cycles as well as providing insights into the importance of predator-prey relationships for parasite transmission.

27. Exposure to a cyanobacterial toxin increases larval amphibian susceptibility to parasitism

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Anthropogenic activities are promoting the proliferation of aquatic primary producers in freshwater habitats, including cyanobacteria. Among various problems stemming from eutrophication, cyanobacterial blooms can be toxic due to the production of secondary compounds, including microcystins such as microcystin-LR (MC-LR); however, it is unknown

whether cyanotoxins can affect the susceptibility of aquatic vertebrates such as fish and larval amphibians to parasites or pathogens even though infectious diseases can significantly affect natural populations. Here, we examined how exposure to environmentally relevant concentrations of MC-LRs affected the resistance of larval amphibians (northern leopard frog, *Rana pipiens*) to infection by a helminth parasite (the trematode *Echinostoma sp.*), and whether this was manifested by reductions in host anti-parasite behaviour. Exposure to a relatively high (82 mu g L-1) concentration of MC-LR caused over 70% mortality, and tadpoles that survived exposure to the low MC-LR (11 mu g L-1) treatment had significantly higher infection intensities than those in the control; however, anti-parasite behavior was not affected by treatment. Our results indicate that MC-LR can have both direct and indirect negative effects on larval amphibians by increasing their mortality and susceptibility to parasitism, which may have implications for other aquatic vertebrates in eutrophic habitats dominated by cyanobacteria as well.

28. *Toxocara cati* is one cause for nematode *larva migrans* in kiwi (*Apteryx* spp.)

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Multiple cases of visceral and neural *larva migrans* have been diagnosed in kiwi (*Apteryx* spp.), but the specific organisms involved have not yet been identified. Histomorphology of the larvae suggests origin from the nematode genus *Toxocara*. PCR was undertaken using DNA extracted from archival formalin-fixed, paraffin-embedded lung and/or brain sections from five recent cases of histologically diagnosed nematode *larva migrans* in kiwi, using several different primer sets targeting the ITS-2 and 18S regions of nuclear ribosomal DNA. Sanger sequencing was performed on positive PCR products, including from organisms present in both the lung and brain of one bird. The results were subjected to BLAST analysis and all were found to align most closely with sequences from *Toxocara cati* present in NCBI**.** One case was PCR negative for all the nematode primer sets tested, although successful extraction of DNA was confirmed by use of kiwi-specific primers. This negative result may reflect either the quality or quantity of nematode DNA present in the section, or potentially the presence of a different organism not recognised by the primer sets used so far.

29. Comparing the accuracy of mini-FLOTAC and centrifugal faecal flotation for the detection of coccidia (*Eimeria* spp.) in kiwi (*Apteryx mantelli*).

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Coccidia (*Eimeria* spp.) in brown kiwi (*Apteryx mantelli)* cause significant morbidity and mortality in crèches and captive rearing facilities. Monitoring this parasite is crucial for successful management of kiwi. This research compares the sensitivity of centrifugal faecal flotations (CFF) with the mini-FLOTAC. We hypothesised that the mini-FLOTAC would detect higher oocyst counts. Kiwi faecal samples (n=10) were homogenised in $MgSO_4$ (SG 1.28) and distributed between three replicates of CFFs and mini-FLOTACs. For CFF, 0.5g of faeces were examined using standard methods. Mini-FLOTACs were prepared according to the manufacturer's protocol at a 1:20 dilution of faeces. Any oocysts present were then quantified using light microscopy at 100-200x magnification. A one-way ANOVA showed that the mini-FLOTAC method detected significantly higher [p=0.0281] oocyst loads than CFF. This increased sensitivity likely represents a more accurate estimation of parasite load and should be considered for use in routine diagnostics.

30. Abomasal nematode species differ in their response to exsheathment triggers

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Infection of ruminants by gastrointestinal nematodes is initiated with the exsheathment of the infective third stage larva. Exsheathment of various species can be achieved *in vitro* using carbon dioxide (CO_2), and recently it was shown that a rapid temperature change (heat shock) also played an important role in exsheathment in *Haemonchus contortus*. The current study set out to evaluate the role of CO_2 and heat shock in other abomasal species.

In CO_2-saturated rumen fluid, significantly higher exsheathment was obtained for all species in response to heat shock compared to slow temperature changes. In artificial buffer the effect of heat shock was species-dependent. Exsheathment of *H. contortus* and *Ostertagia leptospicularis* was similar to rumen fluid, but in *Ostertagia ostertagi* and *Teladorsagia circumcincta* exsheathment was significantly lower and/or slower in artificial buffer, and there was no benefit of heat shock.

The requirement for CO_2 was subsequently investigated using CO_2-depleted rumen fluid. Exsheathment occurred in *O. ostertagi*, *T. circumcincta* and *O. leptospicularis* (respectively 46 %, 22 % and 15 %). Exsheathment of these species also occurred in a CO_2-free rumen fluid-like buffer. For all species exsheathment was significantly higher in presence of CO_2, and for *H. contortus* exsheathment could only be achieved under CO_2-saturated conditions. Interestingly, the exsheathment response was dependent on the composition of the artificial buffer, indicating the involvement of co-factors, in addition to heat shock and CO_2.

Overall, even though these species all exsheath in the rumen, there appear to be significant differences in their response to exsheathment triggers. The data suggest an important role

for both heat shock and CO_2 *in vivo*, but *in vitro* their effects are dependent on the nematode species as well as the surrounding medium.

31. Relationships between CarLA IgA, productivity and parasites in deer

Richard Shaw & Jamie Ward

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Farmed red deer and wapiti-crossbreds are commonly infected with a range of parasites under New Zealand pastoral farming conditions. These include lungworm and gastro-intestinal nematodes. Very little is known about immune responses in deer to parasitic nematodes. Previous studies have shown that deer produce CarLA specific IgA (CARLA) responses in saliva following larval challenge. The CARLA response is heritable, h^2= 0.35 at 10 months of age and there is no unfavourable relationships between this trait and animal production traits.

A trial carried out in 2017 compared the CARLA responses in suppressively and minimally drenched deer from weaning until 10 months of age. The deer were produced from red and wapiti cross sires with high and low CARLA estimated breeding values (eBVs).

A subset of animals were saliva and faecal sampled fortnightly. In these animals faecal larval counts (lung worm L1s) were reduced in high CARLA deer throughout the trial. Faecal egg counts were reduced in high CARLA animals in autumn but were higher in spring. Presently CARLA is included in DEERSelect as research estimated breeding values (rBVs)

32. Identifying the genetic mechanisms that evoke parasitic exsheathment.

Nik Palevich

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In parasitic nematodes that invade the gastro-intestinal tracts of ruminants, larval exsheathment marks the transition from the free-living to the parasitic stages. Our aim is to determine the interplay between the genes and metabolic pathways that trigger infective parasitic larvae to naturally exsheath and become parasitic. *Haemonchus contortus* is one of the most common pathogenic nematodes infecting New Zealand small ruminants. The genome of a NZ *H. contortus* field strain, NZ_Hco_NP, has been sequenced to provide a complete catalogue of genes to be used as the reference dataset. At its current state the chromosome-level assembly of the NZ_Hco_NP genome contains 23,265 genes and is 561 Mb in size. To investigate larval exsheathment, a closed *in vitro* system has been developed that results in a high percentage of exsheathment (~90 %) in *H. contortus* L3's by effectively reproducing the two basic components of an anaerobic environment, predominantly CO_2 and rumen temperature of 39 °C. To improve our understanding of the fundamental genetics of

larval exsheathment, the transcriptomes of 100 samples of *H. contortus* L3's at different stages of the *in vitro* exsheathment process (47 time points over 24 h) have been sequenced using RNA-seq. The preliminary transcriptome data has revealed significant differences in the overall gene expression profiles of the parasite populations at different stages of the exsheathment process. The transcriptome analyses have revealed a total of 11,894 genes are either up- or down-regulated to some extent once triggers are applied. Interestingly, only 872 genes have been found to be statistically significantly differentially expressed (logFC ≥ 2, FDR ≥ 0.05) and are proposed to cause exsheathment. In its entirety this project has thus far identified the genes and metabolic pathways involved in larval exsheathment and will facilitate the identification of novel compounds that can be used to either trigger premature exsheathment or inhibit the process all together.

33. Liver fluke serum ELISA and milk production analysis in nine herds supplying Westland Milk Products

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Fasciolosis, infection with the liver fluke trematode parasite *Fasciola hepatica* causes liver pathology affecting livestock production. A 2017 study, using a commercial bulk milk ELISA antibody test (IDEXX Fasciolosis Verification), identified that the prevalence of *F. hepatica* in 430 seasonal milking herds supplying Westland Milk Co-Op was 57% (248/430) and had an in-herd prevalence of 20% or greater. An in-herd prevalence of 25% infection or greater is considered to cause production loss. Thus, the current study aims to calculate the milk production loss caused by infection with *F. hepatica* to determine the economic impact of infection.

Nine herds representing low, medium and high parasite burden as determined from the previous study were selected. In six large herds, 150 cows were sampled representing 24 to 38% of each herd. In the remaining three herds, all cows (136 to 169) were sampled. Cows were sampled once in the autumn of 2018 with a vat milk sample collected on the same day. Individual serum samples were analysed using an in-house antibody ELISA to determine the antibody titre with the expectation that a greater antibody response in the cow indicates a higher *F. hepatica* burden. Vat milk was analysed using the IDEXX Fasciolosis Verification test. Additionally, milk production parameters (volume, protein, fat, somatic cell count, days in milk and production per day) collated from four herd testing events over the lactation were used to determine the milk production performance of each cow.

I will present the preliminary data assessing the serum antibody and vat milk ELISA results of each herd. Milk production parameters of each cow will be assessed against the serum antibody optical density ratio to determine any correlation. This data will help to determine the economic impact of liver fluke infection in lactating dairy cows and indicate areas for further study.

POSTER ABSTRACTS

1. The RNA cargo of extracellular vesicles from *Trichomonas vaginalis*

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Trichomonas vaginalis is the causative agent of trichomoniasis, one of the most common non-viral sexually transmitted infection in humans worldwide. This extracellular protozoan parasite colonizes the urogenital tract by interacting with the host cells and surrounding microbiota. The communication between parasite\ and host might be mediated by the release of extracellular vesicles (EVs). *T. vaginalis* is known to produce EVs which carry proteins and RNA. In this study, we focused on the characterization of their small RNA cargo. *T. vaginalis* strain B7RC2 was used for EV isolation, purification and further experiments. Nanoparticle tracking analysis, polyacrylamide gel electrophoresis, and transmission electron microscopy were used to characterize EVs. For RNA deep sequencing, six libraries of small RNA size (15-50 nt) from three biological replicas times two technical replicas were constructed. Sequencing data analysis included reads quality check, adapters trimming, reads mapping against the reference genome, alignment of mapped reads into clusters revealing the most abundant clusters of transcripts and the most abundant fragments. We showed that *T. vaginalis* releases typical membrane-bound vesicles with an average size of ~100 nm, as previously reported. The electropherograms revealed that these vesicles are enriched for small RNA while the concentration of DNA was barely detectable. The population of small-size RNAs were consistent among libraries, particularly of tRNA fragments which were the most abundant RNA biotype in all samples. We identified individual sequences from the top 30 transcript clusters as being mostly tRNA fragments, particularly 5'-tRNA halves. RNAs from T. vaginalis EVs are delivered to host cells and tRNA halves can be readily identified from vesicles and host cells upon delivery of EVs. Our study indicates that RNAs from *T. vaginalis* EVs (particularly tRNA halves) might play a role in communicating with host cells.

2. Parasites and pathogens of cultured paua (*Haliotis iris*) in New Zealand

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Paua (*Haliotis iris*) is endemic to New Zealand and supports a small but growing aquaculture industry, which is potentially threatened by pathogens. This work aims to investigate the diseases of paua, to evaluate their health condition and identify the main pathogen threats. A combination of quantitative and semi-quantitative approaches were used to analyse tissues from 86 cultured paua adults (mean size 66.5±7.0 mm). Detailed observation of histological sections resulted in the identification of a number of parasites and pathogens, including *Perkinsus olseni* (prevalence 4.7%), ectocommensal ciliates (prevalence 55.8%), some of which were disintegrating due to possible viral hyperparasitism (prevalence 25.6%) and rickettsial inclusions (prevalence 9.3%) in a range of organs (gills, muscle, digestive gland and esophageal pouch).The prevalence and intensity of parasites varied greatly from one paua to another, and haemocytosis and brown cells indicative of immunological challenge were also observed. The trophozoite (multi and single cells) stages of *Perkinsus olseni* were observed both in swollen gills (prevalence 2.3%) and adductor muscle tissues (prevalence 3.5%). The highest prevalence (51.2%) of ciliates was recorded in gills. *Perkinsus olseni* appears to be the most important parasite and it was associated with severe mortalities. The ciliates are often associated with water quality issues and, rather than being significantly pathological, they are a useful indicator of culturing conditions. Rickettsia could possibly become a disease threat if the paua are subject to environmental challenge and overcrowding, so it is important to establish infection baselines such as this work has done. This study was carried out to gain an understanding of the characteristics of pathogen identification and immunological responses in New Zealand cultured paua (*Haliotis iris*). Thus, we envisage that this presentation will provide valuable information of the health condition of paua and lead to improved production.

3. Uncovering hidden diversity: The distribution and genetic diversity of microsporidians in New Zealand freshwater amphipods

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The phylum Microsporidia consists of intracellular parasites that infect some protozoans and almost all metazoan phyla including many species of economic importance for agriculture, livestock industry, and aquaculture. Considering their worldwide distribution and diversity (>1,500 described species), very little is known about them in New Zealand. In this project, we investigated the genetic diversity of microsporidians from several New Zealand freshwater amphipod species, which are important component of aquatic ecosystems, throughout both the North and South Islands by screening using a PCR based method. Partial small subunit ribosomal RNA region was amplified and a blast search of other available sequences was conducted. As a result, all microsporidian species discovered from this study were new species. Most of them belong to the genus *Dictyoecoela*, which is the most widespread among diverse amphipod species in Europe, Asia and North America. Both Maximum Likelihood and Bayesian trees showed that the *Dictyocoela* species from New Zealand form a distinct clade, different from other species that have been reported from the rest of the world. In addition to the genus *Dictyocoela*, several unique lineages (probably new genera) were discovered,

suggesting a hidden diversity of microsporidians in New Zealand that could be uncovered with more research effort.

4. Investigating effects of gastrointestinal nematodes on earthworms using a metabolomic approach

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Gastrointestinal nematodes in livestock are detrimental to animal health, reducing animal growth rates and costing New Zealand's pastoral sector $700 million dollars a year alone. While anthelmintic drenches can reduce parasite numbers, increasing resistance to anthelmintics worldwide may reduce their efficiency. Hence, finding alternatives for reducing parasitic nematodes in livestock are essential.

Previous studies have shown that parasitic nematode numbers are reduced when earthworms are present in the soil. The mechanism for this reduction is yet unknown but could be caused by either digestion in the earthworm gut or simply by mechanical abrasion from soil particles during passage through the earthworm gut. The objectives of this study was to A) determine gastrointestinal nematode mortality during passage through the earthworms gut and B) measure metabolites in the earthworms to gain insight into the mechanism of nematode mortality.

Earthworms (*Lumbricus rubellus*) were divided into two groups and fed either sheep dung infected with the gastrointestinal nematode *Haemonchus contortus* or non-infected sheep dung for 8 days. Nematode numbers were counted in earthworm casts. Polar metabolites were extracted from the posterior part of the earthworms and analysed by ^{1}H NMR spectroscopy.

Earthworms caused mortality of gastrointestinal nematodes during their passage through the earthworm gut: Nematode numbers in earthworm casts from feeding on infected sheep dung were reduced by 97-100%. Thirty metabolites were identified and quantified in the posterior earthworm extracts with alanine, succinate, glutamate, glutamine and glucose being the most abundant. No statistically significant difference in metabolite concentrations were found between earthworms fed infected and non-infected dung. These results suggest that the mechanism causing nematode mortality in the earthworm gut is likely to be of a mechanical nature (e.g. abrasion) rather than biochemical since metabolites in the host were not affected.

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