

Abstracts of papers presented at the 42nd annual conference of the New Zealand Society for Parasitology, Royal Society of New Zealand, Wellington, 19 – 21 October 2014

Symposium 1 “Living with parasites”

Regulation of the immune response by helminth parasites

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Type 2 immune responses are associated with protection against helminth infections, as well as the pathology of allergic diseases that are initiated against innocuous antigens. Despite some of the key factors for Type 2 immunity having been identified over 30 years ago, the underlying mechanisms for initiating and maintaining these responses remain to be elucidated. Two of the integral cytokines associated with Type 2 immunity are interleukin IL-4 and IL-13, and observing when and where these cytokines are produced is important to understanding how to contribute to the overall response. The 4C13R transgenic dual reporter mouse has been created to allow identification of IL-4 and IL-13 producing cells by the production of two intracellular fluorescent molecules, AmCyan and DS-Red respectively. This technology allows for the analysis of *in situ* IL-4 and/or IL-13 production by the relevant differentiated immune cell types without any effect on the endogenous cytokine genes or their effector activities in the mouse. Using this reporter system we have identified cells of both the adaptive and innate branches of the immune system in both the skin and lung. Studies will be presented that reveal the dynamic behaviour of these cell types in the context of immune responses regulated by helminth parasites.

Faecal microbiota transplantation for recurrent *Clostridium difficile* infection.

Dr Brendan Arnold

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Clostridium difficile is a gram-positive, spore-forming anaerobic bacillus. It is responsible for approximately 10% of hospital-associated diarrhoea. *C. difficile* infection can range from asymptomatic colonization to severe diarrhoea, pseudomembranous colitis, toxic megacolon, intestinal perforation, and even death. It has strong associations with hospitalisation and prior antibiotic use. Its incidence has increased rapidly in the past decade, to epidemic levels in some international settings. Recurrence is a common management problem and occurs in up to 20% of patients after initial treatment with metronidazole or vancomycin. Repeated and extended courses of vancomycin are often prescribed, but many patients either do not respond or promptly relapse once therapy is ceased. This is due to the persistence of *C. difficile* spores in conjunction with a profound disturbance of the intestinal flora. Faecal microbiota transplantation (a.k.a. “stool transplantation”) has now been shown to be a highly effective method for restoring the diversity of the intestinal microbiota, and is gaining acceptance as the most effective treatment for recurrent *C. difficile* infection. This presentation will briefly review the history of faecal microbiota transplantation, the evidence supporting its efficacy, and our experience with the procedure at Wellington Regional Hospital.

Gastrointestinal parasites: fact and fiction

Dr Ian Wilson

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The relationship between parasites, disease and ill health spans a continuum from accepted science through pseudoscience and beyond to quackery.

I will review the common and not so common parasites that present in my clinical practice and contrast that with my observations of the role that parasites play in alternative medicine.

Giardia and Cryptosporidium are almost exclusively the parasites which cause diarrhoea in those patients who have not travelled overseas. A thorough clinical history can help identify patients at risk of parasite infection.

Conventional laboratory testing is discussed in comparison to the commercial "over the counter" comprehensive stool analysis.

Maggot (*Lucilia sericata*) debridement therapy in equine wound management

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Maggot debridement therapy (MDT) has undergone a resurgence in human medicine recently coinciding with an increasing incidence of multiresistant bacterial wound infections. Its use in veterinary medicine has been more sporadic without clear indications when its use might be an advantage over conventional treatments. A rising incidence of multiresistant infections is also being reported in horses and other pets, including MRSA. Pressure from the medical colleagues for tighter control of antibiotic uses supports a more holistic approach in dealing with wound infections that makes MDT attractive. The equine patient presents several unique challenges for the practitioner when dealing with deep and distal limb wounds compared to smaller animals that could indicate a further niche for MDT as an adjunct to conventional therapy for certain wounds. There is already good evidence for its use in chronic foot wounds associated with laminitis and deep infections. However, more controlled studies for maggot use in the veterinary field are required before it becomes more widely accepted as part of the standard wound management armoury rather than a last resort.

You want to put maggots on my wound!

Catherine Hammond

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Larval therapy is not a new concept and been used for centuries, however, it is only more recently that access to sterile maggots has been available. Their purpose in the clinical setting is to assist with debridement of chronic wounds. The larvae are very specific in their diet, acting like micro-surgeons, they munch their way

through slough and necrosis while leaving viable tissue intact. Therefore, they are very precise and efficient in their debridement. What's more they are less likely to cause pain, are convenient for patients, speed up the debridement process, inexpensive and easy to access throughout New Zealand.

This presentation will provide an overview of using larval therapy in the clinical setting of a community specialist wound service.

Contributed Papers

Survey of *Toxoplasma gondii* sero-prevalence in the domestic cat population in New Zealand

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Toxoplasmosis is caused by the protozoan *Toxoplasma gondii*, and is a potentially fatal disease that can affect any warm-blooded species. Over the last five years, we have identified both fatal and chronic infections in the endangered Hector's dolphins and several species of native birds. In addition, current studies are suggesting the *T. gondii* is playing a significant role in poor reproductive performance in farmed deer. As the definitive host, cats are the only source of infectious oocysts, which they shed in their faeces. Although assumed that there is a high rate of *T. gondii* infection in the New Zealand domestic cat population, there has been no seroprevalence studies performed in New Zealand. Thus, we collected 200 domestic cat serum samples from around the country and screened them for *T. gondii* antibodies using two commercial assays; Eiken Latex Agglutination Test (LAT), Chekit ruminant *T. gondii* IgG ELISA, and an in-house IgG Western blot. Using the Western blot as a gold standard, the sensitivity of the LAT and ELISA were equivalent at 95%, however, the specificity was markedly better with the ELISA than the LAT (63% and 35% respectively). While the negative predictive values of the LAT and ELISA tests were high, 90% and 94%, respectively, the positive predictive values were relatively low, 52% and 66%, respectively. Therefore, based on the Western blot results, the overall prevalence was 43% cats sampled, slightly (but not statistically significantly) higher in the South Island. These results suggest that almost half the domestic cat population in New Zealand has been exposed to *T. gondii*. Once infected, these cats may shed oocytes into the environment and provide a possible source of infection in both marine and terrestrial environments, posing a risk not only for marine species but also for humans, livestock and terrestrial endemic species.

Immunomodulators from gastrointestinal helminth parasites

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The incidence of helminth parasite infections worldwide is inversely correlated with allergic and autoimmune disease prevalence. There are ongoing epidemiological and clinical studies looking into the downregulation of immune responses by the active induction of regulatory processes by helminths. *Heligmosomoides polygyrus* is a widely used laboratory mouse model of gastrointestinal helminth infection, and can survive

chronically in its host through the induction of regulatory lymphocytes and antibodies, that dampen the immune response against it. Through proteomic examination of the excretory-secretory products of the adult stage of the worm, several candidates for its immunomodulatory properties have been studied including a homologue of the regulatory cytokine TGF- β , and several novel proteins which are currently under investigation.

ILCs and CD4 T cells co-operate to maintain AAM activation in *Nippostrongylus brasiliensis* lungs

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Establishing sterilizing immunity to helminth nematodes through vaccination is currently a major global health objective. Amongst the helminths infecting humans, hookworms currently infect an estimated 1 billion people, and are considered to be the leading cause of anaemia worldwide. To date, the gut immune response has been considered as the principal source of protection against geohelminths but data is emerging that other tissue sites including skin and lung could also be important. To date, little information is available concerning the specific components of the immune response that confer resistance or immunity to hookworms, principally due to the absence of an adequate model. We use the closely-related rodent parasite *Nippostrongylus brasiliensis* to model the early stages of hookworm infection that may confer subsequent immunity. Using gene deficient mice, truncated infection studies and fluorescent labelling of the worms, we show that the lung is the major protection site against *Nippostrongylus brasiliensis* infection. Furthermore, we have identified a novel developmental defect in the worms occurring during the moult 3 process which is strongly dependent on STAT6-mediated immune pathways and that is associated with the acquisition of protective immunity in the lung. We further show that this protection is mediated by alternative activation of interstitial macrophages, themselves maintained by CD4 T cells and ILC2s. The implications of these findings in the development of a vaccine against hookworm will be discussed.

Presence of *Toxoplasma gondii* DNA in commercially grown shellfish

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Toxoplasma gondii is a parasite of significant medical and veterinary importance worldwide and infects approximately 43% of New Zealanders with prevalence increasing with age. *T. gondii* infection can be life-threatening in immune-suppressed or congenitally infected patients, may alter personality attributes and is also a risk factor for the development of schizophrenia and depression in humans. Until recently, it was thought that most people and mammals became infected by accidental ingestion of the parasite in undercooked meat, soil or cat litter. However, our studies and those from overseas suggest that waterborne infection may be a more significant transmission pathway in many mammalian species, including humans, than previously recognized. Therefore, we hypothesise that *T. gondii* oocysts, shed by domestic cats (the only known definitive host in New Zealand), enter the marine environment in freshwater runoff and are concentrated by natural processes in coastal sediment. These oocysts are then consumed directly or indirectly by marine invertebrates, which are then ingested by marine mammals and humans. We sampled live commercially grown greenlip mussels from

five supermarket stores (four in Palmerston North and one in Raglan). DNA was extracted from mussel hemolymph and tested for the presence of *T. gondii* DNA using nested PCR. On average, 21% of mussels (12/56) tested positive for *T. gondii* DNA. 45% of mussels tested from one store were positive, whereas two other stores had fewer numbers positive (25% and 33%) and no mussels were positive from two stores. Genotyping is underway but proving difficult. The results of this study suggest that farmed shellfish could be an important route of infection for humans. Further studies are needed to determine if the shellfish are infected prior to harvesting through environmental contamination and whether the presence of *T. gondii* DNA equates to viable parasite able to be transmitted to mammals.

Recombinant *Haemonchus contortus* enolase mimics the increase in permeability of Caco-2 cell monolayers induced by adult worm excretory/secretory products

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Pathophysiological changes in the abomasum coincide with the presence of adult worms in the lumen and are likely to be initiated by worm chemicals (Excretory/Secretory (ES) products) acting on the surface mucosa. It is known that ES products increase the permeability of Caco-2 cell monolayers, but the active components remain unknown. Enolase, an enzyme released into ES products *in vitro* by many pathogens and nematodes, is a good candidate, as it binds to several host proteins in addition to its primary role in glycolysis. Recombinant *H. contortus* enolase was expressed in *E. coli* and adult *H. contortus* ES products were prepared by incubation in Dulbecco's Modified Eagle's Medium. Purified recombinant *H. contortus* arginine kinase (*HcAK*) was used as a control worm secreted enzyme and bacterial lysate solution without enzyme as a control for the expression system. Caco-2 cell monolayers were grown to confluence on Transwell plates and had a minimum Transepithelial Resistance (TEER) of 400 Ω .cm². Both ES products and 10 μ g.ml⁻¹ enolase significantly ($p < 0.001$) reduced TEER after 2h to 81.1 \pm 1.0% ($n = 10$) and 82.9 \pm 1.1% ($n = 10$) respectively. Corresponding control values were (a) *HcAK* 100.0 \pm 0.6% ($n = 4$); (b) negative control 98.4 \pm 0.3% ($n = 4$) and (c) culture medium 98.9 \pm 0.3% ($n = 8$). Immunohistological staining of the transwell membranes for the tight junction proteins ZO-1 and occludin confirmed that the junctions remained intact in control medium, but these proteins were internalised from disrupted junctions after exposure to ES products. These experiments demonstrated increased permeability of epithelial tissues exposed to worm chemicals, which could be mimicked by the secreted enzyme enolase. Active permeabilisation of the abomasal surface mucosa would explain how cells deep in the glands could be targeted by adult worms within a day of transplantation into naïve hosts.

***Nippostrongylus brasiliensis* blood feeding and the implications for human and ruminant hookworms**

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Nippostrongylus brasiliensis (Nb) is a natural parasite of rodents, and has been extensively used as a model of type 2 immune diseases, but not as a parasite disease model *per se*. The One health initiative proposes to unite veterinarian and medical research. Here we go one step further by proposing *Nippostrongylus brasiliensis* as a laboratory model to study vaccine development for both the human hookworm *Necator americanus* and the ruminant hookworm *Haemonchus contortus*.

Nb was ruled out as a good model of hookworms a few decades ago, under the assumption that this parasite was not blood-feeding. Contrary to this initial assumption, we show that *Nb* ingest blood at all stages in a host, forming a characteristic dark pigment in the worm. Furthermore, we demonstrate that blood-feeding is one of the first cues of development for the infective L3.

Thus, *Nippostrongylus* vaccine development research could be translatable to blood-feeding parasites of both human and veterinarian importance.

Future of Parasitology

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There are various aspects to be discussed: Sociology, Funding, New lines of Research, New Technologies Global Food Security. The important outcome is to bring the public into science work, to package the science so as to be understood by the public and to weigh anticipated outcomes on Benefits rather than on Impact Factor. **SOCIOLOGY**: There will be larger teams, with open sharing of data and more International work. There will be convergent science fields with emphasis on quantitative biology, functional genomics and the other “omics”. There is a need to establish networks, bring back-up personnel together and start collaborating with mathematicians. New Parasitologists will need to find a suitable niche, and to understand and work with cultural barriers within NZ and throughout the world. You need to be known for something! There will be many more non-Anglo researchers. There will be more women parasitologists. There is a need for passionate women to drive large collaborative research programmes. There is a need for a common simple language to translate research findings for the general public. STEM education to create a more scientifically and technologically engaged public, and a more publicly engaged science sector. **FUNDING**: Who are your competitors? – work with them! Government Competitive funding can be a minor source. There are philanthropists, crowd funding, Biotechnology big pockets. Either ask broad questions with a less-defined outcome or be realistic about your aim, keeping it small and achievable, but pointing out the opportunities that will open. **NEW LINES OF RESEARCH**: Effects on Parasitology of Climate change, Carbon pricing, Sustainable energy, Biofuels, Marine farming. **NEW TECHNOLOGY**: Delivery of biologics. Forward genetic screens, Mass spectrography for multiple antibody interactions, Nano-biotechnology, Nano diamonds, Genomic epidemiology(RNAi), B.t. crystal proteins to target nematodes. **GLOBAL FOOD SECURITY**: to cope with human population doubling within 40 years.

Symposium 2 “Advances in Parasiticide Chemistry”

Insecticide resistance in sheep blowflies: the Australian and New Zealand experiences compared

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Any attempt to protect sheep against ectoparasites by applying insecticides to their fleece creates an environment where selection for fitness to withstand the insecticide occurs within the genetic architecture of the parasites. Those with genes or point mutations that aid in detoxifying the active ingredient of a dip will survive to reproduce, while parasites without such a genetic shield are eliminated. Thus resistant populations gradually appear and will predominate while the selective agents remain.

Australia and New Zealand share a heritage of insecticide resistance in their blowfly populations that dates back around 60-70 years. Australia (merinos predominately, with a high lanolin component, an almost exclusively single species flystrike fauna, single dipping and a generally arid climate in sheep-raising regions) has always shown evidence of resistance in *Lucilia cuprina* before New Zealand. This country, with a high cross-bred component in its sheep flock (lower lanolin levels), two dominant flystrike-causing species, multiple dipping and a wet-temperate climate, has detected insecticide resistance, after, and sometimes long after, it was seen across the Tasman.

Up until now there has always been another new chemical group available to allow the continued reliance on chemical warfare against blowflies, but this may not always be the case, especially given the cost of developing new products. Out of ten major chemical groups used historically, only four are currently fully effective.

All is not gloom however, as it is still possible to protect sheep against flystrike, albeit in a more labour-intensive fashion, by reducing their attractiveness to gravid female flies, by employing fly traps and utilising forages and topographical features to deter fly activity, without necessarily abstaining from insecticide use.

Advances in Commercial Parasitology

Sarah Weston

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Bayer has a proud history in parasitology having developed active ingredients such as praziquantel, toltrazuril and flumethrin. Today, Bayer continues this tradition with a state of the art parasiticide screening cascade which in its early stages utilises electrophysiological technologies co-developed with Bayer crop science to investigate activity at ion channels specifically of animal health interest. New chemistry takes a long time and investment to bring to market and does not always deliver as hoped. Advances in formulations can mean faster entry into new markets and new claims that give strategic advantage over competitors. Bayer's overarching theme is "Science for a better Life" and one of the areas where this is applied within animal health is in science

based marketing approaches developed to support the chemistries and formulations once they are launched and throughout their lifecycle.

Use of an injectable persistent anthelmintic formulation in calves with a mixed nematode infection demonstrates two separate reductions in faecal egg output over time

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The immune system of cattle becomes fully competent during the latter stages of pregnancy. Despite this, parasitic nematodes appear to be able to evade the immune response often for the duration of the first season at grass. The challenge of the naïve host with the burden of parasitic worm larvae often experienced under intensive grazing conditions appears to either overwhelm the innate immune response or modulate the acquired immune response so that survival, growth and reproduction of the parasite can occur.

This prospective controlled faecal egg count, larval culture and worm count study involved the use of an injectable moderately persistent macrocyclic lactone formulation in a group of calves infected with both a natural and artificial mixed burden of strongyle parasites. From the time point when the persistent efficacy of the anthelmintic was deemed to have ceased the faecal egg count began to rise which was indicative of a period of rapid re-establishment of the parasite population. There followed a marked fall in faecal egg output coincident with a peak in the rate of liveweight gain. It is hypothesised that after the treatment had eliminated the resident susceptible nematode population the pharmacokinetic profile of the formulation prevented parasite re-establishment for a period of time sufficient for the host to mount an immune response to the incoming infection facilitating a period of natural parasite control by the host. The use of anthelmintic formulations that facilitate the host's ability to control nematode parasites may allow the treatment interval between anthelmintic administrations to be extended beyond that currently governed by their pharmacokinetic profile, reducing anthelmintic use and allowing for a period of refugia for anthelmintic susceptible parasites, a mechanism to slow the development of anthelmintic resistance.

Contributed Papers

The effect of long and short-acting moxidectin on the CARLA response under field conditions

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The saliva CarLA specific IgA (CARLA) in immune sheep is modulated in part by the number of L3s ingested. Anecdotal evidence suggests that the use of anthelmintics has minimal direct effect on the saliva CarLA specific IgA (CARLA) response in sheep however this has not been empirically tested. Utilising lambs grazing under field larval challenge, we tested the effect of using long and short-acting drench formulations (Moxidectin) on the CARLA response. For most of the trial, treatment with either oral or injectable moxidectin had minimal effect on the measured CARLA response. The results from this trial suggest that saliva sampling

for CARLA after treatment with moxidectin and probably other anthelmintic families will have little if any impact of the CARLA response. Changes in weather over winter, principally in soil temperature are likely to have an impact on day-to-day CARLA responses.

The use and limitations of diagnostic tests including worm counts, faecal egg counts and larval cultures in evaluating nematode parasitism and effectiveness of therapy.

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Worm counts including the identification to genera and species are considered the definitive test for assessing the effectiveness of an anthelmintic treatment, or determining a treatment effect such as its persistent activity (persistence). Typically worm counts are quite laborious, expensive, and normally require the sacrifice of the animal. They usually are not a "total" worm count but an aliquot (such 2% or 5% sample) of a known amount of both the contents and washings of a gastrointestinal compartment, so worm genera present in low numbers can be missed. Faecal egg counts are the most commonly performed diagnostic test to determine anthelmintic effectiveness in the field, but this fails to identify worm genera and there is not always a good correlation between egg counts and worm counts except in relatively young immunologically naïve animals such as lambs. Conventional larval cultures in combination with egg counts do assist to identify worm genera present including those that have survived treatment, but do not give a guide as to how many such larvae are present. Quantitative larval culture where the larval numbers recovered from a known amount of faeces (often pooled by treatment group, typically 50g of faeces) and shown by worm genera has increasingly been found to be a very useful tool. The quantitative larval culture test has greater sensitivity than current egg count techniques and it has been found that it can detect low number of worms that it may not be found in a 2% worm count. This greater sensitivity allows detection of small numbers of worms surviving (early detection of resistance), and even subtle differences between treatments. It is also particularly useful in detecting low egg producing worm genera such as *Ostertagia* in yearling or older cattle post treatment, or detecting resistant worm strains in older more immune sheep. Such cultures when conducted at regular intervals over time after treatment are useful to demonstrate persistent activity, particularly when virtually all the worm population can be removed initially (virtually 100% therapeutic kill) and there is evidence of ongoing infection. The time of the protection period against incoming larvae is determined by the time that worm genera re-establish egg production and is inferred or back calculated based on known prepatent periods (time from infection to egg production) for the particular worm genera. Examples of the sensitivity of quantitative larval culture relative to common conventional diagnostic methods, as well of examples of its use in determining persistent activity periods in sheep (moxidectin oral) and pour ons in cattle are shown. Determining persistent activity and the importance of establishing the correct retreatment intervals for a product on farm is discussed.

Infectivity of sheep and cattle gastrointestinal nematodes in deer

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Red deer can be infected with some gastrointestinal nematodes (GIN) of cattle and sheep but it is unknown to what extent. Two indoor studies were conducted to determine the establishment rate of sheep and cattle GIN in young deer. Group sizes were n=5 in both. All animals were effectively treated when housed and then infected two weeks later. After four weeks they were killed for total worm counts. Establishment rates were assessed comparing worm counts to the infective dose which were identified morphologically.

Sheep-origin GIN: The establishment rates (%) in sheep and deer respectively were *Haemonchus contortus* (18.6, 10.5, p<0.05), *Teladorsagia circumcincta* (35.5, 1.0, p<0.0001), *Cooperia curticei* (30.7, 0.1, p<0.0001), *Trichostrongylus* spp. (74.9, 1.0, p<0.0001) and *Oesophagostomum*+*Chabertia* spp. (19.9, 4.8, p<0.05). No *T. colubriformis* or *T. vitrinus* were seen in deer but were present in all sheep. No *C. ovina* were seen in any deer but were present in four of five sheep in low numbers.

Cattle-origin GIN: The establishment rates (%) in cattle and deer respectively were *H. contortus* (8.0, 19.0, p=0.18), *Ostertagia ostertagi* (31.0, 0.7, p<0.001), *Cooperia* spp. (72.0, 2.3, p<0.001) and *Trichostrongylus* spp. (19.0, 25.3, p=0.12). The majority (>98%) of *Trichostrongylus* spp. were *Trichostrongylus axei* in both hosts. In cattle >98% of *Cooperia* were *Cooperia oncophora* but in deer there were similar proportions of *Cooperia oncophora*, *Cooperia punctata* and *C. curticei*. Small numbers of *Oesophagostomum venulosum* were also present with 3X as many proportionally found in deer as in cattle (p<0.05).

Some sheep- and cattle-origin GIN can establish in red deer. The establishment of *H. contortus* and *T. axei* could allow sufficient burdens to build up to be clinically significant. *O. venulosum* is able to establish reasonably well in deer but it is considered of limited pathogenic significance. Importantly, almost no *Teladorsagia/Ostertagia* species or small intestinal species established in deer.

Cross Grazing with Sheep or Cattle for Gastrointestinal and Pulmonary Nematode Control in Deer

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The aim of this trial was to determine the effectiveness of an organised cross-grazing system between deer and sheep or cattle in controlling deer nematode parasitism.

This was a replicated study over two years and in two locations. There were four treatment groups of 19-20 deer at each location: deer cross-grazing with calves (DC); deer cross-grazing with lambs (DS); deer-only grazing (DD); and deer grazing on their own being "suppressively treated" with anthelmintics (SP). The key outcome was the number of anthelmintic treatments (NT). The decision to treat deer was based on "trigger" criteria including, faecal egg counts ≥ 250 epg or faecal larval counts ≥ 100 lpg or when the individual growth rate was < 80 % of the mean of the SP group. In addition, two sets of three parasite-free "tracer" deer per treatment were introduced, to quantify the species of parasites cycling in each treatment. The NT for the DS and DD groups was significantly greater than for the DC group (p<0.0001). In tracer animals there were significantly fewer *Trichostrongylus* spp. from the DC, DD and SP (p<0.0001) than in the DS group, and

significantly fewer *Ostertagia*-type nematodes in the DS ($p=0.017$) and SP ($p=0.004$) than in the DD group. The DC and SP groups had significantly higher daily liveweight gain ($p<0.001$) than the other two groups. Results from this study demonstrated that cross-grazing with alternative ruminant species offered some advantages over a monoculture of young deer. However, the advantages in the ability to control different parasites species in young deer varied between the use of sheep or cattle.

The effect of formulation and route of administration on control of ML resistant *Ostertagia* in cattle

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A trial comparing the effects on the route of administration of abamectin in 72 yearling cattle was conducted in Nov 2013 on a Waikato farm with known ML resistant *Ostertagia* and *Cooperia* worm strains. Abamectin treatments included commercial oral, pour on (PO) and injectable (Inj) formulations, all dosed at standard label dose rates. Two different types of abamectin pour on (vegetable oil or solvent based) and two combined abamectin+levamisole pour on formulations (Eclipse pour on, Duo pour on) were also compared. Based on combined larval recovery at Day 10 and 19 post treatment (N=8 per group) the most effective formulations in reducing numbers of *Ostertagia* in larval culture were half dose Duo PO (21 Ost larvae/50g), Aba Inj, Duo PO (full), Eclipse PO (full), Aba PO (solv), Aba PO (oil), Eclipse PO (half), Aba oral (1001 Ost larvae/50g). Controls 3300 Ost larvae/50g. The egg reductions generally followed this trend. A second trial was conducted 7 days later using faecal positive animals from the first study reallocated by egg count into 3 groups and treated with separate solvent based Aba and Lev pour ons. These were either applied simultaneously at a full dose, or both applied at a half dose, or both at a full dose but with the Lev applied 24 hours later. The order and numbers of *Ostertagia* larval recovered post treatment at Day 16 post treatment was similar to those observed for Duo PO. The half dose was most effective (22 Ost larvae/50g), followed by full dose but Lev application delayed by 24 hours (41 larvae/50g), followed by the full dose applied simultaneously (51 larvae/50g). A possible explanation for these observations, differences between routes of administration, and the impact of formulation on pour on effectiveness including implications in the selection for ML resistance worm strains is discussed.

An epidemic of *Theileria* associated bovine anaemia in NZ cattle

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In late 2012 outbreaks of *Theileria* associated bovine anaemia (TABA) were reported in dairy and beef cattle herds located in multiple regions of New Zealand. For most of these outbreaks no other causes of anaemia were identified. As a result, *T. orientalis* in combination with other unknown risk factors was considered to be the aetiological cause.

Strain typing of *T. orientalis* was carried out on samples collected from outbreak herds and one of the strains was identified as *T. orientalis* Ikeda type. The Ikeda type has been determined to have been responsible for recent outbreaks of anaemia in cattle in Australia. As in New Zealand, *T. orientalis* strains were considered to be largely non-pathogenic in Australia prior to recent outbreaks. Analysis of data from the New Zealand outbreaks showed that there was a greater likelihood of *T. orientalis* Ikeda type being present in cattle from herds experiencing outbreaks of anaemia compared to non-diseased herds. In addition, individual animals within an affected herd were more likely to be anaemic if the Ikeda type was present compared with animals with endemic strains of *Theileria*.

Since the initial diagnosis of TABA cases have continued to occur. Initial predictions were that the epidemic was going to be significantly greater this spring this year than last. Thus far there have been a greater number of cases; however, less than that predicted from computer modelling. Possibly some of the disparity may relate to under reporting or non-detection. On some farms there have been significant impacts particularly where cattle have been shipped from areas where there is not an endemic population of the tick vector. This presentation will describe some the tools developed for control and the spatial and temporal trends in the outbreak to date.

A cross-sectional survey of equine parasite control practices on stud farms in New Zealand

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There is a lack of specific data regarding parasite control practices and anthelmintic resistance status on stud farms in New Zealand. The objective of this study was to describe the current parasite management and control practices used for horses on stud farms in this country. Data were collected using an online survey, which was available from April to May 2014. A link to the survey webpage along with details of the survey was sent to Thoroughbred and Standardbred breeders via e-mail, and was available on the Standardbred Breeders' Association and Harness Racing New Zealand webpages.

In total, 127 respondents completed the survey, of which 80% of respondents were involved Thoroughbred breeding industry. Only one respondent reported that they did not drench their horses to control parasites. Most stud masters (86%; 97/113) treated horses with oral pastes and 64% (73/113) of stud masters spent more than \$50 per horse per year on treatments. The median number of treatments per year for youngstock, dry mares and wet mares was 6 (IQR 5-8), 4 (IQR 3-6) and 3 (IQR 3-6.5), respectively. Overall, 49% (55/112), 33% (37/112) and 34% (38/113) of stud masters were using interval drenching for youngstock, dry mares and wet mares, respectively. In total, 57% (58/102), 42% (42/99) and 38% (38/102) of stud masters were drenching youngstock, wet mares and dry mares every 6-8 weeks. 28% (31/112) of respondents were rotating anthelmintics randomly, whilst 17% (19/112) were rotating every 2-3 months. Most respondents (65%; 73/113) reported getting advice from veterinarians regarding anthelmintic treatments for their horses, and 20% (21/107) of respondents had previously conducted a faecal egg count on their horses. The results showed that control

practices such as frequent drenching at short intervals and rapid rotation of products were common on stud farms in New Zealand.

Off label use of anthelmintics in equines – a case study

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Anthelmintics are frequently given off-label to horses. One example is the use of injectable moxidectin given orally to horses. This had been the main anthelmintic used in a group of horses on one property for some time. Fifteen out of 29 adult horses on this property were given oral doses of the long acting moxidectin injectable for sheep at 400µg/kg and resampled three weeks later. At this time faecal egg counts were only reduced by 72%, suggesting either that the pharmacokinetics were sub-optimal or that anthelmintic resistance may be an issue. To check whether resistance was an issue, 26 of the horses were later treated with ivermectin in a registered paste formulation at 200µg/kg and resampled 7 and 22 days later. The ivermectin was 100% and 99.5% effective at reducing egg shedding at both time points respectively.

These findings show that the inappropriate use of anthelmintic formulations may be associated with inadequate efficacy. Given the history, regular 'underdosing' of the animals with a persistent anthelmintic had likely occurred on this property, but overt resistance did not yet appear to be an issue. Interestingly, on the day the horses were treated with moxidectin and 12 weeks later with ivermectin, the average egg counts were 1400 and 400 epg respectively and 0/15 and only 4/26 had egg counts less than 200epg. These findings are at odds with the general observation that in any group of horses the majority of the egg output stems from a minority of animals (Kaplan and Nielsen, 2010) and may suggest that after using an ineffective anthelmintic for a prolonged period of time, the level of pasture contamination had become high ensuring that most animals were becoming heavily parasitized, or were at least now shedding reasonable numbers of eggs.

Kaplan RM, Nielsen MK. An evidence-based approach to equine parasite control: it ain't the 60s anymore. *Equine Veterinary Education* 22, 306-16, 2010

Skin lesions in New Zealand grey mullet caused by the parasite *Myxobolus*

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In November 2013 the Animal Health Laboratory, Ministry for Primary Industries, received a grey mullet (*Mugil cephalus*) presenting reddish-white granular lesions across its body. Gross pathological examination revealed cyst-like lesions on the distal portion of the scales and proximal part of the fins. Wet preparations presumptively identified spores as *Myxobolus* sp. DNA sequencing of the 18S rRNA gene further identified these spores as *Myxobolus episquamalis*. This is the first report of *Myxobolus episquamalis* infecting

M. cephalus from New Zealand, however, the myxosporidian has a cosmopolitan distribution so the finding is not unexpected.

Diet and Diversity: Breadth of Diet Influences Tapeworm Richness in Sharks

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Host diet is often thought to have a large influence on parasite diversity. The expected pattern is that hosts with broad diets will harbour more parasite species than those with restricted diets. However, this pattern has been supported by few empirical studies. The present study investigated the influence of diet breadth on tapeworm species richness in sharks by compiling available records in literature. A linear mixed-effects model was used to analyse the effect of host diet breadth on tapeworm species richness relative to several other potentially important factors, including host size, phylogeny, trophic level, latitudinal range, depth range, and depth mid-point. Tapeworm species richness was found to be positively associated with breadth of diet in sharks, and in the analyses, breadth of diet was a better predictor of tapeworm species richness than any of the other host characteristics examined. Overall, this study provides support for the notion that a host species' diet can have important consequences for the diversity of its trophically transmitted parasites.

Sharp global declines in shark populations: implications for marine tapeworm biodiversity

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Globally, there are widespread declines of top predators in oceanic ecosystems, particularly sharks. Despite shark species being described at unprecedented rates, shark populations are declining on average by 11% per year globally. Hence, there is a likelihood of shark species going extinct prior to their discovery. Considering that only 30% of shark species have been examined for parasites, the tapeworm biodiversity of sharks is likely to exceed the 1800 estimated unknown tapeworm species yet to be described from known sharks. The objective of this study was to assess the impact of steep global declines in shark populations on tapeworm discovery and biodiversity. Generalized linear and linear mixed models were used to: (1) calculate the average and 95% confidence intervals for discovery rate of tapeworms from sharks; (2) estimate the global tapeworm diversity from shark hosts; (3) identify predictors influencing the year of discovery of tapeworm species from shark hosts; and (4) identify the predictors influencing the time lag between descriptions of sharks and their tapeworms. Data indicates that we are currently in the midst of an increasing rate of tapeworm discovery and that the cumulative frequency distribution curve for these parasites in sharks is far from reaching an asymptote. Furthermore, larger tapeworms tend to be discovered prior to small ones and host features are most important in explaining variation in time lag between sharks and their tapeworm parasites. Unless further global biosystematics and conservation initiatives are undertaken in the near future, we are at risk of losing small host specific tapeworm taxa prior to their discovery, in large part due to the demise of their shark hosts. This potential loss of biodiversity may hinder efforts to better understand the ecological roles these tapeworms play in our marine ecosystems and how they contribute to the resilience of marine food webs.

Poster

***Trichostrongylus askivali* – a new record for alpacas in New Zealand**

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A range of different species of gastrointestinal parasites have been identified as being present in alpacas in New Zealand. During the period between July and September 2013, three young alpacas from 2 separate properties were submitted for necropsy. All were identified as having gastrointestinal parasitism as a primary diagnosis. A range of different nematode species was found in all three including *Haemonchus contortus*, *Camelostrongylus mentulatus*, *Teladorsagia circumcincta*, *Trichostrongylus axei* and *Cooperia oncophora*. An additional species was found in low numbers in C3 of all three being *Trichostrongylus askivali*. On one property a range of 10 animals of varying ages were faecal sampled and all with a positive egg count were cultured to retrieve 3rd stage larvae. Forty eight larvae which were identified morphologically as being *Camelostrongylus*, *Teladorsagia* or *Trichostrongylus* were selected for PCR analysis. Using a primer set developed to be specific for the ITS2 region of *T. askivali* (Tapia-Escárate unpub) 3 larvae were identified as *T. askivali*. These results confirm the presence of this species and indicate that a further species has been confirmed as a parasite of alpacas within New Zealand although only at low levels so far. *T. askivali* was originally described from red deer in Scotland but has subsequently been found in a range of deer species around the world. It was only relatively recently first identified in New Zealand red deer. Interestingly there was no history of deer being farmed on either of the two properties involved in these cases.