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CONFERENCE ABSTRACTS

ABSTRACTS

1

Equine parasite control - trends, traditions, and evidence

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Strategies for parasite control in horses are undergoing major changes these years. Traditional approaches are abandoned and more surveillance-based strategies are being implemented instead. These changes are largely driven by scientific evidence documenting the widespread occurrence of anthelmintic resistance in equine parasites across the world. The pharmaceutical industry has not introduced new anthelmintic drug classes with new modes of action for treatment of equine parasites since ivermectin in the early 1980s, and it remains unknown when such new formulations will reach the equine market. The following section outlines the basic premises of traditional deworming programs applied in horse populations and presents the evidence behind recommended surveillance-based programs.

The interval-dose program was introduced by Drudge and Lyons in 1966. At that time, the first modern paste-based anthelmintics had become available making it feasible to perform treatments at regular intervals. Drudge and Lyons identified *Strongylus vulgaris* to be the major target of their program, but devised their recommendations to also provide control over small strongyle parasites. In essence, their recommendation was to apply anthelmintic treatments to the entire herd every other month year-round. Questionnaire surveys performed over the past decades have illustrated that the interval-dose approach is widely used (Relf et al. 2012), and this high treatment intensity is believed to be the main reason for the current levels of anthelmintic resistance observed in equine parasites. The advent of new anthelmintic drug classes led parasitologists to recommend rotating between these to counteract development of resistance. Despite the intuitive logic behind this approach, available evidence from the sheep industry suggests that drug rotation does not reduce or delay the development of anthelmintic resistance (Barnes et al. 1995). Instead, work performed in the New Zealand sheep industry has suggested a benefit from combining two nematodicidal anthelmintics in the same formulation. If no resistance has developed to any of the two drugs, resistance development can be delayed significantly by combining drug classes (Leathwick 2012). The long-term consequences of this approach have yet to be evaluated, and it remains unknown if combination dewormers will be applied for equine parasite control. One possible issue of concern is the recent association of anthelmintic resistance with P-glycoprotein (PgP) transporters, which are independent of drug class and could potentially mediate multidrug resistance (Beech et al. 2011).

Surveillance-based regimens have been recommended for equine parasite control since the early 1990s to reduce anthelmintic treatment intensity and delay further development of resistance. In horses, the approach has become to routinely perform fecal egg counts from all horses and treat those that are exceeding a predetermined cutoff egg count value (Duncan and Love 1991). Egg counts are overdispersed in equine populations, with a

minority of horses is shedding the large majority of eggs (Relf et al. 2013). Furthermore, adult horses maintain a considerable degree of strongyle egg shedding consistency over time (Nielsen et al. 2006a). Taken together, this provides a basis for a targeted treatment approach aiming at controlling strongyle egg shedding on the herd level, while reducing anthelmintic usage. Evidence generated from adult horse populations suggest that selective treatment with an efficacious anthelmintic at a threshold of 200 EPG will leave about half of the horses untreated and provide over 95% overall reduction of egg shedding (Kaplan and Nielsen 2010). However, at least four issues separate horses from sheep. 1) In horses, egg counts cannot be directly linked to the size of the luminal worm burden. 2) Migrating and encysted strongyle larvae can represent up to about 80% of the total strongyle worm burden in horses (Chapman et al., 2003), and egg counts will not reflect these. 3) Horses harbor many more different species shedding strongyle eggs than any other domesticated animal species, and the mere counting of eggs do not offer any information about the more pathogenic large strongyle species. 4) In contrast to common approaches for ruminant parasite control, anthelmintic treatment in horse populations invariably involves routine treatments of adult animals.

Prescription-only restrictions of anthelmintic drugs were introduced by law in Denmark in 1999 to enforce veterinary involvement in parasite control. Effects have been remarkable with equine veterinarians applying a high degree of fecal egg count surveillance and clearly reduced treatment intensity (Nielsen et al. 2006b). A European Union directive has led to similar legislations in several other European countries, and surveillance-based parasite control has become a general trend.

Currently, there are several unanswered questions regarding the selective therapy principle in horses. No studies have yet documented disease incidence and health risks under such regimens. Further, there is no documentation for this strategy in foals and young horses. In addition, it remains unknown to which extent development of anthelmintic resistance can be delayed. Experience from Denmark has identified some possible risks with selective therapy. In one survey a majority of Danish horse owners declared to perform selective therapy in foals. This has never been recommended and raises concerns as this age group is more at risk for developing parasitic disease (Nielsen et al., in press). A recent study documented a significant association of the pathogenic *S. vulgaris* with usage of selective therapy (Nielsen et al. 2012). However, it remains unknown to which extent occurrence of this parasite on a farm constitutes an elevated health risk for the horses present.

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2

Does *Toxoplasma* play a role in deer abortions in New Zealand?

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Deer farms in New Zealand experience reproductive efficiency (calves weaned/hinds mated) in the last decade averaging 70% in Rising-2-year-old (R2) and 84% in mixed age (MA) hinds. Loss of foetus during pregnancy was considered to be one of the contributing factors but had not been quantified. Historically, *Toxoplasma* is known for causing abortion in sheep and infecting the foetus in normal pregnancy in deer. In a pilot clinical study on 4 large deer farms, *Toxoplasma* was suspected as a cause of mid-term abortions (2% to 16%) after *Toxoplasma* DNA was identified in non-pregnant uteri (2/15) on two farms and foetal brains (8/9) from aborted foetuses on 1 farm, in association with herd-level serolgocial evidence. A three-year deer foetal wastage study was started in 2012 aiming to establish mid-term abortion incidence and test the hypothesis that *Toxoplasma gondii* was a potential causal agent. Pregnancy scanning and blood sampling were performed in May-June and repeated late August – October. Preliminary (Year 1) data are presented. The mid-gestation abortion rates on 55 deer farms in year 1 averaged at 2.8% (0-17%) in R2 and 1.3% (0-4%) in MA hinds. *Toxoplasma* serology was carried out using a commercially available ELISA test after validating it against western blot assay (gold standard test). The average seroprevalence in non-pregnant R2 hinds at scan-1 and aborted R2 hinds at scan-2 were 16.8% and 17.8%, respectively. In MA hinds it was 22.6% in non-pregnant hinds (scan-1) and 17.8% in aborted hinds (scan-2). Additionally, *T. gondii* DNA was detected in uteri of 6% (4/70) of slaughtered R2 hinds that were scanned pregnant at scan-1 but lost their foetuses thereafter. These data indicate that exposure to *Toxoplasma* is common and may play a role in abortion on deer farms. However, further longitudinal study data and analysis will be needed to determine whether the association between *Toxoplasma* and deer abortions is casual.

3

Do sheep gastrointestinal nematodes establish in red deer?

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Increasingly deer are being cross grazed with other ruminants. This raises the potential for some nematode parasites to cross infect the other ruminant. To identify the establishment rate of sheep "S" gastrointestinal nematodes (GIN) in red deer "D", a group of red deer (n=5) and a group of sheep (n=5) were housed, effectively treated to remove their existing nematode burdens and then adapted to the pen conditions for 20 days. All animals were approximately 5 months old at the start of this experiment. Subsequently they were infected with a mixed larval dose of sheep GIN, and 28 days later, both groups were euthanized for worm counts. Based on the morphological identification of the larvae given to the animals the mean establishment rates (%) of GIN for sheep and deer respectively were for *Haemonchus contortus* 19"S"/11"D", *Oesophagostomum*+*Chabertia* 22"S"/5"D", *Trichostrongylus* spp. 74"S"/1"D", *Teladorsagia circumcincta 36*"S"/1"D" and *Cooperia curticei* 31"S"/0"D". By comparing establishments rates based on worm counts with those in sheep it was determined that the establishment (%) for *Trichostrongylus axei* would be 67"S"/12"D", *Oesophagostomum venulosum* 22"S"/6"D", *Trichostrongylus vitrinus* 74"S"/0"D", *Trichostrongylus colubriformis* 76"S"/0"D" and *Chabertia ovina* 24"S"/0"D". These establishment rates were significantly lower ($p<0.05$) for all the genera in deer. However, if pastures were heavily contaminated with *H. contortus, Oesophagostomum venulosum* and *T. axei* these may still establish substantial infections in young deer.

4

Strongylus vulgaris-specific antibodies: Monitoring naturally infected horses

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Strongylus vulgaris is regarded as the most pathogenic helminth parasite infecting horses. Migrating larvae cause pronounced endarteritis and thrombosis in the cranial mesenteric artery and adjacent branches, and thromboembolism can lead to ischemia and infarction of large intestinal segments. A serum ELISA assay measuring antibodies against an excretory/secretory antigen termed SvSXP has been developed and validated in our laboratory and allows detection of *S. vulgaris*-specific antibodies during the six-month-long prepatent period. A population of horses has been maintained at University of Kentucky without anthelmintic intervention since 1979, and *S. vulgaris* has been documented to be highly prevalent.

In 2012, 12 foals were born in this population, and were studied during a 12-month period (March-March). Weekly serum samples were collected to monitor *S. vulgaris* specific antibodies with the ELISA assay. Nine colts underwent necropsy at different time points between 90 and 300 days of age. At necropsy, large strongyle parasites and *Parascaris equorum* were identified to species and stage and enumerated. Initial statistical findings indicate a significant interaction between foal age and ELISA results ($p<0.042$).

All foals had initial evidence of maternal antibodies transferred in the colostrum, but then remained ELISA negative during their first three months of life. Foals born in February and March became ELISA positive at about 12 weeks of age, while those born in April and May went positive at about 15 and 21 weeks, respectively. This could be explained by age-dependent differences in parasite exposure. One foal remained ELISA-negative throughout the course of 30 weeks during the study. A significant association was found between ELISA values and larval *S. vulgaris* burdens ($p=0.0387$) as well as a three-way interaction between *S. vulgaris*, *S. edentatus*, and *P. equorum* burdens ($p<0.001$). More detailed analyses are needed to analyze these findings further. A plateau with a subsequent decline in ELISA values corresponded with *S. vulgaris* larvae leaving the bloodstream and migrating back to the intestine.

5

CarLA IgA and FEC responses in Angora goats

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The high incidence of anthelmintic resistance in goats in New Zealand means that farmers have to adopt different and generally less favoured practices to remain viable. The CARLA® Saliva test may offer goat farmers a new and easy to use option to improve the natural immunity of goats to internal parasites, as is now the case for NZ sheep farmers.

Young Angora goat bucks farmed together for approximately one year were assessed for a number of important traits including parasite resistance as measured by the CARLA® Saliva test and faecal egg counts (FEC). Expression of protective immunity to parasite challenge as measured by the CARLA® Saliva test was not seen until the bucks were approximately 12 months of age. In general the expression of the CARLA response or the level of FEC was poorer in the bucks than in mixed aged does located in the same environment. The correlation between these 2 traits was weak in the bucks but negative and thus favourable in the mixed aged does. The CARLA responses and FEC data were reasonably repeatable from one sampling to another once the animals were at least 12 months of age. The association between the CARLA or FEC results and production traits of live-weight and fleece-weight was weak.

6

Peptide mimics of the larval carbohydrate antigen CarLA

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Phage display was used to identify peptide mimics of the immunologically protective nematode glycan (CarLA) by screening a constrained C7C peptide library for ligands that bound to an anti-CarLA mAb (PAB1). Characterisation of these peptide mimotopes revealed functional similarities with an epitope that is defined by PAB1. Mimotope vaccinations of mice with three selected individual phage clones facilitated the induction of antibody responses that recognised the purified, native CarLA molecule. Furthermore, these mimotopes are specifically recognised by antibodies in the saliva of nematode-immune animals which shows that antibodies to the PAB1 epitope form part of the mucosal polyclonal anti-CarLA antibody response of nematode immune host animals.

This demonstrates that the selected peptide mimotopes are of biological relevance. These peptides are the first to mimic the PAB1 epitope of CarLA, a defined larval glycan epitope which is conserved between many nematode species. Potential future applications include the development into a nematode vaccine or a second generation CarLA saliva test.

7

CarLA IgA responses in commercial sheep flocks

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We investigated the use of the CARLA® saliva test to improve the performance of ewe flocks managed under sustainable drenching practices. We followed the CARLA® levels and performance of 4880 replacement ewe lambs from 2010-2013. The ewes were managed in large-flock, hill country environments on eight farms ranging from the central North Island to Otago. Management included conventional and organic systems. Key findings were:

1) At all ages, there were large differences in CARLA® antibody levels between individual ewes in a flock.
2) High CARLA® lambs tended to have higher CARLA® antibody levels throughout their life
3) Ewes which were "CARLA® positive" as lambs consistently had lower FEC as adults.
4) On farms with the highest larval challenge levels, high CARLA® hoggets and two-tooths had better, growth, condition score, and NLB than animals with lower CARLA levels

The project identified three areas of application for CARLA®; (1) as a management tool to monitor pasture challenge; (2) as a ram selection tool and; (3) in ewe culling or selection of ewes to enter low drench system.

8

Parasite-induced immune modulation of dendritic cells with the larval antigen CarLA

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CarLA is a glycan antigen expressed on the surface of many strongyloid nematodes. Studies showed that when an infected animal developed an antibody response against CarLA, the animal was protected from re-infection with the parasite. Never-the-less, the antibody response in sheep is frequently slow to develop and surprisingly, CarLA proved unsuccessful as a candidate antigen in vaccine trials in livestock. To investigate the immune modulatory properties of CarLA, we examined its effect on dendritic cell (DC) activation (one of the first steps in a protective immune response). We showed that the presence of CarLA alone causes insignificant changes in levels of activation surface proteins on human monocyte-derived DC and secreted cytokines were low or not detected. DC can become activated in vivo in response to pathogen products. To explore the effect of CarLA on this process, we have set up an in vitro culture system using a titration of lipopolysaccharide (LPS), a toll-like receptor-4 ligand, aiming to determine whether CarLA can suppress DC activation. We confirmed variable sensitivity to LPS activation by human donor cells, with 1ng/ml LPS being sufficient for maximal up-regulation of activation receptors and co-receptors on DC from some donors while others require 100ng/ml to reach the same level. We see striking suppression by CarLA of this up-regulation in some donors with as much as 40% fewer activated cells. This effect is not present in all donors and this donor-to-donor difference is reproducible. We can detect CarLA associated with DC using a monoclonal antibody to CarLA, PAB1. CarLA-PAB1 signal varies widely between donors, pointing to likely phenotypic differences that may reveal more about the impact of CarLA. Cytokine secretion from DC activated by LPS with CarLA is also modified in susceptible donors with reduction in IL-8, IL-6, TNF-alpha, IFN-alpha.

9

Repurposing human drugs for new anthelmintic discovery

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The discovery of field-derived resistance to the anthelmintic monepantel less than 3 years after launch highlights the urgent need to start identifying the next generation of drenches. Identifying new uses for existing human drugs ("repurposing") has been a successful approach in finding new drugs for treating schistosomiasis. An advantage of repurposing such registered drugs is that their pharmacokinetic and safety profiles are already known thus potentially decreasing the cost and time of bringing them to market. We screened the Microsource Spectrum Collection, which includes over 1500 registered human drugs, using a larval development assay with the sheep parasites *Teladorsagia circumcinta* (resistant to benzimidazoles, macrocyclic lactones and levamisole/morantel tartrate) and *Haemonchus contortus*. We identified 43 compounds with activity at 10 µM and tested the lead compound in mice for efficacy against *Heligmosomoides polygyrus*.

11

Cattle tick biology, ecology and distribution and implications for Theileriosis in New Zealand

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The cattle tick, *Haemaphysalis longicornis* is the main vector for *Theileria orientalis* Ikeda in New Zealand, with other blood-sucking arthropods occasionally implicated. The tick alone, however, is essential for the completion of the protozoan's life cycle. Transmission from tick to tick is transstadial with each stage acquiring merozoites after feeding on an infected host. Piroplasms are passed on to the next tick stage during moulting. There is no transovarial transmission, so larvae must become infected by feeding.

The tick life cycle is entrained by day length and temperatures above the developmental threshold, with survival depending upon a suitable moisture balance maintained through atmospheric vapour pressure. In Northern Hemisphere parts of its range the tick is active for about 8 months each year, and each stage has a circumscribed activity period with little or no overlap. In New Zealand, any stage can be present during the year (excepting June), although there are stage-specific peaks of activity.

Distribution of the tick within New Zealand can be defined almost entirely by meteorological measures of moisture availability. One such, the median March average vapour pressure, could be a useful parameter for predicting changes in tick distribution with climate change. In March, larval numbers are at their peak, and as this stage is the most vulnerable to dehydration, it determines the tick's distributional limits. Other measures of moisture such as saturation deficit and soil moisture deficit are useful indicators, but not as precise a fit as vapour pressure.

Anecdotally deer appear to be the livestock species most at risk to ticks, and the longer pastures associated with their farming encourage tick proliferation. It is possible that this farming system (together with climate warming) may eventually allow cattle tick to establish in parts of New Zealand that are currently unsuitable because of high summer dryness.

13

Study of vertical transmission of *neospora caninum* in experimentally infected sheep

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There is lack of transmission studies with *Neospora caninum* in sheep under natural farming conditions. This study was designed to examine the potential for vertical transmission in sheep. The study was conducted in two parts over two successive years. In Year 1, 50 ewes were randomly divided into 2 groups of 25. Prior to mating Group A was inoculated with *N. caninum* tachyzoites and Group B (control) was left uninoculated. When lambs were 8 weeks of age they were euthanized. In Year 2, ewes from Year 1 were mated with two rams seronegative for *N. caninum*. At about Day 120 of gestation some ewes were inoculated with *N. caninum* tachyzoites as follows: 16 previously inoculated in Year1 (Group C); 17 not inoculated in Year 1 (Group E). In addition, 7 ewes previously inoculated in Year 1 and 5 uninoculated in Year 1 were not inoculated in Year 2 and were designated as Group D and F respectively. All lambs were euthanased at 12 weeks of age. No lambs in Group A or B were positive by ELISA, PCR of tissues or histology of brain in Year 1 at 8 weeks of age. For lambs in Year 2 at 12 weeks of age the number respectively positive by ELISA, Western Blot, PCR of tissues and histology of brain in: Group C were 5/12, 12/12, 0/12 and 5/12; Group D were 0/11, 5/11, 0/11 and 2/11; Group E were: 9/10, 10/10, 2/10 and 9/10; and Group F were 0/7, 0/7, 0/7 and 0/7. Inoculation prior to pregnancy in Year 1 did not result in vertical transmission of Neospora to lambs in Year 1 but without further inoculation there was evidence of vertical transfer in Year 2 (Group D) where both serology and histology indicated some vertical transfer had occurred. For those ewes reinoculated in Year 2 (Group C) there was evidence of vertical transfer in about half of the lambs indicating that inoculation in Year 1 did not provide protection from reinoculation in Year 2. Inoculation of previously uninfected ewes (Group E) indicates that this is an effective model to induce vertical transfer and the absence of infection in Group F lambs (controls) indicates no horizontal infection was occurring.

14

Echinococcus control requires dog population management

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Cystic echinococcosis in humans is caused by *Echinococcus granulosus* and alveolar echinococcosis is caused by *E. multilocularis*. Both species can be acquired by humans from ingestion of the eggs of *Echinococcus spp.* worms living in the dog intestine. The carnivore is the definitive host. Dog population management is an essential component in the control of dog transmitted zoonotic diseases, especially in developing countries. The degree of transmission of zoonoses is a function of the infected biomass, and reduction in the biomass, especially of unowned dogs, not only reduces the risk of transmission but also makes disease control more practical. The biomass of owned infected dogs can also play a significant role in human echinococcosis.

Euthanasia is likely to be the most effective procedure in developing countries where resources are scarce. Both euthanasia and fertility control are on-going events, with no predictable end point. If no fostering/rehoming facilities are available, humane euthanasia may be preferable to long-term kennelling, for reasons of animal welfare. An important part of Echinococcus spp. control can be most effectively managed by cost-effective humane euthanasia of unwanted dogs, combined with keeping less female dogs so that replacements keep pace with requirements. Cost-effective humane euthanasia can now be achieved using the correct formulation of PAPP (para-aminopropiophenone) . PAPP is registered in New Zealand for the control of stoats and feral cats, and registration is proceeding in Australia for foxes and wild dogs.

PAPP acts by rapidly forming methaemoglobin (the ferric state of haemoglobin) which is unable to release bound oxygen. This creates a lethal deficit of oxygen in cardiac muscle and the brain. Death in stoats and feral cats usually occurs within 2 hours after eating a lethal dose. The animals become lethargic and sleepy before they die. Death in dogs takes 2-4 hours.

15

One Health Approach to Otters, Toxoplasma Oocysts and Oceans

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The zoonotic protozoan parasite, Toxoplasma gondii, is a significant cause of mortality in Southern sea otters, a threatened species which have been identified as a sentinel for detecting disease threats to people and animals in coastal environments. Highlights from recently published studies by the co-authors on the transport of T. gondii in terrestrial and aquatic systems, as well as exposure and shedding by domestic and wild cats in coastal areas of high risk exposure for sea otters will be presented. These studies reveal the potential role of domestic felids, land-use change, run-off, aquatic aggregates, and invertebrate vectors in transmission of environmentally resistant T. gondii oocysts to susceptible hosts, including sea otters and humans. Our progress to date has shown how a One Health approach, incorporating tools and perspectives from diverse fields and stakeholders has contributed to an advanced understanding of T. gondii and is addressing transmission at the rapidly changing human–animal–environment interface.

16

Avian Malaria in New Zealand Robins (*Petroica Australis*) and Other Passerines in the Waimarino Forest

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In 2011, 20 New Zealand Robins from the Waimarino forest in the central North Island of New Zealand were captured to evaluate the suitability of this area as a donor site for robin translocations. *Plasmodium relictum*, a species of avian malaria that played a major role in the extinction of many native Hawaiian bird species was found in 1 of these 20 birds. Avian malaria could have a huge impact on New Zealand's geographically isolated and immunologically naive native wild bird populations.

The aims of this study are to explore which *Plasmodium* species occur in the Waimarino forest, their prevalence in various passerine species using molecular and microscopic techniques and determine if there is a correlation between the level of infection (parasite load) and physical parameters, like PCV, weight, tarsus and leg measurements.

Samples tested positive for *Plasmodium* by nested PCR were 4 out of 100 North Island robins, 28 out of 35 Blackbirds, 7 out of 33 Silvereyes, 3 out of 3 Song thrushes and 1 out of 1 Dunnock. Birds from other species tested negative for *Plasmodium* were 5 Fantails, 3 Tomtits, 3 Whiteheads, 2 Grey Warblers, 1 Bellbird, 1 Chaffinch and 1 Goldfinch.

All 188 samples will also be screened using a real-time PCR (qPCR) to quantify parasite load and compare its detection sensitivity with conventional nested PCR, and using microscopic techniques.

It is expected that the outcomes of this study will provide critical information for the management of native bird species in the Waimarino Forest, the bordering Wanganui National Park and other conservation regions throughout New Zealand. In addition, the results of a quantification of parasitism by qPCR will be used to compare the level of infection with physical parameters of birds, giving new insight in the implications of avian malaria infections.

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Avian Malaria in New Zealand

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Avian malaria parasites of the genus *Plasmodium* have the ability to cause extreme morbidity and mortality in naïve hosts, and their impact on the native biodiversity is potentially serious. So far, 17 different strains of avian malaria parasites have been found in 35 bird species in New Zealand. Despite the common asymptomatic nature of the infection, deaths in NZ birds caused by *Plasmodium* spp. have been recorded in South Island saddleback, yellow- eyed penguins, mohua (yellowhead), hihi and great spotted kiwi. Recent outbreaks of avian malaria in endangered New Zealand birds causing fatalities include an outbreak in captive New Zealand dotterel chicks in 1996, an outbreak in yellowhead / mohua in 2004 and mortality in a brown kiwi at a ONE (operation nest egg) facility in 2010/2011.

The main objective of this study is to examine the possibility and extent of pathogen pollution of vector borne diseases due to wildlife translocations using the New Zealand saddleback (*Philesturnus carunculatus*) and its infections with different strains of *Plasmodium* spp. as a model.

During the period 2012-2013, nine sampling trips have been done and archived material from five different locations has been examined. So far, PCR was performed on 156 blood samples, with 37 (23.7%) being positive for *Plasmodium* spp. Eighteen of these samples have been sequenced so far, and it was possible to amplify a *P. relictum* strain formerly not known in NZ. In addition, one of the birds from Hen Island showed a strain (KOKAKO01), which is so far only known from one kokako from Tiritiri Matangi Island and a kokako from the Bay of Plenty. This finding might be the first evidence for pathogen pollution due to wildlife translocations after saddlebacks from Hen Island have been translocated to Tiritiri Matangi Island via Cuvier Island.

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Four cases of fatal toxoplasmosis in three species of endemic New Zealand birds

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Mammals were introduced to New Zealand with the arrival of Maori and Europeans. Prior to this time, New Zealand's only native land mammals were three species of bat and the wide range of unique avifauna occupied the ecological niches normally filled by mammals. With no native felines, the introduction of the domestic cat (*Felis catus)* in the mid-19th century allowed *Toxoplasma gondii* to cycle which then exposed the native avifauna to this new pathogen.

To our knowledge, this is the first report of fatal toxoplasmosis in endemic New Zealand birds. Between 2009 and 2012, two kereru, one North Island brown kiwi, and one kaka were submitted for necropsy examination. On gross post mortem the kiwi had marked hepatosplenomegaly while the kaka and two kereru had swollen, slightly firm deep red lungs. Histologically, there was extensive hepatocellular necrosis in the liver of the kiwi while the kaka and kereru showed fibrinous bronchointerstitial pneumonia. In the kiwi, protozoal organisms were present within the Kupffer cells of the liver and within the macrophages of the pulmonary interstitium of the lung in the kiwi kaka and two kereru. The diagnosis of toxoplasmosis was confirmed with immunohistochemistry and PCR. Genotyping revealed an atypical Type II isolate of *T. gondii* was present in all four cases. This study provides the first evidence that *T. gondii* is causing mortality in these endemic species and suggests further research is needed to determine that full extent of morbidity and mortality caused by this parasite in New Zealand's unique avifauna.

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Evolution of parasitism along convergent lines: from ecology to genomics

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From hundreds of independent transitions from a free-living existence to a parasitic mode of life, separate parasite lineages have converged over evolutionary time to share traits and exploit their hosts in similar ways. Here, we first summarise the evidence that, at a phenotypic level, eukaryotic parasite lineages have all converged toward only six general parasitic strategies: parasitoid, parasitic castrator, directly-transmitted parasite, trophically-transmitted parasite, vector-transmitted parasite, or micropredator. We argue that these strategies represent adaptive peaks, with the similarities among unrelated taxa within any strategy extending to all basic aspects of host exploitation and transmission among hosts, and transcending phylogenetic boundaries. Then, we extend our examination of convergent patterns by looking at the evolution of parasite genomes. Despite the limited taxonomic coverage of sequenced parasite genomes currently available, we find some evidence of parallel evolution among unrelated parasite taxa with respect to genome reduction or compaction, and gene losses or gains. Matching such changes in parasite genomes with the broad phenotypic traits that define the convergence of parasites toward only six strategies of host exploitation is not possible at present. Nevertheless, as more parasite genomes become available, we may be able to detect clear trends in the evolution of parasitic genome architectures representing true convergent adaptive peaks, the genomic equivalents of the phenotypic strategies used by all parasites

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Epizootic mange in kakariki; biosecurity indiscretion or assisted self-introduction?

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Tiritiri Matangi Island in the Hauraki Gulf region of Auckland is an open sanctuary with a reintroduced population of kakariki (red crowned parakeets, *Cyanoramphus novaezelandiae*). These birds originally came from captivity in the 1970's. For the past decade, there have been increasing reports of feather loss in this species on the island. In 2008, beak and feather disease virus (BFDV) was detected nearby on Hauturu island, and therefore it was suspected the feather loss might have been due to a spread of this pathogen. However, a 2-year study into health and disease of red crowned parakeets on Tiritiri Matangi has revealed a different source of the clinical signs. During four cross sectional studies from 2011-12, feather loss increased from 8% (95%CI:2.3%-18.8%) up to 52% (95%CI:38.6%-65.2%) of the population. Skin biopsies from all birds in the second year of the study found a skin mite associated with thickening of the skin (acanthosis) and excessive keratin production (hyperkeratosis). Mites were also found in asymptomatic birds, suggesting a subclinical or carrier status. Whole mites were cleared in Hoyer's medium, with larval and female forms of *Procnemidocoptes jansseni* identified. This mite has only been formerly described in a lovebird from Zambia (Fain 1966), raising questions as to how it came to be present in wild kakariki in New Zealand. Another skin mite *Hemimyialges macdonaldi*, also reported to cause mange, was found in low numbers from skin as well as on hippoboscid flies removed from several birds. The relationship between the cosmopolitan *H.macdonaldi,* the dominant mite *P.jansseni*, and the clinical signs of mange requires further investigation.

New Zealand is host to a unique avifauna, which has been significantly affected by the combined impacts of habitat modification and introduced mammalian predators. Many now thrive only on offshore islands or predator-free mainland sanctuaries, with ongoing conservation efforts reliant on re-introductions and translocations between these sites. These assisted movements, which may include periods of captive management, introduce specific biosecurity risks and potential for artificial spread of pathogens and parasites. Historically these activities took place in the absence of extensive or strategic disease screening, or prior to our current understanding of, or capacity to detect, key diseases. Results from this study will feed into captive and wild management of kakariki, specifically identifying new risks for translocations and re-introduction programs. The study also highlights the importance of epidemiological approaches to studying disease syndromes in wild populations.

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Interspecific disease transmission in kiwi

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Like other oceanic islands in the world New Zealand wildlife has suffered the results of colonisation, in particular the introduction of animals, habitat transformation/destruction, and the introduction of exotic diseases. Kiwi is a group of five species of birds endemic to New Zealand. Kiwi are unusual in that they belong to a group of birds called Paleognathous that includes moa (13 sp.), cassowary and emu, rhea (2 sp.), elephant bird, and tinamous (50 sp.); all the other birds in the wold belong to the Neognathous superorder. Second kiwi are nocturnal ground insectivores, a niche which today is mostly occupied by mammals, including species which have been translocated to NZ such as rats and hedgehogs. While predation by large mammals and competition for food are two outcomes of this coexistence, a third one, which we are just now learning about, is sharing of parasites. In this talk I introduce and discuss cat, sheep, cattle and rat parasites that are now affecting kiwi.

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The effect of season and habitats on abundance of Ixodes anatis; A North Island Brown Kiwi ectoparasite.

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Knowledge of the structure of host-parasite relationships enables a better understanding and predicting of the likely spread of vector-borne diseases. Host burrows are the key habitat for their ectoparasites and can influence host parasite interactions and communities. A four month study was conducted from May to August 2013 on Ponui Island in the Inner Hauraki Gulf to evaluate the effect of area, habitat (forest, scrub and pasture) and burrow types (tree, soil and surface), on abundance of the different life stages of Brown Kiwi ticks, Ixodes anatis. In total, 7600 ticks were collected from 64 burrows which were sampled monthly. Immature ticks predominated over adult ticks and accounted for 98.25% (7125 larvae and 342 nymphs) of the samples collected. Abundance of larvae in samples decreased and nymphs increased as the months progressed. This increase coincided with onset of the breeding season of Brown Kiwi. Forest habitats and tree burrows had a higher tick abundance, predominantly larvae. Tick number decreased as the temperatures dropped suggesting an effect of temperature on tick abundance.

This study indicated that season, burrow location, habitat and type all have an effects on tick abundance and we suggest that a combination of these factors associated with the activity of the hosts influences tick development and reproduction. This study opens up new avenues to investigate parasite ecology of “host specific” kiwi parasites.

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Update on Reversion . Limitations of egg counts and worms counts in determining the true drench resistance status of a parasite in Sheep

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The latest efficacy studies on a North Auckland sheep farm which has used triple drenches such as Triton since 2001, is now providing information as controversial as the pioneer formulations themselves. Triple active formulations using ivermectin or abamectin in combination with oxfendazole and levamisole continue to be highly effective at 97-100% confirmed in worm counts. Quite dramatically the dual combinations now appear almost equally effective including oxfendazole+ levamisole (98-99%) and abamectin+levamisole (≥99%) including in hoggets and lambs. The previous efficacy of dual actives such as oxfendazole + levamisole varied from 87-90% in May 05 and Mar 03 respectively. The efficacy for the individual actives appears lowest in young weaned lambs (3-5 months). Ivermectin's efficacy in lambs in late 2012 was 71% against adult *Haemonchus contortus* (*H.c*) and 84% adult *Teladorsagia spp* (*Te*). This is still much higher than seen in initial studies where ivermectin efficacy in hoggets in 2000 was 57.5-65.6% for *H.c* and 38.5-88.7% for *Te* but still contrasts with hoggets on this farm where reductions appear ≥97% (2012). It is speculated that hoggets on this farm are now more immune earlier, possibly as a result of selective breeding, and so respond better to treatment and making the worm strains appear more sensitive than they genetically are. Implications are discussed.

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Can combination anthelmintics be used once resistance to multiple actives has developed?

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In 2010 a programme to evaluate Best Practice Parasite Management practices on commercial farms in New Zealand was initiated. Sheep farms were selected on the basis of having significant problems involving resistance to 2 or more anthelmintic classes. The aims were to manage parasitism, maintain or improve on existing production figures, and ensure that the efficacy of anthelmintics did not decline further.

A management programme was designed specifically for each farm by a parasitologist, a farm consultant, a local veterinarian and the farmer. Strategies implemented included minimising unnecessary treatments whilst maintaining a strategic preventive drenching programme, maintaining refugia of susceptibility and the use of combinations of different anthelmintic classes. Regular monitoring included multiple faecal egg counts combined with an annual faecal egg count reduction test.

Resistance levels varied between farms with some (n=3) having significant resistance to all of the older anthelmintic classes, whilst all except 1 of the remainder had resistance to 2 classes. In most cases the resistant parasite was *Teladorsagia* (*Ostertagia*) *circumcincta*. On all farms combination anthelmintics were used almost exclusively, the exceptions being strategic use of new actives or moxidectin.

Uptake of resistance management practices by farmers was high as long as strategies were simple, practical and the rational for use understood. Monitoring indicated that in most cases parasite load was acceptable and farmers were happy with animal performance. The effectiveness of anthelmintics against *T. circumcincta* over time showed a significant positive slope indicating that overall, efficacy increased over the duration of the study. The use of combination anthelmintics in conjunction with other resistance management strategies appears to have resulted in meaningful improvements in treatment efficacy despite significant multiple-resistance being present initially. The hypothesis that combinations cannot be usefully employed once resistance has developed is not supported by the results of this study.

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Anthelmintic use strategy, parasite fitness and the potential for reversion towards susceptibility

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The rotation of different anthelmintic classes, on an approximately annual basis, has been widely promoted as a strategy to delay the development of anthelmintic resistance. One reason for recommending this practice is the expectation that resistant genotype worms have a lower ecological fitness than susceptible worms, and so reversion toward susceptibility can be expected in the years when an alternative class of anthelmintic was used. It is generally considered that the routine use of combination anthelmintics will negate this opportunity for reversion, and this has been used as an argument against the use of combinations.

A simulation model was used to investigate whether, in the presence of a fitness cost associated with resistance, an annual rotation of two classes of anthelmintic was more effective at slowing the development of resistance than continuous use of two classes in combination. Simulations were run in which the fitness cost associated with each resistance gene was varied from 0-15% and the rate at which resistance developed was compared for each of the drug-use strategies. Other factors evaluated were the initial frequency of the resistance genes and the proportion of the population not exposed to treatment (i.e. in refugia).

As the fitness cost associated with resistance increased, resistance developed more slowly and in some cases resistance did not develop at all (i.e. the gene frequency declined); this was more pronounced when a combination was used compared to a rotation. The results indicate that the optimal drug-use strategy to maximise the benefit of any fitness cost associated with resistance is the use of combinations of different anthelmintic classes, and that reversion towards susceptibility is likely at lower fitness costs when combinations are used. The mechanism underpinning these results is presented elsewhere (Leathwick 2013).

Leathwick DM. 2013. Managing anthelmintic resistance – parasite fitness, drug use strategy and the potential for reversion towards susceptibility. Veterinary Parasitology, 198, 145-153.

26

Lack of efficacy of monepantel on two separate New Zealand farms

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In 2009, monepantel was released onto the New Zealand market at a time when many goat farmers were struggling to cope with gastrointestinal nematode parasites that were resistant to combinations containing all three of the older anthelmintic classes. At least two farms in the Manawatu began to use monepantel soon after its release. On one farm, monepantel was used as the sole anthelmintic drench given to both goats and sheep resident on the property. On the other farm where there were no sheep, monepantel was given to some goats, whilst other goats continued to be treated (sub-optimally) with a triple combination. Problems arose on the first farm, 20 months after the first use of monepantel, when animals became sick and post-treatment egg counts were shown to be high. Faecal egg count reduction tests showed that monepantel given to goats at 1.5 and 3x the sheep dose rate, and to sheep at the recommended dose rate (2.5mg/kg) showed little evidence of efficacy. These findings were confirmed in a slaughter study using sheep, in which monepantel had negligible/zero efficacy against both *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. On the second farm, two egg count reduction tests in goats showed that monepantel at 1.5x the sheep dose was unable to provide efficacy > 95%, suggesting that resistance was present on this farm as well. Although only involving 11 and 6 goats, the reductions in arithmetic mean egg counts in the two tests were 10 and 90%. Larval cultures suggested that both *T. circumcincta* and *T. colubriformis* were involved. Thus of two farms where monepantel has been used routinely and where efficacy has been examined, monepantel has failed on both. Although sheep were present on at least one of the farms, it is likely that treatment of the goats has been pivotal in the rapid development of resistance involving more than one nematode species.

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A survey investigating gastro-intestinal nematode egg output of sheep following treatment with long acting anthelmintics on farms in new zealand

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Introduction: The development of bolus anthelmintic delivery technology and novel chemistry has given sheep farmers the option of treating animals with long acting (LA) anthelmintic products prior to or during periods of high larval challenge or low immunity. The aim of these treatments is to control parasitic nematode infections present at time of treatment, prevent the establishment of new infections by killing incoming larvae and to significantly reduce the subsequent exposure of other sheep to incoming third stage infective larvae. This technology has been embraced by farmers in New Zealand. However, the major downside of treatment with LA products is that they are widely considered to be a high risk activity in terms of selection for anthelmintic resistance[1].

Materials and methods: A reduction in the claimed period of protection is usually the first indication of development of resistance to long acting anthelmintics[2]. To obtain an indication of the level of resistance to these anthelmintics and their ability to achieve their desired aims, a survey was conducted on sheep farms in New Zealand. This survey assessed the presence and level of gastro-intestinal nematode (GIN) egg output from sheep at various time points following treatment with LA products and prior to expiry of the claimed period of persistency. Coprocultures were conducted on all samples - regardless of faecal egg counts (FEC's) - to determine the species composition of GIN burdens. Farmers were also required to complete a brief questionnaire regarding drench history and farming practices to identify potential risk or protective factors for GIN egg output prior to expiry of the claimed persistency period of LA products.

Results: Faecal samples were collected from in lamb ewes and ewe hoggets at between 50 and 70 days post administration from 10 randomly selected animals from treated flocks. These samples were submitted to a local diagnostic laboratory for determination of FEC's and coproculture. Of the 30 flocks tested, 19 had positive FEC's during the claimed period of persistence.

Conclusions: The results of this survey support the incorporation of "exit" treatments using an active (or actives) from a different anthelmintic family to the active present in the LA product, along with adoption of other risk mitigation strategies to facilitate more sustainable use of LA anthelmintic products. These results also indicate that "primer" drenches using an active (or actives) from a different anthelmintic family to that present in the LA product may also be required on some farms at the time of administration of the LA

product to provide adequate control of GIN already present in the sheep at the time of LA product treatment.

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30

Comparison of orals and ML pour on formulations in Cattle

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Increasingly farmers are being encouraged to use single active oral formulations of macrocyclic lactone (ML), instead of pour on formulations of the same active. In this presentation the benefits of oral and pour ons are discussed including some attributes that continue to make pour ons an attractive option. ML pour on formulations because of differences in formulation vary widely in activity.

Trials that demonstrate this variability are presented. This includes trials on one farm where the pioneer products of Genesis® Pour on (Aba), Cydectin® Pour on(Mox) and Ivomec® Eprinex (Epr) were compared with new VetMed (VM) pour on formulations of the same ML active (shown in brackets with efficacy) at 14 and 25 days post treatment. The efficacy for the pioneers were 44.17-71.67% (VM Aba 71.77-91.61%), 50.85-25.42 (VM Mox 80.33-80.33%), and 95.45-95.45% (VM Epi 97.14-97.14%). A comparison was then made between oral Oxf+Lev (VM Dual), Abamectin oral, and Abamectin Pour on (VM Aba) these gave 97.5% (45 larvae/50g, 100% *Ostertagia)* , 89.5% (60 larvae/50g, 100% *Cooperia*) and 80% (40 larvae/50g, 100% *Cooperia)* respectively. On a second farm efficacy of 71.43% for Eclipse® Pour on and 87.89% for Boss ®Pour on is presented, with 160-180 larvae/50g recovered in larval culture. The larvae were identified as 100% *Ostertagia*. Emergence of dual and even triple resistant *Ostertagia* in NZ cattle is discussed.

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The establishment of *Cooperia oncophora* in cattle

C. W. Sauermann, D. M. Leathwick, I. Scott, W. E. Pomroy

The establishment rate of *Cooperia oncophora* related to host age and previous exposure was investigated in young calves. Three groups (n=16) of similar aged calves were kept on a feed pad. One group was kept as an uninfected control, the other two groups received trickle infections with an ivermectin-susceptible isolate of 2,000 or 10,000 infective stage larvae per week. At intervals over a period of 11 months two animals from each group were challenged with 15,000 infective stage larvae of an ivermectin-resistant isolate, 25 days later orally treated with ivermectin (0.2mg/kg; Ivomec®) and 5 days later slaughtered for worm counts. On 3 occasions additional calves (n=2) were included with the high trickle infection rate group but also received an ivermectin treatment before challenge to remove the existing worm burden. Two further groups (n=4) of similar aged calves were introduced at the beginning and the end of the experiment to determine the effect of larval age on establishment rate. There were significant differences in *C. oncophora* establishment between the control and two trickle infection groups. The establishment rate in the two trickle infection groups declined rapidly compared to the control group. In the animals receiving the high trickle infection with anthelmintic treatment before challenge the establishment rate was not significantly different to the control group. The establishment rate in the control group declined over time which was similar to the decrease recorded in the groups to determine the effect of larval age at the beginning and end of the experiment. The findings indicate that an existing nematode burden has a strong effect on the establishment of incoming larvae in the trickle infected groups but this effect was not observed if the existing burden was removed before the final challenge. The decline in establishment rate in the control group was due to the age of the larvae and not the age of the calves per se.

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Implementation of resistance management practices on commercial sheep and cattle farms in New Zealand

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In 2010 a programme to evaluate Best Practice Parasite Management practices on commercial farms in New Zealand was initiated. Sheep farms were selected on the basis of having significant problems involving resistance to 2 or more anthelmintic classes. The aims were to manage parasitism, maintain or improve on existing production figures, and ensure that the efficacy of anthelmintics did not decline further.

A management programme was designed specifically for each farm by a parasitologist, a farm consultant, a local veterinarian and the farmer. Strategies implemented included minimising unnecessary treatments whilst maintaining a strategic preventive drenching programme, maintaining refugia of susceptibility and the use of combinations of different anthelmintic classes. Regular monitoring included multiple faecal egg counts combined with an annual faecal egg count reduction test.

In each case the farmer has significantly changed parasite management practice. Compared to pre-programme, all now routinely apply a range of additional strategies that are considered best practice. Farmers have quickly understood the rationale and principles that constitute best practice, and have been very adept at identifying practical techniques for applying these that most suit them and their farming system.

On every farm “hot spots” - paddocks, grazing blocks and practices with a high risk of resulting in a population of highly selected resistant worms – were able to be identified. Simple but effective changes in management were able to be identified to reduce or eliminate this risk.

A comprehensive programme of monitoring has assisted farmers to quickly build confidence around changing practice, and reducing the number of treatments. All farms have changed to using combination oral treatment of cattle. Stock performance has been maintained, expenditure on parasite treatment has been decreased, effective products are now administered and the number of treatments has declined compared to pre-programme.

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A survey evaluating farmer's use of parasite-related tools

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In late 2012 a survey was sent to approximately 12,000 NZ sheep and beef farmers. Included amongst the questions were those on farmer demographics, whether they had utilised FEC, FECRT and Toxovax in the past 3 years and how useful they found a range of information providers. A total of 1009 usable responses were returned with good representation of regions, farm sizes and farm types within NZ.

Respectively, 36%, 21% and 65% of farmers had used FEC, FECRT and Toxovax. Farmers were more likely to have used a FECRT if they were <60 years of age and/or were educated above High School level. They were more likely to have used FEC and/or Toxovax if they were <40 years of age and/or educated above High School level. Out of 16 possible information providers, veterinarians received the highest average usefulness score.

The figure of 21% of farmers having undertaken a FECRT is greater than previous estimates. In part this may reflect population sample bias of the survey, or alternatively may indicate that FECRT is becoming more commonly utilised by farmers. Further, the greater use of these tools by younger farmers indicates that future uptake may be improved as the younger generation of farmers come through. Veterinarians are highly-regarded as a source of information and as such are likely to be considered by farmers as a reliable source of parasitological advice although it was not determined how frequently they were used to do so.

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The prevalence of anthelmintic resistance on Australian sheep farms (2009 - 2012)

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Aim: This study was conducted in response to the need for up-to-date information on anthelmintic resistance in the Australian sheep industry to help inform management decisions, advisory practices and future research.

Methods: Providers, including government and private parasitology laboratories, pharmaceutical companies and veterinarians who were known to have conducted Worm Egg Count Reduction Tests (WECRT's) between 2009 and 2012, were asked to submit all test results that conformed to standards set by the World Association for the Advancement of Veterinary Parasitology. Of those submitted, 390 tests met these criteria.

Results: The percentage of farms on which <95% efficacy was found in any of *Haemonchus*, *Teladorsagia* or *Trichostrongylus* spp. (and the number of farms tested) for the four broad-spectrum drench groups was:

benzimidazoles 96% (80); levamisole 96% (111); macrocyclic lactones (represented by ivermectin) 84% (92) and aminoacetonitrile derivatives (monepantel) 0% (4).

Against the same criteria, and compared with the findings above, the results for moxidectin 57% (136) and three-way combinations - containing abamectin, levamisole and a benzimidazole 28% (50) - clearly show that drench effectiveness needs to be assessed at an individual farm level. We will also present the range of efficacies found by active.

Conclusion: These findings contrast sharply with the results of a national drench resistance survey conducted in 1994 which found no evidence of ML resistance[1]. The current study highlights the urgent need to better manage existing anthelmintic resources and to promote adoption of appropriate non-chemical control measures on individual farms as part of an Integrated Parasite Management (IPM) approach.

Acknowledgements:

Greg Croft, Lewis Kahn, Paul Nilon, David Hucker, Matt Playford, Nicole Swan, Brown Besier, Elizabeth Patrick and Novartis Animal Health

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Cost-effectiveness analysis: using the incremental net benefit approach to evaluate therapeutic treatment interventions.

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Although clinical evidence is the accepted method of demonstrating the benefit of an intervention, there is growing awareness among clinicians that an intervention needs to be cost-effective. This presentation addresses statistical analysis of cost-effectiveness data, and the inevitability of collecting not only clinical, but also economic data during clinical trials. Measuring costs and effectiveness at the level of the individual subject (or patient in human studies) has permitted the use of conventional statistical inference to quantify uncertainty due to measurement and sampling error, and numerous publications dealing with statistical analysis of cost-effectiveness data have been produced. Initial efforts were centred on producing confidence intervals for incremental cost-effectiveness ratios, but due to concerns regarding ratio statistics, the incremental net benefit concept has been proposed as an alternative. This presentation addresses statistical issues related to cost-effectiveness comparisons of treatment groups when measures of effectiveness and cost are determined at the subject level in randomised control trials, and where subjects are randomised to intervention or standard of care treatment groups. The concept is explored according to the willingness-to-pay principle, as well as application of the incremental net benefit evaluated at a particular value where treatment increases success but also increases costs. Inference is made regarding the difference between treatment arms using the principle of cost-minimization by applying the incremental net benefit approach for any selected value that the user is willing to pay. Lastly, a Bayesian cost-effectiveness acceptability curve is applied to determine the probability that a treatment is cost-effective compared to standard-of-care.

Posters

Immunomodulation of human monocyte-derived dendritic cells by *Haemonchus contortus* Excretory/Secretory (ES) products

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Parasitic helminths are powerful immunoregulators of the host to ensure their establishment and survival in a hostile environment. Many immunomodulators, including some acting on host antigen presenting cells, are present in ES products of different life cycle stages of numerous helminths, including nematodes. Parasite control strategies, such as vaccination, may depend on understanding and preventing the interaction between antigen presenting cells and helminth immunomodulators.

This experiment investigated whether human monocyte-derived dendritic cells (mdDCs) can be modulated by ES products from adult *H. contortus*. ES products were prepared by incubation of adult worms in RPMI for 4 hours. Monocytes collected from four healthy human donors were transformed into mdDCs during *in vitro* culture. Changes in the expressions of CD305, CD32, galectin, CD80, CD86, MHCII and CD40, stimulated with ES products, with and without LPS, were assessed by multicolour flow cytometry.

Adult *H. contortus* ES products modulated mdDCs in 3 experiments, each using cells from 4 donors. ES products neither increased CD86 expression by mdDCs, nor reduced stimulation by LPS. ES products (but not LPS) markedly up-regulated CD32 (Fcγ-receptor II), but reduced expression in the presence of LPS. Expressions of CD80, MHCII, galectin and CD40 were increased by either ES products or LPS, but together, the effect of LPS was reduced by ES products. All effects on CD305 were weak, but qualitatively similar. The effects of ES concentration were complex, as the higher and lower were similar, but the intermediate concentration was ineffectual.

ES products contained concentration-dependent immunomodulators, suggesting there was a mixture of stimulators and inhibitors. Although the roles of CD32 isoforms are not understood, the increase in surface expression of CD32 by ES products may contribute to the induction of tolerance to chronic infection by up-regulation of CD32. Effects of ES products on mdDCs appear to involve interaction with pathways which can be induced by LPS.