

# **New Zealand Society for Parasitology**



**Annual Meeting No. 45**

**26<sup>th</sup> October 2017**

**Massey University,  
Palmerston North**

**Programme and Abstract Book**

Supported by



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**Programme for New Zealand Society for Parasitology Meeting 25 and 26  
October**

**Meeting to be held in Ira Cunningham Lecture Theatre (ICLT), Massey  
University, Palmerston North**

Wed 25<sup>th</sup>

Parasite Advisor's Day

Thursday 26<sup>th</sup>

*Half-day session updating information and recent findings on Theileria  
orientalis*

9-9.20: Andy McFadden, Diagnostic and Surveillance Services, Ministry for Primary  
Industries. The continuing impact of *Theileria orientalis* Ikeda in NZ cattle herds.  
(This will provide an update on the NZ situation, esp with regards the South Island)

9.20-10.00: Cheryl Jenkins, Elizabeth Macarthur Institute, New South Wales Dept of  
Primary Industries, Camden, NSW, Australia – Theileriosis in Australia - recent  
developments. Cheryl will explain the Australian *Theileria* story and recent research  
findings from Australia

10.00-10.20: *Morning Tea – IVABS Foyer*

10.20-10.40: Rebecca Hickson/Michaela Gibson, Massey University – effects of  
*Theileria* on bull fertility and libido

10.40-11.00: Rebecca Hickson, Massey University – The effects of *Theileria* on calf  
growth rates

11.00-11.40: Kevin Lawrence, Massey University – Summary of recent research to  
include: distribution modelling of ticks and potential distribution of *Theileria*  
associated bovine anaemia, review of submission histories and clinical pathology  
associated with infection and the results from the experimental infection in bulls

11.40-12.00: Dave Rankin, Wanganui Vet Services – A veterinary practitioners  
experience with *Theileria* on the tick fringe zone of New Zealand

*LUNCH – Your own expense – either at Student Centre or Wharerata Staff  
Club.*

1.00-1.15: Kiliana Bekelaar, Tania Waghorn, Michael Tavendale, Dave Leathwick,  
AgResearch - Heat shock, but not temperature, is a biological trigger for the  
exsheathment of third stage larvae of *Haemonchus contortus*

1.15-1.30: Richard Shaw and Mary Wheeler, Hopkirk Research Institute,  
AgResearch Ltd - Genetic and phenotypic relationships between CARLA IgA, IgG,  
faecal egg counts and productivity in a commercial Angora goat herd

1.30-2.00: Harry Taylor, Te Puke Veterinary Centre – Coccidia in kiwi – the  
periodicity of oocyst shedding over a 24 hour cycle

2.00-2.15: Alicia Coupe, Massey University – Diagnostic Tools for use with *Toxoplasma*.

2.15-2.30: Kandarp Patel/Laryssa Howe – *Toxoplasma* in Deer

2.30-2.45: Paul Hughes, Taihape Veterinary Services - A novel application of an anthelmintic mixture for use against gastrointestinal parasites of red deer (*Cervus elaphus*)

2.45-3.00: Andrew Dowling, PGG Wrightson/Massey University, A survey of liver fluke in Westland.

3.00-3.20: *Afternoon Tea – IVABS Foyer*

3.20-3.35: Preet Singh, Massey University – The pharmacology of abamectin in sheep and goats

3.35-3.50: Nick Cave/Vicki Erceg, Massey University – *Giardia* in working dogs – summary of recent cases

3.50-4.05: Kevin Lawrence, Massey University – The effect of a mid-lactation treatment with eprinomectin on milk production

4.05-4.20: Bill Pomroy, Massey University – A report on a postal survey on flystrike and lice on sheep

4.20-4.30: Caroline Kriechbaum, Kristene Gedye, Bill Pomroy, Massey University – Sarcoptic mange in hedgehogs – results of a survey and potential implications for other animals

4.30-4.40: Ian Scott, Massey University – An update on prevalence of gastrointestinal parasites of dogs

4.40-4.55: Rakesh Sehgal, Postgraduate Institute of Medical Education and Research, Chandigarh - Comparison of three molecular diagnostic techniques for the diagnosis of amoebic liver abscess

4.55-5.10 Laryssa Howe, Massey University – review of WAAVP

#### *Posters*

Juan Carlos Garcia-R, Nigel French, Anthony Pita, Niluka Velathanthiri and David Hayman, Massey University. Local and global genetic diversity of protozoan parasites: Spatial distribution of *Cryptosporidium* and *Giardia* genotypes

Juan Carlos Garcia-R and David Hayman, Massey University. Origin of a major infectious disease in vertebrates: The timing of *Cryptosporidium* evolution and its hosts

**ANNUAL GENERAL MEETING**                      **5.20pm ICLT**  
**CONFERENCE DINNER**                      **7pm**    **Manawatu Golf Club, 19 Centennial Drive, Palm Nth**

### **Continuing impact of *Theileria orientalis* Ikeda in NZ cattle herds**

Andrew McFadden, Diagnostic and Surveillance Services | Operations Branch,  
Ministry for Primary Industries - Manatū Ahu Matua, New Zealand

The Ministry for Primary Industries has carried out several investigations where the impact of *Theileria* Associated Bovine Anaemia has been apparent. Two specific investigations where this has been the case were in a large dairy farm in the South Island characterised by subclinical anaemia and in a dairy-beef calf enterprise where ill-thriftiness was a significant part of the syndrome, along with mortality of calves that had failed to thrive. In both of these cases significant effort was required to characterise the involvement of *Theileria orientalis* Ikeda. Thus, given these experiences it is likely that there are a number of other farms impacted that are not identified. TABA remains a hidden disease that can easily be dismissed as being relatively unimportant; however, the case studies presented demonstrate its continued importance in the cattle sectors of NZ.

**Theileriosis in Australia - recent developments.**

Cheryl Jenkins, Elizabeth Macarthur Institute, New South Wales Dept of Primary Industries, Camden, NSW, Australia

Cheryl will explain the Australian *Theileria* story and recent research findings from Australia

## **The effects of *Theileria orientalis* Ikeda on bull fertility and libido**

Michaela Gibson/Rebecca Hickson,

Institute of Veterinary Animal and Biomedical Sciences, Massey University

*Theileria orientalis* is a blood-borne parasite that is prevalent in New Zealand and other countries. The Ikeda type has been found to be more pathogenic than previously discovered types such as Chitose and Buffeli. Little is known about how Ikeda-type affects the reproductive performance of bulls. The aim of this experiment was to examine the effects of *Theileria orientalis* Ikeda on the fertility and libido of bulls. A group of 17 bulls were used in the experiment with 10 being infused with *Theileria* Ikeda-infected blood from two donor cows and the remaining 7 bulls used as controls. All 10 of the treatment bulls were successfully infected with *Theileria* and became clinically anaemic (Haematocrit below 24) between days 47 and 84 post transfusion. Semen and libido was tested every 2 weeks throughout the experiment. There was no observed change in wave motion score of semen between infected ( $7.51 \pm 0.18$ ) and control ( $7.08 \pm 0.35$ ) treatment groups ( $P=0.2935$ ) along with no change in forward motion between infected ( $7.82 \pm 0.16$ ) and control ( $7.64 \pm 0.2610$ ) treatment groups ( $P=0.5579$ ). The percentage of normal sperm ( $P=0.0032$ ) was lower in the infected bulls ( $91.9 \pm 0.05$ ) compared to the control group ( $94.25 \pm 0.06$ ) although the density of sperm in an ejaculate ( $P=0.0044$ ) was higher in infected bulls ( $1.45 \times 10^{10} \pm 6.88 \times 10^6$  sperm per mL) compared to control bulls ( $1.14 \times 10^{10} \pm 9.82 \times 10^6$  sperm per ml). Time to first mount ( $P=0.7374$ ) and gap between first and second mount ( $P=0.2204$ ) was not significantly different between infected and control groups. The number of mounts was similar between infected ( $2.33 \pm 0.28$ ) and control ( $2.36 \pm 0.17$ ) treatment groups ( $P=0.9269$ ) and there was no interaction with time ( $P=0.2221$ ). However, there was a significant effect of treatment on order of service with infected bulls coming in to the yard later in the herd on Day 55 and was statistically significant ( $P=0.02$ ). In conclusion, changes in fertility occurred in infected bulls but were not drastic enough to indicate a decrease in overall fertility. The only measure of libido affected was order and it is unknown how this would affect pregnancy rates in a herd situation.

**The effects of *Theileria orientalis* Ikeda on calf growth rates**

Carol Hewitt and Rebecca Hickson

Institute of Veterinary Animal and Biomedical Sciences, Massey University

**Summary of recent *Theileria orientalis* (Ikeda) research conducted at Massey University**  
Kevin Lawrence, Institute of Veterinary Animal and Biomedical Sciences, Massey University

**Vertical transmission of infection in pregnant dairy cows**

The aim of this study was to establish if vertical trans-placental transfer of infection from infected dams to their calves occurred. Dairy cows (n=98) and their calves were blood sampled at calving and screened for *T. orientalis* genotypes using a multiplex Buffeli, Chitose and Ikeda specific TaqMan assay. The cows were sourced from one dairy herd which had suffered a serious *T. orientalis* (Ikeda) outbreak the previous season. The results found 57/98 dams positive but all calves negative for *T. orientalis* (Ikeda) infection. From this it was concluded that vertical trans-uterine transmission of *T. orientalis* (Ikeda) infection is unlikely in dairy cows and not important in the epidemiology of *T. orientalis* (Ikeda) infection.

Lawrence, K. E., Gedye, K., McFadden, A. M. J., Pulford, D. J., & Pomroy, W. E. (2016). An observational study of the vertical transmission of *Theileria orientalis* (Ikeda) in a New Zealand pastoral dairy herd. *Veterinary Parasitology*, 218, 59-65.

**Predicting the environmental suitability for *Theileria orientalis* transmission**

The aim of this study was to estimate the relative environmental suitability for *T. orientalis* transmission in New Zealand using the Maxent (maximum entropy) modelling program. Maxent is used extensively in ecological studies for modelling species distributions and has also been used a small number of times to model arthropod vectored disease transmission. The Maxent model uses climate and geography variables to fit a probability distribution for environmental suitability by using maximum entropy, which means fitting a distribution to the data with the minimum of assumptions about the data. The environmental variables included in the final model were elevation, 30-year average climate data (median vapour pressure for the months February, March and April; median soil moisture deficit days for January) and farm density (cattle and deer). The results predicted that 99% of North Island cattle farms, 64% South Island cattle farms and 89% of New Zealand cattle farms overall could potentially be suitable for *T. orientalis* transmission.

Lawrence, K. E., Summers, S. R., Heath, A. C. G., McFadden, A. M. J., Pulford, D. J., & Pomroy, W. E. (2016). Predicting the potential environmental suitability for *Theileria orientalis* transmission in New Zealand cattle using maximum entropy niche modelling. *Veterinary Parasitology*, 224, 82-91.

**Predicting the expansion of habitat suitability within New Zealand for the tick *Haemaphysalis longicornis***

The aim of this study was to predict the spatial distribution of habitat suitability of New Zealand for the tick *H. longicornis* using a simple rule-based climate envelope model, to validate the model against published data and use the validated model to project an expansion in habitat suitability for *H. longicornis* under two alternative climate change scenarios for the periods 2046-2065 and 2081-2100, relative to the climate of 1981–2010. The model showed good agreement with two published distributions and predicted that 75% of cattle farms in the North Island, 3% of cattle farms in the South Island and 54% of cattle farms in New Zealand overall have habitats potentially suitable for the establishment of *H. longicornis* ticks. The climate change projections showed limited expansion of tick habitats on the West Coast of South Island and slight reduction in the East Cape, North Island.

Lawrence, K. E., Summers, S. R., Heath, A. C. G., McFadden, A. M. J., Pulford, D. J., Tait, A. B., & Pomroy, W. E. (2017). Using a rule-based envelope model to predict the expansion of habitat suitability within New Zealand for the tick *Haemaphysalis longicornis*, with future projections based on two climate change scenarios. *Veterinary Parasitology*, 243, 226-234.



### **Clinical histories of cattle affected with bovine anaemia associated with *Theileria orientalis* Ikeda type infection**

The aim of this study was to determine the most commonly used words in the clinical histories of animals naturally infected with *Theileria orientalis* Ikeda type; whether these words differed between cases categorised by age, farm type or haematocrit (HCT), and if there was any clustering of the common words in relation to these categories. Clinical histories were transcribed for 605 cases of bovine anaemia associated with *T. orientalis* (TABA), that were submitted to laboratories with blood samples which tested positive for *T. orientalis* Ikeda type infection by PCR analysis, between October 2012 and November 2014. Agglomerative hierarchical clustering, using Ward's method, was then performed on the coordinates from the correspondence analysis. The six most commonly used history words were jaundice (204/605), lethargic (162/605), pale mucous membranes (161/605), cow (151/605), anaemia (147/605), and off milk (115/605). The proportion of cases with some history words differed between categories of age, farm type and HCT. The cluster analysis indicated that the recorded history words were grouped in two main clusters. The first included the words weight loss, tachycardia, pale mucous membranes, anaemia, lethargic and thin, and was associated with adult ( $p < 0.001$ ), severe anaemia ( $p < 0.001$ ) and dairy ( $p < 0.001$ ). The second cluster included the words deaths, ill-thrift, calves, calf and diarrhoea, and was associated with young ( $p < 0.001$ ), normal HCT ( $p < 0.001$ ), beef ( $p < 0.001$ ) and moderate anaemia ( $p < 0.001$ ). The conclusions drawn from this analysis were that two potentially different disease syndromes associated with *T. orientalis* Ikeda type infection may exist in New Zealand cattle, one is consistent with the affected cattle suffering from a severe regenerative extravascular haemolytic anaemia and the other is associated with ill thrift and diarrhoea, particularly in young beef cattle.

Lawrence, K. E., Forsyth, S. F., Vaatstra, B. L., McFadden, A. M. J., Pulford, D. J., Govindaraju, K., & Pomroy, W. E. (2017). Cluster analysis of the clinical histories of cattle affected with bovine anaemia associated with *Theileria orientalis* Ikeda type infection. *New Zealand Veterinary Journal*, 65(6), 305-312.

### **Clinical haematology and biochemistry profiles of cattle naturally infected with *Theileria orientalis* Ikeda type**

The aim of this study was to present the haematology and biochemistry profiles for sick New Zealand cattle naturally infected with *Theileria orientalis* Ikeda type over the period 2012 to 2014. The analysis showed that haematology and biochemistry changes, associated with *T. orientalis* Ikeda type infection, were consistent with extravascular haemolytic anaemia being the main pathogenesis of disease. Sick adult dairy cattle were significantly more likely to be severely anaemic than calves. The conclusions from this study were that calves appeared to be less likely to develop severe anaemia than adults and that deliberately exposing young cattle to *T. orientalis* Ikeda type infection was a viable option for controlling disease in endemic unstable areas of New Zealand.

Lawrence, K. E., Forsyth, S. F., Vaatstra, B. L., McFadden, A. M. J., Pulford, D. J., Govindaraju, K., & Pomroy, W. E. (2017). Clinical haematology and biochemistry profiles of cattle naturally infected with *Theileria orientalis* Ikeda type in New Zealand. *New Zealand Veterinary Journal*, accepted for publication.

### **Experimental infection of Friesian bulls with *Theileria orientalis* (Ikeda) and effects on the haematocrit, live weight, rectal temperature and activity**

The objectives of this study were to infect a group of mature dairy Friesian bulls with *T. orientalis* (Ikeda) and measure the effects on the haematocrit, rectal temperature, weight and activity over a follow-up period of 20 weeks. A random sample of 10 of the 17, 2-year-old, Friesian bulls were inoculated with *T. orientalis* (Ikeda) using intravenous injection of 30ml of

infected blood from 2 acute cases of TABA. After infection the bulls were blood sampled, had rectal temperatures taken and weighed 3 times weekly for 13 weeks and then once weekly for a further 7 weeks. Activity pedometers were attached to the bulls from days 8 to 59 and from days 64 to 123. The bulls were kept on a property where ticks had not been reported and the bulls were also treated with flumethrin pour-on tickicide every 4 weeks. The first bull was confirmed Ikeda PCR positive on day 19 after infection, 8/10 bulls were positive by Day 26 and by Day 33 all 10 were positive (additional testing to be completed). On a group basis the maximum *Theileria* DNA for the infected group peaked on Day 52 at 710fg/ $\mu$ l (155750 DNA copies/ $\mu$ l), and on an individual basis the *Theileria* PCR DNA peaked on Day 50.8 with an average DNA yield = 1360 fg/ $\mu$ l (298050 DNA copies/ $\mu$ l). Gene sequencing confirmed that all the infected bulls and none of the control bulls were uniquely infected with only the *T. orientalis* Ikeda type. Overall 6/10 (60%) of infected bulls became clinically anaemic (HCT  $\leq$  0.24 L/L) with the lowest HCT recorded by an individual bull = 0.19 on Day 54. On a group basis, the lowest average HCT was on Day 59 (average HCT = 25.0) for the infected group and on an individual basis was at an average of 68.7 days (average HCT = 22.8). The HCT of the infected bulls was significantly lower for the period 47 to 80 days post infection. The Least Squares means difference plot showed an almost symmetrical fall and rise in HCT over Days 25 to 125 as well as an increased HCT for the first 3 to 24 days in the infected group. On average there was 18 days between the peak in *Theileria* DNA and the HCT nadir for individual animals. There was no pyrexia recorded in the infected bulls. Over the entire study period there was no significant difference in the weight gain of the two bull groups, however the control bulls were on average heavier from day 70 to day 138, the convalescent period for the infected bulls. The control bulls on average took 189 steps per day less than the control bulls and took fewer steps than control bulls on 101/111 sample days. The study confirms the findings from Jenkins and Bogema (2016) that the infection intensity peaks roughly 18 days before the lowest HCT is reached.

*Gibson, M., Lawrence, K. E., Hickson, R. E., Gedye, K., Hoogenboom, A., Fermin, L., & Pomroy, W. E. (2018). Experimental infection of Friesian bulls with Theileria orientalis (Ikeda) and effects on the haematocrit, live weight, rectal temperature and activity. Article in preparation, targeting Veterinary Parasitology.*

## **A Veterinarian practitioner's experience with *Theileria* on the tick fringe zone of New Zealand**

David Rankin, Wanganui Veterinary Services, Box 911 Wanganui, 4500, New Zealand

I will outline the progression of the disease in our practice, the variance of clinical cases, briefly how we deal with these and lastly how I believe I can predict the next seasons' outbreak by looking at the diseases epidemiology.

The first cases of *Theileria* were diagnosed in the spring of 2013 with our initial farmer having over 100 clinical cases out of 550 and more than 25 deaths. The 2014 spring saw this farmer have around 50 clinical cases and no deaths; we also had about another six farmers with between 2 and 10 cases. The 2015 season we saw the original farm have around 25 cases but we had a significant outbreak on another farm of around 90 clinically affected out of 1000 and 20 deaths another 6 farmers had smaller outbreaks, that season we also had poorer than expected growth rates in one of our heifer grazing blocks which had 850 heifers grazing of which around 800 had seroconverted to be positive for *Theileria* and it was the first time I diagnosed *Theileria* in a 3 to six week old beef calf which had died. The 2016 season again saw my original farm with about 20 cases, the standard few other farms with the odd case, a significant outbreak with around 70 clinical cases out of 500 and two deaths and lastly a case of spring calving heifers all having to be dried off early in the Autumn due to being infected. In the 2017 season we are still seeing a few farms with the odd case but are in the process of dealing with a 600 cow herd in which we have treated 25 cows with one death thus far.

The clinical cases vary from the classic jaundiced anaemic cow around calving with a death rate of around 25% if left untreated, poor growth rates in rising yearlings, deaths in beef calves and cows and poor production in cows and heifers in stress periods.

We are dealing with most outbreaks by proactively using buparvaquone, in some herds we still either dry off clinicals or reduce stress by only milking them once a day and only walking them short distances, blood transfusions in severe clinical disease along with buparvaquone, strategic use of an alternate species such as sheep to produce safe zones for beef calves and strategically using natural immunity to prevent further outbreaks.

Predicting the following seasons outbreak likelihood involves understanding that for cattle to be infected you firstly need enough infected cattle to then infect enough ticks which then infect the naive cattle population. This then involves evaluating individual farms to estimate their approximate tick burden to see if they would be exposed pre-calving (high risk of clinical disease) or from 4 weeks post calving (high likelihood of protective immunity developing). The risk factors start with proximity to deer farms or is the farm a converted deer farm (high tick burdens), is the farm flat or hilly (flat well grazed farms have low tick burdens), does the farmer send his cattle off for winter grazing and is his herd likely to already have been exposed and lastly does the farm have more than one species of livestock present (as if you have a high sheep to cattle ratio you will have less ticks carrying theileria).

In conclusion, we find treating cattle with clinical theileriosis with buparvaquone to be very effective, getting herds immune to the disease reduces the severity of future outbreaks and lastly trying to eradicate ticks and therefore *Theileria* leaves your herd exposed to the risk of a large outbreak especially if you graze in-calf cows off for the winter.

**Heat shock, but not temperature, is a biological trigger for the exsheathment of third stage larvae of *Haemonchus contortus***

Kiliana Bekelaar, Tania Waghorn, Michael Tavendale, Dave Leathwick

AgResearch Ltd, Grasslands Research Centre, Private Bag 11008, Palmerston North, New Zealand

Gastro-intestinal nematodes are a major issue in ruminants, and resistance to current treatment methods is continuously increasing. Understanding the processes involved in the transition from the free living to the parasitic life stage of gastro-intestinal nematodes may aid in identifying new targets amenable to future intervention, either inside of the host or out on pastures. This transition to parasitism is initiated by exsheathment and is triggered by the sudden change in environment after ingestion by the host. We investigated the involvement of temperature and found that there are very specific requirements. The exsheathment response of *Haemonchus contortus* is highly dependent on the rate and magnitude of the temperature change. This greatly enhances our understanding of exsheathment and implicates rapid temperature change, but not temperature itself, as an important factor in triggering exsheathment.

**Genetic and phenotypic relationships between CARLA IgA, IgG, faecal egg counts and productivity in a commercial Angora goat herd**

Richard Shaw and Mary Wheeler

AgResearch Limited, Hopkirk Research Institute, Private Bag 11008, Palmerston North 4442,  
New Zealand

Gastrointestinal nematode (GIN) infections are a major problem for small ruminant production and especially so in goats. In part this is thought to be due to goats evolving as browsers as opposed to grazers like sheep and cattle. Goats tend to have weak immunity to parasite infection presumably by altered immunity due to avoidance of infective L3s. Breeding for nematode resistance is a sustainable means to control GIN that is slowly being taken up by sheep farmers in NZ and overseas. The practice is not thought to be that widespread amongst goat farmers.

A commercial Angora goat herd where animal pedigree was recorded was used to assess heritability and relationships between the host resistant traits CarLA-specific antibody (IgA & IgG) and faecal egg counts (FEC) and productivity (live weight and fleece weight). Angora goats produced a strong CARLA antibody response by 7-8 months of age which was still present at 18-20 months of age when testing of all animals occurred. CarLA IgA and IgG are moderately heritable in young Angora goats at levels similar to estimates determined previously in sheep. FEC had a lower heritability estimate but this in the range of previously published values for Angora goats and sheep. In most cases the genetic and phenotypic correlations between the traits measured were favourable. Issues with incorporating these traits into Angora flocks will be discussed.

## **The circadian periodicity of oocyst shedding of *Eimeria* spp. affecting brown kiwi**

Harry S. Taylor, BVSc MSc (*Con. Bio.*)

Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston  
North. [harry.s.taylor@gmail.com](mailto:harry.s.taylor@gmail.com)

Operation Nest Egg is an important tool for kiwi conservation however as hatcheries and rearing centres result in a high density of young, immunologically naive kiwi being reared in semi-captive conditions, environmental build-up of pathogens, such as coccidia, occurs with a resultant increased risk of disease. Coccidia are protozoal parasites that cause weight loss, diarrhoea and death and significant morbidity and mortality from coccidia has been reported in numerous populations of kiwi, in particular at crèches. At present much of what we base testing and treatment on is extrapolation from the extensive knowledge of *Eimeria* spp. in commercial chickens, however with the kiwi's unique evolutionary history and drastically different biology and ecology differences in parasite interactions are likely too. Understanding the host/parasite interaction is key to developing effective management plans and it is imperative that our understanding improves to enable appropriate disease management to reduce coccidia's detrimental impact on kiwi and ensure the success and sustainability of ONE. It is well documented in other bird species that coccidial oocysts are shed in the faeces at different times of the day in order to maximise the potential for intra-specific host ingestion and/or increase their survival rate. Due to the nocturnal lifestyle it is likely that this pattern is different for kiwi, thereby impacting the accuracy of faecal oocyst counts depending on the time of the day the sample was collected. Here I will share the outcomes of research into the shedding cycle of coccidia spp. in kiwi and discuss the practical and theoretical implications of what we found.

## Validation of PCR methods for detection of *Toxoplasma gondii* in shellfish

Alicia Coupe, Laryssa Howe, Elizabeth Burrows, Abigail Sine, and Wendi D. Roe.  
IVABS, Massey University, Palmerston North.

The zoonotic protozoan parasite *Toxoplasma gondii* is now recognised as important marine pathogen with oocysts carried to coastal waters in land-sea runoff. Currently there are no reliable methods to detect *T. gondii* directly in seawater to assess the extent of contamination in marine ecosystems. However, filter-feeding shellfish may be able to serve as biosentinels of *T. gondii* contamination. Worldwide, a variety of PCR-based molecular methods have been used to confirm presence of *T. gondii* DNA in a number of marine shellfish species. However, there is no standardised PCR method available for detection of *T. gondii* in shellfish, and comparative studies assessing performance of popular PCR assays are lacking. Therefore, the primary objective of this study was to evaluate analytical sensitivity and specificity of two nested-PCR assays targeting the *dhps* and B1 genes, and two real-time assays targeting the B1 gene and a 529bp repetitive element, for detection of *T. gondii* tachyzoites and oocysts. These assays were subsequently validated for *T. gondii* detection in green-lipped mussel (*Perna canaliculus*) haemolymph using oocyst spiking experiments. While PCR assays validated in this study can be used to show presence of *T. gondii* DNA, they do not distinguish between unsporulated and sporulated oocysts, and only sporulated oocysts can be infectious. An additional RT-PCR targeting the sporozoite-specific SporoSAG gene of *T. gondii* was therefore employed to investigate presence of sporulated oocysts in mussel haemolymph. Testing samples in triplicate, all assays could reliably detect 50 oocysts spiked into mussel haemolymph, but the lowest limit of detection was 5 spiked oocysts using the real-time repeat element assay. The nested PCRs were most sensitive for tachyzoites; the nested *dhps* had a sensitivity of 10 tachyzoites and the nested B1 consistently detected a single tachyzoite. Specificity was evaluated by testing DNA from closely related protozoans, *Neospora caninum* and *Hammondia hammondi*. Both nested PCRs were specific to *T. gondii*. However, both real-time assays cross-reacted with *N. caninum*. Using the RT-PCR assay, SporoSAG mRNA was detected in total RNA extracted from 1000 oocysts but not from 1000 tachyzoites. Furthermore, RNA from sporulated oocysts was detected in 4 out of 7 haemolymph samples previously found to be positive for *T. gondii* by nested *dhps* PCR. These studies suggest that the real-time repeat element assay may be preferable for future green-lipped mussel studies. The SporoSAG RT-PCR may be a useful tool to detect sporulated oocysts in shellfish and evaluate health risks for consumers, but assay sensitivity and specificity remains to be determined.

## Investigation of association between *Toxoplasma gondii* and early pregnancy and abortion rates in New Zealand farmed red deer

K. Patel<sup>1</sup>, L. Howe<sup>1</sup>, G. Asher<sup>2</sup>, C. Heuer<sup>1</sup>, P. Wilson<sup>1</sup>

<sup>1</sup>Institute of Veterinary, Animal, and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand

<sup>2</sup>AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel 9053, New Zealand

Reproductive performance in farmed red deer in New Zealand is suboptimal. This study examined association between *Toxoplasma gondii* sero-status, as determined by ELISA, mid-pregnancy abortion, and DNA evidence from maternal and fetal tissue, in young (R2) and adult (MA) hinds on 85 deer farms. At ultrasound pregnancy scanning early in gestation (Scan-1), 31.1% of 861 R2 and 28.3% of 357 MA hinds were sero-positive for *T. gondii*. There was no association between Scan-1 sero-status and non-pregnancy at animal (R2  $P=0.05$ , MA  $P=0.43$ ) or herd level (R2  $P=0.37$ ). *Toxoplasma gondii* DNA was detected in 16% of placenta and 22% fetal brain samples from aborting R2 hinds and 10% of uteri from non-pregnant hinds at Scan-1. Later in gestation at Scan-2, the sero-prevalence in R2 hinds aborted since Scan-1 (34.3%) was significantly higher than in non-aborted R2 hinds (23.5%) (OR=1.6,  $P=0.032$ ,  $n=714$ ). However, no difference in sero-prevalence was observed in aborted (32.9%) or pregnant (31.4%) MA hinds ( $P=0.21$ ,  $n=372$ ). The within-herd sero-prevalence at Scan-2 was positively associated with daily abortion rate in R2 herds having aborted hinds ( $P=0.0003$ ) but not in MA herds ( $P=0.07$ ). *Toxoplasma gondii* DNA was detected in 16% of uteri, 10% coyledons, and 1/5 fetal brains from aborted hinds at Scan-2 and in 15% of uteri from hinds not rearing a calf to weaning. In R2 hinds, 7.9% of abortions could be attributed to *T. gondii*. These findings provide serological and molecular evidence that *T. gondii* causes abortion in deer, possibly in all three trimesters.



**A novel application of an anthelmintic mixture for use against gastrointestinal parasites of red deer (*Cervus elaphus*)**

P.L.Hughes

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**AIMS:** Clinical investigation of a novel mixture of proprietary anthelmintics delivering 0.5mg/kg moxidectin, 9.06mg/kg oxfendazole, 15mg/kg levamisole and 0.08 mg/kg selenium on a bodyweight basis, per os to weaner red deer.

**METHODS:** There were three components to this clinical investigation.

- a) Efficacy study. Six weaner mixed sex red deer were treated orally with a 50/50 mixture of Exodus Pour-On (Merial Ancare, Auckland, NZ) and Oxfen C Plus (Merial Ancare, Auckland, NZ) (Ex/Ox ) at a dose rate of 1ml/5kg bodyweight. Six similar weight herd mates were untreated. Eleven days later all 12 deer were tranquilised and euthanised and the gastrointestinal (G/I) tracts sent to Gribbles Laboratory (PN) for total worm counts. The lungs were examined grossly.
- b) Residue analysis. Eight rising one- year- old mixed sex red deer were sent to a deer slaughter plant (DSP) where tissue samples were collected and sent to R J Hill Laboratory for residue analysis. Six of these deer were treated with the Ex/Ox combination at 1ml/5kg orally 39 days prior. Two deer had not been treated. Samples were taken from muscle, liver, kidney and fat from all eight deer. These 32 tissue samples were analysed for residues to fenbendazole (including sulfone), oxfendazole, levamisole and moxidectin.
- c) Stability profile. The Ex/Ox combination was analysed by Labtec Scientific and Technical Services to determine physical and chemical stability after six weeks in ambient storage.

**RESULTS:**

- a) Efficacy study. Abomasal worm counts for the six untreated deer revealed an arithmetic mean burden of 2,566 *Ostertagia*-type worms and 300 *Trichostrongylus axei*. No worms were detected either in the small intestines of these animals or in the abomasa of the treated group. No lungworm were detected grossly.
- b) Residue analysis. Moxidectin was the only anthelmintic compound to show residues and the concentrations measured were well below the maximum residue limit (MRL) published by the Ministry of Primary Industries (MPI).
- c) Stability profile. Analysis of the Ex/Ox product after six weeks storage at 2-8°C and room temperature indicate good physical and chemical stability.

**CONCLUSIONS:**

- a) Efficacy study. The Ex/Ox combination orally dosed at 1ml/5kg bodyweight demonstrated a high efficacy against abomasal parasite burdens in weaner mixed sex red deer on a property that previously had severely parasitized animals.
- b) Residue analysis. The Ex/Ox combination orally dosed to rising one-year-old red deer at 1ml/5kg bodyweight in this clinical investigation had residue concentrations well below the recognised MRL for all three active compounds 39 days post treatment.
- c) Stability profile. The mixture was stable for 6 weeks and hence the mix- and- use- immediately rule may not need to apply to the Ex/Ox combination.

**CLINICAL RELEVANCE:** These on farm clinical investigations support the hypothesis that the Ex/Ox combination used can be an effective and practical anthelmintic option for use in red deer against an industry background of widespread gastrointestinal parasite resistance to the registered alternatives.

## **Prevalence of liver fluke on farms of suppliers to Westland Milk Products**

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Fasciolosis is thought to be a common parasite on the West Coast of the South Island of New Zealand and an in-herd prevalence of 25% is considered to be production limiting. The aim of this study was to determine the prevalence and intensity of *Fasciola hepatica* infection in dairy cows in this region using the presence of antibodies in a bulk milk sample from dairy farms. Samples of vat milk from 430 suppliers to Westland Milk Products were collected on two occasions during March 2017. Bulk milk samples were analysed for antibodies to *F. hepatica* using a commercial ELISA test (IDEXX Fasciolosis Verification, IDEXX Europe BV, Hoofddorp, The Netherlands) which measures the antibody present to the f2 antigen which is highly specific for *F. hepatica*. The colour reaction is read as an optical density and the S/P% is calculated with reference to both negative and positive controls with this being used to correlate the percentage of cows infected in each herd. This test is able to estimate the infection level in the herd in 4 categories; >50% infection; 20-50% infection; <20% infection level; no infection. The test sensitivity is 97.5% with a specificity of >99%. All herds were tested in either the first (n=389) or second week (n=41) and a subset of 99 herd samples from the first week were also retested in the second week to determine the repeatability of a result from a herd. Autumn was chosen as this is thought to be the time of year when most cows will have seroconverted to infection. A short survey was also posted to each supplier with 157 returned completed.

Of the 430 farms tested 133 (31%) had an S/P% ≤30 indicating no infection, 49 (11%) had an S/P% of 30.1-80 indicating <20% cows were infected, 62 (14%) had an S/P% of 80-150 indicating 20-50% cows were infected and 186 (43%) had an S/P% of >150 indicating >50% cows were infected. Overall, 298/430 (58%) farms had a level of infection with *F. hepatica* that is considered to be production limiting. Suppliers were identified as coming from 18 different regions. Canterbury was one region and all farms from here tested negative (n=35). Of the remainder 9/18 regions had >50% farms in the strong infection group. For the 99 samples which were tested on both occasions the Kappa analysis by level of infection was 0.7 which indicates a substantial agreement between the two test points.

From the survey: 85% of all farmers have been aware of liver fluke on their farm in the last 5 years, including 58% whose farms tested negative: 70% of all farmers treated their cows with a flukicide during the dry period (non-lactating) of which 94% treated all of the herd: 33% of farmers whose herd had tested negative also treated all of the herd. For younger cows, 65% and 68% of all farmers specifically treat R1 and R2 heifers respectively at least once against liver fluke including 50% and 47% of the farms which tested negative. In this study 248/430 (58%) of farms on the West Coast tested positive for liver fluke at a level that is considered to cause production losses. Of the 70% of farms who treated cows, 94% chose whole herd treatment.

These results indicated that liver fluke is a common infection on the West Coast and further study is required to determine when infection occurs and the degree of production loss attributed to infection with *F. hepatica*.

## Comparative pharmacokinetics of abamectin in sheep and goats.

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**Background:** The objective of this study project was to measure the pharmacokinetic (PK) parameters of the macrocyclic lactone anthelmintic abamectin in goats and then manipulate the dose rate for goats so the PK parameters more closely resemble those of sheep. It has been established that goats metabolise anthelmintics more rapidly than sheep which means goats are usually effectively under dosed when treated at sheep dose rates, yet most farmers still use dose rates established for sheep when treating goats. Underdosing is an accepted risk factor for developing anthelmintic resistance as it allows resistant, and even some partly resistant parasites, to survive. Despite this risk, there has been no study to date which has precisely quantified the degree of underdosing in goats when using sheep dose rates by testing the same commercial product in both animals in both species under the same conditions. Similarly, no effort has been made to develop a goat-specific dosing regimen to provide levels of protection similar to those achieved for sheep.

**Methods:** Six adult Romney sheep were orally drenched with Genesis Hi Mineral (abamectin 1g/L) at 0.2-mg/kg body weights. Blood samples were collected from jugular veins at 0 (before drug administration), 3, 6, 9, 12, 15, 18, 24, 72, 120 and 168 hours after the administration of drug. Six adult Saanen goats were used both for 0.2 and 0.4 mg/kg dose experiments after a wash out period of two months. The plasma concentration of abamectin was analysed by high performance liquid chromatography (HPLC) with fluorescence detection.

**Results:** The HPLC method used for analysis of abamectin in sheep and goat plasma was linear from 0.8 to 192 ng/mL. The correlation coefficient was 0.9967. The inter- and intra-day variation ranged from 4.23 to 9.30 and 0.07 to 6.7%, respectively. The overall recovery of abamectin ranged from 75 to 83%, for sheep and goat plasma. Goats orally dosed with 0.2 mg/kg abamectin had a significantly lower C<sub>max</sub> (11.5 ± 1.6 ng/mL) as compared to goats dosed with 0.4mg/kg abamectin (20.2± 5.9 ng/mL). The C<sub>max</sub> was significantly higher in sheep (20.1±7.3 ng/mL) as compared to goats dosed orally at 0.2 mg/kg.

There was no significant difference in the other pharmacokinetic parameters.

**Conclusion:** The difference in the sheep and goat pharmacokinetics suggest that goats given abamectin at sheep dose rates are potentially under dosed and are at risk of selecting their nematodes for anthelmintic resistance. A comparative efficacy study between these two dosing regimens is required to substantiate the anthelmintic resistance problem due to under dosing in goats.

***Giardia intestinalis* at the New Zealand Police Dog Breeding Centre**

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A clinical outbreak of *Giardia intestinalis* at the NZ Police Dog Breeding Centre has been investigated. Genotyping detected assemblage types B, C and D are involved. The C and D assemblages appear to show resistance to treatment with Fenbendazole (50mg/kg once daily x10 days). Subsequent treatment with metronidazole (20-25mg/kg twice daily x10 days) and fenbendazole (50mg/kg once daily x10 days) has resulted in elimination of the *Giardia* from affected dogs. Treatment also involved environmental management and bathing of affected individuals on days 0, 5 and 10 of treatment.

**The effect of mid-lactation treatment with topically applied eprinomectin on milk production in nine New Zealand dairy farms**

Lawrence, K. E., Tulley, W. J., Scott, I., & Pomroy, W. E. Institute of Veterinary Animal and Biomedical Sciences, Massey University, New Zealand

The aim of the study was to evaluate the effect of mid-lactation eprinomectin treatment on milk production in New Zealand. A random sample of cows from nine commercial dairy herds in the Manawatu region of New Zealand was treated once with pour-on eprinomectin at the dose rate of 0.5mg/kg during the afternoon milking in the period 6 Jan 2013 to 24 Jan 2013. The treatment cows (n=540) were matched to control cows (n=540), within their respective herds, on age and calving date. The outcome measure used for the study was 24-hour test-day energy-corrected milk production per cow and the change in milk production, recorded at the first two herd tests after eprinomectin treatment, was analysed using a linear mixed effects model. The study found a small but significant increase in energy corrected milk production of 0.35kg/day (95% CI 0.05–0.64) at the first and second herd tests after treatment. There were no significant interactions found between treatment and age or between treatment and the bulk tank *Ostertagia* antibody measured on the day of treatment. A concurrent North Island drought may have affected the response to treatment. The small effect of eprinomectin treatment on milk production found in this trial is, we believe, not sufficient to advocate whole herd treatment of dairy cows in New Zealand. With the real risk of developing widespread ML-resistance whole herd eprinomectin treatment represents a true short-term gain for long-term pain.

*Lawrence, K. E., Tulley, W. J., Scott, I., & Pomroy, W. E. (2017). The effect of mid-lactation treatment with topically applied eprinomectin on milk production in nine New Zealand dairy farms. Veterinary Parasitology: Regional Studies and Reports, 10, 95-101.*

## **A report on a “Survey of aspects of sheep management: flystrike and lice”**

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There has been minimal research conducted around the various ectoparasites of sheep in recent years. A questionnaire was sent out with the special edition of Country Wide Sheep in October 2016. This was sent to 14000 farmers. It comprised 30 questions on aspects of management of flystrike, louse control, tailing and castration. A total of 1254 questionnaires were returned. For flystrike, it is notable that overall 83% of farmers consider it “important” or “very important”. Even at the bottom of the South Island there are still farmers who consider it “important/very important” although that percentage is lower than further north. For two-tooth and older sheep there is a small percentage (3%) who report no flystrike but the majority still report flystrike despite adopting control programmes to prevent this occurring. Overall 15% report >2% of these older age groups flystruck during this calendar year. For lambs/ewe hoggets there is an even smaller percentage (1%) who report no flystrike compared with the predominance of farmers who still report flystrike despite the majority adopting control programmes to prevent this occurring. Overall 21% report >2% of this age groups flystruck during this calendar year. Interestingly, flystrike is still reported, on at least some lambs, by the majority of farmers (88%) in the bottom of the South Island.

For lice, 76% of farmers consider them to be either “important” or “very important”. There is a general trend for this opinion to be stronger at the bottom of the South Island than further north in New Zealand.

Methods to apply insecticides show that automatic jetting races are the most popular but even so were used by less than half the respondents. Backline applications were the next most popular. The use of shower dips has declined and indicated that only 9% of respondents used this method and only a very small percentage still used a plunge dip.

Veterinarians were the most favoured source of information about ectoparasites but even then were only considered “useful” or “very useful” by 71% of farmers indicating room for improvement by many. The least helpful source was farm consultants where the responses indicated that 36% of farmers indicated they were of no use at all.

## **Hedgehogs are a potential reservoir host for scabies in dogs and humans**

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Sarcoptic mange affects many species of mammals, with wild animals at times acting as a source of infestation for domestic animals. This study examined hedgehogs for *Sarcoptes scabiei* to ascertain if hedgehogs in New Zealand are a potential reservoir host for sarcoptic mange. Dead hedgehogs were collected from veterinary clinics, rescue centres, members of the public and from road-kill. The bodies were kept frozen until the time of examination, when they were thawed and material was collected, via brushing, for microscopic examination. Both *Caparinia* and *Sarcoptes* mites were identified on microscopic examination with *Sarcoptes* the most common, being found on over 70% of animals examined (n=38). The numbers of mites recovered after brushing the head and body ranged from 1 to 5659 (median = 341 mites) with only six animals (22.2%) having fewer than 10 *Sarcoptes* mites found. Concurrent hyperkeratosis was seen on most infected hedgehogs. *Caparinia* mites were seen on fewer animals and generally in very low numbers. Polymerase chain reaction (PCR) testing, I (cox 1) gene, was undertaken on material from two hedgehogs. Both *Caparinia* and *Sarcoptes* mites were identified on microscopic examination. These findings indicate a significant change in the mite populations on hedgehogs in New Zealand. It appears hedgehogs have acquired sarcoptic mange since their arrival in New Zealand and this may account for the recent decline in hedgehog numbers. The source of sarcoptic mange is assumed to be one of the three primary hosts of *Sarcoptes* in New Zealand; namely, humans, dogs, or pigs. PCR targeting the cytochrome oxidase subunit of two hedgehogs showed close alignment with sequence derived from a pig in one case whilst in the second case was most similar (although two single nucleotide polymorphisms were present) to sequence derived from dog. More work needs to be undertaken to identify the source(s) of the *Sarcoptes* found on hedgehogs in New Zealand, in order to better understand which, if any, other mammalian hosts would most likely be susceptible to infestation.

## Surveys of nematode parasitism in dogs and cats in New Zealand

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There have been a number of attempts over the years to assess the prevalence of gastrointestinal nematodes in dogs and cats, the vast majority involving the examination of faecal samples for eggs, and many were never published or presented only to a limited audience (e.g. in newsletters). The following tables present summaries of all known coprological surveys, from the late sixties onwards.

Surveys fall primarily into two categories, those that specifically examined healthy animals and those that relied on the submission of samples to a diagnostic laboratory, in which case sampled animals may have been affected by a condition for which nematodes or other GI parasites (e.g. coccidia) were a suspected cause, or were healthy animals (e.g. health screen of newly acquired pet). Some surveys specifically looked at working farm dogs or included pound/shelter dogs.

**Table 1 Summary of findings of nematode eggs in faecal samples from dogs**

Source	MU	Fielding Vets	BAHL	MU	O'Connell	Mark M	Merial	MU	PNCC Pound	Mngumi
Survey type	Diagnostic	Healthy	Diagnostic	Healthy	Healthy	Healthy	Healthy	Diagnostic	Healthy	Healthy
Date	1969-75	1975	90-91	1996	2010	2013	2014-15	2006-15	2006-15	2016
n	654	202	232	45 (pet)	170 (farm)	185 (farm)	54	348	797	259 (farm)
Any nematode			24.1	15.6	19.4	21	19	11.5	37.9	21.7
Toxocara	9.9	8.4	3.4	8.9	5.3	9	11	6	12.3	13.9
Hookworm	13	36	6.5	6.7	11.8	10	4	3.4	23.5	4.3
(Ancylostoma)#		3.5								
Trichuris	16.4	21.8	17.7	2.2	4.7	9	6	2.3	11.7	3.5

#in the Fielding survey, hookworm eggs were differentiated on the basis of size, for this survey, hookworm = *Uncinaria stenocephala*.

**Table 2 Summary of findings of nematode eggs in faecal samples from cats**

Source	MU	BAHL	MU	Merial	MU
Survey type	Diagnostic	Diagnostic	Healthy	Healthy	Diagnostic
Date	1983-90	90-91	2006-7	2014-15	2006-15
n	155	54	70	98	274
Any nematode	23.2		1.4	14	6.9
Toxocara	21.9	5.6	1.4	11	6.6

Taken together, these surveys suggest that GI nematodes are less common than they used to be, especially in pet dogs, *Trichuris vulpis* in particular, but that some populations of dogs (farm and pound/shelter) remain relatively wormier.

### Sources:

MU Diagnostic – samples submitted from the Massey University Veterinary Clinic to the Parasitology lab.

Fielding Vet Club – McDiarmid E, Somerville GF and Charleston WAG (unpublished).

BAHL – Canine and feline parasitological examinations at the Batchelar Animal Health Laboratory. Newsletter.

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## Comparison of three molecular diagnostic techniques for the diagnosis of amoebic liver abscess

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**Objective:** Amoebiasis is one of the most common parasitic infection in humans. *Entamoeba histolytica* is the main aetiological agent. Molecular techniques are considered as reliable method for diagnosis of infectious agents in clinical samples as these methods have high sensitivity and specificity. The present study was planned to compare three molecular diagnostic techniques for the detection of *E. histolytica* from clinical samples of amoebic liver abscess (ALA).

**Patients and Methods:** A total of 37 pus samples were collected from suspected patients of ALA who attended emergency medical OPD/Liver clinic of the Postgraduate Institute of Medical Education and Research, Chandigarh from January 2016 to November 2016. Pus aspirate of liver abscess were obtained by ultrasound guidance. Three molecular diagnostic techniques viz. conventional PCR, Real Time PCR (Taqman assay) and Loop Mediated Isothermal Amplification assay (LAMP) was performed targeting the gene of the small subunit of ribosomal RNA of *E. histolytica*.

**Results:** The mean age of the recruited patients was 44.2 years with M:F ratio of 33:4. Of the 37 samples, Real time PCR was able to detect DNA of *E. histolytica* in all 37 samples and conventional PCR and LAMP assay were able to pick 34 specimens and missed 3 samples. The conventional PCR missed 2 samples which were picked by LAMP assay, similarly the LAMP assay missed 2 different samples which were picked by conventional PCR; one sample was negative by both conventional PCR and LAMP assay.

**Conclusion:** Real Time PCR showed a higher sensitivity as compared to conventional PCR and LAMP assay. However, LAMP proved to be simpler, cost effective and has the potential to be used in field setting and low resource laboratories.

## Posters

### **Local and global genetic diversity of protozoan parasites: Spatial distribution of *Cryptosporidium* and *Giardia* genotypes**

Juan Carlos Garcia-R, Nigel French, Anthony Pita, Niluka Velathanthiri and David Hayman  
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Cryptosporidiosis and giardiasis are recognized as important diseases due to their long-term health effects in humans and economic impact in agriculture and medical care. Molecular analysis is essential to identify species and genotypes causing these infectious diseases and provides a potential tool for monitoring. Here, we describe the species, subtype families and assemblages of *Cryptosporidium* and *Giardia* parasites present in New Zealand to compare the current variation with those found in other countries around the globe. Species identified in New Zealand also occurred worldwide and there are not endemic genetic variants in the country compared with other gp60 genotypes and gdh assemblages. This indicates that isolation did not promote divergence due to high gene flow of historical and current human activity (travel and trade) and persistence of large host population sizes. However, genetic variation is underestimated by data biases (e.g. neglected submission of sequences to genetic databases) and low sampling. New genotypes are likely to be discovered as sampling efforts increase according to accumulation prediction analyses. Overall our study represents a comprehensive overview for exploring local and global protozoan genotype diversity and advances our understanding of the importance for surveillance and potential risk associated with these infectious diseases.

### **Origin of a major infectious disease in vertebrates: The timing of *Cryptosporidium* evolution and its hosts**

Juan Carlos Garcia-R and David Hayman  
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*Cryptosporidium* species are parasites that have been found in all vertebrate groups and display some host specificity in their infections, although most of their diversity is associated with mammal and bird species. It is therefore possible to assume that *Cryptosporidium* parasites evolved intimately aside with vertebrate lineages. Here we propose a scenario of *Cryptosporidium*–Vertebrata coevolution testing the hypothesis that the origin of *Cryptosporidium* parasites follows that of the origin of modern vertebrates. We use calibrated molecular clocks and cophylogeny analyses to provide and compare age estimates and patterns of association between these clades. Our study provides strong support for the evolution of parasitism of *Cryptosporidium* with the rise of the vertebrates about 600 million years ago (Mya). Interestingly, periods of increased diversification in *Cryptosporidium* coincides with diversification of crown mammalian and avian orders after the Cretaceous-Palaeogene (K-Pg) boundary, suggesting that adaptive radiation to new mammalian and avian hosts triggered the diversification of this parasite lineage. Despite evidence for ongoing host shifts we also found significant correlation between protozoan parasites and vertebrate hosts trees in the cophylogenetic analysis. These results help us to understand the underlying macroevolutionary mechanisms driving evolution in *Cryptosporidium* and may have important implications for the ecology, dynamics and epidemiology of cryptosporidiosis disease in humans and other animals.