BAVP

Bristol

14-15 April 2016

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Programme

Thursday 14th April

Time	Speaker	Title
1.00 – 2.00	Lunch – rear foyer, Bristol Museum	
Session 1	**Helminth epidemiology**	
2.00	Marisol Collins, University of Liverpool.	The *Hy*Data Project: Investigating the distribution of *Echinococcus granulosus* (sensu lato) in the UK
2.20	Martha Betson, University of Surrey.	Molecular epidemiology of *Ascaris* and *Trichuris*
2.40	Catherine McLeonard, University of Liverpool.	Controlling the uncontrollable: predicting the risk of lungworm outbreaks in dairy herds in the UK?
3.00	Ludovica Beltrame, University of Bristol.	Simulating the risk of Liver Fluke infection using a mechanistic hydro-epidemiological model
3.20	Tea / coffee – mezzanine (up the stone steps)	
Session 2	**Arthropods and arthropod-borne diseases**	
4.00	Richard Wall, University of Bristol.	Tick and tick-borne disease surveillance in the UK: the Big Tick Project
4.20	Roger Daniel, Animal and Plant Health Agency.	Inter-current tick-borne fever infection and *Bibersteinia trehalosi* septicaemia in a five week old lamb
4.40	Mark Eisler, University of Bristol.	Vector-borne diseases of African livestock: modelling the hard way
5.00	Close	
7.30	Dinner – Jamie's Italian (opposite Will's Memorial Building)	

Friday 15th April

Time	Speaker	Title
Session 3	**Detection and management of anthelmintic resistance**	
9.00	Gerald Coles, University of Bristol.	A fresh look at anthelmintic resistance in sheep
9.20	Jonathan Love, University of Strathclyde.	Probability distributions of faecal egg count data and their impact on investigating anthelmintic efficacy
9.40	Hannah Rose, University of Bristol.	Attitudes of horse owners to faecal egg count directed treatment strategies
10.00	BAVP AGM	
10.30	Tea / coffee - mezzanine (up the stone steps)	
Session 4	**New therapies and aetiologies**	
11.00	Esther Rawlinson, Merial Animal Health.	Prevention of the establishment of *Angiostrongylus vasorum* infestation in dogs through monthly oral administration of milbemycin oxime/afoxolaner
11.20	Hany Elsheikha, University of Nottingham.	The inhibitory effect of monensin (a Wnt signalling inhibitor) on the growth of *Toxoplasma gondii* infecting human brain cells in vitro.
11.40	Siân Mitchell, Animal and Plant Health Agency.	*Neospora caninum* as a cause of arthrogryposis in a lamb
12.00	Lunch – rear foyer, Bristol Museum - and close	

Abstracts

Session 1 – Helminth epidemiology

The *Hy*Data Project: Investigating the distribution of *Echinococcus granulosus* (sensu lato) in the UK

Authors: Marisol Collins[1], John McGarry[1], Eleni Michalopoulou[1], Michael Rogan[2], Arjen Brouwer[3], Phillip Jones[1]

Institute(s): [1]Institute of Infection and Global Health, University of Liverpool

[2]Cestode Research Group, University of Salford

[3]The Welsh Assembly Government

The tapeworm, *Echinococcus granulosus*, maintains a life cycle that includes dogs as the primary host and sheep as the main secondary host. However, many other species, including cattle, horses and humans can become infected as accidental secondary hosts. Within such hosts, the larval stages of *E.granulosus* can form large 'hydatid cysts' in the liver, lungs and other sites leading to tissue damage and disease. Dogs typically become infected by scavenging or being fed raw infected livestock meat and offal, and will carry the parasite in the intestines without signs of disease. Tapeworm eggs passed in faeces of dogs are orally ingested by the intermediate host during grazing thus completing the lifecycle of the parasite. Humans can become infected through accidental ingestion of eggs in contaminated soil, water or food, or by direct contact with an animal host.

Worldwide, *E. granulosus* presents a significant risk to human health and is an important source of economic loss for livestock industries, associated with poor growth, reduced meat and milk production and rejection of organs at meat inspection. In the UK, human and livestock cases of hydatidosis have historically been restricted to focal farming areas of south Powys in Wales, Herefordshire and the Hebridean Scottish Islands. However, a recent pilot study, utilizing hydatid data from cattle raised throughout the UK has provided strong evidence that the parasite is more widely distributed than previously thought.

This presentation describes the *Hy*Data project, a multi-centre study investigating the national distribution of *E.granulosus* in high-risk dog populations (hunting hounds, farm dogs and pet dogs in rural areas), livestock (cattle, sheep) and horses at slaughter. The study, which runs from 2016 to 2018, aims to build the most comprehensive picture of *E.granulosus* distribution in the UK to date and explore the associated risk factors for animal and human infection.

Molecular epidemiology of *Ascaris* and *Trichuris*

Martha Betson[1], Peter Nejsum[2], J. Russell Stothard[3]

Institute(s): [1] University of Surrey, [2] University of Copenhagen, [3] Liverpool School of Tropical Medicine

The giant roundworm *Ascaris lumbricoides* and the whipworm *Trichuris trichiura* are important soil-transmitted helminth infections of humans. Two parasitic worms of pigs, *A. suum* and *T. suis*, are closely related to *A. lumbricoides* and *T. trichiura*, respectively. Natural *A. suum* and *T. suis* infections are found in pigs worldwide, though their distribution is

dependent on farming practices. Interestingly, *A. lumbricoides* and *A. suum* are virtually indistinguishable based on their morphology, and the same is true for *T. trichiura* and *T. suis*. This raises two questions: 1) Do the *Ascaris* parasites infecting humans and pigs belong to the same or different species? 2) What is the contribution of zoonotic transmission from pigs to the overall burden of *Ascaris* and *Trichuris* infection in humans? We have been addressing these questions through phylogenetic and population genetic analysis of *Ascaris* and *Trichuris* using nuclear and mitochondrial markers.

In a study of over 500 *Ascaris* worms collected from humans and pigs across the globe, we found marked genetic segregation between worms originating from humans and those originating from pigs, suggesting two separate *Ascaris* species in humans and pigs. All human *Ascaris* worms sampled in Europe were of pig origin, and there was evidence of cross-transmission of *Ascaris* between humans and pigs in Africa. In more recent work, PCR-RFLP analysis was carried out on *Trichuris* worms sampled from humans and pigs in Ecuador. Nearly all worms exhibited expected restriction patterns. However, two pig-derived worms showed a "heterozygous-type" ITS-2 pattern and 18S patterns, indicating genetic exchange between *T. trichiura* and *T. suis*. In contrast, phylogenetic analysis of the mitochondrial large ribosomal subunit partitioned all worms by host species.

Our studies provide new insights into the epidemiology of *Ascaris* and *Trichuris*. Understanding the level of cross-transmission between humans and pigs is important for adoption of appropriate control strategies.

Controlling the uncontrollable: predicting the risk of lungworm outbreaks in dairy herds in the UK?

Catherine M[c]Leonard[1], Rob Christley[1], Jonathan Read[2], Jan van-Dijk[1]

[1] University of Liverpool, UK; [2]Lancaster University, UK

The bovine lungworm, *Dictyocaulus viviparus*, causes devastating financial losses to the dairy industry. While many herds maintain an endemic status, the true prevalence of the parasite on UK dairy farms is unknown. In non-endemic herds the threat of outbreaks after reintroduction can have even more devastating effects. Therefore, there is a need for a simple, cheap, herd-level presence-absence test. Published work has shown that, in contrast to *Ostertagia* and *Fasciola*, bulk milk ELISA testing may lead to many false negative diagnoses. Currently, the only indications of lungworm abundance stem from the Veterinary Investigation Diagnosis Analysis (VIDA) surveillance database, which suggests that the incidence of lungworm has increased since the 1990s, particularly in adult cattle.

We present an analysis of the available database, including disease seasonality, gross spatial distribution and host factors. Aiming to improve prevalence data by designing the herd-level test, we also present an analysis of lungworm abundance in endemic herds. The test will be validated for UK herds, using milk recording samples collected by NMR milk laboratories. Subsequently, it will be used not only to identify farm management related risk factors for lungworm outbreaks but also to validate a temporally explicit mathematical model of parasite abundance.

Simulating the risk of Liver Fluke infection using a mechanistic hydro-epidemiological model

Ludovica Beltrame (1), Toby Dunne (1), Hannah Rose (2), Josephine Walker (3), Eric Morgan (2), Peter Vickerman (3), and Thorsten Wagener (1)

(1) Department of Civil Engineering, University of Bristol; (2) School of Veterinary Sciences, University of Bristol; (3) School of Social and Community Medicine, University of Bristol

Liver Fluke *(Fasciola hepatica)* is a common parasite of livestock, responsible for considerable economic losses throughout the world. Risk of infection is strongly influenced by climatic and hydrological conditions, which characterise the host environment for parasite development and transmission. Despite on-going control efforts, increases in fluke outbreaks have been reported in recent years in the UK, and have been often attributed to climate change. Currently used fluke risk models are based on empirical relationships derived between historical climate and incidence data. However, hydro-climate conditions are becoming increasingly non-stationary due to climate change and direct anthropogenic impacts such as land use change, making empirical models unsuitable for simulating future risk.

In this study we introduce a mechanistic hydro-epidemiological model for Liver Fluke, which explicitly simulates habitat suitability for disease development in space and time, representing the parasite life cycle in connection with key environmental conditions. The model is used to assess patterns of Liver Fluke risk for a catchment in the UK under current and potential future climate conditions. Comparisons are made with a widely used empirical model employing different datasets, including data from regional veterinary laboratories. Preliminary results suggest that mechanistic models can achieve adequate predictive ability and support adaptive fluke control strategies under climate change scenarios.

Session 2 - Arthropods and arthropod-borne diseases

Intercurrent tick-borne fever infection and *Bibersteinia trehalosi* septicaemia in a five week old lamb

Roger Daniel, Kate Pugh, Nick Torrens, Amanda Carson, Mark Wessels,

Wales Veterinary Science Centre, APHA

High mortality was recorded in lambs, in a flock of 300 ewes grazing mountain pasture. Deaths occurred at a rate of one per day over a two-week period, which prompted post mortem examination. The first post mortem conducted at the local practice was inconclusive. A second post mortem conducted at the newly opened Wales Veterinary Science Centre revealed a pneumonia, splenomegaly and lymphadenopathy. *Bibersteinia trehalosi* was cultured from lung tissue but the severity of the lesions suggested a further underlying infection. The newly introduced PCR for *Anaplasma phagocytophilum,* the causal organism of tickborne fever, was employed. A positive result was obtained. Further details of the pathology, and the role of *A. phagocytophilum,* will be presented; and the potential role for this new diagnostic tool to investigate disease in tick infested areas.

Session 3 - Detection and management of anthelmintic resistance

A fresh look at anthelmintic resistance in sheep.

Gerald Coles and Eric Morgan, School of Veterinary Sciences, University of Bristol, Langford House, Bristol BS40 5DU.

In 1992 the WAAVP published methods for the detection of anthelmintic resistance and defined resistance as a less than 95% reduction in egg counts with 95% confidence limits less than 90%. This was based on suggestions from Australia, but were they correct? In a recent study in southern Italy where little anthelmintic treatment is used the efficacy of albendazole was 100%, levamisole 99.3% and ivermectin 99.9% . So should the definition of resistance be revised? In order to detect very few eggs in faeces the McMaster slides should be abandoned and the much more sensitive method for egg counts, Mini-FLOTAC, used. It is now generally accepted that once anthelmintic resistance has occurred on a farm it will not disappear. In 1998 we reported the presence of levamisole resistance *Trichostrongylus colubriformis* in lambs purchased from central Wales. Since then the farmer has practiced 'farmer's eye' (a form of TST) for treating the lambs, i.e treating those lambs with a dirty back end and those with a poor wool condition. In October 2015 using 20 lambs the efficacy of levamisole on the farm was 99.1%. Reversion to susceptibility is possible but the full implication of using 'farmer's eye' requires research. The way farmers use anthelmintics is the most important issue in the development of anthelmintic resistance. Over a 10 year period only one action of farmers in the south-west of England to control anthelmintic resistance had changed significantly, running of anthelmintic tests, up to 23%. Only 9% were quarantining introduced sheep correctly. We need to rethink how we communicate effectively with sheep farmers.

Probability distributions of faecal egg count data and their impact on investigating anthelmintic efficacy

Johnathan Love, Louise Kelly, Chris Robertson, Ian Nanjiani, Mike Taylor and Hannah Lester.

University of Strathclyde, Westpoint Research and VParsT Ltd.

Over the past 60 years, the use of anthelmintics has been increasingly under threat due to parasite populations becoming resistant to products in use.The Faecal Egg Count Reduction Test (FECRT) has been the most widely used field-based method for determining anthelmintic efficacy and as an indicator of the presence of anthelmintic resistant nematodes in livestock, based on the World Association for Advancement in Veterinary Parasitology (WAAVP) guidelines. These guidelines make recommendations on both test performance and statistical analysis and data interpretation in an effort to quantify levels of resistance present at the farm level. Whilst aimed primarily at sheep, the guidelines are also used for FECRT in cattle. However, due to differences in levels of faecal egg outputs between sheep and cattle and the limits of detection in faecal egg count (FEC) methods; the guidelines applied to cattle have come under much review. A collaborative project involving the authors has been set up in which one of the aims is to evaluate a range of analytical and statistical methods on extensive field data collected over 3 grazing seasons in which cattle were screened, treated and monitored for parasitic nematode infections using several FEC methods and sensitivities. This investigation has shown that for cattle FECs obtained using a counting technique with high precision (1epg); distributions associated with the Negative binomial are of better representation and hence, arithmetic mean estimates should be used when calculating percentage reductions for a FECRT. However, if cattle FECs are obtained with less precise

counting techniques (30 or 15epg), then it is recommended to take the arithmetic group mean and divide this by the proportion of non-zero counts present in FEC data (zero-inflated distributions), otherwise anthelmintic efficacy could be exaggerated.

Attitudes of horse owners to faecal egg count directed treatment strategies

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Due to the increasing threat of anthelmintic resistance in equine cyathostomins in the UK, faecal egg count (FEC) directed treatment strategies have been encouraged and now form a key part of industry guidelines on the prescription and responsible use of anthelmintics in horses. However, many horse owners use anthelmintics at set intervals, regardless of risk or need. A recent study drew on key theories of behaviour – the Theory of Planned Behaviour and the Health Belief Model - to investigate attitudes of Belgian farmers towards the use of diagnostics prior to treatment of cattle for gastrointestinal nematodes. In the present study the theoretical framework was extended to identify determinants of the use of FEC-directed targeted treatment in horses in the UK. Potential drivers and barriers to the uptake of FEC-directed strategies were identified which could be targeted to encourage the use of FECs.

Session 4 - New therapies and aetiologies

Prevention of the establishment of *Angiostrongylus vasorum* infestation in dogs through monthly oral administration of milbemycin oxime/afoxolaner

Wilfried Lebon[1], Eric Tielemans[1], Steffen Rebhein[2], Pascal Dumont[1], Frédéric Beugnet[1], Philippe Jeannin[1], Lénaïg Halos[1], Esther Rawlinson[3] *
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Context: Lungworm infestation in dogs is mainly due to *Angiostrongylus vasorum* and may result in severe clinical signs. A blinded, randomized and negative controlled study was conducted to evaluate the efficacy of monthly oral administration of NexGard Spectra® chewable tablets, a combination of milbemycin oxime (MO) and afoxolaner, to prevent the establishment of *Angiostrongylus vasorum* in dogs in an experimental model mimicking natural infestation

Methods: Twenty beagle dogs were orally infested seven times every two weeks (on Days -7, 7, 21, 35, 49, 63 and 77), with approximatively 30 third stage larvae (L_3) *A. vasorum* collected from infested snails. Ten of those dogs were treated monthly four times with NexGard Spectra (on Days 0, 28, 56 and 84) at a dose as close as possible to the minimum recommended dose (0.5 mg/kg MO, 2.5 mg/kg afoxolaner), the other ten dogs remained untreated. In order to determine the worm burden in lungs, the dogs were necropsied on day 90(+/-2) and worms were collected using the reverse lung perfusion method. Excretion of L_1 larvae was also assessed by faecal examination from Day 45 to 90(+/-2).

Results and discussion: The model resulted in adequate infection as all dogs from the control group harboured worms. Means of 66.4 (range of 39-95) and 3.4 (range of 0-24) *A. vasorum* were found in the untreated group and treated group respectively, the percentage reduction was 94.9%. The faecal larval count was consistent with the worm count and the percentage reduction in the treated group was 99.6%.

Conclusion: This study supports monthly oral administration of milbemycin oxime/afoxolaner (NexGard Spectra®) for the prevention of the establishment of *Angiostrongylus* in dogs.

The inhibitory effect of monensin (a Wnt signalling inhibitor) on the growth of *Toxoplasma gondii* infecting human brain cells in vitro

Mica Taylor[1] and Hany M. Elsheikha[1*]

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Central to the pathologic progression of human cerebral toxoplasmosis is the interaction of *Toxoplasma gondii* with the blood-brain barrier (BBB) endothelium. We tested the hypothesis that inhibition of Wnt signalling by monensin reduces the growth of *T. gondii* infecting human brain microvascular endothelial cells (hBMECs) or microglial cells. Two separate methods (Sulforhodamine B staining and microscopic parasite count) were used to assess the antiparasitic effect of monensin on *T. gondii* tachyzoites. Monensin significantly inhibited *T. gondii* growth at 0.1μM, with limited cytotoxicity to hBMECs or microglia cells. The effects of *T. gondii* on the integrity of the BBB were assessed by transendothelial electrical resistance (TEER) measurements in an *in vitro* human BBB model. *T. gondii* infection significantly decreased hBMEC' resistance, which was rescued when cells are treated with monensin, probably attributed to the anti-*Toxoplasma* activity of monensin. These findings suggest that the Wnt signalling may underline some of the pathogenesis in toxoplasmosis and that potentially new therapeutics targeting this Wnt and related pathways may have clinical utility in treating *T. gondii* infection.

***Neospora caninum* as a cause of arthrogryposis in a lamb**

Sian Mitchell, Sandra Scholes - Animal and Plant Health Agency

The submission of a lamb showing arthrogryposis in 2015 as part of surveillance for Schmallenberg virus infection, detected zoites and tissue cysts in the brain and spinal cord that were identified as *Neospora caninum* by immunohistochemical staining. A PCR for *N. caninum* was positive on brain tissue and the dam had antibody to *N. caninum* at the time of lambing. No other similar cases occurred on this farm. The source of the infection was not established. This case confirms that *N. caninum* infection can be a differential for SBV- like arthrogryposis in lambs.

BAVP 2016 attendee list

Swaid Abdullah, University of Bristol

Simon Allen, Gower Bird Hospital

Gerardo Arias Robledo, University of Bristol

Hind Azaylaee, University of Bristol

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Gerald Coles, University of Bristol

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