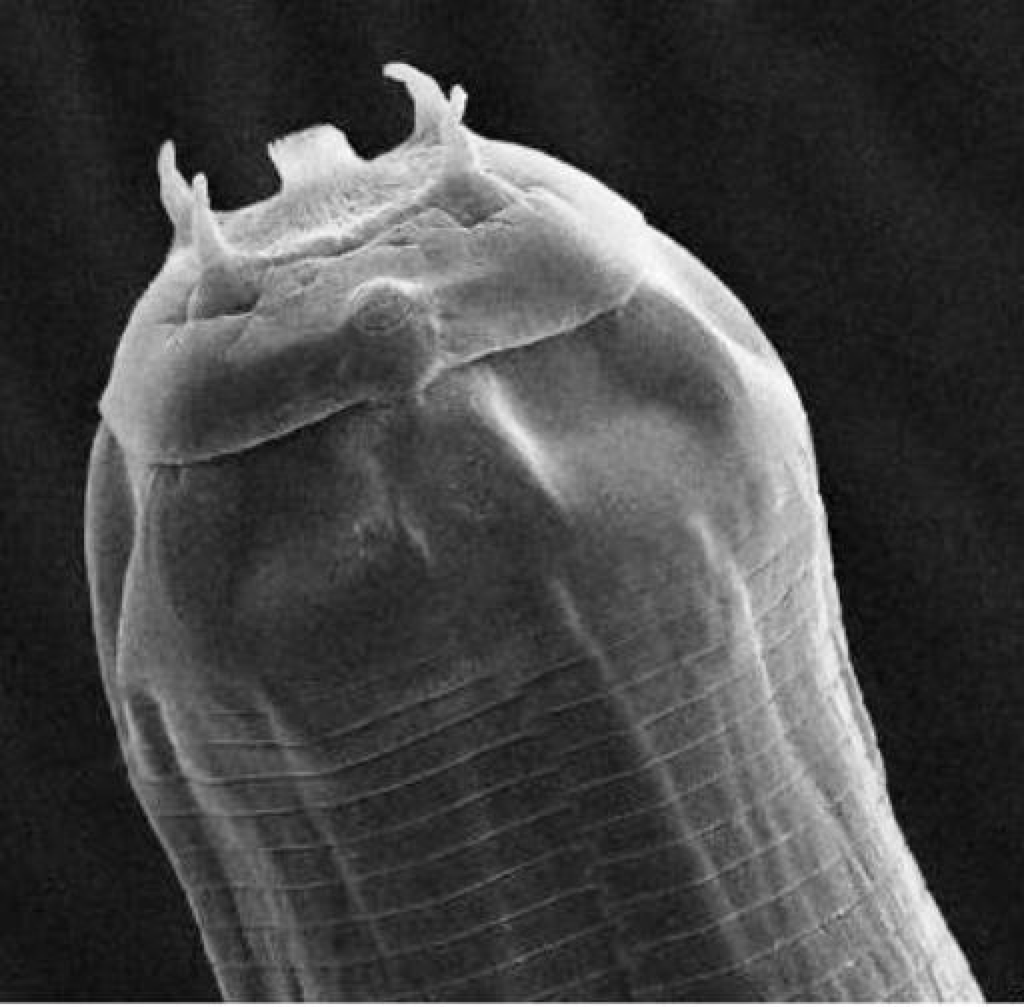

Proceeding

Equine Parasite Drug Resistance Workshop

July 31 and August 1, 2008

Faculty of Life Sciences
University of Copenhagen - Denmark

Proceeding
International Equine Parasite Drug Resistance Workshop

An international workshop meeting for developing guidelines for the diagnosis of drug resistance in equine parasites

Edited by Ray M. Kaplan and
Martin K. Nielsen

Faculty of Life Sciences
University of Copenhagen
July 31-August 1, 2008

Proceedings, International Equine Parasite Drug Resistance Workshop
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Background and Goals

In 1998, an International Workshop on Equine Cyathostomin Nematodes was held in Athens, Georgia,USA. The purpose of that meeting was to provide a forum dedicated to reviewing new developments in the biology and control of equine cyathostomin parasites. The problem of anthelmintic resistance was addressed, but only on a conceptual basis, and no broad-based conclusions or recommendations were developed. Since that time, the problem of anthelmintic resistance (AR) has dramatically increased, and is now recognized as a serious problem in the control of equine gastrointestinal nematodes, particularly in the cyathostomins. Benzimidazole resistance is practically ubiquitous in much of the world, resistance to pyrantel is increasing in prevalence, and in 2005, the first case of avermectin/milbemycin (AM) resistance in the cyathostomins was reported. Also of particular concern are the worldwide reports of resistance to AM anthelmintics in *Parascaris equorum*.

Some useful advances in the molecular and *in vitro* diagnosis of anthelmintic resistance have been made, but so far no test has proven useful at the farm level. Lacking any real alternative, fecal egg count reduction tests (FECRT) are considered the practical gold standard for field diagnosis of AR in nematodes of horses. However, no standards exist for study design, data analysis, or data interpretation for FECRT. Consequently, there is no standard for making a diagnosis of resistance. This prevents any real comparison of data reported from different regions of the world, and prevents us from knowing what the true prevalence of resistance is for any particular drug. Interpretation of FECRT data in horses is complicated by many factors, among which are: treatment groups tend to be small, low and zero pretreatment counts are common, distribution of counts is highly aggregated, variation between counts may be large, management practices among farms are variable, and efficacy of the major drug groups differ in known susceptible worm populations. These complicating factors are not insurmountable; newer statistical modeling methods provide a framework for developing data analysis tools that can provide a statistical approach for evaluating FECRT. There is an urgent need for the equine parasitology community to address this issue and develop recommended standards that can be used both by researchers and field veterinarians. In this workshop we will discuss the issues involved in the design, analysis and interpretation of the FECRT in horses. In addition, we will discuss the current state of anthelmintic resistance in equine parasites, the most recent advances for *in vitro* diagnosis of anthelmintic resistance in cyathostomins, and we will highlight research needs to develop sustainable methods for control of equine parasites. The outcome of the workshop will serve as a basis for a set of guidelines to be published in the journal *Veterinary Parasitology*. The World Association for the Advancement of Veterinary Parasitology (WAAVP) has appointed an Equine Parasite Guideline

Committee to futher develop and finally approve the guidelines suggested on the workshop.

The meeting consists of a series of presentations from invited speakers, with blocks of time reserved for discussion. Suggested guidelines will be discussed point by point and then workshop participants will break up into groups. Each group will evaluate in detail a portion of the guidelines and the relevant issues that were raised during the discussions. Groups will then prepare a written report that will be submitted to the WAAVP equine parasite guidelines committee shortly after the conclusion of the meeting. These reports will be taken into full consideration by the committee, which will then produce the final set of guidelines for publication in the special issue of *Veterinary Parasitology*.

Anticipated Outcomes

1. A bringing together of equine parasitologists and other interested individuals for a focused meeting to discuss current and important issues relating to parasite control in horses. This will be the first such meeting since 1998, 10 years ago.
2. A comprehensive proceedings will be produced that will be published in a special issue of *Veterinary Parasitology*. This has already been arranged with the Senior Publisher of the journal, Ken Plaxton. Included in these proceedings will be full-length review papers for each of the major presentations made during the workshop. This will be the first publication reviewing many of the currently important issues in equine parasitology in a single volume, since the special issue of *Veterinary Parasitology* published in 1999 as an outcome of the International Workshop on Equine Cyathostomin Nematodes held in Athens, Georgia in 1998.
3. A set of guidelines on the diagnosis of resistance in equine parasites that is fully sanctioned by the WAAVP will be published in this same special issue of *Veterinary Parasitology*. These guidelines will form what is hoped will become the basis for accepted practices used in the performance of the Fecal Egg Count Reduction Test in horses. This will permit international standardization of methods and interpretation of results, allowing a direct comparison of data from studies in geographically diverse locations.

We are very pleased with the large number of delegates from five continents representing both the academic sector, equine veterinary practice and the pharmaceutical industry. We look forward to a fruitful meeting and wish all delegates a nice stay in Copenhagen.

July 2008,

Ray M. Kaplan, Jane E. Hodgkinson, Stig Milan Thamsborg and Martin K. Nielsen
Conference organizers

Committees and Speakers

Conference organization committee:
Martin Krarup Nielsen, University of Copenhagen, Denmark
Ray M. Kaplan, University of Georgia, USA
Jane E. Hodgkinson, Liverpool School of Tropical Medicine, UK
Stig M. Thamsborg, University of Copenhagen, Denmark

WAAVP Equine Parasite Guideline Committee:
Ray M. Kaplan (chair), University of Georgia, USA
Nick Sangster, Charles Sturt University, Australia
Jacqui Matthews, Moredun Research Institute, UK
Georg von Samson-Himmelstjerna, School of Veterinary Medicine Hannover, Germany
Marcelo Molento, Federal University of Paraná, Brazil
Anand Vidyashankar, Cornell University, USA

Invited speakers:
President, adjunct professor, Craig R. Reinemeyer, East Tennessee Clinical Research, USA

Professor Nick Sangster, School of Agricultural and Veterinary Sciences, Charles Sturt University, Wagga Wagga, Australia

Professor Jacqui Matthews, Division of Parasitology, Moredun Research Institute, Midlothian, Scotland, UK

Professor Georg von Samson-Himmelstjerna, Department for Parasitology, School of Veterinary Medicine Hannover, Germany

Professor Marcelo Molento, Department of Veterinary Medicine, Federal University of Paraná, Brazil

Associate Professor Anand Vidyashankar, Department of Statistical Science, Cornell University, USA

Assistant Professor Martin Krarup Nielsen, Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark

Associate Professor Ray M. Kaplan, Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, USA

Program Schedule

Thursday July 31st 2008
Morning session 8:30-12:00

I: 8:30-9:00:	Welcome, introductory remarks, and historical perspectives *Martin Nielsen, Jane Hodgkinson,* and *Ray Kaplan*
II: 9:00-9:30:	Anthelmintic resistance in equine parasites – potential clinical consequences and implications for control *Georg von Samson-Himmelstjerna*
IV: 9:30-10:00:	Avermectin/milbemycin resistance in cyathostomins - current situation *Marcelo Molento*
10:00-10:30	Break
V: 10:30-11:00	Anthelmintic resistance in non-strongylid parasites of horses – current situation *Craig Reinemeyer*
11:00-12:00	Discussion
12:00-1:30:	Posters and lunch
VI: 1:30-2:15:	Advances in the in vitro diagnosis of anthelmintic resistance in cyathostomins *Jacqui Matthews*
2:15-2:45:	Discussion
2:45-3:15:	Break
VII: 3:15-4.00:	Sustainable equine parasite control: perspectives and research needs *Martin Krarup Nielsen*
4:00 – 4:45:	Discussion
6:00:	Evening Social

Friday August 1st 2008

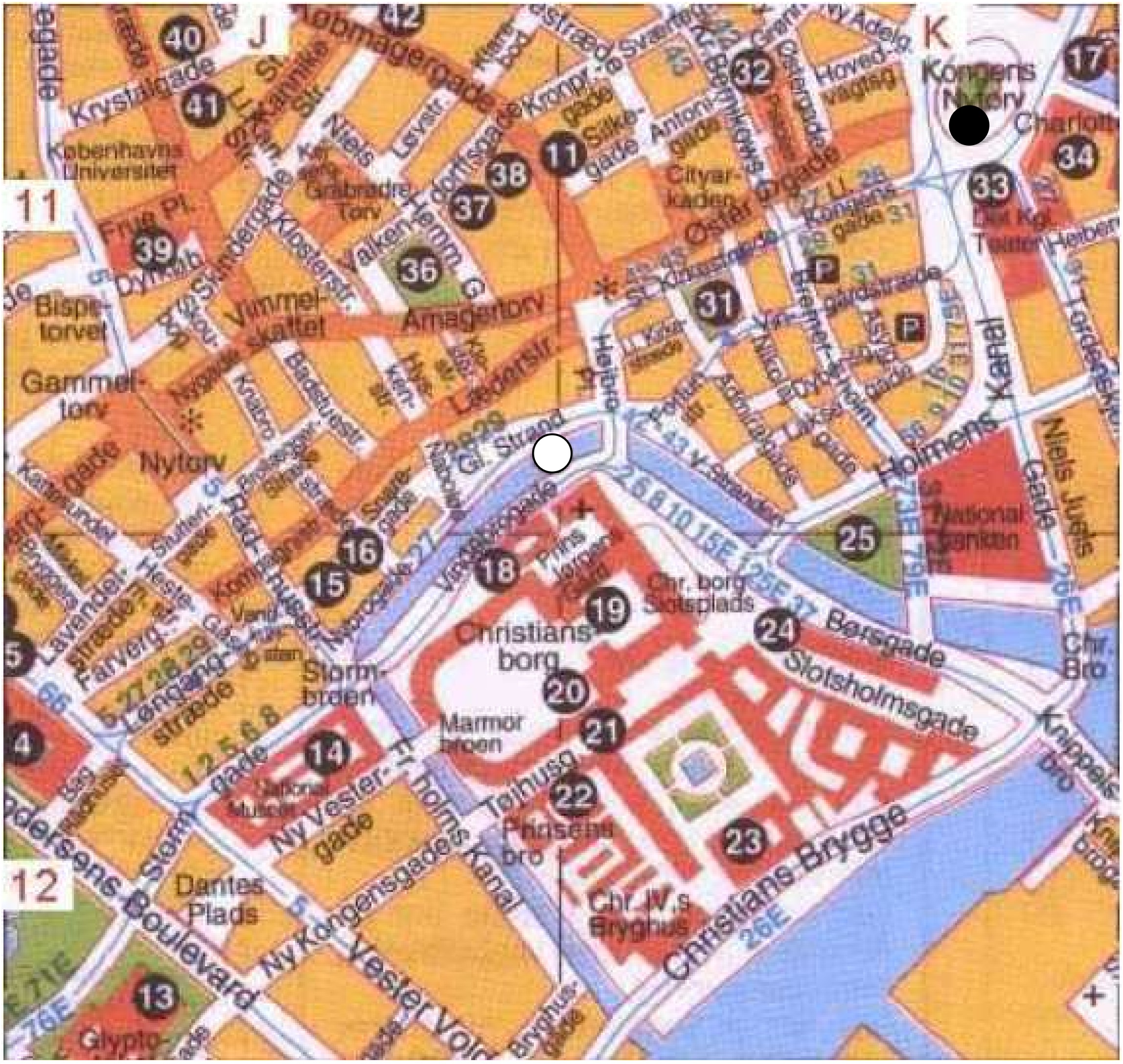
VIII: 8:30-9:00:	Biological considerations in evaluating drug efficacy and resistance in equine strongyle parasites using fecal egg count data *Ray Kaplan*
IX: 9:00-9:45:	Statistical considerations in evaluating drug efficacy and resistance in equine strongyle parasites using fecal egg count data: Part 1 *Anand Vidyashankar*
9:45 – 10:00:	Break
X: 10:00-10:30:	Statistical considerations in evaluating drug efficacy and resistance in equine strongyle parasites using fecal egg count data: Part 2 *Anand Vidyashankar*
10:30-11:00:	Discussion
11:00-11:15:	Break
XI: 11:15- 11:45:	Presentation of suggested guidelines for detection of anthelmintic resistance in equine parasites – preliminary work of the WAAVP guidelines committee *Nick Sangster*
XII: 11:45-12:30:	Discussion of guidelines point by point
12:30-2.00:	Posters and lunch
2:00-3:30:	Finish initial discussion of guidelines; breakout into small groups for review of guidelines
3:30-3:45:	Break
3:45-4:30:	Finish small group reviews of guidelines
XIII: 4:30-5:15:	Short presentations by each group summarizing major points brought up during small-group discussions
5:15-5:30:	Concluding remarks *Stig Milan Thamsborg* and *Ray Kaplan*

Conference sponsors

We are deeply grateful to our sponsors making this meeting possible:

The Abildgaard Foundation
Network on Veterinary Parasitology in the Nordic and Baltic region

Copenhagen down-town area



- ○ Gl. Strand. Meeting point for the Thursday evening social
- ● The Kongens Nytorv Metro Station

We will meet at Gammel Strand in the central part of historic Copenhagen. The boat leaves at 6 pm, so be there in time. Gammel Strand is situated just 5-10 minutes walk from the Metro Station, Kongens Nytorv.

The Metro leaves every other minute from two stations near the meeting venue; Forum and Frederiksberg. Both within five minutes of walk from Campus.

Abstracts, Key-note presentations

1. Anthelmintic resistance in equine parasites – potential clinical relevance and implications for control.

Georg von Samson-Himmelstjerna. Institute for Parasitology, School of Veterinary Medicine, Hannover, Germany.

During the past two decades anthelmintic resistance in equine parasites has been found exclusively in the group of small strongyle species, i.e. the cyathostomins, and the ascarid species *Parascaris equorum*. Cyathostomins are represented by more than 50 morphologically distinct species and usually constitute multi-species infections with often more than 5 different species parasitizing different sections of the large intestine, showing some degree of species specific tropism. The worms occur world-wide, basically on all horse farms and infect all age classes of horses. This class of worms can cause a broad range of different clinical symptoms including diarrhoea, chronic weight loss, colic and as larval cyathostominosis acute, often fatal diarrhoea. Noteworthy, for the cyathostomins to date no clear species specific pathogenicity has been described. The ubiquitous nature and the possibly severe disease these worms can induce make them a key target of current worm control programmes. Traditional control strategies mainly rely on the so called 'strategic' application of anthelmintics, currently represented by three major drug classes, i.e. the benzimidazoles (BZ), the tetrahydropyrimidines (PYR), macrocyclic lactones (ML). Following decades of routine and frequent anthelmintic application, nowadays the majority of cyathostomin populations on horse farms in many industrialized countries must be considered to have developed resistance against BZ-type anthelmintics. The exclusive reliance on this drug class may thus increase the threat of clinical disease by resistant cyathostomins. Consequently farms should routinely, e.g. once a year, examine the efficacy of their anthelmintic treatments. More recently the horse ascarid species *P. equorum* was reported to have developed resistance against ivermectin and moxidectin, two representatives of the ML-class, currently the most often used drug class in horses. This worm species is mainly found in foals and younger horses and less often in adults due to the development of immunity during the course of infection. Compared with the cyathostomins, *P. equorum* often results in clinically more drastic consequences, e.g. obstruction and/or penetration of the small intestine, usually leading to peracute death. Several factors can influence the rate at which anthelmintic resistance develops in a worm population, high treatment frequency being one of the most prominent. Modern control strategies should therefore attempt to significantly reduce the requirement for anthelmintic treatments. Several pasture, farm and worm control management practices found to be associated with worm prevalence and presence of anthelmintic resistance will be discussed in this context.

2. Avermectin/milbemycin resistance in cyathostomins – current situation.

Marcelo B. Molento. Lab. of Parasitic Diseases, Dept. of Veterinary Medicine, Federal University of Paraná, Curitiba, PR, Brazil. molento@ufpr.br

In many countries the horse is a symbol of affluence, in others a necessity of life, in all a pleasure. Although recommendations for controlling common internal parasites of the horse (particularly small and large strongyles) involves the use of a combination of management practices, many continue to deworm foals and racehorses at 30 to 60-day intervals and mares at 60 to 90 day intervals. Avermectins and milbemycins (macrocyclic lactones, ML) are potent compounds against all major nematode parasites, but continuous usage has led to the development of resistance in many of the important species of ruminant parasites and *Parascaris equorum* in horses. The general exception to this has been the cyathostomins, where resistance has been much slower to develop. However, the diagnosis of ML resistance in the cyathostomins was imminent, and the first reported case was made in 2005 by Trawford et al., where they found that moxidectin failed to fully control cyathostomins in donkeys. In 2007 we confirmed for the first time a reduction in the efficacy of MLs against cyathostomins in a Thoroughbred yard. Furthermore these were multi-resistant cyathostomins (resistant to all 3 major classes of anthelmintics). The trial used groups of six naturally infected horses with faecal egg counts > 200 EPG that were treated with abamectin 2%, ivermectin 1.8 and 2% and moxidectin 2%, as well as other commercially available (non-ML) anthelmintics. Horses were weighed, given the manufacturer's recommended dose orally and faecal samples were collected from the rectum on days 0, 7, 14, 21 and 28 post-treatment. Abamectin 2%, ivermectin 1.8 and 2% and moxidectin 2% showed reduction in faecal egg counts of 84, 5, 65 and 16%, respectively against cyathostomins after 28 days after treatment. Parasite control in horses is becoming a serious problem in Brazil with many reports of deaths due to colic episodes even after anthelmintic treatment. When viewed together, the recent report of ML resistance in the UK Donkey Sanctuary, various reports around the world of reduced egg reappearance periods following ML treatments, and the recent data showing full resistance in Brazil, it is highly likely that ML resistance in the cyathostomins has finally reached the point where it is a problem of worldwide concern. To limit the spread and development of ML-resistant cyathostomins the equine industry must implement new strategies for worm control, and the veterinary parasitology community must develop and implement new sensitive analytical and diagnostic tools for detecting resistance in its earliest stages.

3. Anthelmintic resistance in non-strongylid parasites of horses – current situation.

Craig R. Reinemeyer, East Tennessee Clinical Research, Inc., Rockwood, TN, USA.

In 2002, parasitologists in the European Union and Canada observed that macrocyclic lactone (M/L) treatments did not achieve historically-expected levels of fecal egg count reduction for *Parascaris equorum*. Since that time, putatively M/L-resistant equine ascarids have been reported in numerous countries, based on failures of FECR. Kaplan *et al*. confirmed M/L resistance in *Parascaris* through classical efficacy testing in 2006, and resistance was shown to involve both ivermectin and moxidectin. Development of M/L resistance in *Parascaris* was not surprising, considering that 1) *P. equorum* is the dose-limiting parasite for most broad spectrum equine anthelmintics, and 2) for several years, many breeding farms have practiced early, exclusive, and excessively frequent treatment of foals and weanlings with macrocyclic lactone dewormers. These control practices select for resistance by simultaneously minimizing *refugia* within the foal and in the environment. Benzimidazoles and pyrimidine salts still demonstrate high efficacy against most M/L-R populations of *Parascaris*, but an isolate from Lexington, KY is reported to be concurrently resistant to M/L's and pyrantel salts. Given the prospect of multiple drug-resistance in *Parascaris*, perhaps consideration should be given to intentional infection of foals with standardized inocula of characterized isolates. Carefully-timed, chemical termination of induced infections could invoke absolute immunity with little or no environmental contamination. Numerous anecdotal reports have surfaced in recent years of equine pinworm (*Oxyuris equi*) infections which persist despite frequent treatment with macrocyclic lactone anthelmintics, even at elevated dosages. Recent, controlled trials have demonstrated that some adult pinworms survive treatment with ivermectin or moxidectin, but it is yet unknown whether this phenomenon represents anthelmintic resistance or merely confirms the incomplete efficacy of all anthelmintic classes against adult pinworms. In the future, it must be recognized that anthelmintic resistance is biologically inevitable for every anthelmintic used against a target parasite. Efforts to delay the development of resistance, whether by chemical or non-chemical means, must be implemented prophylactically while we still have therapeutic options.

4. Advances in the in vitro diagnosis of anthelmintic resistance in cyathostomins.

Jacqueline B. Matthews[1/2], Ailie Robinson[1], Claire McArthur[1] and Frank Jackson.[1]

[1] Division of Veterinary Clinical Studies, Royal (Dick) School of Veterinary Studies, University of Edinburgh. [2] Moredun Research Institute, Pentlands Science Park, Midlothian EH26 0BL

The larval migration inhibition assay (LMIA) is one of several in vitro assays that has been used to asses anthelmintic sensitivity in parasitic nematodes. This assay applies the basic principle that infective third stage larvae (L3) will decrease their migratory activities in the presence of increasing concentrations of a specific anthelmintic drug. The assay has a number of advantages over other in vitro systems such as the egg hatch assay and the larval development assay in that it can be performed using storable L3 and it is relatively straightforward in its execution. The LMIA is particularly useful for assessing potential resistance to nematode-paralysing anthelmintics such as the macrocyclic lactones. Despite the fact that a number of LMIA protocols have been published in the last 30 years or so, no definitive procedure has been established. Various methods have involved the use of motility meters, agar blocks, commercial filter plates, migration chambers and individual mesh filters in different combinations. Common to most procedures is the fact that the L3 are incubated in a series of drug concentrations prior to being transferred to the migration chamber in the same range of concentrations of the drug. They are incubated in the chamber for a sufficient length of time to ensure that all larvae have an equal chance of migrating, but a short enough period to reduce the opportunity of reverse migration back through the apparatus. The percentage migration is calculated for each sample, compared to L3 migration in the absence of drug and then the data subjected to statistical analysis. From this, a larval population's sensitivity to a particular anthelmintic is determined. The assay has been described using a number of ruminant parasitic nematodes but, thus far, there have been no publications detailing its use to assess anthelmintic sensitivity in cyathostomins. The current paper describes an attempt to standardise the LMIA for use with cyathostomins in combination with macrocyclic lactone anthelmintics.

5. Sustainable equine parasite control: Perspectives and research needs.

Martin K. Nielsen, University of Copenhagen, Faculty of Life Sciences, Department of Large Animal Sciences, Denmark.

Clinically important equine parasites are ubiquitous in managed horse populations. The traditional approach to parasite control is frequent administration of anthelmintics to all horses on a farm, however, increasing levels of anthelmintic resistance is forcing horse owners and veterinarians to shift this control paradigm. Treatment regimens involving routine deworming of all horses throughout the year are now being replaced by more sustainable approaches, which take in to account the importance of maintaining adequate parasite refugia. The selective therapy principle has been recommended for more than 15 years, but there still is limited experience with this approach. The relative magnitude of the faecal egg count for an individual horse is a consistent trait, and this provides a reliable basis for selective therapy. But no studies have evaluated consequences of selective therapy on the long-term, and such studies are strongly needed to validate this approach. Importantly, it remains unclear how selective therapy may affect the prevalence and intensity of other parasites of significant pathogenic potential (e.g. *Strongylus vulgaris*), which have become uncommon due to years of intensive chemotherapy. Consequently, a selective approach requires vigilant surveillance of the parasite fauna and intensity. This places huge demands for reliable diagnostic tools. Also noteworthy is the fact that the majority of equine nematode parasites are more pathogenic during their larval stages, when they cannot be detected by traditional egg counting techniques. Consequently, parasite-specific diagnostic tools capable of assessing prepatent parasite burdens, and in differentiating between strongyle species of different pathogenic potentials would be of great value to the equine clinician. Tools for detecting infections with the tapeworm *Anoplocephala perfoliata* are laborious, difficult to interpret, and at present there is no established method to evaluate treatment efficacy. Thus, better diagnostic tools are needed for tapeworms as well. Biological control, especially the predacious fungi have demonstrated good potential as an adjunct for strongyle control and such a product could easily have a market in equine establishments. In summary, there is general agreement that the traditional treat-all at frequent interval approach should be abandoned, and that optimal parasite control can be maintained with far fewer anthelmintic treatments. But because of the potential for undesirable consequences, better diagnostic techniques and more evidence documenting the long-term consequences of selective therapy programs are needed to further develop and validate systems for sustainable equine parasite control.

6. Biological considerations in evaluating drug efficacy and resistance in equine strongyle parasites using fecal egg count data.

Ray M. Kaplan, Department of Infectious Diseases, College of Veterinary Medicine University of Georgia, Athens, Georgia USA.

Anthelmintic resistance (AR) is a serious problem in the control of equine gastrointestinal nematodes, particularly with the cyathostomins. Benzimidazole resistance is near ubiquitous in much of the world, resistance to pyrantel appears to be increasing in prevalence, and recent reports suggest that avermectin/milbemycin (AM) resistance is also beginning to emerge. Though some necropsy studies have been performed to quantify efficacy (and diagnose resistance) using worm counts, all studies on prevalence rely on the fecal egg count reduction test (FECRT). However, accurate quantification of resistance prevalence and especially accurate diagnosis of emerging AM resistance with the FECRT is hampered by a lack of accepted standards for study design, data analysis, and data interpretation. Also important is the fact that fecal egg count (FEC) data are surrogate measurements, which are subject to many sources of variability. Interpretation of FECRT data in horses is complicated by many biological factors, among which are: treatment groups tend to be small, low and zero pretreatment fecal egg counts (FEC) are common, distribution of FEC are non-parametric and tend to be highly aggregated, variation in FEC measurements may be large, management practices among farms are variable, breeds, age and sex of animals vary among farms, and the expected efficacy of the major drug groups differ in known susceptible worm populations. Though usually ignored, these biological issues can greatly impact the accuracy of inferences made on efficacy, and therefore conclusions relating to the presence or absence of resistance. Consequently, there is an urgent need for the equine parasitology community to address these issues and develop recommended standards for the FECRT that can be used both by researchers and field veterinarians. Newer statistical modeling methods provide a framework for developing data analysis tools that can provide a statistical approach for evaluating FECRT, and thus greatly improve the accuracy of the inference made on the data. Furthermore, Monte Carlo simulations provide a means to test the outcome of using various analysis models, and to test the effect that our biological and statistical assumptions have on these outcomes.

7. Statistical considerations in evaluating drug efficacy and resistance in equine strongyle parasites using fecal egg count data.

Anand N. Vidyashankar, Department of Statistical Science, Cornell University, Ithaca, NY, USA.

Fecal Egg Count Reduction Test (FECRT) is the practical gold standard for evaluating drug resistance and efficacy in horses, but analyses of FECRT data are fraught with many statistical issues that are often not considered. In particular, egg counts from various fecal samples exhibit variability and other distributional patterns that need to be taken into account to accurately assess drug efficacy, and consequently to assess drug resistance. A number of gaps continue to exist in the theoretical and practical understanding of resistance, and many of these can be addressed using various computational approaches. Newer statistical modeling methods and simulation approaches provide a framework for developing data analysis tools that can provide a statistical approach for evaluating FECRT. Bootstrap analysis methods which have demonstrated optimal Type I error rate and high power to detect differences between the presumed and true efficacy without the need to know the true distribution of pre-treatment egg counts provide one such approach. In this talk, we will describe how statistical and biological information can be combined with simulations to describe various facets of fecal egg count data, and how this can improve our understanding of drug efficacy and resistance. These methods will be illustrated using several different data sets.

8. Draft guidelines for detection of anthelmintic resistance in equine parasites.

Nick Sangster, School of Animal and Veterinary Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga NSW 2678, Australia.

The diagnosis of anthelmintic resistance in cyathostomin nematodes of horses is important for future parasite control. This is especially true for monitoring emerging resistance to pyrantel and the macrocyclic lactone drugs. Methods based on faecal egg count reduction are the most widely accepted and practical in the field. While such methods are well described for several host species, parasitism in horses presents some particular challenges. These include variable and, often, low egg counts, not knowing the efficacy of drugs against susceptible parasites to use as comparators and that normally small numbers of animals are available for testing on a farm. A committee comprising Ray Kaplan, Jacqui Matthews, Georg von Samson-Himmelstjerna, Marcelo Molento, Anand Vidyashankar and myself has been formed to consider these issues and make recommendations. Our

approach has been to review trial data from a large number of farms and to use mathematical simulations to estimate the ‘true’ parameters and apply this knowledge to real data. Using this approach we believe improved statistical confidence in diagnosis of resistance can be achieved by modifying sampling and counting procedures and by using novel statistical approaches. Some elements of our statistical approach are to compare reductions within individual horses, tailor different efficacy cut off values for different drug classes test these against calculated confidence limits

Abstracts, Poster presentations

Anthelmintic Resistance

9. Equine anthelmintic resistance and rotational deworming regimens.

Megan Blanek[1], Heidi A. Brady[1] and Wade T. Nichols[2]. [1]Department of Animal and Food Sciences, Texas Tech University, Lubbock, Texas, U.S.A. [2] Intervet/ Schering-Plough, Millsboro, Delaware, U.S.A.

Three studies examined parasite resistance and anthelmintic efficacy on a horse farm in West Texas. In Study One, three fenbendazole (FBZ) treatments (5 mg/kg BW, 10 mg/kg BW, or a larvicidal 50 mg/kg BW) were administered to horses treated only with FBZ for 18 months prior. Resistance to FBZ was documented by larval hatch assay and fecal egg count reduction (FECR) tests. In all treatment groups, FECR were not greater than 90%. In Study Two, the efficacy of moxidectin (MOX), ivermectin (IVM), and the larvicidial FBZ dose were studied in these horses. Mean FECR was 99.91%, 98.73%, and 84.3%, respectively. Animals treated with MOX and IVM had significantly higher FECR than the FBZ group ($P < 0.05$). When FECR was analyzed by age, there were no differences between treatment groups in the mature horses, while the MOX and IVM groups were significantly higher than the FBZ group in the young horses ($P < 0.05$). Study Three determined the efficacy of a year-long quarterly rotation. A farm-wide study including the animals from Study One and Two showed a quarterly rotation of pyrantel pamoate (Strongid, Pfizer) (June), Ivermectin+Praziquantel (Equimax, Pfizer) (September), fenbendazole larvicidal dose 50 mg/kg BW (Power-Pac, Intervet) (December), and Moxidectin (Quest, Fort Dodge) (March) effectively reduced or broke resistance based on FECR tests. The mean FECR for the treatments were 95.86%, 100%, 97.84%, and 100% respectively. These studies indicate that neither frequent use nor larger doses of FBZ will alleviate a FBZ resistance problem in the horse. However, Study three demonstrated that a rotational regimen can effectively break or reduce FBZ resistance and that FBZ can be successfully implemented in a rotational scheme subsequent to prior resistance.

10. Evaluation of 6-way rotational anthelmintic regimen on a previously documented fenbendazole-resistant farm in Texas.

Heidi A. Brady[1] and Wade T. Nichols[2]. [1]Department of Animal and Food Sciences, Texas Tech University, Lubbock, Texas, U.S.A. [2] Intervet/ Schering-Plough, Millsboro, Delaware, U.S.A.

It is increasingly apparent that parasite resistance to current equine anthelmintics is an area of great concern world-wide. Various rotational schemes between classes have been suggested with the goal of the prevention of parasite resistance, although few studies have examined these plans. We have gathered a significant amount of information on the parasite load and control in a group of Quarter Horses at the Texas Tech Ranch Horse Center

(2003-2005). Resistance to fenbendazole in this herd was experimentally produced by the use of fenbendazole (FBZ) for 18 months without rotation. The implementation of a quarterly rotation program of pyrantel pamoate, ivermectin, larvicidal FBZ, and moxidectin effectively reduced resistant populations based on fecal egg count reductions (FECR) of 95.9 %, 100 %, 97.8 %, and 100%, respectively. In May of 2005 until November 2006, we began a farm-wide 6-way rotational plan at the Texas Tech Horse Center at New Deal, TX. This rotation consisted in 2005 of pyrantel pamoate (Strongid, Pfizer) (May), fenbendazole (Safeguard; Intervet) (July), moxidectin (Quest, Fort Dodge) (September), ivermectin + praziquantel (Equimax, Pfizer) (November), and in 2006 of fenbendazole 50 mg/kg BW (Power-Pac, Intervet), (January) moxidectin (March), pyrantel pamoate (May), fenbendazole (July), ivermectin + praziquantel (September), and Moxidectin (November). The FECR using the rotation scheme in 2005 was 86%, 98 %, 100 % and 100 % effective, respectively. Effective control of parasite loads was evidenced by very low pre-treatment fecal worm counts. By January of 2006, the majority of animals had pre-treatment parasite worm loads of 0, so FECR could not be analyzed. Pre-treatment worm counts in positive animals were very low, and only exceeded 100 eggs/g in 2 animals. The rotational regimens used continued to successfully control parasites in this herd, despite prior resistance. The majority of the horses in 2006 had 0 or negligible egg counts. This is in great contrast to parasite loads measured when resistance was documented in 2003, when many of the eggs in the fecal samples were too great to count. This study showed that resistance to fenbendazole in a herd can be broken or effectively controlled by using a rotation of different anthelmintics and that fenbendazole can be effectively implemented in a rotational scheme despite prior resistance.

11. Comparative evaluation of drug resistance tests in equine strongylidosis.

Mihai S. Cernea[1], Laura C. Cernea[1], V. Cozma[1], S. Răileanu[1], A. Gut[2], R. Silberg[1], L. Madeira de Carvalho[3]. [1]Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania. [2]Faculty of Automation and Computer Science, Tehnical University of Cluj-Napoca, Romania. [3]Facultade de Medicina Veterinaria, Universidade Tecnica de Lisboa, Portugal.

Equine strongylidosis study is a world - wide highly important topic due to the serious consequences of this disease and drug resistance. In this context, research conducted between 2002 and 2008 on equines populations in Romania, focused on identification through *in vivo* and *in vitro* tests of strongyls resistance. Identification of strongyle species obtained by fecal cultures coming from 1992 equines (26 horses populations, from 13 counties) revealed dominance of Cyathostomims (82.10-93.03%) followed by *Strongylus vulgaris* (4.17-10.10%). *In vivo* analysis by fecal eggs count reduction test - FECRT of resistance to benzimidazole, conducted between 2003-2008,on 992 horses belonging to 22 populations, revealed installation of resistance phenomenon in 66.66% of cases. *In vitro* strongyle resistance to anthelmintic drugs was detected by use of egg hatch assay (EHA) and larval development assay (LDA). Strongyle resistance to benzimidazoles was detected in 77.28% of the populations taken in to study. Statistical interpretation of data was carried by use of a mathematical resistance evaluation model devised in such a manner as to be able to determine the following characteristics: egg-hatch or larval development percentage, lethal concentration (LC); minimal inhibitory concentration (MIC) and resistance factor (RF). Our analyses establish that the correlation between FECRT and *in vitro* tests was 86.25%, quantified through a biomathematical model. EHA and LDA tests data interpretation needed identification of equines populations that were never provided anthelmintic treatment. Such an equines population has been identified in the Danube Delta Natural Reserve, from Romania.

12. Macrocyclic lactone resistance in UK cyathostomins?

Gerald.C.Coles[1], Alexandra Dudeney[2], Claire Campbel[2]. [1]School of Clinical Veterinary Science, University of Bristol, Langford House, Bristol, UK., [2]Department of Anatomy, University of Bristol, Southwell Street, Bristol, UK.

As failure of MLs has been reported in Thoroughbreds in Brazil, the possibility that ML resistance might be developing in UK horses was investigated over two winters. Owners were asked to treat their animals with ivermectin and send in faecal samples 28 days later. This would indicate if there was an early egg re-appearance. Egg counts were performed by the FLOTAC method that is accurate to 1 epg. The activity was very high in local non-Thoroughbred yards with four yards with zero epg in all horses and the remaining yards having a mean egg count of 1-4 epg. However, two Thoroughbred yards had mean egg counts of 14 and 17 epg. In the second winter 12 Thoroughbred yards were tested from 436 yards contacted by post or phone. In five the mean epg was >5 epg. These yards were treating every 4-8 weeks with an ML, more often than those with the lower or negative egg counts. The egg counts were too low to be detected with conventional egg counting procedures (McMaster, FECPAK). Whilst some of the egg counts might be due to misdosing, we interpret the data to indicate that resistance to the MLs may be starting to develop. Further widespread testing is warranted using sensitive egg counts 28 days after treating with ivermectin or full faecal egg count reduction tests.

13. How do we slow the development of ML resistance in cyathostomins?

Gerald.C.Coles[1], Marcelo Molento[2]. [1]School of Clinical Veterinary Science, University of Btistol, Langford House, Bristol, UK . [2]Laboratório de Doenças Parasitárias, Universidade Federal do Paraná, R: dos Funcionários, 1540 Curitiba, PR, Brazil.

With no new anthelmintic types in sight for treating horses, and triple resistance already present in Brazil, advice must be given to horse owners and particularly to Thoroughbred owners on how to slow the development of resistance. We have suggested (Molento et al. 2008, Vet.Rec162, 384) the following four actions: 1. Only treat horses with egg counts >200 epg. Adult mares may not requirement treatment until the count exceeds 500 epg. This will increase numbers of worms in refugia on the pasture although it will make control less efficient. 2. Establish the resistance status of each yard on at least an annual basis and use all anthelmintic types that are effective, 3. Quarantine all animals coming onto a yard by treating with ivermectin and running a sensitive egg count 28 days later. Animals with a positive egg count should not be kept or should be grazed separately. 4. Wherever possible non-chemical methods of control should be used, especially picking up faeces and/or grazing with sheep. Once we have agreed validated tests to detect resistance the major question is how we persuade owners that these strategies must be adopted and that they have to pay for the cost of the testing required.

14. Use of a novel Bayesian statistical model to improve detection of anthelmintic resistance in cyathostomins.

Matthew J. Denwood [1], Sandy Love [1], Jacqui B. Matthews [2], Stuart W.J. Reid [1] and Giles T. Innocent[1]. [1]Institute for Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow, Bearsden Road, Glasgow, Scotland, G61 1QH. [2] Division of Veterinary Clinical Studies, Royal (Dick) School of Veterinary Studies, University of Edinburgh & Moredun Research Institute, Pentlands Science Park, Midlothian, EH26 0BL.

The faecal egg count reduction test (FECRT) is currently the gold standard method for demonstrating anthelmintic efficacy on farms, but the statistical processes underlying the test are generally poorly understood. A novel method of data analysis using Bayesian Markov chain Monte Carlo techniques was used to analyse simulated FECRT data alongside the method currently recommended in the WAAVP guidelines for the detection of anthelmintic resistance, and the results from the two methods compared. First, 100 datasets were simulated with randomly selected group sizes of between 5 and 25, mean faecal worm egg counts of between 50 and 500 eggs per gram, and a prevalence of infection of 75%. Post-dosing data were simulated with an 80% reduction in the population, or true, mean faecal egg count. Each of the datasets were analysed using the WAAVP guidelines and the Bayesian technique to calculate a mean or median reduction and a lower 95% confidence interval for each method. Using the WAAVP method, 90 of the datasets were diagnosed as confirmed resistant, 1 as suspect resistant, and 9 as drug susceptible. Using the Bayesian method, 98 of the datasets were diagnosed as confirmed resistant, 2 as suspect resistant and 0 as drug susceptible. The root-mean-square error was smaller using the Bayesian method, and the 95% confidence intervals were also on average smaller and, therefore, more informative. The Bayesian method also outperformed the WAAVP method when other true reductions between 75% and 95% were used to simulate the data. By taking into account the source of the true variability in faecal egg counts, the use of a modeling approach appears to derive more reliable and more informative results than estimating confidence intervals based on the simple arithmetic mean and variance. Use of an unsuitable statistical test to analyse FECRT data will result in potentially erroneous conclusions being made about the true anthelmintic efficacy on farm. More appropriate

statistical tests should therefore be used, particularly with the small sample sizes and high proportion of zero counts commonly encountered in equine clinical practice.

15. Evaluation of anthelmintic efficacy in horses in Nicaragua – with special focus on *Strongylus vulgaris*

Jenny Lindbom[1], Line L. Andreasen[1], Martin K. Nielsen[2], Luz A. Luna[3], Jesper Monrad[1], Niels C. Kyvsgaard[1]. [1] Department of Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen, Denmark. [2] Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark. [3] UNAN, Léon, Nicaragua.

Horses and mules are indispensable for transport of people and goods in Nicaragua and many other developing countries. Few studies of strongyle parasites have been made in Nicaraguan horses and the objectives of this study was to provide data on; infection level and prevalence of different strongyle species in the area, efficacy and re-infection rate of commonly used anthelmintic drugs, species composition after treatment and infection level of the untreated horses during the study period. The studied horse population was used for working purposes. The age ranged from 1 to 20 years, and none of the horses had been treated with anthelmintics at least three months prior to the study. The study was performed as a clinical trial where a total of 172 horses were randomly treated with either ivermectin (IVM) or fenbendazole (FBZ). A third group of 33 horses was kept untreated as control. The weight of horses was estimated by girth tape measurement, and ranged from 113 to 297 kg with a mean of 230 kg. Both treatment groups were dosed corresponding to 340 kg of bodyweight to rule out the risk of underdosing. Faecal samples were collected at day 0, 14, 27, 42, 55 and 70 post treatment and were analysed for faecal egg counts. At each occasion of faecal sampling, larval cultures were performed from 12 horses in each group for subsequent identification of *Strongylus vulgaris* third stage larvae. The three groups of horses were found uniform before treatment, since analysis of variance found no statistical differences between the groups in terms of faecal egg counts, *S. vulgaris* prevalence, age, weight and gender of horses. Treatment efficacies, measured as percent faecal egg count reductions (FECR) 14 days post-treatment, were 100 %(IVM) and 94 %(FBZ) Using the methods given by Kaplan (2004), the IVM and FBZ treatments were evaluated as fully efficacious and it can therefore be concluded that there was no evidence of resistance to these anthelmintics in the horses tested. Using the cut off-value of 100 strongyle eggs per gram (EPG) of faeces for the arithmetic mean of horses, egg reappearance periods (ERP) were approximated to 56 and 27 days for IVM and FBZ, respectively. Using 200 EPG as cut off-value yielded ERPs of 58 and 38 days, respectively. The larval cultivations revealed that cyathostomins comprised 98.4 % of the total strongyle burden and S. *vulgaris* nearly 1%. The prevalence of *S*. *vulgaris* was determined to 46%. In all treatment groups *S*. *vulgaris* was not detected until day 70 (IVM and FBZ) post treatment. In conclusion, both drugs were found efficacious with the expected levels of FECR and ERP. The overall prevalence of *S. vulgaris* was high compared to western countries, and none of the two treatments were fully

efficacious against larval stages, since this parasite was detected in both treatment groups long before reinfection could have occurred.

16. Evaluation of a larval migration inhibition assay in equine cyathostomin nematodes.

Alycia Monopoli[1], Anand N. Vidyashankar[2], and Ray M. Kaplan[1], [1]Department of Infectious Diseases, College of Veterinary Medicine University of Georgia, Athens, Georgia USA, [2]Department of Statistical Science, Cornell University, Ithaca, NY. USA.

Presently there are no in vitro diagnostic assays for anthelmintic resistance validated for use in equine parasites. Assays such as the egg hatch test (EHT) and the larval development assay (LDA), which have proven quite useful with ruminant parasites, have so far demonstrated limited usefulness with the cyathostomins, and none of these tests have been properly validated. The larval migration inhibition assay (LMIA) is another in vitro assay that has recently gained increased attention as a diagnostic test for resistance, particularly with the avermectin/milbemycin (AM) class of anthelmintics. In this study we used two different isolates of cyathostomin nematodes with no known prior exposure to AM drugs in an LMIA. Third-stage larvae from these isolates were evaluated for their ability to migrate while varying a number of different assay parameters in order to optimize the consistency of the dose-response measured for 3 different avermectin drugs (ivermectin, eprinomectin, and doramectin). Factors favoring migration included: using exsheathed larvae, DMSO concentrations $\leq$0.5%, a saline media, and a 2-hour migration time. Overlay of dose-response curves created using the log-dose logit model showed fairly good consistency among the replicates. Eprinomectin (EPR), and doramectin (DOR) had smaller confidence intervals than ivermectin (IVM), suggesting that these two drugs yield more consistent results than IVM. Additionally, the 95% CI for the mean LC50 values showed no significant difference between EPR and DOR. However, when mean LC50 of the two AM-naive isolates were compared, the two isolates showed significantly different responses. These data demonstrate that the LMIA yields fairly consistent dose-response data for EPR and DOR when used with avermectin-sensitive equine cyathostomin parasite larvae. The LMIA appeared to work well under the conditions tested in this study, and the assay exhibits several features indicating it has potential as a diagnostic assay for AM resistance in cyathostomes. However, the fact that two different AM-naïve worm isolates had significantly different LC50 values suggest that many more isolates need to be tested. Until the assay is further optimized and tested with known avermectin-resistant isolates it cannot be determined if it will be a viable option for detecting avermectin resistance in cyathostomins.

17. Prevalence of pyrantel resistant strongyles and *S. vulgaris* on Danish horse farms using selective therapy.

Martin K. Nielsen[1], Stig L. Petersen[2]. [1] Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark. [2] EquiLab Laboratory, Jørlunde Overdrev 7, DK-3550 Slangerup, Denmark.

Resistance of equine nematode parasites to anthelmintic drugs is a serious problem, and resistance has been documented in equine parasites to all drugs on the market. Resistance patterns may vary between geographic regions, since treatment traditions can vary widely. In Denmark, anthelmintic drug usage has been restricted by prescription-only legislation since 1999, and prophylactic treatments are disallowed. Danish parasite strategies now involve considerable levels of parasite surveillance and a decreased treatment intensity. However, no studies have been performed to evaluate levels of anthelmintic resistance under this treatment policy. In addition, it has been hypothesized that the most pathogenic of equine nematodes, *Strongylus vulgaris*, may be reemerging due to the low treatment intensity. A study from 1998 raised suspicion that pyrantel resistant cyathostomin parasites were developing in Denmark, but this has not been confirmed. From March 17 to May 1, 2008 individual faecal samples were taken from horses on horse farms all over the country. Faecal egg counts (FEC) were determined with a modified McMaster technique with a 20 eggs per gram detection limit. Horses with FEC of 200 or higher were treated with pyrantel pamoate paste. Farms with at least six horses receiving treatment were included in the study. Horses studied aged between 1 and 33 years (mean=6.9, median=5) and 49% were geldings, 48% mares and 3% stallions. Individual larval cultures were done on all pretreatment samples for identification of *Strongylus vulgaris*. Sixty four farms with a total of 1644 horses were included in the study. Control samples were performed on a total of 614 horses. Farm mean faecal egg count reductions (FECR) were calculated with 95% confidence intervals. Pyrantel resistance was declared, when the farm mean FECR and the lower confidence limit (LCL) were below 90% and 85%, respectively. Twelve farms (18.75%) fulfilled these criteria, and an additional two were suspected resistant, with mean FECRs just above 90% but LCLs below 85%. *S. vulgaris* was detected on 31 of the farms (48.44%) and in 78 (4.75%) of individual horses. This study documents pyrantel resistance on Danish horse farms, despite the restricted treatment policies. However, the overall efficacy of pyrantel is still high, and the drug worked well on the majority of farms. Pyrantel can therefore be recommended for anthelmintic treatments if horse farms are routinely screened for resistance on a yearly basis. The prevalence of *S. vulgaris* was much higher than reported in other Danish studies, and programs for parasite control may need revision in order to achieve better control over this pathogenic parasite.

18. Use of the larval migration inhibition assay to investigate suspected macrocyclic lactone resistant cyathostomin populations.

Ailie Robinson[1], Claire McArthur[1], Faith Burden[2], Leila Goss[2], Andrew Trawford[2], Frank Jackson[1] & Jacqui B. Matthews[1,3]. [1]Parasitology Division, Moredun Research Institute, Bush Loan, EH26 0PZ, UK. [2]The Donkey Sanctuary, Devon, EX10 0NU, UK. [3]Division of Veterinary Clinical Science, R(D)SVS, University of Edinburgh, Midlothian, EH25 9RG.

Resistance to all three classes of broad spectrum anthelmintic has now been recorded in cyathostomins, highly pathogenic parasitic nematodes of Equidae. The relatively recent emergence of resistance to the macrocyclic lactone (ML) class is particularly worrying as we now rely heavily upon these drugs, especially for their efficacy against mucosal larval stage cyathostomins. The large population sizes and high levels of genetic diversity that occur in nematodes will facilitate resistance-conferring genes to spread quickly through cyathostomin populations. This will be augmented by frequent animal movement between establishments and shared grazing. For these reasons it is imperative to recognize resistance as early as possible. The currently used methods for identification of drug resistance (i.e. faecal egg count reduction test) are not sufficiently sensitive. It is possible to determine nematode sensitivity to paralyzing anthelmintics (e.g. ML) by assessing motility. Although this concept has been utilized by a number of research groups for sheep nematodes, there has been little effort to establish standard methodologies and no work has been published for Cyathostominae. This poster details the optimization and validation of a larval migration inhibition assay which determines cyathostomin sensitivity to ML anthelmintics. The migration assay was used to establish LMI50 values in cyathostomin populations that had been shown to exhibit different sensitivities to MLs in vivo.

19. Survey on anthelmintic resistance in equine cyathostomes in Italy, UK and Germany: preliminary results.

Donato Traversa[1]*, Piermarino Milillo[1], Georg von Samson-Himmelstjerna[2], Albert Boeckh[3]. [1]Dept of Comparative Biomedical Sciences, University of Teramo, Italy. [2]Institute for Parasitology, University of Veterinary Medicine Hannover, Germany. [3]Fort Dodge Animal Health, Princeton, NJ, USA.

In the past decade, increasing numbers of drug resistant cyathostome populations have been reported in a range of European countries. Nonetheless, geographically and numerically broader investigations, together with an evaluation of the cyathostome species, are necessary to evaluate anthelmintic resistance in horses. A two-year project has started in February 2008 with the purposes to evaluate, on a large scale, the prevalence of anthelmintic resistance on horse farms by a Fecal Egg Count Reduction Test (FECRT) and to identify to species the resistant cyathostomes by an innovative biomolecular diagnostic test. From February to May 2008, a total of 788 horses from 12 and 31 horse yards were enrolled in UK and Italy respectively. In particular 7 (UK) and 22 (Italy) yards were subjected

to a FECRT using fenbendazole, pyrantel, ivermectin and moxidectin, while 5 (UK) and 1 (Italy) using pyrantel, ivermectin and moxidectin. Fenbendazole resistance was found on 85.7% and 30.4% of the farms examined in UK and Italy respectively, and suspected in 17.4% of the Italian farms. Resistance to pyrantel was found and suspected respectively in 18.2% and 9% of UK farms, while in Italy it was found in 13% and suspected in 8.7% of the yards. In one out of the 22 Italian farms resistance to ivermectin was suspected. No resistance was seen to moxidectin. These preliminary results show that declared and suspected single (in 3 and 8 yards in UK and Italy respectively) and multiple (in 3 and 4 yards in UK and Italy respectively) drug resistance in cyathostomes are spread in UK and Italy. In the next months of 2008 and in 2009 more yards will be enrolled in UK and Italy and the recruitment will start also in Germany. A DNA-based assay (i.e. Reverse Line Blot) will be used to identify at the species level the resistant cyathostomes in each of the three countries. This study will elucidate the prevalence and distribution of individual susceptible/resistant cyathostomes, thus providing information on geographical spread of resistance to anthelmintic drugs and pivotal information for future control plans.

Epidemiology and Control

20. Simultaneously raising sheep as a strategy to control horse parasites.

Gisane Lanes de Almeida[1], Marcelo Beltrão Molento[2], José Osvaldo Jardim Filho[1], Walter Nunes Flores[1]. 1. Federal University of Santa Maria, Brazil. 2 Lab. of Parasitic Diseases, Dept. of Veterinary Medicine, Federal University of Paraná, Curitiba, PR, Brazil. molento@ufpr.br. Aceguá, RS

Equines harbour a variety of parasitic organisms on their helminth fauna and there are a few species/genus of interest: *Parascaris equorum*, *Anoplocephala perfoliata*, *Oxyuris equi*, *Cyathostomum sp.* and *Strongylus sp*. The possibility to raise animals under alternate and/or in consortium management may benefit all species that are involved mainly by reducing pasture contamination. Scientific literature on sheep and horse consortium is limited and horses may benefit from this because sheep have the habit to graze at low pasture levels ingesting horse helmith infective larvae. The objective of this work was to determine the efficiency of raising Hampshire Down sheep as an alternative method to control parasite infections together with Thoroughbred horses in Rio Grande do Sul, Brazil. Thirty-five foals born between July and October of 2004, from both sexes were used in an area of 65.2 hec. from July 2004 to February 2005. The area was divided in seven lots being homogeneously and randomly occupied by all horses. Fecal samples were collected and processed for parasite egg counts per gram (EPG) with larval identification. A sheep flock of 112 animals was maintained together with the horses with free access to all areas. The data demonstrated that the average EPG were lower for horses from the lots 3 and 4 (0.0 and 16.7) when compared to the 1, 2, 5, 6 and 7 (400.0; 162.5; 683.3; 400.0; 533.3) areas respectively ($P<0.05$). The flock stayed longer (up to 6h) on the plot 3 and 4 when compare to the other areas (less than 2h). It was observed a disperse distribution of the animals and sheep consumed pasture close to the horses' fecal pat (4 cm). Although small strongyles were not highly prevalent (42%) at the beginning of the trial, the effect imposed by the larval clearance done by the sheep is suggested. Coproculture data revealed the presence of 94% *Trichostrongylus sp*., 3% *S. equinus* and 3% cyathostomins, at the end of the trial, indicating the possibility of cross infection in the areas occupied by the two species of animals. The present data indicates that the presence of sheep along with horses might be beneficial to the latter, reducing pasture contamination and the number of treatments, which would consequently improve their sanitary conditions and lessen parasite population selection pressure.

21. Helminths in horses: use of selective treatment for the control of strongyles.

Sonja Matthee[1] and Melodie A McGeoch[1,2]. [1]Department of Conservation Ecology and Entomology, [2]Center for Invasion Biology, Private Bag X1, University of Stellenbosch,7602, South Africa.

The current level of anthelmintic resistance in the horse-breeding industry is extremely high and therefore more emphasis is being placed on studies that focus on the judicious use of anthelmintic products. The aims of the study were to: 1) establish if there is variation in the egg excretion pattern of strongyles between the different age classes of Thoroughbred horses in the Western Cape Province (WCP), 2) test if a selective treatment approach successfully reduces the number of anthelmintic treatments and maintains acceptably low helminth burdens in adult Thoroughbred horses, and 3) evaluate the efficacy of subsampling large horse herds for faecal egg counts (FECs) to monitor the strongyle burden. In 2001 the FECs of 4 adult mare, 5 yearling and 3 weanling herds from 8 different farms were compared in the WCP. Within the mare herds there were generally fewer egg-excreting individuals with lower mean FECs compared with the younger age classes. Individual faecal samples were collected every 3–4 weeks from 52 adult Thoroughbred mares from 1 farm in the WCP during a 12-month period (2002/2003). Animals with strongyle FECs ≥100 eggs per gram (epg) were treated with an ivermectin-praziquantel combination drug (Equimax oral paste, Virbac). The mean monthly strongyle FEC for the entire group was <300 epg throughout the study and the number of treatments was reduced by 50 %. Resampling methods showed that an asymptote to mean FEC was reached at 55 animals for each of the pooled weanling, yearling and mare egg counts. Resampling within 4 different mare herds recorded asymptotes of between 24 and 28 animals. Subsampling entire herds for FECs therefore provided an effective approach to treatment management. This study demonstrates that selective treatment is both a practical and an effective approach to the management of anthelmintic resistance.

22. Efficacy of an equine rotation deworming program.

Wade T. Nichols[1], Jenifer Edmonds[2], Matt Edmonds[2], Ed Johnson[2], Wendy Vaala[1], Mike Brown[3]. [1]Intervet/Shering Plough, Millsboro, Delaware, USA. [2]Johnson Research, Parma, Idaho, USA. [3]West Texas A&M University, Canyon, Texas, USA.

This study evaluated a year-long six treatment rotational deworming program in pastured horses with naturally acquired parasite burdens against matched controls. Internal parasite control with respect to efficacy and resistance was examined via serial animal selection with parasite identification and enumeration. Effect of parasite control on animal health and pasture contamination was also examined. A total of 85 horses were initially purchased as potential study candidates. Following an acclimation period of 36-66 days depending on arrival date, individual fecal samples were obtained for fecal eggs/gram (EPG) analysis. All horses grazed together during the acclimation period and grazed both pastures for equal

days. Based on d -10 EPG counts, animal age, body condition score, health, and ease of handling, 78 horses were selected for the study and randomized to treatment. Pasture was randomly assigned to treatment and the selected horses in each treatment were allowed to graze together in their respective treatment pastures. Both treatments were given an equal amount of pasture/horse. The treatments consisted of a control (C) which never received an anthelmintic throughout the entire year vs. a year-long rotational anthelmintic program (R). The year-long rotational deworming program consisted of Moxidectin on d 0 (August), Ivermectin+Praziquantel (October), Larvalcidal dose of Fenbendazole (December), Pyrantel Pamoate (February), Moxidectin+Praziquantel (April), Fenbendazole (June). On d 0, 12 horses were sacrificed to establish a baseline of parasitism. At each 60 day interval 3 horses were sacrificed prior to receiving the selected anthelmintic to establish the baseline of parasitism for that particular 60 d period within the rotation treatment. Then 14 d later three additional horses were sacrificed to establish anthelmintic efficacy. In addition three control horses were sacrificed at this same time period to establish parasite infection. Analyzing the data from d 0 – 365 indicated that R tended to increase final body weight and average daily gain over C, as well as, improve body condition score (1218 vs. 1098 (P=.12) and 0.27 vs. 0.16 (P=.17) respectively). R significantly (P<.001) reduced the geometric mean EPG which basically were 0 by study end vs. C which increased from 15.2 to 59.5 epg. R significantly (P<.01) reduced total number of parasites found in the digesta and gastrointestinal tissues. R also significantly (P<.02) reduced Cecum, Ventral Colon, and Dorsal Colon pathology. R reduced larvae pasture contamination to 0 by the end of the 365 d period. This is in contrast to C, which increased larvae pasture contamination over the same time period. When analyzing the data at 60 d time periods, each anthelmintic significantly reduced epg of feces, total parasites found in the digesta and gastrointestinal tissues, and most pathology scores were improved in comparison to C. The larvalcidal dose of Fenbendazole significantly controlled the early L3 and developing larvae in the Cecum, Ventral Colon, and Dorsal Colon to a greater extent than all other anthelmintic treatments. This trial strongly indicates that the use of a year-long anthelmintic rotation program is very efficacious in controlling parasitism, maintaining gut health, and improving gain and condition in horses, and reducing larvae pasture contamination.

23. Long term strongyle control in horse farms in Greece.

Elias Papadopoulos[1]*, Georgios Arsenos[2], Socrates Ptochos[1], George C. Fthenakis[3].
[1]Laboratory of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, Aristotle University, Thessaloniki Greece. [2]Laboratory of Animal Production and Husbandry, Faculty of Veterinary Medicine, Aristotle University, Thessaloniki Greece. [3]Department of Obstetrics and Reproduction, Veterinary Faculty, University of Thessaly, Karditsa Greece.

Strongyles are the most important intestinal parasites of horses worldwide and can cause severe clinical disease; hence, most equine parasite control programs are focused on strongyles. The objective of this study was to investigate the efficacy of a control program for strongyles, implemented in athletic horses over three years. The study was performed in six

equestrian clubs located in Northern Greece. In total, 143 athletic horses, which represent ≈4% of athletic horses in Greece, were included into the study. All animals belonged to breeds or crossbreeds used for sports and were kept under similar husbandry and management conditions; they were stabled in designated facilities and had very little access to pasture daily. Throughout the study period, faecal samples from individual horses were obtained every three to six months. All samples were examined for faecal eggs per g (epg) counts, by using the modified McMaster technique. Horses with >100 epg were treated with moxidectin (EQUEST™ oral gel) at the recommended dose rate of 400 μg per kg bodyweight, according to the faecal egg count reduction test (FECRT). Every horse that received treatment was sampled again 2 weeks later, in order to confirm the efficacy of the treatment. In all cases and throughout the study, treatment was 100% effective. Moreover, there was no evidence of developing resistance strains. Mean epg at start of the study was 59.9; thereafter (at 6-monthly intervals), mean epg were 19.7, 16.0, 16.9, 19.7, 15.1 and 16.9 epg. Coprological examination of individual animals reduced significantly the number of anthelmintic treatments. The approach of targeted selective treatments in athletic horses, based on epg counts, was effective in keeping the strongyle population low and delaying development of anthelmintic resistance. Moxidectin was effective according to the FECRT results, despite its long-term use. The results suggest that targeted use of anthelmintics is useful to controlling equine strongyles.

Anoplocephala spp**., *****Parascaris*****, Miscellaneous parasites and other topics**

24. Quantitative prevalence of *Anoplocephala sp*. and egg shedding consistency in horses.

Jonatas C. Almeida; Marília M. Augusto, Thaís G. Silva, Paula S. Toledo, Damaris F. Souza, Joaquim Antunes, Marcelo B. Molento. Laboratory of Parasitic Diseases, Department of Veterinary Medicine, Federal University of Paraná, Curitiba, PR, Brazil. molento@ufpr.br

There are three species of equine tapeworms (*Anoplocephala perfoliata*, *A. perfoliata* and *Paranoplocephala mamillana*) in Brazil with an average prevalence of 16% in horse yards. The objective of this work was to determine the quantitative prevalence of *Anoplocephala sp.* in 28 one-year old animals, 25 two-year old animals and 28 thoroughbred mares raised in São José dos Pinhais, PR during January 31st and June 15th of 2007. Repeatability values for the number of parasite eggs in feces were evaluated at 28-day intervals. Coproparasitological tests were processed using the modified centrifugal-flotation technique with 30g of feces. Repeatability was calculated after getting the average count from the herd, extracting the variance (d^2) that was associated with the number of observations, which resulted in the phenotypic variance ($S^2p = ed^2 / n\text{-}1$). Environmental (S^2ep) and genotypic variance ($S^2p = S^2g + S^2ep$) was calculated. Repeatability was determined by the sum of the variances: $r = (S^2g + S^2ep) / S^2p$. The animals were included in three repeatability categories: 0.0 to 0.4, which was considered low; 0.41 to 0.7: good; and 0,71 to 1.0 optimal. All mares showed persistent lower EPG values than the foals (P=0.04). The prevalence results indicated 50, 18 and 40% of *Anoplocephala sp.* in mares, one-year old and two-year old foals, respectively. The frequency of anthelmintic treatments did not influence the results due to long treatment intervals for foals (above 60 days) and mares (above 90 days). The egg shedding consistency data indicated that both foal generations (2005 and 2006) had average values above 62% and that the mares showed values higher than 44%. These optimal conditions reveal an interesting management scenario, which could be incorporated into breeding programs with the goal of reducing anthelmintic usage and parasite population selection towards resistance.

25. The prevalence of *Parascaris equorum* of horses in Marmara Region, Turkey.

Veli Y. Cirak *, E. Gülegen. Dept of Parasitology, Faculty of Veterinary Medicine, University of Uludag, 16059 Gorukle-Bursa, Turkey.

A cross-sectional survey was carried out to determine the prevalence of parascariosis on 15 horse farms in the southern part of Marmara Region in Turkey. Briefly, data on parasite control practices on these farms were as follows: Helminth control is based on the application of anthelmintic drugs. Macrocyclic lactones such as ivermectin or abamectin are the widely used anthelmintics with a treatment frequency of 2 to 12 times per year. Few farm

managers had an adequate knowledge of strategic timing of treatment, the different potency of the anthelmintics used and pasture management for helminth control. Post-treatment faecal egg counts were never determined. A total of 510 horses of different breed, sex and ages were coprologically investigated for *Parascaris equorum*-infections. *Parascaris*-eggs were found in 71 (14%) horses and on 8 farms (53%), at least one animal was found to be infected with *P. equorum*. As recently resistance in *P. equorum* against both ivermectin and moxidectin was detected on a horse farm in this region, this survey will continue to assess the effectiveness of macrocyclic lactones on the studied farms.

26. L3 subpopulations of *Cyathostomum sensu latum* : Adventures with an alternative approach to study cyathostomin infection of horses in Portugal and Romania.

Luís M. Madeira de Carvalho[1], Mihai S. Cernea[2], Sónia Martins[3], Sérgio Sousa[4], Sofia Gersão[1], Laura C. Cernea[2]. [1]Interdisciplinary Centre of Research in Animal Health, Faculty of Veterinary Medicine, Technical University of Lisbon (CIISA/FVM/TUL), Pólo Universitário do Alto da Ajuda, Av. Universidade Técnica, 1300-477, Lisbon, Portugal. [2]Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania. [3]Cooperativa Agrícola da Murtosa, Aveiro, Portugal; [4]Escola Universitária Vasco da Gama, Estrada da Conraria, Quinta de S. Jorge de Milréu, 3040-714 Castelo Viegas, Coimbra, Portugal.

A prevalence/abundance analysis of different morphological types of *Cyathostomum sensu latum* L3 was performed in faecal samples of domestic and feral horses and donkeys in Portugal and Romania, from 2000 till 2008. Eight morphological types of *Cyathostomum sensu lato* L3 were recorded: five with 8 cells (A, B, C, D and G) and other three with 6, 7 and 9 cells (E, F and H). The most abundant in Portugal belonged to *Cyathostomum* type A (65-98%), followed by type D (3,9-9,7) and C (2,3-20,6). The larval types with lower abundance were B (1,4-2,2) and E to H (0,3-3,2), rarely referred in the literature. Type A L3 was the most prevalent (93-100%), but C and D were prevalent too (31-100%). Feral and non treated horse populations showed higher diversity of larval types, being B the more relevant (19-85%). Mann-Whitney test revealed significant differences in the abundance of subpopulations, namely for type A (p<0,001), which is more abundant in farm dewormed horses. In Romania the pattern was similar, being Type A the most abundant (59-71%), followed too by D (11,3-20,3%) and C (16,8-23,7%). B and H were found between 0,7-1%. Identification of horse cyathostomin L3 larval stages remains an interesting field of study, v.g. type A with 8 intestinal cells, according to the literature originated by highly prevalent species, v.g., *Cylicocyclus nassatus, Cylicostephanus longibursatus, Cyathostomum catinatum*. Conclusions: a) There are different subpopulations of *Cyathostomum s.l.* and type A L3 is dominant, followed by C and D. b) There are significant differences between horses regularly dewormed or not; c) This method allows an indirect evaluation of the impact of control measures on cyathostomins biodiversity.

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27. Parasites detected in equines in Albania.

Elias Papadopoulos[1]*, Anastasia Diakou[1], Rezart Postoli[2], Bejo Bizhga[2], Dhimiter Rapti[2], Georgios Arsenos[3], Margarita Papazahariadou[1]. [1]Laboratory of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, Aristotle University, Thessaloniki Greece. [2]Faculty of Veterinary Medicine, Agricultural University of Tirana, Albania. [3]Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, Aristotle University, Thessaloniki Greece.

The objective of the present study was to investigate the prevalence of parasites in equines in Albania, where there is still a considerable number of working equines and particularly donkeys. Moreover, recently, there is an increasing interest for athletic horses used for sport or pleasure. The number of equines in Albania has been reduced dramatically over the last years as a result of modernisation in agriculture, transportation and communication. Parasites play an important role in the health status and welfare of these animals but to date, there is very little data available regarding their parasite fauna. A total of 336 (68 horses, 190 donkeys and 78 mules) faecal and 110 blood sera samples were tested using standard coprological methods and ELISA. The study was undertaken for a period of a year. The results showed that an average of about 45.8% of equines, used to obtain faecal samples, were infected with one or more parasitic elements. In particular, amongst the examined equines, 36.8% of the horses, 46.8% of the donkeys and 51.3% of the mules were infected. Strongyles were found in 41%, *Anoplocephala* spp. in 3.8%, *Dictyocaulus arnfieldi* in 7.6% and *Parascaris equorum* in 1.9% of the animals. Strongyles were significantly more prevalent during the period of October to December compared to the rest of the year. The results from blood sera analyses revealed the following specific antibodies: anti-*Echinococcus granulosus* 17.3%, anti-*Toxoplasma gondii* 10.9%, anti-*Leishmania infantum* 9.1% and anti-*Trichinella spiralis* 6.4%. In the course of this study it was found that in most cases, treatment of infected equines is still sporadic and there is not a national or regional control programme for parasites. In conclusion the results of this study showed a high prevalence of parasites in equines in Albania. Therefore, strategic control programmes should be applied in order to reduce the parasitic infection of these animal species whereas at the same time to take measured that will delay the development of any anthelmintic resistance.

28. Resistance to pyrantel in *Anoplocephala perfoliata* on a Standardbred farm in Canada?

Andrew S. Peregrine[1*], Lise Trotz-Williams[1], Christopher J. Proudman[2]. [1]Dept of Pathobiology, Ontario Veterinary College, University of Guelph, Ontario, Canada. [2]Equine Division, University of Liverpool, Leahurst, Wirral, United Kingdom.

Between April 2006 and September 2007, ceco-colic intussusceptions were diagnosed in four Standardbred horses aged 1-3 years on a well-managed farm in southern Ontario, Canada. Throughout this period there were approximately 80 Standardbred horses on the

farm. Since all four horses with ceco-colic intussusceptions were infected with *Anoplocephala perfoliata*, blood samples were collected from all horses on the farm in October 2007 and examined for antibody to a 12/13 kDa *A. perfoliata* secretory protein; 61 of 67 (91%) horses were seropositive, and the mean optical density (OD) of all horses was 1.391 (median=1.538). Amongst the seropositive horses, 4 (6% of total) and 57 (85% of total) had OD values indicative of moderate (0.201-0.600) and high (>0.600) infection intensity, respectively. This is in contrast to a recent study of 234 horses in southern Ontario of multiple breeds, 117 with colic and 117 controls; 131 (56%) were seropositive to *A. perfoliata*, and the mean OD of all horses was 0.509 (median=0.243). Amongst the seropositives, 65 (27.9% of total) and 66 (28.1% of total) had OD values indicative of moderate and high infection intensity, respectively. The reason for the high prevalence, and high intensity, of *A. perfoliata* infection amongst horses on the Standardbred farm is unclear. In November each year, all yearlings and adult horses on the farm were treated for tapeworms; prior to 2006 this was carried out with double-dose pyrantel. Since neither underdosing nor use of outdated product appear to have been a concern, it would appear likely that the problem was associated with resistance to pyrantel in *A. perfoliata*. However, in the absence of a sensitive diagnostic test for current infection with *A. perfoliata*, this is highly problematic to prove ante mortem.

29. Evaluation of the association between *Anoplocephala perfoliata* infection and colic in horses in Canada.

Lise Trotz-Williams[1], Peter Physick-Sheard[2], Heather McFarlane[1], David L. Pearl[2], S. Wayne Martin[2], Andrew S. Peregrine[1*]. [1]Dept of Pathobiology and [2]Dept of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada.

Infection with *Anoplocephala perfoliata* has been associated with certain types of colic in horses in the United Kingdom and The Netherlands. However, this association has not been evaluated in North America. The objectives for this case-control study were to determine if infection with *A. perfoliata* is associated with risk of colic (as defined in this study) in horses in Ontario, Canada, and to identify risk factors for exposure to *A. perfoliata*. Cases were horses in southern Ontario diagnosed with any type of colic by veterinarians in primary health care practices; controls were horses from the same stables, matched by age, breed and sex where possible. Data collected from 117 pairs of horses over a 16-month period were examined for evidence of an association between the risk of colic and *A. perfoliata* infection. Management data for the horses were also evaluated for evidence of association with *A. perfoliata* infection. Tapeworm infection status was defined on the basis of results of coprological examination using sucrose centrifugation or examination of blood samples for antibody to a 12/13 kDa *A. perfoliata* secretory protein. Fifty-six percent of 234 horses were seropositive for *A. perfoliata*; eggs of *A. perfoliata* were found in samples from 6% of the horses. Using mixed effects logistic regression with a random effect for matched pair, horses dependent on pasture for a large part of their diet were shown to be significantly more likely to have ELISA optical density levels above 0.600 compared to other horses (odds ratio =

6.38; $p = 0.029$). However, neither McNemar's chi-squared statistic nor conditional logistic regression showed any statistical evidence for an association between tapeworm infection (defined either serologically or coprologically) and colic.

Author Index