PROCEEDINGS

American Association of Veterinary Parasitologists

38th Annual Meeting

July 17-20
Minneapolis, Minnesota
1993
American Association of Veterinary Parasitologists
Founded 1936
Affiliated with the American Veterinary Medical Association

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*** Provided honorarium for the Distinguished Veterinary Parasitologist Award

The American Association of Veterinary Parasitologists gratefully acknowledges the above Corporations for their loyal support and sponsoring of special sectors of the 1993 AAVP Conference.
Registration - 38th Annual Meeting
Marriott - City Center, Minneapolis, Minnesota
Pre-function Area Ballrooms 1-2
Saturday 2:00 - 7:00 PM

Social Program

*Saturday, July 17, 1993*
Marriott - City Center, Pre-function Area Ballrooms 1-2
AAVP Mixer
7:00 - 10:30 PM

*Sunday, July 18, 1993*
Marriott - City Center, Ballrooms 1-2
Ciba Animal Health Sponsored Social
6:00 - 7:30 PM

Speaker Ready Room
Marriott - City Center
Bauer Audio Video Speaker Ready Room

AAVP Spouse Meeting Room
Sunday & Monday, July 18-19, 1993
Marriott - City Center, Grays Bay Suite
8:00 AM - 5:30 PM
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American Association of Veterinary Parasitologists
Program 38th Annual AAVP Meeting
Minneapolis, Minnesota

Saturday Afternoon, July 17, 1993
Minneapolis Marriott City Center
Grand Portage Ballroom 3-4

2:30 Registration (Prefunction Area)

3:00 Opening Remarks:
President Ronald Fayer
Vice President and Program Chairman
Charles Courtney.

Session A1: Invited Presentations
Arthropods and Disease, Grand Portage Ballroom 3-4. Moderator:
A.A. Marchiondo


4:00 (2) Culicoides as Vectors of Bluetongue Virus in the New World. E.C. Greiner.


5:00 Coffee (Sponsored by Miles, Inc.)


6:00 (7) Breeding Perendale Sheep with Resistance or Susceptibility to Internal Parasites Following Experimental Infection. T.G. Watson, B.C. Hosking, A.P. Hurford and D.L. Johnson.

6:15 (8) In Vitro Assessment of Two Species of Nematophagous Fungi (Arthrobotrys oligospora and A. flagrans) to Control the Development of Infective Cyathostome Larvae from Naturally-Infected Horses. J. Bird and R.P. Herd.

6:30 (9) Larvicidal Toxicity of Bacillus thuringiensis (strain YBT-1953) to Haemonchus contortus Larvae (Nematoda). Yao Baoan, Sun Ming, Wang Qianian and Yu Ziniu.


6:00 (13) Comparison of Treatment Strategies With Ivermectin for Control of Gastrointestinal Nematodes of Cattle in Louisiana. J.C. Williams and S.D. Broussard.

Saturday Evening, July 17, 1993
Minneapolis Marriott City Center
Grand Portage Ballroom Prefunction Area

7:00-10:00  AAVP Social

Sunday Morning, July 18, 1993
Minneapolis Marriott City Center

Session A3: Ruminants, Grand Portage Ballroom 3-4. Moderators: J.C. Williams and A.M. Zajac


10:00 Coffee (Sponsored by Miles, Inc.)

Session A4: Molecular Biology 1, Grand Portage Ballroom 3-4. Moderators: R.H. Fetterer and T.G. Geary


11:45 Lunch
Session B3: Equine, Grand Portage


8:45 (32) Comparison of Strongid C (Daily) and Strongid P (Every 4 Weeks) in Yearling Thoroughbreds and Strongid C (Daily) on Barren and Foaling Mares. R.P. Herd and G.A. Majewski.

9:00 (33) Control of Strongyles in Ponies Receiving a Daily Feed Ration Containing Pyrantel Tartrate (Strongid C). O. Siocombe and M.C. Lake.


10:00 Coffee (Sponsored by Miles, Inc.)

Session B4: Canine, Grand Portage


11:30 (42) Evaluation of Topical Formulations of Moxidectin and Temephos Alone or in Combination for Activity Against Heartworms, Hookworms, Ascarids, Ticks, and Fleas in Dogs. T. McTier, P. Supakorndej and J.W. McCaill.

11:45 Lunch

Sunday Afternoon, July 18, 1993
Minneapolis Marriott City Center
Grand Portage Ballroom 3-4

1:15 Presidential Address: Ronald Fayer
Moderator: George Conder.

1:30 Awards
Awards Chairman: J.P. Dubey.
Session A5: Joint AAVP/American Heartworm Society Presentation, Grand Portage Ballroom 3-4. Moderator: M. Soli


3:00 (45) Evaluation of Elisa-Based Adult Heartworm Antigen Test Kits Using Well Defined Sera from Experimentally and Naturally Infected Cats. T.L. McTier, N. Supakorndej, J.W. McCall and M.T. Dzimianski.


3:45 Coffee (Sponsored by Miles, Inc.)

Session A7: CIBA Symposium, Grand Portage Ballroom 3-4. Moderator: R.C. Lynn


4:30 (53) Lufenuron: Dose Titration in Dogs and Cats. B.L. Blagburn.


5:00 (55) Lufenuron: Safety Studies. W.R. Campbell.


Sunday Evening, 18 July, 1993
Minneapolis Marriott City Center
Grand Portage Ballroom 1

6:00 CIBA Sponsored Social (Heavy Hor D'oevres)
Monday Morning, July 19, 1993
Minneapolis Marriott City Center

Session A8: Molecular Biology 2, Grand Portage Ballroom 3-4. Moderators: H.R. Gamble and R.D. Klein


9:00 (59) Genetic Changes at the beta-Tubulin Genes of *Haemonchus contortus* Associated with Resistance to Benzimidazoles. R.N. Beech and M.E. Scott.


10:00 Coffee (Sponsored by Miles, Inc.)

Session A9: Protozoa, Grand Portage Ballroom 3-4. Moderators: J.P. Dubey and D.S. Lindsay


10:30 (64) Paromomycin Effectively Prevents Cryptosporidiosis in Experimentally Infected Calves. R. Fayer and W. Ellis.

10:45 (65) *Cryptosporidium* and *Giardia* Infections in Farm Animals. L. Xiao and R.P. Herd.


11:30 (68) Studies to Determine the Relationship of Myxozoans Found in Catfish Ponds to Proliferative Gill Disease (PGD) in Channel Catfish. L.M. Pote, T.L. Lin, B. Bellerud and E.F. Chenney.

12:00 Lunch


8:00 (69) Prevalence of Helminthic Infection in Cats in Patiala, India. M. Johal.


9:30 (75) Efficacy of the Preventic (9% Amitraz) Collar for Control of Rhipicephalus sanguineus and Dermacentor variabilis Infestations on Dogs. B.L. Blagburn, J.L. Vaughan, D.S. Lindsay and T.A. Miller.

9:45 (76) Evaluation of a 1% and 2% (S)-Methoprene Collar Against the Hatch of Cat Flea Eggs When Worn by Dogs and Cats. W.A. Donahue and R. Young.

10:00 Coffee (Sponsored by Miles, Inc.)


11:00 (80) A Systematic Approach to Classification of Heartworm Disease to Define Specific Populations. P.A. Tanner, D.M. Keister and N.J. Meo.


11:30 (82) Clinical Field Trial for the Treatment of Mature and Immature Dirofilaria immitis Infestation in Dogs with Severe Heartworm Disease. P.A. Tanner, H. Winograd and D.M. Keister.


12:00 Lunch

Monday Afternoon, July 19, 1993
Minneapolis Marriott City Center
Grand Portage Ballroom 3-4

1:30 Business Meeting
President Ronald Fayer

Session A10: Miles Symposium, Grand Portage Ballroom 3-4. Moderator: R.B. Grieve

2:30 (84) Human Toxoplasmosis. T.J. Curiel.

3:00 (85) What Is the Usefulness of a Vaccine for Feline Toxoplasmosis? J.K. Frenkel.

3:30 Coffee (Sponsored by Miles, Inc.)

Session A11: Toxoplasmosis, Grand Portage Ballroom 3-4. Moderators: B.L. Blagburn and E.C. Greiner

3:45 (86) Immunization of Cats With Tissue Cysts, Bradyzoites and Tachyzoites of the T-263 Strain of Toxoplasma gondii. A. Freyre, J.L. Fishback, L. Choromanski and I. Popiel.
4:00 (87) Activity of Diclazuril Against Toxoplasma gondii in Cell Cultures and Mice. D.S. Lindsay and B.L. Blagburn.


Tuesday Morning, July 20, 1993
Minneapolis Convention Center

Session A12: AVVP/AVMA Symposium Parasite Problems Practitioners Encounter in Food Animals, Room 102 EF. Moderator: Ronald Fayer

9:30 Chairman's Opening Remarks. Ronald Fayer, President AVVP.


10:15 (99) Parasite Problems in Swine Production. J.D. McKean.

10:55 Coffee (Sponsored by Miles, Inc.)

11:10 (100) Parasites of Poultry. D. Schwartz and M.D. Ruff.

11:50 (101) Parasites of Farm-raised Fish. P.H. Klesius.

12:30 Summary. Ronald Fayer.
PSOROPTIC SCABIES IN WILDLIFE AND DOMESTIC ANIMAL HOSTS. W.M. BOYCE*. UNIVERSITY OF CALIFORNIA. DAVIS, CA 95616.

Although Psoroptes sp. mites infest a variety of potentially sympatric domestic and wild ungulates such as cattle, llamas, goats, bighorn sheep, deer, and elk, it is not clear whether or not mites are transmitted between these hosts. For example, recent evidence from New Mexico suggests that one interbreeding population of mites may be infesting both sympatric bighorn sheep and mule deer. However, in contrast, a herd of domestic cattle has remained free of mites for at least 4 years in spite of their sympatric relationship with mite infested bighorn sheep. Until further information is available, it would be prudent to consider the possibility that infestations may be transmitted between host species, and to refer to infestations as being caused by Psoroptes sp., rather than attempting to make a species-level identification based on host identity or location of mites on the host.

CULICOIDES AS VECTORS OF BLUETONGUE VIRUSES IN THE NEW WORLD. E.C. GREINER, UNIVERSITY OF FLORIDA, GAINESVILLE, FLORIDA 32611

Evidence that bluetongue viruses (BTV) cycle in new world ruminants outside of the geographic distribution of the proven vector, Culicoides variipennis grew in the 1980s. The prevalence of antibodies to BTV in domestic ruminants in the Caribbean basin and Central America usually ranges from 50 to 90%. Because C. variipennis does not occur in southern Florida, the Caribbean islands, and most of Central America south of Mexico, studies were begun to determine the vectors of these viruses in this region. Insect light traps were operated near sentinel ruminants to search for correlations between population trends of potential vectors and patterns of seroconversion and virus isolation in the ruminants. Culicoides insignis, C. pusillus, C. furens, and C. filarifer/ocumarensis were the most commonly collected species. Aspirating Culicoides spp. from ruminants demonstrated that each of these in addition to C. lahillei actually feeding on these hosts. Virus isolations have been made from pools of wild C. insignis, C. filarifer/ocumarensis, and C. pusillus. Finally, C. insignis has been experimentally infected with BTV and has transmitted BTV to sheep via bite. Therefore, the evidence incriminates C. insignis as the primary vector of BTV in middle America.
ECOSYSTEM DYNAMICS OF LYME DISEASE IN CASTLE ROCK STATE PARK IN NORTHWESTERN ILLINOIS. T. SLAJCHERT*, U.D. KITRON, C.J. JONES AND A. MANNELLI. UNIVERSITY OF ILLINOIS, URBANA, IL 61801

Ixodes dammini, the primary vector of Lyme disease, was first found in northwestern Illinois in 1987. Since the tick's discovery, the number of cases of endemically acquired Lyme disease diagnosed in humans and pets in Illinois has been gradually increasing. Aspects of Lyme disease vector ecology have been examined in Castle Rock State Park, the largest I. dammini focus in the state. Comprehensive studies have been made of tick population dynamics, host preferences of immature ticks, prevalence of ticks on deer and potential reservoirs for Borrelia burgdorferi. Additional analysis of tick habitat has been performed using a geographic information system (IGIS). Based on our results, some aspects of the ecosystem dynamics of Lyme disease in Illinois differ significantly from those of the larger Lyme disease foci of the northeastern United States. The significance for controlling Lyme disease in the Midwest will be discussed.

GENETIC CONTROL OF TRICHOSTRONGYLE EPG VALUES IN PASTURED BEEF CALVES: FACTORS INFLUENCING EXPRESSION. L.C. GASBARRE* AND C.J. DAVIES. USDA, ARS, LPSI, HELMINTHIC DISEASES LABORATORY. BELTSVILLE, MD 20705

Previous studies have shown that host genetics significantly affects the number of trichostrongyle eggs passed in the feces of beef cattle. This effect is evident after the calves have been on pasture for several months. In addition to affecting fecal EPG values, the sire of the calf also significantly influences serum antibody levels against the parasites. These two effects appear to be under the control of different genes or sets of genes. During the first grazing exposure the majority of calves regulate egg output, while a few calves continue to show increased EPG values. Certain sires produce these high EPG calves at higher than expected frequencies. Genes of the major histocompatibility complex exert a very minor effect on EPG values, and a stronger but still insignificant effect on serum antibody responses against antigens derived from whole adult worms. The differences between calf EPG value are evident under pasture conditions, but are not significant when calves are experimentally challenged with a secondary bolus infection. These results indicate that either the response is restricted to the primary immune response, or that the response functions only under conditions of natural transmission. Finally, the sex of the calf also significantly affects fecal EPG values. Bull calves excrete significantly more eggs than heifer calves, and this trait appears earlier than the sire effect on EPG values.

Periparturient egg rise (PPR) is a principal means for some nematode parasites to survive overwinter and to provide transmission of infective larvae from ewes to lambs during the spring. Routine laboratory propagation techniques have probably selected for those characteristics that would not promote PPR in conventional laboratory strains of *H. contortus*. The selection process herein included inoculation of helminth-free pregnant ewes during mid-gestation, collection of nematode eggs during lactation, and storage of infective larvae at 4°C for four months. After ten generations, the two strains, the stock (BPL) and PPR, were compared for reproductive, morphological, and parasitological differences in lambs and pregnant ewes. After lambing, ewes inoculated with the PPR strain had significantly higher fecal egg concentrations. Lambs inoculated with the PPR strain had higher egg concentrations, higher total daily egg production, fewer adult worms, larger female worms, and higher fecundity. Repeated selection in the appropriate host, after prolonged storage of the inoculum, has produced a novel PPR-strain of *H. contortus*.

Development of resistance to *Haemonchus contortus* by Saanen goats. T.G. WATSON*, B.C. HOSKING, AgResearch, Ruakura Agricultural Centre, Private Bag 3123, Hamilton, New Zealand

General opinion is that unlike sheep, goats do not develop an effective resistance to infection by nematode parasites. An experiment was carried out to determine if Saanen kids can acquire resistance to *Haemonchus contortus*.

Twenty-five buck kids reared without exposure to nematodes were randomised into 5 similar groups on the basis of source, age and liveweight. Each kid was given 350 infective larvae per kg liveweight 0, 4 and 15 weeks into the study. Infections in Group ND were not abbreviated with anthelmintic. Those in the DD group were abbreviated with levamisole (LEV) prior to each challenge (3 and 14 weeks). Kids in the PD group were treated only at 3 weeks and those in the SD group only at 14 weeks. Animals in Group C were infected and slaughtered 4 weeks later to determine the infectivity of the parasite culture whereas all other animals were killed 10 weeks after the last challenge.

Mean numbers of worms recovered did not differ between the groups. Establishment rate for the primary infection (Group C) was 24.0%. Based on the size of the challenge after anthelmintic abbreviation recovery rates were 7.0%, 20.0%, 17.1% and 15.2% for ND, DD, PD, SD, respectively. The tendency was for lower male:female ratios when infections were not abbreviated 0.42 versus 1.38, 0.90 and 1.06.

Results from this experimental study suggest that existing worm burdens may be required to regulate establishment of newly ingested larvae. Populations appear to be self-regulating. This can be one explanation for the continued susceptibility of goats and the dependency farmers have on anthelmintics to control nematode parasite burdens.
Breeding Perendale Sheep with Resistance or Susceptibility to Internal Parasites Following Experimental Infection. T.G. WATSON*, B.C. HOSKING, A.P. HURFORD and D.L. JOHNSON, AgResearch, Ruakura Agricultural Centre, Private Bag 3123, Hamilton, New Zealand

Selective mating of Perendale sheep to generate lines with increased or reduced susceptibility to Haemonchus contortus and Trichostrongylus colubriformis following experimental challenge commenced in 1986. Five years later changes were assessed by monitoring faecal nematode egg count (FEC) of selected breeding ewes and their progeny. In the absence of drenching, mature flock ewes were monitored 4 times during the periparturient period. Ewe and ram lamb progeny were screened on 5 occasions between weaning (November 1990) and May 1991. Lambs were given anthelmintic at sample dates. Progeny were run as single sex mobs subsequent to weaning. All stock were grazed across pasture contaminated previously with mixed parasite populations.

Mature ewes selected as lambs with Low FEC shed lower numbers of eggs than High FEC line ewes 2 weeks pre- and 3, 6, and 9 weeks post-parturition. At the periparturient peak, approximately 3 weeks after lambing, Low FEC line ewes were shedding over 3 times fewer nematode eggs than their High FEC line counterparts.

Lambs from the Low FEC line had significantly lower FECs on 4 or 5 sample dates (P<0.01). At the final sample date (April/May), there was a 10-fold difference in mean FEC. Although ram lambs had significantly higher egg counts than ewes differences between line were consistent with the selection criteria. Bulked faecal cultures demonstrated that Haemonchus and Trichostrongylus were the dominant genera.

IN VITRO ASSESSMENT OF TWO SPECIES OF NEMATOPHAGOUS FUNGI (ARTHROBOTRYS OLIGOSPORA AND A. FLAGRANS) TO CONTROL THE DEVELOPMENT OF INFECTIVE CYATHOSTOME LARVAE FROM NATURALLY-INFECTED HORSES. BIRD, J.* and R.P. HERD. DEPARTMENT OF VETERINARY PREVENTIVE MEDICINE, 1900 COFFEY RD., COLUMBUS, OH 43210-1092

The ability of two species of nematophagous fungi, Arthrobotrys oligospora and A. flagrans, to control the development of infective larvae in feces from naturally-infected horses was assessed in vitro. The horses were from a farm where it had been previously established that cyathostomes accounted for 100% of the strongyle egg output. The feces from these naturally-infected horses were mixed with spores of each fungal species at four concentrations: 0 (control), 1, 10, and 100 spores per egg. Five replicates for each group were incubated for 8 days. Infective larvae were harvested using a Baermann technique, counted and the treated groups compared to the controls. The percentage reduction in infective cyathostome larvae was calculated for each fungal concentration. A fungal concentration of 1 spore per egg resulted in 40.5% and 32.1% reduction for A. oligospora and A. flagrans, respectively. A concentration of 10 spores per egg resulted in 87.4% and 90.49% reduction, while 100 spores per egg resulted in 95.9% and 93.8% reduction for A. oligospora and A. flagrans, respectively.
LARVICIDAL TOXICITY OF BACILLUS THURINGIENSIS (STRAIN YBT-1953) TO
HAEMONCHUS CONTORTUS- LARVAE (NEMATODA). YAO BAOAN*, SUN MING, WANG
QIANIAN AND YU ZINU. HUAZHONG AGRICULTURAL UNIVERSITY, WUHAN, 430070,
PEOPLE’S REPUBLIC OF CHINA.

The third-stage larvae of the ruminant parasite Haemonchus contortus were recovered in a
Baermann apparatus maintained in PBS from rectal feces of goat after incubation at 25°C for
7 days.

A toxin from the bacterium Bacillus thuringiensis (strain YBT-1953) was lethal to nematode
third-stage larvae. Exposure of the third-stage larvae of the ruminant nematode Haemonchus
contortus to the toxin from the B.t. (strain YBT-1953) at 25°C.

Treatment of third-stage larvae of Haemonchus contortus with 10.0 ug total protein/ml of B.t.
toxin yielded 92.0% mortality after 72 hours. The LD$_{50}$ was about 2.5ug total protein/ml in
vitro.

The larvicidal toxin from B.t. may facilitate microbial control of parasitic nematodes.

EFFICACY OF A DOSE AND MOVE PROGRAM OF PARASITE CONTROL FOR SHEEP
IN SOUTHWESTERN VIRGINIA. A.M. ZAJAC*, G.A. MOORE, C.D.
THATCHER, D. NOTTER, S. UMBERGER. VIRGINIA TECH, BLACKSBURG, VA
24061.

In the southeastern U.S., Haemonchus contortus is the predominant
trichostrongyle of sheep and producers must often administer
frequent dewormings to prevent losses. As an alternative, the
efficacy of a dose and move program for lambs was examined during
2 grazing seasons. In the first year of the study lambs and ewes
were dewormed orally with ivermectin at the start of the grazing
season and turned out onto pasture grazed by sheep the previous
year. Lambs were retreated in July and moved to a sheep pasture
from which a cutting of hay had been taken. In the second year
of the study the same protocol was followed except that weaned
lambs were used for the experiment. An equivalent set-stocked
control group was treated monthly with ivermectin drench. Fecal
egg counts and lamb weights were determined at 2 week intervals
and serum pepsinogen levels were evaluated monthly. In both
years of the study additional anthelmintic treatments were
required in the dose and move group to prevent severe
haemonchosis. However, comparable weight gains for both
experimental and control groups were achieved with fewer
treatments in the dose and move group.

Day-night pairs of NOAA satellite thermal infrared images from the AVHRR were processed to produce temperature maximum (Tmax), temperature minimum (Tmin) and diurnal temperature difference (dT) maps of the lower Nile River valley. Patterns were seen that reflected the schistosomiasis prevalence regions described by Scott in 1937. Temperature difference image subsets of the Nile delta for 16 AUG 90 and 14 FEB 91 were analyzed in more detail. Values of dT at specific locations were derived using the median of 5 X 5 pixels centered on the latitude and longitude of 41 survey sites listed in 1937, 1983 and 1990 schistosomiasis surveys of the Nile Delta. A Spearman correlation coefficients revealed an inverse relationship between site dT values for 16 AUG 90 and 14 FEB 91 and prevalence of S. mansoni in 1937 and 1983 surveys. For S. haematobium, a positive association of site dT values and prevalence was seen for 1937 only. A significant association was observed between 1937 S. mansoni prevalence and that observed in 1983 and 1990; S. haematobium prevalence in 1937 was not correlated with the later surveys. Results suggest dT values reflect hydrologic regime variables that can be used to predict environmental risk of snail-borne diseases on a regional scale, particularly if thermal-moisture domains and prevalence can be related to soil types, water table and climate by GIS methods.

Cow/calf Parasite Control With Mid-summer Use of Ivermectin - A Summary of Seven Years of Data. J.A. Hawkins*, C.E. Couvillion, and R.R. Evans. Merck AgVet and Mississippi State University, Starkville, MS 39759.

A series of trials was performed (1982-1984, 1987-1989, and 1990) at the Prairie Research Unit of the North Mississippi Research and Extension Center, Mississippi State University, to determine the value of anthelmintic use in mixed breed commercial beef cows and calves in a herd with no obvious signs of parasitism. Several different treatment schedules with thiabendazole paste and ivermectin injection were used. The primary objectives of the trial were to: 1. Determine if anthelmintic use was beneficial in improving productivity, and 2. Determine if the mid-summer use of ivermectin injection provided better GI parasite control than treatment at other times (i.e. spring and fall). Treated calves from treated cows weighed significantly (p<0.05) more at weaning (221.7 kg vs. 204.6 kg) and had significantly higher gain (ADG) from birth to weaning (809 g vs. 737 g) than untreated calves from untreated cows. Cows and calves that were treated with ivermectin in July had significantly (p<0.01) lower fecal egg counts in September or October than either untreated calves from untreated cows or calves subject to any treatment schedule that did not include mid-summer ivermectin.
COMPARISON OF TREATMENT STRATEGIES WITH IVERMECTIN (IVM) FOR CONTROL OF GASTROINTESTINAL NEMATODES OF CATTLE IN LOUISIANA. J.C. WILLIAMS* AND S.D. BROUSSARD, DEPARTMENT OF VETERINARY SCIENCE, LOUISIANA AGRICULTURAL EXPERIMENT STATION, LSU AGRICULTURAL CENTER, BATON ROUGE, LA 70803.

In a third yearly experiment (1991-92), strategic IVM treatment of weaner-yearling beef heifers at 3 intervals was compared with 3 treatments at 6-week intervals in fall-winter with or without a 4th treatment in spring. Three groups of 11 heifers were grazed on separate pastures from Nov 1991 through Sep 1992 and treated with IVM as follows: group 1-Nov 18, Mar 10, and Jun 2; group 2-Nov 18, Dec 30, Feb 11; group 3-same as group 2, but 4th IVM on Jun 2. All IVM treatments were SC at 200 μg/kg. Winter conditions were mild and heavy rainfall was prevalent in Jan-Feb and Jun-Aug. Group 1 egg counts were highest through Mar, although pasture larval counts were highest for group 2 in Dec and for groups 2 and 3 in Jan. Larval counts for group 1 were highest in Mar and Apr. Egg counts for all groups were consistently low after Mar (<100 EPG). Both egg and larval counts increased slightly during Jun-Sep with those of group 1 generally lowest and those of group 3 highest. Mean liveweights of group 3 were highest from Jun-Sep, but not significantly higher than group 1. Weights of group 2 cattle remained lowest from Jun. A high rate of gain by group 1 and weight loss in group 3 resulted in a final highest mean weight for group 1 (371 kg). Final weights for groups 2 and 3 were 342 kg and 362 kg, respectively. Total worm counts from representative cattle at trial end were generally low, but higher in groups 2 and 3 than in group 1. Impact of Ostertagia was minimal; Haemonchus was high in groups 1 and 3 and Cooperia spp. in group 2. The effect of concentrated treatment at 6-week intervals in fall-winter, with or without spring treatment, was similar or less effective than treatment at 3 long intervals in parasite control and growth promotion during 3 years of investigation.


Sixty-four (182 kg) weaned Angus steer calves were used to study the effect of a 1% injectable formulation of moxidectin on calf performance over a 112-day winter-spring grazing period beginning 1 February. Steers were randomly allotted to eight groups of eight steers based on weight and pretreatment nematode eggs per gram of feces (epg). A completely randomized design with four replications of two treatments was used. The treatments were carrier only and moxidectin 1% injectable at 0.2 mg moxidectin/kg body wt. The split pasture technique was used in that four 4.86 ha pastures were divided into two 2.43 ha paddocks with both of the animal treatments appearing in each pasture. Pastures were dormant bermudagrass pastures that had been no-till interseeded with cereal rye the previous October. Average daily gain over the 112-day study of treated steers (0.96 kg) exceeded (P<.0006) that of control steers (0.84 kg). Total epg were reduced (P<.0001) within seven days after the initial treatment. Total epg were increasing among treated animals by day 56; consequently, a second treatment was administered which again reduced epg of treated animals compared to control steers. Moxidectin treatment effectively reduced epg and resulted in a 13.4% improvement (P<.0006) in gain over the 112-day study.

The anthelmintic efficacy of pour-on ivermectin for cattle was evaluated using 10 yearling red deer harboring both natural and artificially induced gastrointestinal nematode infections. The red deer were randomly allocated into a control group of 5 animals and a treatment group of 5 animals to which pour-on ivermectin for cattle was applied topically at 500 mcg/kg body weight. Adverse reactions were not observed. Approximately 14 days after treatment, necropsies were performed and the nematode parasites were recovered by standard techniques. Specimens of the following genera were recovered in numbers adequate for meaningful efficacy calculations: Haemonchus, Spiculopteragia, Trichostrongylus, and Oesophagostomum. The overall mean efficacy was > 99%.


Eye worms (Thelazia spp.), once thought to be rare in cattle in North America, have been reported in cattle in several states as well as several Canadian provinces. Recent studies in Alberta have shown a much higher prevalence than previously reported. The importance of this parasite as a contributing element to eye diseases of cattle remains controversial.

Sixteen crossbred beef cattle from a Southern Alberta herd with a demonstrated high prevalence of Thelazia spp. were ranked by weight and formed into replicates of two animals each. Within replicates, animals were randomly assigned to serve as untreated controls or to receive IVOMEC® Pour-On applied at the rate of 1 ml/10 kg (500 mcg/kg). Fourteen days after treatment the animals were sacrificed and the eyes and surrounding tissues excised and examined. Thelazia spp. were recovered from all eight untreated control animals; a total of 86 worms were recovered from these animals. Only two worms, one from each of two animals, were recovered from ivermectin-treated animals.
STUDIES ON THE ANTELMINTIC EFFICACIES OF NETOBIMIN IN CATTLE. T.A. YAZWINSKI, C. TUCKER AND H. FEATHERSTON. UNIVERSITY OF ARKANSAS. FAYETTEVILLE, AR. 72701.

Three studies were conducted in the evaluation of netobimin for use in cattle; 2 titration control trials and one long-term administration prophylaxis trial. The control studies were done in the spring of 1983 and 1990, with each involving natural infections. For both studies, efficacies against adult nematodes were ≥ 98.8% at dose rates ≥ 5 mg/kg BW. Efficacies against Ostertagia EL₄ & LL₄ forms were inversely proportionate to the extent of arrestment seen in the control calves at necropsy. At dose rates of 10 mg/kg BW or greater, however, reductions of these worm burdens were ≥ 95.1%.

The long term (56 day) administration study was started on April 8, 1991, with the utilization of 16, parasite-free calves at day 0. The eight control calves were sham dosed with water whereas the treated calves received daily oral doses of 243 mg of netobimin in 10 ml of water. All calves grazed a common, 5 hectare pasture until day 56, followed by 14 days on concrete prior to necropsy. Percent reductions of adult forms at necropsy ranged from 65.6% (N helvetianus) to 100% (H placei). Numbers of Ostertagia EL₄ were not altered as a result of netobimin administration.

For the above studies, netobimin was easily administered and induced no untoward effects in the treated animals.

THE RESIDUAL EFFECT OF MOXIDECTIN (1% INJECTABLE) AGAINST LUNGWORM AND GASTROINTESTINAL NEMATODES IN CATTLE UNDER FIELD CONDITIONS IN THE NETHERLANDS. M. EYSKER*, J.H. BOERSEMA, P VAN DOMMELEN, H. VAN DEN HURK AND F.N.J. KOOYMAN, UNIVERSITY OF UTRECHT, P.O. BOX 80.165, 3508 TD UTRECHT, THE NETHERLANDS.

An experiment was done on the residual efficacy of a 1% injectable of moxidectin against lungworm and gastrointestinal nematodes in calves under field conditions.

On May 8th 12 calves were infected experimentally with 20 lungworm larvae and turned out on a pasture with large numbers of overwintered gastrointestinal nematode larvae. On June 20th these calves were divided into three groups; a control group which remained on the pasture and two groups which were treated with moxidectin. One treated group was moved to aftermath and the other remained on the pasture, but separate from the control group. In addition two groups of 4 calves, one treated with moxidectin and one non treated, were turned out on the pasture of the control group.

The results demonstrate a 100% residual effect of three weeks against Ostertagia and of at most 5 weeks against Dictyocaulus. For Cooperia oncophora the results indicate a residual effect of approximately 90% during 2-3 weeks.

Various techniques were examined to determine optimum conditions for exsheathing infective larvae of 3 important ruminant parasites (Haemonchus contortus, Ostertagia ostertagi, and Trichostrongylus colubriformis). In repeated experiments, separate samples of $1 \times 10^5 - 1 \times 10^6$ infective larvae, 1-2 months old, of each parasite were incubated in each of 4 exsheathing media (distilled water, Earle's Balanced Salt Solution + carbon dioxide, nematode washing buffer + carbon dioxide, or sodium hypochlorite) for 1 or 18 hours. In each case, percentage of larvae exsheathed and infectivity for jirds was determined. Results of these studies indicate that no single exsheathing technique, of those studied, is optimum for every parasite. In addition, caution must be used in drawing conclusions from in vitro studies using exsheathed larvae, since techniques which routinely provide high percentages of exsheathment also appear to reduce viability.


An effective method to control gastrointestinal helminthiasis in dairy calves in North West Europe is a move to aftermath in July combined with anthelmintic treatment. The rational of this is evasion of the midsummer increase in pasture infectivity while immediate contamination of the aftermath is avoided. This method has not been proven effective against lungworm but may be so when low primary infections are acquired immediately after turnout.

In 1992 an experiment was done at the University of Utrecht which involved moxidectin treatment (1% injectable) 7 weeks after turnout in May. The results indicate an effective control of Ostertagia ostertagi and lungworm infections. Challenge infections demonstrate a sufficient development of immunity against lungworm. Due to the extremely high faecal egg counts (>6,000 EPG) before the treatment and the less than 100% efficacy of moxidectin against Cooperia oncophora the control of this species was less satisfactorily.
A novel series of 3-carbonitrile pyroles were active in a recently developed in vitro test designed to detect potential antifluke activity. Fresh or frozen livers from gerbils treated with the test compounds, primarily in diet but also by gavage or injection, were fed to fasted free-living flatworms, Dugesia tigrina or Phagocata morgani. Observations on the planaria following feeding were made using a 0-9 rating system with 9 representing the most severe reaction (death within 48 hours). Reactions of treated planaria were compared with those of planaria fed livers from untreated gerbils.

The most interesting analogs were Br substituted in the 2, 4 and 5 positions with various substitutions on the ring nitrogen. Examples of analogs active against P. morgani at 100PPM diet were the methyl (5.8 rating), hydroxymethyl (6.8), p-chlorobenzoyl (8.5), methoxymethyl (7.0), and isopropoxymethyl (8.6). Control ratings were less than 1.

In vivo efficacy against Fasciola hepatica has been confirmed in rats, sheep and cattle.

Long term stability of ivermectin resistance following initial diagnosis. T.G. WATSON*, B.C. HOSKING, P.F. McKEE, AgResearch, Ruakura Agricultural Centre, Private Bag 3123, Hamilton, New Zealand

The first case of ivermectin (IVM) resistance in New Zealand was reported on a goat property in 1989. As with subsequent diagnoses in this country, Ostertagia spp. were involved. Following faecal egg count reduction testing (FECRT) a slaughter study confirmed multiple anthelmintic resistance with drug efficacies of 93.5%, 79.6% and 43.8% for IVM, morantel and oxfendazole, respectively.

Total spelling of all anthelmintics the farm has not been a management option. Since isolation, a strict drench rotation has been adopted - levamisole (LEV) 1988-89, IVM 1990 and combination 1991. IVM has been used very strategically as animal health demanded, 2 occasions in 1988, 6 in 1990 and twice in each of 1991 and 1992.

Surveillance monitoring of anthelmintic status using lambs in 1992 has indicated that LEV remains highly effective (99.3%) against the resistant parasites and there has been a subtle shift downward for IVM (87.5%). The newer milbemycin/ivermectin, moxidectin (MOX), removed 99.9% of the Ostertagia spp. population.

Stability of resistance is an important issue to address when designing and evaluating management options to delay or restrict resistance and minimise the impact on farming profitability. These results suggest that annual rotation and strategic use of IVM have not had a significant effect on the field isolate over the past 5 years. Currently in the field, there appears to be a significant difference in efficacy between IVM and MOX despite their close structural similarities. It is proposed that MOX will not be used on the site but efficacies will be monitored at regular intervals.
LOCALIZATION AND CHARACTERIZATION OF PHENOL OXIDASE IN TRICHURIS SUIS FEMALES. D.E. HILL* AND R.H. FETTERER, USDA AGRICULTURAL RESEARCH SERVICE, BELTSVILLE, MD 20705.

Previous studies have demonstrated the presence of a tanning process exclusively in adult female Trichuris suis that is most likely mediated by an enzyme of the phenol oxidase type. The observed tanning was hypothesized to be associated with the egg shell or reproductive tract. The present study characterizes and localizes the phenol oxidase enzyme in female T. suis. Phenol oxidase (PO) was partially purified from adult females by ammonia sulfate precipitation followed by ion exchange or affinity chromatography. Kinetic parameters and pH and temperature optima determined for the T. suis enzyme are similar to those of PO from other organisms. Antisera specific for PO, prepared by immunizing rabbits with phenol oxidase purified by SDS-PAGE, was used to localize the enzyme in histological sections of adult female worms and for ELISA assays to localize phenol oxidase in anatomical regions of individual worms. These observations indicate that phenol oxidase is found almost exclusively in the posterior portion of the female and is anatomically associated with the anterior portion of the reproductive tract. PO may be a key element for egg shell formation in T. suis.

THE CUTICULAR PROTEIN SYNTHESIS IN ASCARIS SUUM LARVAE. R.H. FETTERER* AND D.E. HILL. HELMINTHIC DISEASES AND BIOSYSTEMATIC PARASITOLOGY LABORATORIES, LIVESTOCK AND POULTRY SCIENCES INSTITUTE, USDA/ARS, BELTSVILLE, MARYLAND 20705

The development of Ascaris suum larvae from the late third-stage (L3) to the fourth-stage (L4) in a stationary culture system is a simple and useful model for the study of a number of aspects of helminth biology. However, the underlying biochemical changes associated with development have not been determined. In the present experiment we used radiolabeling techniques to measure the rate of protein synthesis of both cuticular and noncuticular proteins as the larvae developed from the L3 to L4.

The L3 were collected from the lungs of pigs 7 days post inoculation and placed into culture flasks containing methionine-free culture medium. $^{35}$S-methionine was added to flasks at 24 hr intervals from day 0 through day 8 in culture. The incorporation of the radiolabel into the noncuticular, and the collagenous and noncollagenous cuticular proteins was determined. The results demonstrate that the cuticular protein synthesis represents about 10% of the total protein synthesis. A rapid increase in the rate of noncuticular and cuticular collagens occurs after about 5 days in culture which corresponds to a period shortly after the third molt. The increase in the rate of synthesis of noncollagenous cuticular proteins occurs after about 6 days in culture.

Recent studies demonstrated that Ascaris suum excretes volatile fatty acids (VFAs) across the cuticle. Based on preliminary studies, VFA excretion by intact A. suum is Na\(^+\)-independent and insensitive to agents that inhibit their transport in vertebrates. The biochemical basis for these potentially important host-parasite differences are unknown.

The kinetics and directionality of VFA excretion by isolated body wall (cuticle, hypodermis and muscle) of A. suum was characterized in a two-chamber diffusion cell. The initial focus of these studies was development of incubation media that maintained viability of the tissue segments. Throughout the course of 24 hr incubations, VFA levels on the muscle side were greater than those on the cuticle side. The isolated body wall acidifies neutral media and alkalinizes acidic media. The dominant VFAs excreted at early time points (2-4 hr) were 2-methyl butyric and 2-methyl valeric acids. By 24 hr, however, acetic and propionic acids were the dominant VFAs. Furthermore, the rate of excretion of acetic and propionic acids by the isolated tissue segments was stimulated when the muscle side was incubated in ARS fortified with glucose, bicarbonate, vitamins, amino acids, antioxidants, and serotonin. These results suggest that during short term incubations at least (\(\leq 4\) hr), isolated segments of body wall provide a useful model for investigating the factors that affect VFA synthesis and excretion by A. suum.


Ornithine decarboxylase (ODC) is necessary for polyamine synthesis in eukaryotes. Biochemical data show that ODC obtained from Haemonchus contortus is distinct from ODCs obtained from vertebrates. Using oligonucleotide primers that represent conserved regions of known ODCs, we amplified a fragment of an H. contortus cDNA in PCR experiments that was shown by subsequent nucleotide sequencing to encode ODC. This fragment was used to screen 2 H. contortus cDNA libraries, resulting in two distinct classes of clones. Immature adults express a version of ODC that is considerably truncated at the 5' end compared to most other ODCs. Egg stages express this short form plus a form that is comparable in length to other ODCs. Analysis of these sequences and the sequence of a genomic DNA fragment amplified by PCR suggests that both forms of ODC arise from a common locus. The longer form of H. contortus ODC has been functionally expressed in a strain of E. coli that lacks all polyamine biosynthetic pathways.
CHARACTERIZATION OF SURFACE ASSOCIATED PROTEINS FROM ADULT HAEMONCHUS CONTORTUS. MARCIA RHoads* and RAYMOND H. FETTERER. HELMINTHIC DISEASES LABORATORY, LIVESTOCK AND POULTRY SCIENCES INSTITUTE, USDA/ARS, BELTSVILLE MARYLAND 20705.

Male and female adults of H. contortus express a stage-specific set of surface-associated proteins with apparent molecular weight values of 30, 58, 81, 143 and 180 kDa. The 58, 81 and 143 kDa proteins are glycosylated, whereas the 30 kDa protein is not. The binding of wheat germ agglutinin to the 58, 81 and 143 kDa proteins was inhibited by the trimer of N-acetyl glucosamine (N,N,N-triacetylchitotriose) but not by the monosaccharide, indicating the presence of a chitin-like homopolymer. The carbohydrate portion of the 58 kDa protein is N-linked and accounts for 30% of the molecular weight. Under non-reducing conditions, the 58 kDa glycoprotein forms a high molecular weight polymer, unable to penetrate a 10% acrylamide gel. The 143 and 81 kDa surface glycoproteins were not digested by either N- or O-glycanase, possibly indicating unusual modifications to the saccharide-linkage rendering it unsusceptible to glycosidase digestion.

DEVELOPMENTALLY REGULATED METALLOPROTEASES IN HAEMONCHUS CONTORTUS. H.R. GAMBLE* AND L.S. MANSFIELD. USDA, ARS, LPSI, HELMINTHIC DISEASES LABORATORY, BELTSVILLE MARYLAND 20705.

Nematode proteases have various functions including tissue penetration, extracorporeal and internal digestion, inhibition of blood coagulation and regulation of ecdysis. In the sheep abomasal nematode Haemonchus contortus, a metalloprotease has been shown to regulate ecdysis of infective larvae and cysteine proteases which actively degrade fibrinogen have been described from adult worms.

Haemonchus contortus develops in vitro from parasitic third stage to fourth stage larvae (L4) in 48-72 hours, at which time they begin to feed. Coincident with development to the fourth stage, larvae secreted a protease into culture fluids distinct from the previously described metalloprotease which mediates the ecdysis of infective larvae. The purified protease from L4 had a molecular weight of approximately 46 kDa, functioned as an endoproteinase and digested several native proteins of host origin including fibrinogen, fibronectin, and laminin. Adult H. contortus secreted at least four metalloproteases, with apparent differences from both larval proteases. In contrast to the secreted metalloproteases, cysteine proteases were demonstrated predominantly in extracts of adult H. contortus.

The objective of this study was to determine the effect of fecal preservation and storage on the recovery of equine strongyle eggs. Feces were obtained from a horse, mixed thoroughly, and 2 g samples stored in absolute methyl alcohol, 70% ethyl alcohol or 2.5, 5, and 10% formalin at 25 °C, or storage without preservative at 4, -10, or -20 °C. Recovery of strongyle eggs from stored samples was compared to recovery from fresh feces prior to storage. Strongyle eggs were most difficult to recover from feces preserved in ethyl or methyl alcohol. Strongyle eggs were more reliably recovered from samples preserved in formalin at all concentrations, but best in those stored in 5 and 10% formalin. Strongyle eggs were recovered more reliably from samples stored fresh and held at 4 °C than those stored at either -10 or -20 °C. Formalin fixation was the most suitable means of preservation for long term (up to 200 days) storage.


The prevalence of *Anoplocephala perfoliata* infections in broodmares and their foals has been examined over a five year period. Three separate herds were studied. These herds were treated with three distinct anthelmintic programs. One herd received no anthelmintics (CON), a second herd received ivermectin (IVM) at eight-week to two-month intervals, and the third herd received a fast rotation (ROT) of ivermectin, pyrantel pamoate, benzimidazoles and benzimidazole-piperazine combination. All drugs were given at the therapeutic dose level.

Eggs per gram (EPG) data from these mares indicate a significant increase (P<0.005) in both numbers of positive samples and numbers of mares positive for *A. perfoliata* in the IVM herd vs. CON and CON vs. ROT. Significant differences in egg recovery from feces using currently described methods were not seen. However, recovery data from yearlings at necropsy and mares treated with a double dose (13.2 mg/kg) of pyrantel pamoate show no significant differences between groups. Supported in part by MSD AGVET.
ASSAY ON ANTHELMINTIC ACTIVITY OF A PASTE CONTAINING ALBENDAZOLE AND TRICHLORFON AGAINST HORSE SMALL STRONGYLES. A.J. COSTA*; G.J. ARANTES; O.F. BARBOSA; O.T. VASCONCELOS; J.R. PEREIRA. São Paulo State University (UNESP), 14870-000 - Jaboticabal-SP, Brazil.

From each pair of the 14 equines that were randomly chosen for treatment one was kept untreated and served as control. The treated ones received the paste orally at the doses of 10.0 mg/kg of Albendazole and 25.0 mg/kg of Trichlorfon EPGF countings, culture of larvae, biochemical analysis (Alanine transferase, Aspartate transferase, creatinine, urea, glucose) were conducted on the days zero, 1, 3, 5 and 7 post-treatment. On the seventh day all the equines were submitted to necropsy and the results demonstrated that the combination of the drugs was 100% efficient to remove: Cylicostephanus minutus, Cylicostephanus calicatus, Cylicocyclus insigne, Cylicocyclus leptostomus, Cylicocyclus nassatus, Cyathostomum labiatum, Cyathostomum labratum, Cyathostomum coronatum, Cyathostomum pateratum, Cyalocephalus capitatus and Cylicodontophorus euproctus. Efficiency over 99% was observed against Cylicostephanus longibursatus, Cylicostephanus goldi and Cyathostomum catinatum. For the immature small strongyles the efficacy was 31.70%. The treated and untreated animals were parasited by 1880 and 143070 "small strongyles" (general reduction of 98.69%). The hematological examinations did not reveal anything that could be attributed to toxic effect of the drugs used.


Pyrantel tartrate (Strongid C) daily and pyrantel pamoate (Strongid P) every 4 weeks were evaluated in 2 groups of 12 and 11 yearlings from Jan 21 to Sept 3, 1991. Barren mares were given Strongid C daily for the same period, while foaling mares were given Strongid C strategically from April 1 until Aug 16. Yearlings of both groups initially showed a reduction in strongyle and ascarid egg counts, followed by a marked rise of strongyle egg counts in Aug and Sept with mean peaks of 723 epg (Strongid C) and 454 epg (Strongid P). Individual counts rose as high as 1584 epg. By contrast, mean strongyle egg counts of barren and foaling mares were low (<100 epg) throughout the Strongid C medication periods. Pasture larval counts rose in yearling pastures in Sept and Oct, but remained negligible in barren and foaling mare pastures. Larval cultures and pasture larvae were 100% cyathostomes. There was no difference in body condition scores or body weights of yearlings treated with Strongid C or Strongid P.

Fecal egg counts of 23 untreated foals of Strongid C medicated mares remained low until after weaning. Mean strongyle or ascarid counts were less than 100 epg until Sept 3. Treatments with Strongid P on Sept 3 and Oct 1 had little effect on fecal egg counts. Treatment of three weanling groups (n= 7,8,8) with ivermectin (Zimecterin), oxibendazole (Anthelcide), and pyrantel pamoate (Strongid P) on Oct 15 resulted in mean strongyle counts of 5, 53, and 525 epg respectively 2 weeks posttreatment and 6, 96, and 607 epg respectively 4 weeks posttreatment. Although Strongid C medication was highly effective in barren and foaling mares, there was a clear failure of Strongid C and/or Strongid P in yearlings and weanlings. This confirmed earlier studies showing reduced efficacy of anthelmintics in young compared with adult horses (Herd & Gabel, 1990).
CONTROL OF STRONGYLES IN PONIES RECEIVING A DAILY FEED RATION CONTAINING PYRANTEL TARTRATE (STRONGID C®). OWEN SLOCOMBE* AND MARY C. LAKE, DEPT OF PATHOLOGY, ONTARIO VETERINARY COLLEGE, UNIVERSITY OF GUELPH, GUELPH, ONTARIO N1G 2W1

On May 21, 1992, 20 ponies were weighed and assigned to 2 groups each consisting of 9 fillies and one gelding. On May 22 and once daily thereafter until October 19, each pony was given a ration of sweetfeed and in one group the ponies were offered the ration top dressed with pellets containing pyrantel tartrate at the rate 2.64 mg/kg bodyweight. Ponies were carefully monitored during feeding and all spilled pellets were returned to the feed container. On May 29, each group was placed on a separate pasture and remained there until October 19 except that daily the ponies were housed for a short period to receive the feed ration. Every 2 weeks throughout the period described above a fecal sample was taken from each pony and herbage samples from each pasture. Ponies were weighed monthly. On October 19, one filly and the gelding from each group were isolated indoors for 6 weeks and then necropsied.

Ponies readily consumed the daily ration except one pony which refused the medicated ration on one day. Prior to treatment on May 22, the mean strongyle eggs per gram of feces (epg) for each group was greater than 2200. Thereafter, the mean epg for untreated ponies ranged from 1405 to 2294. The mean epg for treated ponies decreased to 16.8 on the day of turnout to pasture and ranged from 0.2 to 4.0 for the remainder of the trial. Pasture larval counts was at a high of 26,790 strongyle larvae/kg dry herbage from the pasture with untreated ponies in August and at that time it was 610 from the pasture with treated ponies. At necropsy, 2 treated ponies had 8403 and 8500 strongyles in the large intestine; the 2 untreated had 56,178 and 82,416. The daily consumption of pyrantel tartrate by the ponies significantly reduced the transmission of strongyles.


The efficacy of daily pyrantel-tartrate (Stongid-C) at the recommended dose in reducing total cyathostome numbers and associated pathology was tested using 16 ponies previously exposed to parasites. Ponies were divided in to 3 groups. A fourth group of 4, age matched ponies reared under parasite free conditions was also used. Two weeks prior to initiation of experimental infections all ponies were treated with ivermectin at .2 mg/kg body weight, and daily for 5 days with oxibendazole at 20 mg/kg body weight. Necropsies were performed on 3 ponies on day 0. The remaining ponies in this group were not experimentally infected and were necropsied at the end of the experiment. All other ponies received 10⁴ small strongyle L₃/day for 6 weeks. One previously infected group received daily Strongid-C during this period. Strongid-C was > 98% efficacious against cyathostome larvae. Marked changes in the large intestinal mucosae were not noted. However, weights of large intestine biopsies suggest an increase in tissue mass associated with increased parasite burdens. Comparisons of parasite numbers in nontreated parasite-free ponies with untreated, previously infected ponies suggest a acquired resistance (82% reduction) against late L₃-L₄ larvae occurred in the previously infected ponies. Supported in part by Pfizer, Inc.

Thirty-two yearling horses with naturally acquired nematode infections were assigned to 8 replicates based on sex and on similarities of fecal egg counts and strongylid larval cultures. Within each replicate, horses were allocated randomly to one of four treatment groups: control (placebo), oral moxidectin gel at 0.3 or 0.4 mg/kg, or ivermectin paste at 0.2 mg/kg body weight. Study parameters included weekly fecal egg counts and strongylid larval cultures from 4 weeks pre-treatment to 2 weeks post-treatment (PT). Total worm counts were performed 14 days PT.

Ascarid and strongylid fecal egg counts were uniformly reduced by 100% at 14 days PT. All treatments had 100% efficacy against adult and larval Habronema muscae, Parascaris equorum, and Oxyuris equi, and against adult stages of Strongylus vulgaris, S. edentatus, and Triodontophorus spp. All removed ≥99.8% of adult and fourth stage larval cyathostomes from the gut lumen. Moxidectin (0.3 or 0.4 mg/kg) and ivermectin (0.2 mg/kg) removed 38.3%, 64.2%, and (30%) of arterial stages of S. vulgaris; 97.7%, 100%, and 98.4% of peritoneal stages of S. edentatus; and 94%, 96.2%, and 99.4% of Gasterophilus spp., respectively. Only moxidectin (0.4 mg/kg) demonstrated moderate efficacy (69.8%) against late third and fourth stage cyathostome larvae encysted in the cecal and ventral colonic mucosa. No regimen was effective against encysted early third stage cyathostome larvae.

EFFICACY OF MOXIDECTIN EQUINE GEL AGAINST INTERNAL PARASITE INFECTIONS IN EQUIDS WITH SPECIAL ATTENTION TO HYPOBIOTIC CYATHOSTOMES. L. XIAO*, R. P. HERD AND G. A. MAJEWSKI, COLLEGE OF VETERINARY MEDICINE, THE OHIO STATE UNIVERSITY, 1900 COFFEY ROAD, COLUMBUS, OH 43210.

Thirty two ponies with natural parasite infections were allocated to four groups of eight animals: control (placebo), oral moxidectin gel at 0.3 mg/kg body weight, oral moxidectin gel at 0.4 mg/kg body weight, and oral ivermectin paste at 0.2 mg/kg body weight. They were housed for at least four weeks before the start of the experiment. Fecal samples were taken at 0 and 2 weeks posttreatment. Animals were necropsied at 2 weeks posttreatment for worm burden determination.

At the doses tested, moxidectin and ivermectin were 100% effective against Habronema muscae, Strongylus spp. and Triodontophorus adults and Oxyuris fourth stage larvae, >97% effective against cyathostome adults and luminal fourth stage larvae, and ineffective (<36% efficacies) against hypobiotic early third stage larvae and encysted late third stage and fourth stage larvae of cyathostomes. While the efficacy of moxidectin against Oxyuris adults was lower at 0.3 mg/kg (93%), moxidectin at 0.4 mg/kg and ivermectin were 100% effective. The only significant difference between moxidectin and ivermectin was their efficacies against Gasterophilus third stage larvae; ivermectin was 98% effective whereas moxidectin was 44% and 57% effective at 0.3 mg/kg and 0.4 mg/kg, respectively. This result suggests that moxidectin may be less lethal to arthropods than ivermectin, and subsequently less ecotoxic.
LABORATORY EVALUATION OF DRONTAL™ PLUS (FEBANTEL/PRAZIQUANTEL/ PYRANTEL) TABLETS FOR DOGS. DWIGHT D. BOWMAN*1, AND ROBERT G. ARThER2. 1CHK R&D, STANWOOD, MI AND 2MILES INC., AGRICULTURE DIVISION, SHAWNEE MISSION, KS

A tablet formulation of febantel, praziquantel and pyrantel pamoate (Drontal™ Plus) was tested as a broad spectrum anthelmintic in laboratory trials using a total of 88 dogs with naturally acquired infections. In one trial, 48 dogs with *Ancylostoma caninum* and/or *Toxocara canis* infections were allocated to one of four treatment groups: Group 1 received treatment with placebo tablets (controls), Group 2 received pyrantel tablets at a dosage of 5 mg/kg, Group 3 received a 2-way tablet formulation to provide 5 mg/kg praziquantel + 5 mg/kg pyrantel and Group 4 received Drontal™ Plus tablets at a dosage of 5 mg/kg praziquantel + 5 mg/kg pyrantel + 25 mg/kg febantel. All dogs were euthanatized 7 days after treatment and examined for all remaining intestinal helminths. Drontal™ Plus tablet efficacy was 100% for *Ancylostoma caninum*, 99.7% for *Toxocara canis* and 94.0% for *Trichuris vulpis*.

In the second study, 40 dogs with naturally acquired *Taenia pisiformis* plus intestinal nematode infections were allocated into 4 treatment groups. Group 1 received treatment with placebo tablets (control), Group 2 received a 2-way tablet formulation to provide 5 mg/kg praziquantel + 5 mg/kg pyrantel, Group 3 received febantel tablets at 25 mg/kg and Group 4 received Drontal™ Plus tablets at a dosage of 5 mg/kg praziquantel + 5 mg/kg pyrantel + 25 mg/kg febantel. Drontal™ Plus tablet efficacy was 100% for *T. pisiformis*, 98.6% for *A. caninum*, 93.0% for *T. canis* and 93.5% for *T. vulpis*. In both trials, the combined nematocidal efficacy of pyrantel + febantel against *T. vulpis* in Drontal™ Plus was greater than the same single dosage of either pyrantel or febantel given alone.


HEARTGARD-30® (ivermectin, Merck) Chewables were used in a dose titration trial in dogs with induced infections of adult *Ancylostoma caninum* and *Uncinaria stenocephala*. Thirty-five hookworm-free dogs were randomly allocated to receive ivermectin chewables once at 0, 6, 12, 18 or 24 µg/kg. Dosages were tailored to body weight and worms were counted in dogs 7 or 8 days after treatment. Efficacy of ivermectin against *A. caninum* reached a plateau of 97.2% (relative to control) at 12 µg/kg, with an ED90 of 8.4 µg/kg. Against *U. stenocephala*, the dose response was linear in the range studied, with an ED90 of 20.8 µg/kg.
ANTHELMINTIC RESISTANCE TO ROUNDWORMS AND HOOKWORMS IN GREYHOUND KENNELS. R.K. RIDLEY* AND M.W. DRYDEN. KANSAS STATE UNIVERSITY, MANHATTAN, KS 66506.

Toxocara canis eggs isolated from feces obtained from a racing greyhound kennel and from an animal shelter, were allowed to develop in 0.1N H₂SO₄. 1000 viable eggs were administered via stomach tube to white mice. After approximately 28 days, the mice were humanely killed, skinned, and ground in a meat grinder. The carcasses were mixed in a palatable, moist dog food and fed to puppies. Eighteen pure-bred puppies of different breeds ranging in age from 9 to 18 weeks were used in these experiments. Puppies infected with Toxocara canis from a greyhound kennel (GK puppies) became patent 35 days to 55 days PI. Puppies infected with Toxocara canis from the animal shelter (AS-puppies) became patent 19 to 35 days PI. After the infections were patent, puppies from both groups were treated with pyrantel pamoate (PYR) once (2.27mg/lb), or 3 times with fenbendazole (FBZ;22.7mg/lb/da). Controls were not treated. GK-puppies treated with PYR had more worms at necropsy than the controls. FBZ was 100% effective when administered for 3 consecutive days. Both PYR and FBZ were 100% effective against AS-puppies.

In another study, two greyhound bitches were bred and whelped. Twenty puppies were allocated into 4 groups of 5 puppies. Group 1 served as controls, Group 2 was given PYR at 2.27mg/lb once, Group 3 was given PYR at 3.78mg/lb once and Group 4 was given FBZ (22.7mg/lb) daily for three days. The %FECR in Groups 2, 3 and 4 was 83.84%, 99.89% and 100% respectively. The %Efficacy in Groups 2 and 4 was 91.84 in both groups. Puppies in Group 3 were not available for necropsy.

EFFICACY OF DRONTAL™ PLUS (PRAZIQUANTEL/PYRANTEL/ FEBANTEL) TABLETS FOR REMOVAL OF ANCYLOSTOMA CANINUM, UNCINARIA STENOCEPHALA and TOXASCARIS LEONINA IN DOGS. LARRY R. CRUTHERS†, ROBYN L. SLONE† AND ROBERT G. ARTHER‡. †PROFESSIONAL LABORATORY AND RESEARCH SERVICES, CORAPEAKE, NC AND ‡MILES INC., AGRICULTURE DIVISION, SHAWNEE MISSION, KS.

Drontal™ Plus is a new tablet formulation containing 3 active ingredients (praziquantel/pyrantel pamoate /febantel) for use as a broad spectrum anthelmintic for dogs. Two well controlled laboratory studies were conducted to evaluate the efficacy of this product against intestinal nematode infections. In each study the dogs were randomly assigned to one of four equally sized treatment groups: Group 1 - Drontal™ Plus (5 mg/kg praziquantel/ 5 mg/kg pyrantel / 25 mg/kg febantel), Group 2 - praziquantel/pyrantel tablets (5 mg/kg of each active), Group 3 - pyrantel alone (5 mg/kg), Group 4 - placebo treated controls. The tablets were administered as a single oral treatment. All dogs were euthanatized 7 days after treatment and examined for remaining intestinal parasites.

In a study using 40 dogs with naturally acquired Ancylostoma caninum and Uncinaria stenocephala infections, Drontal™ Plus provided 99.6 and 99.8% efficacy against the two species, respectively. Praziquantel/pyrantel provided 80.0 and 74.6% efficacy, respectively, and pyrantel alone provided 96.0 and 85.3% efficacy, respectively. At necropsy the control dogs were infected with means of 124.0 and 123.4 A. caninum and U. stenocephala/dog, respectively.

In a separate study, using 48 dogs experimentally infected with Toxascaris leonina, Drontal™ Plus provided 97.8% efficacy while praziquantel/pyrantel and pyrantel alone provided 92.5 and 95.7% efficacy, respectively. At necropsy the control dogs were infected with a mean of 7.75 T. leonina/dog.
EVALUATION OF SINGLE ORAL DOSAGES OF MOXIDECTIN AGAINST HOOKWORMS, ASCARIDS, AND WHIPWORMS IN DOGS. P. SUPAKORNDEJ,* T.L. MCTIER, AND J.W. MCCALL. DEPARTMENT OF PARASITOLOGY, COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF GEORGIA, ATHENS, GA 30602.

In a critical trial, 30 mongrel dogs with natural infections of whipworms (Trichuris vulpis) and other gastrointestinal nematodes and with artificially induced hookworm (Ancylostoma caninum and Uncinaria stenocephala) and ascarid (Toxascaris leonina) infections superimposed on these natural infections were used to determine the minimum effective dose level of moxidectin needed against these nematodes. Infections of T. leonina (100 larvated eggs) and both hookworm species (100 larvae of each) were induced 82 and 47 days, respectively, prior to treatment. The dogs were ranked by T. vulpis egg counts and allocated to 6 groups of 5 dogs each. Each dog in a treatment group received moxidectin as a single oral dose of 0, 25, 50, 100, 150, or 300 mcg/kg of body weight. Fecal egg counts (EPG) were checked periodically before and after treatment. Beginning after treatment and continuing until necropsy 7 days later, total fecal samples from each dog were collected twice daily and checked for expelled worms; the gastrointestinal tract was also examined for worms.

Moxidectin was 100% effective against the adults of A. caninum when given at a single oral dose of 25 mcg/kg; it cleared these hookworms from 21 of the 25 dogs given higher dose levels. The drug appeared to be less effective against U. stenocephala, but all of the dogs were cleared of this species of worms at dose levels of 150 and 300 mcg/kg. Most of the hookworms were eliminated within 24 hours after treatment. Whipworms were less sensitive to the drug than were hookworms, as all whipworms were eliminated from only 2 of the 5 dogs given 300 mcg/kg (61.5% efficacy). An insufficient number of dogs were infected with T. leonina or Toxascara canis to interpret the data with confidence, but the drug appeared to have activity against both species. No adverse reactions to moxidectin were seen.

EVALUATION OF TOPICAL FORMULATIONS OF MOXIDECTIN AND TEMEPHOS ALONE OR IN COMBINATION FOR ACTIVITY AGAINST HEARTWORMS, HOOKWORMS, ASCARIDS, TICKS, AND FLEAS IN DOGS. T. MCTIER,* P. SUPAKORNDEJ, AND J.W. MCCALL. DEPARTMENT OF PARASITOLOGY, COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF GEORGIA, ATHENS, GA 30602.

In a critical/control trial, 33 beagles free of parasites were experimentally inoculated with Dirofilaria immitis, Ancylostoma caninum, and Toxascaris leonina and infested with Rhipicephalus sanguineus and Ctenocephalides felis. The dogs were then allocated to 11 groups and given vehicle (control) or single spot-on formulations of either moxidectin (MOX) or temephos (TEM) or a combination of MOX and TEM. MOX was 100% effective against 1-month-old heartworms when given alone at 0.5 mg/kg or in combination with TEM (5 or 10 mg/kg). TEM (10 mg/kg) plus MOX, at a dosage as low as 0.01 mg/kg was completely effective against D. immitis. Control dogs had an average of 10.6 heartworms (range, 10-12). MOX (0.5 mg/kg) and MOX plus TEM (5 or 10 mg/kg) showed 99 to 100% efficacy against adult hookworms. Lower levels of MOX plus TEM (10 mg/kg) gave substantial but not complete efficacy against hookworms. Control dogs had an average of 57.0 hookworms per dog (range, 35-72). Activity of MOX, TEM, or MOX plus TEM against ascarids could not be adequately evaluated due to low worm recovery. Wide variation in adult tick counts precluded a conclusive determination of efficacy against R. sanguineus. A single application of TEM at 20 mg/kg was 100% effective against adult C. felis by 7 days after each of 4 successive weekly infestations. After the 5th infestation, efficacy dropped to about 90%. At 10 mg/kg, TEM (alone or plus MOX) was 97 to 100% effective against fleas after the first 2 weekly infestations; thereafter, efficacy gradually declined. At 5 mg/kg, TEM plus MOX (0.5 mg/kg) gave substantial reductions after infestations 1 and 2, but complete efficacy was never obtained at this level. MOX (0.5 mg/kg) alone had little, if any, efficacy against fleas.
RECENT CHANGES IN THE AMERICAN HEARTWORM SOCIETY'S RECOMMENDED PROCEDURES FOR THE DIAGNOSIS AND MANAGEMENT OF HEARTWORM INFECTION.
C.H. COURTNEY, UNIVERSITY OF FLORIDA, GAINESVILLE, FL 32611

For years the American Heartworm Society has recommended that a concentration test for microfilariae (modified Knotts or membrane filtration) be used as the primary screening test for canine heartworm infection. Two new developments have led to the reevaluation of these heartworm screening guidelines. First was the discovery of the microfilaricidal action of the macrolides (milbemycin and ivermectin) when used for heartworm prophylaxis. Second was the continued improvement in sensitivity and specificity of the commercial heartworm antigen tests. Because of these new developments, the blanket recommendation that all dogs be screened annually with a concentration test for microfilariae is no longer appropriate. One now must take into consideration a dog's past history of prophylactic medication and/or its intended prophylactic medication before selecting a screening test. Dogs seen by a veterinarian for the first time that are old enough to harbor patent heartworm infection should be tested with both a concentration test for microfilariae and an antigen test before prophylaxis is started. These dogs should be retested within 6-12 months of initiation of prophylaxis. Dogs that were placed on macrolide prophylaxis should be retested for antigens only whereas those on diethylcarbamazine prophylaxis should be retested for both antigens and microfilariae. Annual retesting thereafter is required for dogs on diethylcarbamazine prophylaxis. Regular, but not necessarily annual, retesting, at the discretion of the veterinarian, is recommended for dogs on macrolide prophylaxis.

COMMERCIAL HEARTWORM ANTIGEN TEST KITS DETECT INFECTIONS WITH A SINGLE ADULT FEMALE WORM BUT NOT THOSE WITH NUMEROUS ADULT MALE WORMS ONLY. J.W. MCCALL,1* N. SUPAKORDNEJ,2 T.L. MCTIER,1 M.T. DZIMIANSKI,1 AND R.P. RICKETTS.2
1DEPARTMENT OF PARASITOLOGY, COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF GEORGIA, ATHENS, GA 30602; 2TRS LABS, INC., ATHENS, GA.

The accuracy of ELISA-based adult heartworm antigen detection tests in detecting single-sex infections of low to moderate intensity was studied by IV transplantation of adult (about 12 months old) Dirofilaria immitis into heartworm-naive beagles. The sensitivities of several commercially available test kits (ASSURE/CHN, DiroCHEK®, UNI-TEC™CHW, Synbiotics, Inc.; CITE®Semi-Quant™, IDEXX Corp.) were determined using 3 groups of 4 dogs each with 1, 2, or 3 female worms only, respectively, 1 group of 4 dogs with 5 male worms only, 1 group of 2 dogs with 10 male worms only, and 1 group of 3 dogs with 13 male worms only. At 10 weeks posttransplantation (PTR), all of the test kits detected antigens in all of the dogs with 1 female worm only. In addition, antigenemia was checked with the DiroCHEK® test at 1, 2, 4, and 10 weeks PTR in the dogs given 1, 2, or 3 female worms only. The rate at which detectable antigen appeared after transplantation depended on the number of females present, with infections consisting of 3 females being detected the earliest. With this kit, none of the dogs with 1 female worm was positive for antigen 1 week PTR, whereas all of the 4 dogs given 3 female worms and 1 of the 4 given 2 female worms were positive at this time. At 2 weeks PTR, 3 of the 4 dogs with 2 female worms and 1 of the dogs with 1 female worm were positive. By 4 weeks PTR, all of the remaining dogs given 1 or 2 female worms were positive. None of the test kits detected any of the infections with male worms only, even infections with 13 male worms. These data indicate that male worms alone do not contribute to the antigen(s) detected by these commercial kits.
EVALUATION OF ELISA-BASED ADULT HEARTWORM ANTIGEN TEST KITS USING WELL-DEFINED SERA FROM EXPERIMENTALLY AND NATURALLY INFECTED CATS. T.L. MCTIER,1* N. SUPAKRNDEJ,2 J.W. MCCALL,1 AND M.T. DZIMIANSKI.1 1DEPARTMENT OF PARASITOLOGY, COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF GEORGIA, ATHENS, GA 30602; 2TRS LABS, INC., ATHENS, GA.

Several commercially available adult heartworm antigen detection test kits were evaluated for sensitivity and specificity using well-defined sera from 31 cats with at least one heartworm (Dirofilaria immitis) and 30 heartworm-naive cats. Of the 31 heartworm-positive cats, 6 had naturally acquired infections, 6 had infections experimentally induced by SC inoculation of L3, and 19 had infections established by IV transplantation of live adult worms. For determining specificity, the 30 heartworm-naive cats were selected to include some with various gastrointestinal parasites and some that were free of specific parasites. Of the total of 25 experimentally infected cats, all of the heartworms were at least 7 months old, with the exception of 2 cats with 6-month-old worms. For determining sensitivity (no. detected/total), the data were tabulated according to the number of female worms, regardless of the number of male worms in each cat; those cats (3) with only male worms were negative with all kits, thus they were not included in calculating sensitivity. Overall sensitivity (%) of each of the kits for infections with 1 to 7 female worms was as follows: ASSURE/CHM, 86; ASSURE/CHM (Modified), 93; DiroCHEK®, 93; UNI-TEC®CHW, 82; UNI-TEC®CHW (Modified), 82; CITE®Semi-Quant™, 68; PetChek®, 57; Snap™, 36. In general, the sensitivity of each of these tests increased as the number of female worms increased from 1 to 3 as follows: ASSURE/CH™, 75 to 86; ASSURE/CH™ (Modified), 100 to 100; DiroCHEK®, 100 to 100; UNI-TEC®CHW, 50-100; UNI-TEC®CHW (Modified), 50 to 100; CITE®Semi-Quant™, 25 to 71; PetChek®, 25 to 57; Snap™, 0 to 43. All of the kits were 100% specific.


The suitability of the canine heartworm antigen detection kit (VetRed®) was evaluated in a setting typical of those expected at veterinary hospitals. To determine the suitability of this canine heartworm antigen detection kit, a total of 5 clinics in 4 states used the test according to kit directions.

Investigators conducted comparative sensitivity tests using established microfilarial detection methods such as Difil® or modified Knott's tests along with the VetRed® test. Parallel testing with other antigen diagnostic kits was performed on 60 samples. The adequacy of the directions for use and interpretation of the results were rated on a scoring system of A+ for very adequate, A for adequate, N for neutral, and I for inadequate. An effort was made to have the test conducted by several individuals in order to obtain maximal comments regarding adequacy. The results of 307 tests performed by 26 individuals were obtained. The directions for use of the VetRed® kits were determined to be very adequate or adequate in 99% of the tests. The interpretation of results were determined to be very adequate or adequate in 98.7% of the tests conducted. VetRed® detected 15 more positive samples than did Difil®, or modified Knott’s. When tested in parallel with Dirocheck® or Assure®, VetRed® detected only 1 more positive and demonstrated a 98.3% agreement with these commercially available antigen tests.
WORLD-WIDE EVALUATIONS OF A RAPID IMMUNODIAGNOSTIC TEST FOR THE DETECTION OF DIROFILARIA IMMITIS ANTIGEN IN DOGS. T. TSEGAI, D.E. COCHRAN*, S. MARTIN, RHONE MERIEUX, INC., ATHENS, GEORGIA, USA; AGEN BIOMEDICAL LTD., ACACIA RIDGE, QUEENSLAND, AUSTRALIA.

Since the discovery of very effective microfilaricidal molecules and the development of sensitive heartworm antigen-based diagnostic tests, authorities recommend that veterinarians screen all dogs on macrolide or diethylcarbamazine prophylaxis for the presence of heartworm antigen.

Currently available antigen-based tests use the ELISA technique on serum samples and require at least 20 minutes for results. A rapid and simple agglutination test using whole blood and giving results in two minutes has been evaluated in Italy, Australia, New Guinea and in seven different locations in the United States.

A performance level of 100% specificity and 100% sensitivity for the detection of three or more female worms was obtained by three different investigators located in New Guinea and in seven different locations in the United States. Of the worldwide specificity data base represented by 712 dogs determined to be negative by necropsy or by at least one other in vitro diagnostic method, 688 dogs (96.6%) were confirmed negative by the new test. For sensitivity evaluation, 377 dogs with a known worm population determined by necropsy or by experimental implantation were studied. The new test detected 203 of the 212 (95.8%) dogs infested with three or more adult female worms.

Detailed results are presented and their interpretation is discussed.


Studies were done using 4 monoclonal antibodies (Mab) to F. hepatica produced by immunizing BALB-C mice with excretory-secretory products (ES) boosted by a 26 KDa coproantigen band separated by SDS-PAGE and blotted with nitrocellulose (El Bahi et al., Vet Parasit 45:157-167, 1992). By western blot, each reacted positively with a 26-29 Kd band from ES. Using one Mab (Fayum), western blot assay conditions were optimized and enzyme substrates compared. Chemiluminescence substrate (ECL) was more sensitive than colorimetric substrates using Fayum cell culture supernate. However, using Fayum ascites fluid, TMB could be used to detect a 26-29 Kd coproantigen band in fecal supernates from 3 calves experimentally infected with 28, 30 or 39 adult flukes. Additionally, Fayum detected, in descending order of strength, 26-29 Kd bands in fresh fluke antigen supernates of F. hepatica, Fascioloides magna and Paramphistomum microbothrioides using ECL.

Studies are in progress to determine the time of appearance post-infection of coproantigen and minimum detectable F. hepatica numbers in a time series of fecal samples from a panel of calves experimentally infected with F. hepatica. Supported by the Peace Fellowship Program, Egyptian Cultural and Educational Bureau.
AN ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF
FASCIOLA HEPATICA ANTIGENS IN CATTLE FECES USING A MONOCLONAL
ANTIBODY. M.S. SOLANO* AND R.K. RIDLEY. KANSAS STATE
UNIVERSITY. MANHATTAN, KS 66506

A capture Enzyme-Linked Immunosorbent Assay was developed to
detect Fasciola hepatica antigens in cattle feces. An IgM
murine monoclonal antibody raised against F. hepatica
excretory-secretory products was adsorbed to 96-well
polystyrene plates. This antibody captured F. hepatica
antigens which were then detected by using serum from an
immunized rabbit and anti-rabbit IgG conjugated to alkaline
phosphatase. F. hepatica antigens were first detected in
cattle feces four weeks after experimental infection. Antigen
levels remained detectable during the course of the 17 week
study. Immunoblot results showed that F. hepatica antigens
from cotton rat and bovine feces are very resistant to long
term storage (> 1 year) at -20°C and boiling.

Preliminary investigations indicated that this assay did not
cross-react with samples infected with strongyles, Eimeria
spp., Trichuris sp., or Nematodirus sp. Crude antigens from
Fascioloides magna, Anoplocephala magna, Stichorchis
subtriquetra, Haemonchus contortus, and Moniezia expansa did
not cross react.

SERODIAGNOSIS OF FASCIOLOIDES MAGNA IN CATTLE. J.R. LAURSEN*, AND B.E.
STROMBERG. UNIVERSITY OF MINNESOTA. ST. PAUL, MN 55108.

A sandwich-style ELISA was developed to diagnose cattle infections with
Fascioloides magna, based on the detection of serum antibodies against adult fluke
homogenate (AH) or excretory/secretory (ES) antigens. Experimentally infected
calves sero-converted between weeks 3 and 6 post infection (PI). The assay was
93% sensitive for natural infections with F. magna, and over 95% specific in
uninfected field controls from Minnesota, with both antigens. The test cross-
reacted with 31% and 83% of cattle infected with Fasciola hepatica, and 20% and
50% of cattle with F. gigantica infections, using AH and ES antigens respectively.

Western blot profiles correlated well with the ELISA specificity using AH antigen, but
could not explain the specificity of the test with ES antigen. A 24 KDa band in the
AH antigen, preferentially recognized by rabbit capture antibodies, was also
recognized by sera from infected cattle with positive ELISA values. This band was
not recognized by sera from cattle with negative ELISA values. A 28 KDa band in
the ES antigen, recognized by rabbit capture antibodies and cattle sera with
positive ELISA values, was also recognized by sera from uninfected cattle with
negative ELISA values.
IMMUNITY TO HELIGMOSOMOIDES POLYGYRUS INDUCED BY SUBCUTANEOUS VACCINATION WITH AND AGAINST ADULT WORMS. J.P. TRITSCHLER* AND L.H. SEMPREVIVO. UNIVERSITY OF MASSACHUSETTS, AMHERST, MA 01003.

Experiments were conducted to determine if subcutaneous (s.c.) vaccination with Heligmosomoides polygyrus fourth stage larvae (L4) affected homologous adult worm populations in the gut lumen and if adult worms placed s.c. induced immunity to an oral third stage larvae (L3) challenge. To address the first question, C57BL/6 mice were infected with 100 L3 and 30 days later vaccinated s.c. with 100 L4. Sixty days post-infection (30 days post-vaccination), infected-vaccinated mice were necropsied and the mean intestinal worm burden for the group determined. Control values were obtained from similar age-matched mice which were infected and sham vaccinated. Control mice had a mean adult intestinal worm burden of 83 and experimentals 50, for a reduction of 40% (P<.01). To address the second question, C57BL/6 mature female mice were vaccinated s.c. with 100 adult worms, 100 L4, 50 adult worms and 50 L4 or sham vaccinated. Compared to controls, C57BL/6 mice vaccinated with adults, adults and L4, and L4 alone had reductions in challenge worm burdens of 99%, 100%, and 100%, respectively (P<.001). Unlike all other mice used, mice vaccinated s.c. with adult worms developed scruffy coats. Results presented here indicate that immunity induced by s.c. vaccination with L4's or adults is active against both the larval tissue stages and mature worms in the gut lumen. This research was supported in part by the Massachusetts Agricultural Experiment Hatch Project 665.

Biology of Fleas on Dogs and Cats. M.W. Dryden Department of Pathology and Microbiology. Kansas State University, Manhattan, Kansas 66506.

A review of the veterinary and entomology literature reveals many inconsistencies concerning the biology of fleas on dogs and cats. Such inconsistencies result mainly from inappropriate extrapolations of the known biology and behavior of other flea species and. The most common flea species on dogs and cats in North America is the cat flea, Ctenocephalides felis felis. While eggs and larvae are extremely susceptible to temperature and humidity fluctuations, pupae and pre-emerged adult cat fleas residing in the cocoon are the most resistant and potentially the longest lived of any life cycle stage. Cat fleas newly emerged from cocoons typically survive only 1 to 2 weeks before they must find a host, while pre-emerged adults may live for several months. Female cat fleas have a large reproductive capacity, being capable of producing between 40 and 50 eggs per day during peak reproduction. In order to produce such an egg mass female cat fleas consume up to 15x their body weight in blood daily. Cat Fleas are permanent ectoparasites as adults with females laying their eggs in the pelage of their host. Eggs fall off the animal to be deposited in the environment.
LABORATORY EFFICACY EVALUATION OF LUFENURON AGAINST CTENOCEPHALIDES FELIS FELIS IN DOGS AND CATS. B. L. BLAGBURN.
COLLEGE OF VETERINARY MEDICINE, AUBURN UNIVERSITY, AL 36849

Laboratory studies confirm efficacy of lufenuron against *C. f. felis* in both dogs and cats. Beagle dogs were treated orally at the rate of 10 mg/kg BWT on days 7, 37, 68, and 98 of the study. All dogs were infested with 100 newly emerged, unfed, insectary-reared adult *C. f. felis* on days 0, 2, and 6. The numbers of adult *C. f. felis* on each of the treated and control beagles were enumerated by combing each dog free of adult fleas at weekly intervals until termination of the study on day 119. Reductions in mean flea burdens on treated dogs compared to control dogs exceeded 90% by day 35 of the study and remained within the range of 90-99% for the remainder of the study. Purpose-bred cats were treated orally with a 7% suspension formulation of lufenuron at dosages of either 15 mg/kg, 30 mg/kg or 45 mg/kg of body weight. Cats in the fourth group were treated orally with a placebo suspension without lufenuron. Cats were infested with 75 newly-emerged, unfed *C. f. felis* on days -7 and -3 before treatment and at approximate weekly intervals after treatment. Flea ova were collected from beneath each cat on selected days before and after treatment and placed in an artificial rearing medium. Flea ova and media were held for 35 days in an insectary to determine effects of lufenuron or placebo suspension on emergence of adults of the *F₁* generation. Lufenuron was 100% effective in inhibiting development of *C. f. felis* at all dosages for 11 days after treatment. Thereafter, efficacies exceeded 92% in all dosage groups. On day 32, when the study was terminated, efficacies expressed as mean percentages control for each of the dosage groups were as follows: 15 mg/kg - 95.2%, 30 mg/kg - 98.2%, 45 mg/kg - 99.6%.


Multi-centered, controlled, clinical field trials of an insect development inhibitor, administered orally to pets, were conducted during the spring, summer, and fall of 1990. Efficacy was assessed when used for either preventing the build up of a flea infestation (beginning administration prior to "flea season"), or as an adjunct to control measures when treatment is begun in the midst of a flea infestation. Households consisting only of cats, only of dogs, and combinations of cats and dogs participated. Lufenuron was administered monthly to dogs (10 mg/kg) and cats (30 mg/kg). Monthly counts of the number of fleas on the study animals was used to measure efficacy.

Initiating dosing prior to the onset of a flea infestation, resulted, after four months, in those animals receiving the drug, averaging approximately 3 fleas per dog, compared to 50 per dog in the control group. Cats averaged 5 fleas per cat when dosed with lufenuron, compared to 87 per cat in the control group. Administration to animals already experiencing flea infestations, resulted in a decrease from 67 to 10 fleas per dog and from 21 to 8 fleas per cat, after 60 days, and the numbers continued to decline for the next four months. No adverse reactions were noted in either cats or dogs, and the drug was administered concomitantly with a wide range of therapeutic agents.

Target animal safety studies have been conducted in dogs and cats with Ciba’s new insect development inhibitor, lufenuron. Lufenuron belongs to the benzoylphenyl urea class of chemicals and acts by inhibiting the normal synthesis of chitin. These studies were conducted at many multiples of the single monthly ad usum dose given frequently throughout most studies. Acute (single dose), sub-chronic (multiple doses over a short time period), and chronic (multiple doses over a long time period) studies were conducted in dogs while acute, sub-chronic and reproduction studies were conducted in cats. Sub-chronic studies were also conducted in dogs and cats where commonly used flea adulticides were applied on the lufenuron-treated animals.

Results in all studies indicate that lufenuron is well tolerated by both dogs and cats. In the chronic dog study, up to 5x (50 mg/kg) was given 3 times monthly to young animals until they were one-year of age; no adverse-effects were observed. No compound related effects were noted in the cat studies. The studies in dogs and cats where lufenuron was used simultaneously with other flea adulticides did not indicate any enhanced signs of toxicity.

CLONING AND EXPRESSION OF LARVAL EXCRETORY-SECRETORY PROTEINS OF DIROFILARIA IMMITIS. G.R. FRANK*, C.A. TRIPP AND R.B. GRIEVE. DEPARTMENT OF PATHOLOGY, COLORADO STATE UNIVERSITY AND PARAVAX, INC., FORT COLLINS, CO.

Two proteins were described in the excretory-secretory products (ES) collected from D. immitis during the molt from the third stage to the fourth stage in vitro. During the purification of these two proteins a third protein was identified. Their molecular weights were between 20 and 23 kDa as determined by Tris-glycine SDS-PAGE and the three were designated as 20, 22L and 22U kDa proteins. The 20 and 22L kDa proteins were quite similar based on the difficulty in separation during purification and peptide sequence similarity. The 22U kDa protein was also identified in adults using tryptic mapping and amino acid sequencing. The 20 and 22L kDa proteins were recognized by sera from dogs immune to infection by D. immitis but not sera from infected non-immune dogs as determined by immunoblot analysis. Oligomeric DNA probes were synthesized based on the amino acid sequences obtained and the proteins were cloned from larval cDNA libraries. Expressed recombinant proteins are being evaluated for their potential roles in the protective immune response to D. immitis.
Tubulin is thought to be the parasite protein that is selectively affected by the benzimidazole anthelmintics. Genetic evidence points to β-tubulin as the critical component for benzimidazole pharmacology. This class of anthelmintics typically shows unimpressive activity as macrofilaricides. Whether this is due to inappropriate pharmacokinetics or to the presence of an unusual type of β-tubulin in the filariae has not yet been conclusively demonstrated.

We have previously shown that nematode β-tubulins do not fall into easily recognized classes, as is the case for most metazoan β-tubulins. We have isolated cDNAs encoding β-tubulin from *Dirofilaria immitis* and *Onchocerca volvulus*. Both sequences are 98% similar to the major β-tubulin isotype reported from *Brugia malayi*. The hypervariable COOH terminal region is identical in all 3 species. No evidence for 1 isotype (as is found in *Haemonchus contortus* and *Caenorhabditis elegans*) was obtained in *D. immitis*. Determining if the filarial β-tubulin isotype is pharmacologically distinct from other nematode β-tubulins is now warranted.

One α-tubulin and 3 β-tubulin (β8-9, β12-16 and β12-164) cDNAs were expressed in *E. coli* and characterised by their specific BZ-binding and polymerization (microtubule formation) properties. The role of each of these tubulins in susceptibility/resistance of *H. contortus* to benzimidazole anthelmintics will be discussed.
GENETIC CHANGES AT THE β-TUBULIN GENES IN HAEMONCHUS CONTORCUS ASSOCIATED WITH RESISTANCE TO BENZIMIDAZOLES. R.N. BEECH* AND M.E. SCOTT. INSTITUTE OF PARASITOLOGY, MACDONALD CAMPUS, Mcgill UNIVERSITY, 21,111 Lakeshore Road, ste Anne de bellevue, CANADA, h9x 3V9.

Resistance to benzimidazoles (BZ) in Haemonchus contortus has been linked to changes in β-tubulin. Using the polymerase chain reaction it is possible to amplify sufficient DNA from single adult H. contortus to identify allelic variation at each of the two loci encoding β-tubulin. In an effort to characterize alleles conferring resistance, the genotypes (with respect to the two β-tubulin genes) of individual adult Haemonchus contortus from one susceptible and two BZ resistant strains have been determined. In the susceptible strain both loci were highly polymorphic. In a cambendazole resistant strain, derived from the susceptible strain, one locus was found to be almost entirely homozygous for one of the alleles present in the susceptible strain. The second locus, although showing a reduced variability still retained many alleles. A similar pattern was observed in an independently derived thiabendazole resistant strain. These results suggest that the first β-tubulin locus is closely linked to the genetic factor responsible for BZ resistance. Interestingly, the frequency of the allele associated with BZ resistance approached 50% in the susceptible strain.


The major exsheathment protein (MEP) of H. contortus is a 160,000 kDa protein located in the refractile ring region (20th annulus) of the second molt cuticle. It differs in amino acid composition from known cuticular collagens. Under abomasal conditions, MEP is specifically degraded by a 44 kDa zinc metalloprotease, resulting in opening of the sheath and release of the infective larva. A 200 bp clone encoding a portion of the protein was isolated from a H. contortus genomic expression library using a MEP-binding monoclonal antibody. Sequencing revealed a complete open reading frame encoding a 59 amino acid polypeptide. An oligonucleotide prepared from this sequence was used to identify homologous sequences in a Lambda Zap cDNA library made with mRNA of free-living larval stages. An 1800 bp clone was excised from the phage and expressed as a plasmid for sequencing. Developmental regulation of this gene was shown by northern hybridization of probes to isolated mRNA from the second and third larval stages, but not to mRNA from the fourth stage or adults, and western blot analysis which demonstrated that the encoded protein was expressed in the same larval stages.
A REPEATITIVE DNA PROBE FOR THE SENSITIVE DETECTION OF *Fasciola hepatica* INFECTED SNAILS. R. M. Kaplan*, J. B. Dame, G. R. Reddy and C. H. Courtney. Department of Infectious Diseases, College of Veterinary Medicine, University of Florida, Gainesville, Florida 32611

The epizootiology of *Fasciola hepatica* is directly related to the bionomics of its snail intermediate hosts. To understand the transmission dynamics of *F. hepatica* for a given region, both the seasonal population characteristics and infection prevalence of the snail intermediate hosts must be studied. Previous epizootiologic studies on *F. hepatica* have used microscopic techniques for the detection of infected snails, however, these techniques suffer from problems of efficiency, sensitivity and specificity. A DNA based test for the identification of *F. hepatica* infected snails would solve these problems and enable a level of detection accuracy previously unavailable. Eukaryotic organisms possess highly repetitive DNA sequences which are present in multiple copies and constitute a large percentage of the genome. The relative abundance of this repetitive DNA plus the fact that these sequences rapidly evolve make them both very sensitive and specific targets for DNA based identification. A genomic DNA library was prepared by ligating Sau 3A digested *F. hepatica* DNA into plasmid Bluescript SK+ and transforming into *E. coli* strain, XL-1 Blue. The library was screened using 32p radiolabelled *F. hepatica* genomic DNA to detect clones containing highly repetitive sequences. Ten clones were selected and analysed for specificity to *F. hepatica* using DNA of *Fossaria cubensis*, the snail intermediate host, and using DNA of *Fascioloides magna* and *Paramphistomum liorchis*, trematodes which share the same intermediate host and same enzootic range as *F. hepatica*. Five clones were specific to *F. hepatica*. Preliminary results indicate that as little as 100pg of purified *F. hepatica* DNA can be detected with each of the probes.

A SUMMARY OF PROGRESS TOWARD THE DEVELOPMENT OF A RECOMBINANT VACCINE AGAINST HYPODERMA LINEATUM AN INSECT PARASITE OF CATTLE. J.H. PRUETT* USDA-ARS, KNIPLING-BUSHLAND U.S. LIVESTOCK INSECTS RESEARCH LABORATORY, KERRVILLE, TX 78028.

Cattle acquire resistance to *Hypoderma* infestation as a result of repeated exposure to this parasite. Resistance is primarily manifested as an increase in host-induced larval mortality as the larvae inhabit the back tissues of cattle. Maintenance of the cattle grub population is believed to be dependent upon the naive animal. Therefore, conversion of naive susceptible cattle to the resistant state by vaccination is thought to be a potential method for control of the cattle grub population. Conversion of cattle from a susceptible to a resistant state has been demonstrated with crude and purified proteins of the 1st-instar larvae of *H. lineatum* using Complete Freund's adjuvant (CFA).

Experimentation over the last 4 years has led to the development of a recombinant vaccine formulated with a veterinary acceptable adjuvant that elicits parasite specific immune responses and a level of host-induced larval mortality comparable to the original candidate vaccine formulated with natural larval protein and CFA.
EVALUATION OF THE ANTICOCCIDIAL ACTIVITY OF LAIDLOMYCIN PROPIONATE IN EXPERIMENTALLY INFECTED DAIRY CALVES. BYRON L. BLAGBURN*, DAVID S. LINDSAY, HOWARD R. SPIRES, AND DAVID I. BRANSBY. AUBURN UNIVERSITY, AL 36849, AND SYNTEX RESEARCH, PALO ALTO, CA 94304

The present study was conducted to evaluate the activity of laidlomycin propionate (LP) against experimentally induced coccidiosis (Eimeria bovis) in dairy calves. Twenty-four, holstein bull calves were acquired at birth and raised under conditions that limited exposure to coccidia. Calves were randomly allocated to 3 groups of 8 calves when they were 8 to 12 weeks-old. Group 1 calves were not inoculated with oocysts or treated with LP. Group 2 calves were inoculated with $2 \times 10^5$ sporulated oocysts of E. bovis, but were not treated with LP. Group 3 calves were inoculated with $2 \times 10^5$ sporulated oocysts of E. bovis and fed a ration containing LP at a rate of 12.2 mg/kg of expected dry matter feed intake for 3 days prior to inoculation and continuously until the end of the study. This dosage was previously shown to promote increased weight gains in growing calves. The following parameters were used to determine efficacy of LP against E. bovis: fecal scores, weight gains, and oocyst counts. Calves in each of the inoculated groups developed clinical signs of coccidiosis. Noninfected, nontreated calves remained clinically normal. Mean fecal scores were significantly higher for group 2 (infected/nontreated) than for groups 1 or 3. LP treated calves (group 3) gained more weight than infected nontreated calves (group 2), but less than the noninoculated control calves (group 1). Mean oocyst counts were not significantly different between the inoculated groups. We believe that the selected use rate of LP reduced the severity of disease in treated calves. An increased use rate of LP would likely result in significantly increased weight gains and decreased oocyst production in treated calves.

PAROMOMYCIN EFFECTIVELY PREVENTS CRYPTOSPORIDIOSIS IN EXPERIMENTALLY INFECTED CALVES. R. FAYER* AND W. ELLIS. USDA, ZOONOTIC DISEASES LABORATORY, BELTSVILLE, MD 20705 AND DIVISION OF EXPERIMENTAL THERAPEUTICS, WALTER REED ARMY MEDICAL CENTER, WASHINGTON, D.C. 20307.

In dairy and beef calves under one month of age in the United States cryptosporidiosis is a major cause of diarrhea. Despite numerous attempts to identify effective chemoprophylactic or chemotherapeutic modalities, none has been reported to be both effective and nontoxic. In the present study 16 newborn Holstein calves were experimentally infected with $1.5-2.0 \times 10^6$ oocysts of Cryptosporidium parvum (Cp) per os. Four calves each received 100, 50, 25, or 0 mg paromomycin/kg body weight/day for 11 days beginning 1 day before infection with Cp, groups A, B, C, and D, respectively. Duration and severity of diarrhea, numbers of oocysts and duration of shedding were significantly less in group A than in D. Severity of diarrhea was significantly less in groups B and C than in D. Group A shed no oocysts. Except for 1 calf, groups B and C shed no oocysts during the first week of prophylaxis. Because neither drug toxicity nor inappetence was observed, paromomycin appears to be ideally suited for field testing under commercial rearing conditions where cryptosporidiosis is endemic.

Surveys conducted on two cattle farms and one sheep farm with neonatal diarrhea in Ohio using immunofluorescence assay showed that 29.4 to 81.3% of calves and 81.3% of lambs were infected with Cryptosporidium, and 82.4 to 100% of calves and 6.3% of lambs were infected with Giardia. Ewes in late pregnancy and lactation also had Cryptosporidium (17.4%) and Giardia (30.4%) infection, suggesting that periparturient suppression of immunity to these protozoa may exist. Concurrent infections of both parasites were found in some calves, lambs and one ewe. Positive calves ranged from 11 to 177 days in age for Cryptosporidium and 11 to 164 days for Giardia. A survey of 19 healthy sows and 80 suckling piglets (7-25 days old) on an Ohio farm failed to reveal Cryptosporidium or Giardia infections. However, 8 of 30 weanlings (6 to 8 weeks old) had Cryptosporidium infection, and one had Giardia infection.

Samples from 222 clinically normal horses of different ages from several Ohio and Kentucky farms were examined. Cryptosporidium oocysts were found in 15.0 to 31.0% of foals. No yearlings or mares were positive, and only one of 39 weanlings examined was infected. Giardia cysts were found in all age groups, with highest infection rates in foals (17.2 to 35.0%). Some foals had concurrent infections of both parasites. Chronological study of infection in 35 foals on an Ohio farm from birth showed that foals started to excrete Cryptosporidium oocysts from 4 to 19 weeks and Giardia cysts from 2 to 22 weeks of age. Cumulative infection rates of the two parasites in foals were each 71.4%. Excretion of oocysts or cysts was intermittent and long-lasting.

RECENT PROGRESS ON BOVINE NEOSPOROSIS. P.A. CONRAD1, B.C. BARR1, M.L. ANDERSON1, K. SVERLOW1, J. ROWE1, A. MARSH1, R. BONDURANT1, G. TUTER1, R. BREITMEYER2, C. PALMER2, J.P. DUBEY3, J. REYNOLDS1 AND A.A. ARDANS1.

1SCHOOL OF VETERINARY MEDICINE, UNIVERSITY OF CALIFORNIA, DAVIS, CA 95616, 2CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE, 3ZOONOTIC DISEASES LABORATORY, ARS, USDA

Neospora has been isolated from the brains of aborted bovine fetuses, and recently from congenitally infected calves that were submitted alive with severe limb paresis and protozoal encephalomyelitis. Continuous in vitro growth of these isolates was established in bovine monolayer cell cultures. Antigenic and morphologic comparisons, including ultrastructural studies indicate that these protozoal isolates are representative of the parasites seen in aborted fetuses and infected calves and they are Neospora parasites. Culture-derived tachyzoites of the bovine Neospora were used to inoculate pregnant heifers and confirm that this parasite caused fetal death and pathologic lesions like those seen in naturally infected fetuses. In addition, the parasite isolates were employed to develop an indirect fluorescent antibody test. Cattle with natural or experimental infections of Neospora were shown to produce antibodies to tachyzoite antigens. The significance of our recent serological results and the potential application of this test for the diagnosis of Neospora infections in cattle will be discussed.
NEONATAL PORCINE COCCIDIOSIS FACILITATES BACTERIAL INVASION.
J. A. JARVINEN*, IOWA STATE UNIVERSITY. AMES, IA 50011.

Pigs given 50,000 Isospora suis oocysts po at 1.5 days of age (DA) were sacrificed at 4, 6, 8, and 12 DA and compared to age-matched noninoculated controls (C) to examine the role of I. suis in secondary bacterial invasion. Fecal oocyst shedding (6-12 DA), fibrinonecrotic membranes (6 DA), fecal occult blood (4-12 DA), and decreased weight gain (6-12 DA) occurred only in inoculated pigs (IN). Compared to C, IN had fewer total neutrophils with immature and toxic neutrophils at 4-6 DA, but had more total neutrophils at 8-12 DA. Bacterial abscesses (≥1) occurred in 11% of IN at 4-6 DA, 50% at 8 DA, and 67% at 12 DA; no abscesses occurred in C. Bacteria were isolated from heart, spleen, liver, kidneys, and/or lungs of 80% IN and C at 4 DA. The rate of positive C fell to 20% at 12 DA whereas that of IN rose to 100% at 6-8 DA and declined to 80% at 12 DA. Compared to C, the numbers of bacterial species and colonies isolated from IN was lower at 4 DA but higher at 6-12 DA. Serum endotoxin levels of IN were elevated relative to C at 6-8 DA. Destruction of intestinal epithelium and consumption of neutrophils in neonatal pigs infected with I. suis likely facilitated secondary bacterial invasion.

STUDIES TO DETERMINE THE RELATIONSHIP OF MYXOZOA FOUND IN CATFISH PONDS TO PROLIFERATIVE GILL DISEASE (PGD) IN CHANNEL CATFISH. L.M. POTE*, T.L. LIN, B. BELLERUD AND E.F. CHENNEY. COLLEGE OF VETERINARY MEDICINE, MISSISSIPPI STATE UNIVERSITY. MISSISSIPPI STATE, MS 39762.

Proliferative gill disease (PGD) causes significant economic losses to the catfish industry due to high mortalities. It is now known that the myxozoan Aurantiactinomyxon spp. is involved in PGD; however, the life cycle is not completely known. This organism and several other myxozoan species are routinely isolated from catfish ponds. While Aurantiactinomyxon spp. has been associated with PGD, the significance of other myxozoan spp. present in catfish ponds is not known. These studies were an attempt to determine if Raabeia spp. and Triactinomyxon spp. both myxozoa isolated from catfish ponds, could infect catfish.

Two experiments were done using specific pathogen free (SPF) channel catfish. In both experiments, fish were divided into 4 groups (n=10), each group was housed in a 5 gal aquarium. The groups were a negative control and 3 groups, each exposed to either Triactinomyxon spp. (n=12,000) or Raabeia spp. (n=6,000/tank) placed in the tank on day 1. The fish were necropsied 5 days after exposure and all organs were collected for histology. Organs were found to be negative for myxozoan parasites when examined histologically. The design used in these experiments was one that is routinely used in our laboratory to artificially infect catfish with Aurantiactinomyxon spp. These experiments indicated that Raabeia spp. and Triactinomyxon spp. do not infect catfish or play a role in the etiology of PGD.
PREVALENCE OF HELMINTHIC INFECTION IN CATS IN PATIALA, INDIA. M. JOHAL',
DEPARTMENT OF ZOOLOGY, PUNJABI UNIVERSITY, PATIALA (INDIA), 147002.

A study was conducted at Patiala to determine the prevalence of helminthic infection in cats (Canis familiaris). Fresh fecal samples of 25 cats were examined for the presence of ova by using various floatation techniques. Eighty percent of these were found to be positive for one or other type of infection including 4 species of nematodes and one of trematode and cestode each. Sixty percent of the infected cats carried single infection and 20% had doubled with a combination of ascarid and tapeworm (12%), trichurid and strongyle (5%) and ascarid and Opisthorchis (4%). The individual infection of Toxocara as found to be maximum (44%), followed by Taenia (24%), Trichuris (16%), Opisthorchis (12%), strongyle (8%) and capillaria (4%).

SAFETY OF IVERMECTIN IN CATS WITH PATENT INFECTIONS OF DIROFILARIA IMMITIS.
R. Alva', M. Dzimianski', T. McTier² and J. McCall². ¹Merck Research Laboratories, Rahway, NJ and ²Department of Veterinary Parasitology, College of Veterinary Medicine, The University of Georgia, Athens, GA.

Safety of oral ivermectin in cats with patent infections of Dirofilaria immitis was demonstrated in twelve 7 to 10-month old cats. Patent infections were established in each cat by transplanting at least five adult D. immitis via jugular venotomy. Six cats served as vehicle-treated controls, and six cats received ivermectin in a chewable formulation orally once at ≥24 mcg/kg body weight. Cats were observed frequently for clinical signs. Blood was obtained for determination of circulating microfilariae, hematology and serum chemistry prior to treatment and 1, 3, 5, 7, 9, 11 and 14 days after treatment. Fourteen days after treatment, heartworms were recovered using standard techniques. Tissues were examined for gross and microscopic pathology.

Live heartworms were recovered from all cats. No circulating microfilariae were detected in the ivermectin-treated cats on Days 11 and 14; however, microfilariae were detected in both groups on all other days. No relevant clinical findings were observed. Hematology and serum chemistry results were within normal limits with the exception of elevated liver enzymes and eosinophilia in both groups. Incidental histomorphologic lesions due to the presence of parasitic larvae in tissue were seen in both groups and were similar to lesions described in dogs infected with heartworm. In this study, ivermectin at ≥24 mcg/kg was safe in cats with patent infections of D. immitis.
LABORATORY AND CLINICAL EVALUATION OF PRAZIQUANTEL PLUS PYRANTEL TABLETS FOR CATS. ROBERT G. ARTHERT, DWIGHT D. BOWMAN2 AND LARRY R. CRUTHERS3. MILES INC., AGRICULTURE DIVISION, SHAWNEE MISSION, KS, 2CHK R&D, STANWOOD, MI AND 3PROFESSIONAL LABORATORY AND RESEARCH SERVICES, CORAPEAKE, NC.

A tablet formulation containing praziquantel plus pyrantel pamoate was developed as a broad spectrum anthelmintic for cats. A total of 93 cats with naturally acquired helminth infections were included in two well-controlled laboratory studies to evaluate the efficacy of this product. In each study the cats were randomly allocated to one of three treatment groups to receive either 5 mg/kg praziquantel + 20 mg pyrantel, pyrantel alone at 20 mg/kg or placebo tablets. The cats were euthanatized 7 days after treatment for recovery of all remaining intestinal helminths. The mean group efficacy for praziquantel + pyrantel tablets against Taenia taeniaeformis, Ancylostoma tubaeforme and Toxocara cati was 100, 99.3 and 93.7%, respectively. Praziquantel treatment was 77.1 and 96.12% efficacious against A. tubaeforme and T. cati, respectively. Praziquantel did not interfere with the activity of pyrantel against nematodes and pyrantel did not interfere with the activity of praziquantel against cestodes.

In a separate clinical evaluation, 85 cats infected with tapeworms plus nematodes were included in a study conducted at 6 different geographical locations to further evaluate efficacy and safety. Praziquantel + pyrantel tablets provided 100% efficacy against T. taeniaeformis and Dipylidium caninum and >98% reduction in A. tubaeforme and T. cati fecal worm egg counts 7 - 10 days following treatment.

EFFICACY OF A CHEWABLE FORMULATION OF IVERMECTIN AGAINST ANCYLOSTOMA TUBAEFORME. E.L. Roberson1*, D.O. Bowman2, A.J. Paul3, and L.R. Cruthers4. 1The University of Georgia, Athens, GA., 2CHK, Research Division, Stanwood, MI; 3University of Illinois, Champaign, IL; 4Professional Laboratory Research Services, Corapeake, NC.

Ivermectin at 12, 24 and 36 mcg/kg body weight against adult Ancylostoma tubaeforme was evaluated in four dose titration studies (two with natural infections and two with induced infections). For each study, replicates were formed based on fecal EPG; within replicates, cats were randomly allocated to one of four treatment groups: vehicle control, or ivermectin at 12, 24 or 36 mcg/kg body weight. Cats were necropsied 7 days after treatment for parasite recovery.

Linear-plateau modeling indicated the best fitting model included a plateau beginning at no lower than 15.6 mcg/kg ($R^2 > 99.99\%$). For this model, efficacy predicted at the plateau is 99.40% relative to the predicted control geometric mean of 43.14 worms. The overall treatment effect of ivermectin vs control was highly significant ($p<0.001$); the contrast of ivermectin at 12 mcg/kg vs higher doses was nearly significant ($p=0.0538$); and the contrast of 24 vs 36 mcg/kg was not significant ($p=0.90$). These results indicate that a dosage greater than 15.6 mcg/kg of ivermectin is required for optimal control of A. tubaeforme in cats.
EFFICACY OF A CHEWABLE IVERMECTIN FORMULATION AGAINST INDUCED ANCYLOSTOMA TUBAEFORME INFECTIONS IN KITTENS. A. Paul*1, K. Todd, Jr.1, J. DiPietro1, D. Wallace2 and C. Dauro2. 1University of Illinois, Urbana, IL; 2Merck Research Laboratories, Rahway, NJ.

The effective dosage of a chewable formulation of ivermectin was determined in thirty-two 10- to 24-week old kittens with induced infections of Ancylostoma tubaeforme. Kittens were inoculated with 750 third-stage A. tubaeforme larvae on Day -22. Based on pretreatment fecal egg counts, eight replicates of four kittens each were formed. Within replicates, kittens were randomly allocated to one of four treatment groups: vehicle control, or ivermectin at 12, 24 or 36 mcg/kg body weight. Seven days after treatment, parasites were recovered using standard techniques. All eight controls had adult A. tubaeforme (geometric mean = 147.7 worms). The efficacy of ivermectin was 83.8%, 99.3% and 99.6% at 12, 24 and 36 mcg/kg, respectively. The statistical model which best described the dose response was linear to 24 mcg/kg with a plateau thereafter. Using this model, the estimated reduction from the predicted control mean is 99.42% at the plateau. Twenty-four mcg/kg of ivermectin is the optimal dosage for control of A. tubaeforme infections in cats.

CLINICAL PROPHYLAXIS OF EXPERIMENTALLY INDUCED INFECTIONS OF DIROFILARIA IMMITIS BY MONTHLY TREATMENT WITH HEARTGARD 30® BEGINNING AT FOUR MONTHS PI. J.W. MCCALL,1* T.L. MCTIER,1 N. SUPAKORNEDEJ,2 AND R.P. RICKETTS.2 1DEPARTMENT OF PARASITOLOGY, COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF GEORGIA, ATHENS, GA 30602; 2TRS LABS, INC., ATHENS, GA.

In a preliminary trial, treatment of dogs with ivermectin (6 mcg/kg) monthly for 13 months (14 doses) beginning 4 months after experimental infection with heartworm suppressed infection, whereas treatment with milbemycin oxime (500 mcg/kg) at the same schedule was only partially effective. Nine beagles (male and female) were each given 50 infective larvae of Dirofilaria immitis and allocated to 3 groups of 3 dogs each. One group of dogs was given ivermectin (Heartgard 30®), another group was given milbemycin (Interceptor®), and the remaining group served as a nontreated control. Worm recoveries at necropsy 1 month after the last treatment (18 mos. PI) revealed that ivermectin was 99% effective in suppressing infection. One dog treated with ivermectin was cleared of worms, 1 had 1 dying female worm, and 1 had 1 live male worm only. Milbemycin was 46% effective. All of the 3 dogs treated with milbemycin had heartworms (avg. 15/dog; range 10-25); it appeared that male worms were more sensitive. All of the control dogs had heartworms (avg. 27.7/dog, range 20-34). None of the dogs treated with ivermectin ever developed a patent infection, but 2 of the 3 dogs treated with milbemycin had a few microfilariae at 8 and 9 months PI, respectively. All of the nontreated dogs had patent infections at 8 months PI and monthly thereafter. In general, adult heartworm antigen (ASSURE/CH™ test) levels were negative to low in the ivermectin-treated dogs and moderate to high in the milbemycin-treated and nontreated dogs. At 17 months PI, 2 of the 3 controls were negative for antibody to cuticular antigen(s) of microfilariae on an IFA test, whereas 2 of the ivermectin-treated and all of the milbemycin-treated dogs were at least weakly positive.
EFFICACY OF THE PREVENTIC (9% AMITRAZ) COLLAR FOR CONTROL OF RHIPICEPHALUS SANGUINEUS AND DERMACENTOR VARIABILIS INFESTATIONS ON DOGS.
BYRON L. BLAGBURN*, JOY L. VAUGHAN, DAVID S. LINDSAY AND THOMAS A. MILLER.
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The formamidines are a novel group of acaricidal compounds effective against a variety of ectoparasitic mites and ticks of domestic animals. The present research was conducted to support efficacy claims for a collar containing 9% amitraz labeled to control common tick infestations on dogs. Twelve dogs of mixed sexes, weights and haircoat types were allocated, based on weight, to two treatment groups of 6 dogs each. Each dog in one of the two groups was fitted with a collar containing 9% amitraz; dogs in the remaining group were fitted with collars that contained excipients without amitraz. Dogs in both groups were infested with 50 unfed adults of *Rhipicephalus sanguineus* (EL Labs, Soquel, CA) or *Dermacentor variabilis* (TRS Labs, Athens, GA) at intervals throughout the study. Nondestructive counting techniques were employed to enumerate live adults at selected intervals. Each dog was examined daily for changes in behavior, appetite, excretions, skin irritation, or other abnormalities suggestive of collar irritation or amitraz intoxication. Significant differences in tick counts were observed between amitraz-treated and control dogs on all counting days except those prior to collar application and on days 94, 95, 107 and 108 after collar application. Significant reductions in the numbers of ticks recovered from the amitraz-treated dogs were observed within 24 hours after application to dogs with existing infestations (day 9). The amitraz-treated dogs resisted reinfestation with *R. sanguineus* or with *R. sanguineus* and *D. variabilis* on days 22, 36 and 64 as evidenced by statistically significant reductions in tick burdens. The data obtained on days 94-108 indicated that inadequate amounts of amitraz were being released to provide consistent protection against challenge.

EVALUATION OF A 1% AND 2% (S)-METHOPRENE COLLAR AGAINST THE HATCH OF CAT FLEA EGGS WHEN WORN BY DOGS AND CATS. W. A. DONAHUE*, TEXAS A&M UNIVERSITY, COLLEGE STATION, TX 77843. R. YOUNG, YOUNG VETERINARY RESEARCH, MODESTO, CA 95356.

The ovicidal effect of the insect growth regulator (IGR) (S)-methoprene incorporated into plastic flea collars was evaluated on both dogs and cats. Artificial infestations of adult *Ctenocephalides felis* (Bouche) were utilized for this one year study. Flea eggs were recovered from cages for each animal, held separately, and scored for egg hatch. Comparisons were conducted with a placebo group of dogs and cats and the percent inhibition of hatch was calculated.

The 1% (S)-methoprene dog collar provided 99.5% inhibition of flea egg hatch 4 days after placing collars on the animals. The 1% (S)-methoprene collared group of dogs continued to give 97.9% to 100% inhibition of egg hatch through day 130. Dogs were placed in outdoor runs for approximately 9 hours per day. Residual ovicidal activity for the 2% collar worn by cats was 94% to 100% for 375 days. Recommendations are given for incorporating an ovicidal IGR flea collar into an integrated flea control strategy.
Reports in the literature indicate variable but frequently low infection rates (~4-6%) following experimental infection of dogs with Echinococcus multilocularis or E. granulosus. We developed a system that produced much higher infection rates for E. multilocularis in dogs. Protoscolices were processed under as optimum physiological conditions as possible. Criteria judged to be important included harvesting and processing protoscolices quickly, maintaining them in nutrient medium instead of saline, keeping them warm (37-38°C) until infection of the dogs, and infecting the dogs by natural means. Food was withheld from the dogs for 24 hours prior to infection. Cysts were removed from gerbils and placed into warm, sterile, high-glucose (4,500 mg/L) Dulbecco's Modified Eagle's Medium. The cysts were finely macerated and the solution was filtered through cheesecloth. Protoscolices were quantitated, aliquoted to siliconized conical tubes and kept warm in a water bath. The dogs were fed their dose in small meatballs of moist dog food, each containing a central cavity into which the protoscolices were placed. The dogs were held off food an additional 4-6 hours to facilitate parasite translocation and attachment. Eighteen dogs infected with 102,700-106,250 protoscolices harbored an average of 43,100 worms (range 6,200-75,200) at necropsy 7-30 days later, for an average infection rate of 41.3% (range 6.0-70.8%). Individual infections clustered as follows [worms (inf. rate) (no. dogs)]: 6,200-11,200 (6.0-10.7%) (n=3); 20,800-40,400 (19.6-38.6%) (n=5); 41,900-58,200 (40.3-56.0%) (n=5); and 66,800-75,200 (62.9-70.8%) (n=5).

Presently, there are no anthelmintic drugs approved for treatment and control of Echinococcus multilocularis in carnivores in the United States. We conducted a blinded, controlled anthelmintic trial designed to assess the efficacy of marketed tablet and injectable formulations of praziquantel (Droncit) against adult E. multilocularis in dogs. E. multilocularis was isolated from an Indiana coyote and maintained as a larval infection in gerbils. Thirty-six dogs, studied in 3 replicates of 12 dogs each, were each infected orally with 102,700-106,250 E. multilocularis protoscolices, isolated from infected gerbils. In each replicate, the dogs were randomly and equally assigned to praziquantel tablet, injectable solution, or untreated control groups. Treated dogs received praziquantel at 21 days post-infection (PI), at a dose rate of 5.0-7.0 mg/kg (tablets) or 4.8-5.7 mg/kg (injectable solution). All dogs were necropsied at 28 days PI and their worm burdens evaluated in a biohazard containment facility. Both formulations of praziquantel were 100% effective in clearing E. multilocularis infections from the treated dogs. Untreated control dogs had an average of 41,492 E. multilocularis (range 6,200-75,200), versus 0 worms in the treated dogs. No adverse clinical signs were related to the infections or treatments. It was concluded that praziquantel is a highly effective anthelmintic treatment for E. multilocularis infections in dogs, which should prove useful in the control of this important parasite in the United States.
Parasite Transmission in Greyhounds. M.W. Dryden* and N.H. Gabbert
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Parasite contamination of the environment at greyhound farms and
pre- and post- parturient parasite egg shedding in feces of
greyhounds was investigated. Parasite contamination in exercise
runs was conducted by collecting sand and fecal samples once
monthly at three greyhound breeding farms from May 1991 through
April 1992. Quantitative fecal examinations were performed on four
greyhounds once weekly throughout pregnancy and for at least eight
weeks after whelping. Fecal examinations were also performed
weekly on pups of two litters starting seven days after birth.
While two farms had low to moderate levels of sand contamination,
one farm (Farm 1) had severe environmental contamination. Largest
numbers of eggs were recovered Farm 1 in June, when one run was
found to contaminated with 375 Toxocara canis per 300 g of soil.
Additional parasite eggs recovered from soil samples were
identified as Toxascaris leonina, Trichuris vulpis, Eucoleus
boehmi, and Capillaria aerophila.

Fecal samples from the four greyhound bitches were negative
for T. canis during their entire pregnancy. But fecals from all
bitches became positive for T. canis by four weeks after whelping.
All twenty pups from the two litters became positive for T. canis
between three and four weeks of age.

A Systematic Approach To Classification Of Heartworm Disease To
Rhone Merieux,Inc., Athens, GA 30601

Assessment of the heartworm patient is done to evaluate the
potential of drug toxicity and/or thromboembolic disease post
treatment. In the past, various parameters have been used to
evaluate heartworm infected dogs, however, a systematic approach to
classification has not been widely employed. One system used
separates dogs with heartworm disease into 4 clinical classes:
class 1 (none to mild clinical signs), class 2 (moderate clinical
signs), class 3 (severe clinical signs), and class 4 (caval
syndrome). Physical exam, radiographic lesions, and laboratory
parameters help define and separate the classes. The elements used
are presented in a schematic approach to encourage consistency in
classification. Using this system permits the practitioner to
anticipate some post-therapeutic complications and provide a more
accurate prognosis. The objective is to reduce risk of death from
thromboembolic disease. With the advent of new adulticide
therapies, the system will assist the practitioner in giving
appropriate treatment to the corresponding class of disease. The
scheme has been used in clinical and field trials to separate
populations of heartworm infected dogs for treatment. In these
trials with an experimental adulticide the system was sucessfully
used by practitioners in defining these populations.
ALGORITHMS FOR THE DIAGNOSIS AND TREATMENT OF HEARTWORMS IN DOGS AND CATS

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Diagnostic and treatment protocols for heartworms in dogs have been well documented. Recent advances in diagnostic tools such as antigen ELISA and ultrasound have caused a reassessment of the importance and order in which all tests are performed. The older diagnostic and treatment methods used for dogs have been applied to cats because of the lack of specific knowledge. As more is learned about the biology of heartworms in cats, it is apparent that they respond to larval migration and the presence of adults in a manner uniquely different than dogs. This also has forced a reassessment of the order and importance in which diagnostic tests are performed in cats. Diagnostic algorithms will be presented that will reflect the latest knowledge in the diagnosis and treatment of heartworms in dogs and cats. Algorithms for dogs and cats will be compared to show how each species are different.

Clinical Field Trial For The Treatment Of Mature And Immature Dirofilaria Immitis Infestation In Dogs With Severe Heartworm Disease. P.A.Tanner*, H.Winograd, And D.M.Keister. Rhone-Merieux, Inc., Athens GA, 30601

Previous studies have indicated that a reduced dose regimen of RM340 would partially reduce the heartworm burden in affected dogs. The objective of this treatment is to reduce severely infected dogs’ worm burden, thereby reducing the incidence of life threatening thromboembolic disease when a full regime is given. A multi-centered clinical field trial recruiting 44 client owned heartworm infected dogs was performed using this alternative dose regimen of RM340 during April 1992 through March 1993. Treatments of RM340 were given as deep intramuscular injections into the lumbar musculature and administered at a dosage of 2.5 mg/kg body weight. An initial RM340 injection was followed 1 month later by 2 RM340 injections given 24 hrs. apart. Of the 44 dogs treated, 8 deaths were reported. Of this total, 2 deaths were related to thromboembolism post adulticide treatment. Two deaths were due to trauma or from euthanasia. The remaining 4 deaths were not confirmed to be treatment related. Preliminary results indicate an efficacy rate of approximately 88% seroconversion from an antigen positive status to an antigen negative status. Subjective evaluations of clinical improvement have been good to excellent.
Giardia is a protozoan that can be found in the small intestine of dogs and cats. The importance of giardiasis is exemplified by its high prevalence, seriousness as a disease entity, possible zoonotic potential, and poor efficacy or serious side effects of drugs used in therapy. Metronidazole and quinacrine are the main therapeutic agents available in the United States. Metronidazole (the most commonly used drug) is only approximately 67% effective, can occasionally produce neurologic toxicosis, is relatively expensive, and is suspected of being teratogenic. Quinacrine, although relatively inexpensive and effective, often produces side effects (anorexia, lethargy, and pyrexia) restricting its use.

Albendazole has been shown to be effective and safe in treating giardiasis in mice and humans. In a pilot study, 5 of 7 dogs were cleared of Giardia cysts, (as determined by the zinc-sulfate concentration technique) after receiving a single oral dose (25 mg/kg) of albendazole (Valbazan™, Norden Lab, Lincoln, NE), while 3 of 7 dogs became clear of cysts without treatment (not significant at $P < 0.05$). In a second pilot study, 5 of 5 dogs became clear of cysts after albendazole (25 mg/kg, PO, q 12 h for 4 doses), while 1 of 5 untreated control dogs became clear (significant at $P < 0.05$). In a third study, 18 of 20 dogs became clear after receiving albendazole (25 mg/kg, PO, q 12 h for 4 doses), while none of the 20 control dogs cleared (significant at $P < 0.005$). No signs of toxicity were seen in any dog. In a pilot study, 5 infected adult cats (as determined by visualizing trophozoites in fresh stool samples) remained infected after albendazole (25 mg/kg, PO, q 12 h for 4 doses). It was concluded that albendazole at 25 mg/kg, PO, q 12 h for 4 doses is effective and safe for treating Giardia infection in dogs, but may not be in cats.

Human infection with Toxoplasma gondii (Tg) is an important cause of morbidity and mortality throughout the world. In immunocompetent hosts, the infection is usually not significant, asymptomatic persistence of the organism in the hosts. Seroprevalence rates vary widely depending on the geographic location, ranging from a few percent in low prevalence areas to greater than 80% in hyperendemic foci. Infection occurring in neonates or in other individuals with defective cellular immunity (such as recipients of organ allografts or persons with AIDS), may be life-threatening. In the United States, the prevalence of human disease associated with Toxoplasma gondii has increased markedly in the past decade, due largely to the concomitant upsurge in the numbers of AIDS patients, Clinical disease syndromes associated with Tg infection, and the current state of our understanding of immunity to this protozoan parasite will be discussed.
WHAT IS THE USEFULNESS OF A VACCINE FOR FELINE TOXOPLASMOSIS?
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Cats are of paramount importance in the transmission of Toxoplasma, because one cat can give rise to several million infectious oocysts potentially infecting a similar number of animals, including humans; in contrast, an infected bird or rodent usually infects only one or a few carnivores. A mutant, T-263 was selected because, although it did not give rise to oocyst shedding in kittens, it protected 84% of kittens against oocyst shedding after reexposure to a normal isolate [Am. J. Vet. Res. 52, 759-763, 1993; US Patent 5,045,313]. Use of T-263 as a vaccine in cats would be a useful public health measure to prevent spread of Toxoplasma to other animals and to humans. Animals at risk are: 1. Australian marsupials, lemurs, and neotropical monkeys in zoos where stray or caged felines are present, because such animals do not develop a timely immunity to Toxoplasma (probably because they have not been exposed to, and selected for resistance to Toxoplasma); 2. ewes and goat does exposed to Toxoplasma oocysts, to prevent fetal infection and abortion. Humans at risk are: 1. women about to become pregnant, who are exposed to cats or oocysts in soil, especially when frequent hand washing is not feasible; 2. HIV infected humans without Toxoplasma antibody exposed to cats or oocysts in soil; and 3. heart transplant candidates without Toxoplasma antibody. Carnivores, and humans exposed to raw or undercooked meat, and transplant candidates could be protected by the ts-4 vaccine. The ts-4 mutant, selected by E. R. and L. C. Pfefferkorn protects individual non-felines against illness, but does not persist in the host [J. Parasit. 69,60-65, 1983; US Patent 4,473,549].


Previous studies have demonstrated that oral administration to cats of tissue cysts of the oocyst negative mutant strain of Toxoplasma gondii T-263, induces immunity to oocyst shedding following challenge. The experiments described herein were designed to compare the levels of protection induced by T. gondii T-263 when tissue cysts, bradyzoites released from tissue cysts and tachyzoites are administered to cats. In one experiment, groups of cats received 2 oral doses of intact tissue cysts or released bradyzoites of T. gondii T-263 and were challenged 47 days later with the oocyst producing strain of T. gondii T-265. All cats seroconverted following immunization and none of them shed oocysts following challenge. In a second experiment, groups of cats received tachyzoites of T. gondii T-263 as a single oral dose and either one or two intraduodenal doses; they were challenged 60 days after the last immunization. All cats seroconverted following immunization. Following challenge, all cats shed oocysts except for 2 of 7 cats that received 2 intraduodenal doses of tachyzoites. Thus, orally administered bradyzoites of T. gondii T-263 either contained in intact tissue cysts or liberated from cysts, induced immunity to oocyst shedding. In contrast, tachyzoites did not completely protect against oocyst shedding, even when delivered directly to the duodenum and despite the development of high antibody titers.
ACTIVITY OF DICLAZURIL AGAINST TOXOPLASMA GONDII IN CELL CULTURES AND MICE. DAVID S. LINDSAY*, AND BYRON L. BLAGBURN. AUBURN UNIVERSITY, AL 36849

The present study was done to evaluate the activity of diclazuril, a benzeneacetonitrile anticoccidial, against the RH isolate of Toxoplasma gondii in human fibroblast cell cultures and in female, Hsd:ICR mice. Diclazuril treatment of infected cells resulted in a >99% reduction in tachyzoite numbers at concentrations of 10.0, 1.0, 0.1 and 0.01 μg/ml, >97% reduction at 0.05 μg/ml, and approximately 50% reduction at 0.0025 μg/ml. Pretreatment of host cells with 10.0, 1.0, 0.1, and 0.01 μg/ml of diclazuril for 24 hours before inoculation with tachyzoites caused a 97%, 31%, 0% and 0% reduction in tachyzoite production, respectively. Oral treatment of mice with 10.0 mg/kg or 1.0 mg/kg of diclazuril one day prior to and daily for 10 days after tachyzoite inoculations protected 100 and 80% of mice, respectively, from fatal infection over a 6 week observation period. Oral treatment of mice with 10.0 mg/kg diclazuril did not permit the development of protective immunity because 100% of these mice died within 16 days after challenge. Oral treatment of mice with 1.0 mg/kg diclazuril allowed mice to develop immunity and none died after challenge. Results of this study demonstrate that diclazuril has demonstrable activity against T. gondii at low concentrations in cell cultures and in mice.

SEROLOGIC PREVALENCE OF TOXOPLASMA GONDII IN SWINE FROM 57 MINNESOTA COUNTIES REPRESENTING FOUR GEOGRAPHIC REGIONS. J. VANEK,*1 J. DUBEY,1 P. THULLIEZ,3 M. RIGGS,1 AND B. STROMBERG.1

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Sera from 2956 swine (Sus scrofa) selected from 212 farms from 57 contiguous counties representing four geographic regions of Minnesota were assayed for prevalence of Toxoplasma gondii antibodies using a direct agglutination test containing whole tachyzoite antigen. Three hundred fifty-one of the samples (12%) were positive at a titer of 1:25 or greater. Analysis by region yielded stratum prevalences of 6% (39/662) in the west, 11% (163/1537) in the south, 19% (81/437) in the east, and 21% (68/320) in the north with a significant trend (p < 0.001) toward increasing prevalence from west and south to east and north, and a significant (p < 0.001) dichotomy in prevalence between the southwest (10%) and the northeast halves (20%) of the state.

The lower prevalence of Toxoplasma exposure in that region of Minnesota characterized by large, numerous confinement facilities located on tall grass prairie, and the higher prevalence of exposure in that region characterized by small, dispersed, open facilities in forested areas support either a geographic and/or management association with disease prevalence.

Serum samples from 6965 pigs from 179 swine herds in 68 counties in the State of Illinois were selected from a pseudorabies virus testing program during January to August, 1992. These were tested for anti Toxoplasma gondii antibodies by the agglutination test. Anti T. gondii antibodies were found in 21.8% of 5080 breeding pigs; at dilutions of 1:25, 1:50 and 1:500, 6.3%, 8.7%, and 5.8% had antibodies, respectively. In contrast, only 3.1% of 1885 grow-finish pigs were seropositive; at dilutions of 1:25, 1:50 and 1:500, 1.2%, 1.4% and 0.5% were seropositive, respectively. Compared with a seropositivity of 24.2% in 1330 Illinois grow-finish pigs in the 1983-1984 national survey for Toxoplasma antibody in pigs, the seropositivity for T. gondii in Illinois pigs has declined.

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Sera from 1167 white-tailed deer (Odocoileus virginianus) harvested within the boundaries of ten Minnesota state and county parks during controlled hunts in October and November, 1990, 1991, and 1992 were assayed for prevalence of Toxoplasma gondii antibodies using a direct agglutination test containing whole tachyzoite antigen. Three hundred fifty-two of the samples (30%) were positive at a titer of 1:25 or greater. Stratification by age and sex resulted in stratum prevalences of 15% (24/164) in male fawns, 10% (14/138) in female fawns, 25% (32/128) in male yearlings, 26% (27/103) in female yearlings, 35% (64/185) in male adults, and 42% (191/449) in female adults. There was a significant trend toward increasing prevalence with age (p<0.001), but no association with sex. The adjusted prevalence was 26%.

The parks were stratified into four regions: northeast conifer, west-central prairie, southeast hardwood, and twin cities metropolitan. The adjusted prevalences for these regions were: 24% (northeast), 22% (west-central), 22% (southeast), and 33% (metropolitan).

Two commercial turkey farms were monitored weekly throughout one grow out. Both farms had experienced various degrees of liver condemnations at processing due to the presence of white circular to irregular spots on the liver. Fifty birds were taken weekly from each farm and monitored for the presence of nematodes, protozoans, bacteria, mycoplasma, and viruses. Liver spots on both farms were associated with the appearance of Ascaridia dissimilis larvae. Histomonas meleagridis, Trichomonas gallinarum and Heterakis gallinarum were also isolated from the flocks. A. dissimilis larvae were first observed on Farm A at 2 weeks while Farm B did not experience significant numbers of the larvae until 8 weeks. Poults from Farm B seroconverted to Mycoplasma meleagridis and Newcastle Disease Virus at 12 weeks of age.

Histologic examination of the liver revealed the presence of larvae enclosed in a narrow zone of necrosis surrounded by lymphocytes, macrophages and eosinophils. Large and numerous masses of lymphocytes were present in the mucosa of the ceca.

EVALUATION OF EXPERIMENTAL MENINGEAL WORM INFECTIONS IN THE LLAMA. L.G. RICKARD, E.J. GENTZ, E.G. PEARSON, A. FRANK AND B.B. SMITH. COLLEGE OF VETERINARY MEDICINE, OREGON STATE UNIVERSITY, CORVALLIS, OR 97330 AND MISSISSIPPI STATE UNIVERSITY, MISSISSIPPI STATE, MS 39762

Six llamas were artificially infected with 5 Parelaphostrongylus tenuis larvae each to evaluate the course of infection. Animals were monitored for the development of clinical signs daily from days 0 to 40 and twice daily from days 60 to 140. All pre-infection blood and cerebrospinal fluid (CSF) samples were obtained at days -3 or -2. Blood samples were also obtained on days 40 and at euthanasia. Cerebrospinal fluid from the lumbosacral space was also obtained at days 40 and approximately 20 day intervals thereafter. Additional samples of CSF from the cisterna magna site were obtained only at days 40 and at euthanasia. Fecal samples were evaluated for dorsal-spined larvae prior to infection, weekly from days 40 to 60 and daily thereafter. Clinical signs first appeared in the llamas between days 45 and 53 after infection. Signs progressed at differing rates in the llamas such that 1 llama was euthanized on each of days 70, 84 and 124. A fourth llama died suddenly on day 90. Dorsal-spined larvae were not present in the feces of these animals. Consistent abnormalities were not evident in the CBC or biochemical profiles of any llama. An eosinophilic pleocytosis and elevated CK were inconsistently present in the CSF from both sites in all llamas. Two white-tailed deer controls, receiving 6 or 7 larvae, did not exhibit clinical signs.
CHARACTERIZATION OF A WHIPWORM (TRICHURIS SUIS) INFECTION IN GROWING SWINE. J. URBAN, JR., I. CHUNG, L. MANSFIELD, D. HILL AND N. STEELE. HDL, BPL, & NRRNL, LPSI, ARS, USDA. BELTSVILLE, MD 20705

A study to characterize the basic hematologic, parasitologic, and immunologic parameters of a T. suis infection in swine, as well as pig growth performance, was initiated. Forty eight growers that had been maintained helminth-free in confinement were divided into six groups of eight pigs each. Four of these groups were inoculated with infective T. suis eggs at 0, 50, 150, or 450 eggs/kg body weight. The two remaining groups were transferred to pastures, one contaminated and the other free of T. suis eggs. One week later, all pigs were moved to a confinement facility and paired measurements of daily feed intake and weight gain were taken, along with weekly blood and serum samples. After five weeks of measurement all pigs were necropsied. No T. suis was found in pigs that were uninoculated or from the T. suis-free pasture, while pigs inoculated with 50, 150, or 450 eggs/kg b.w. or exposed to T. suis on a contaminated pasture had 84 ± 40, 211 ± 53, 212 ± 112, and 208 ± 137 adult worms, respectively. There was a significant decrease in average daily gain and an increase in the ratio of feed/gain in pigs given 450 egg/kg or exposed to T. suis on a contaminated pasture compared to uninfected pigs. Values of RBC's, Hct, Hgb, MCV and WBC's for all groups generally fell within normal ranges, but an increased eosinophilia was observed in the pasture exposed groups, and an increase in serum antibody to T. suis antigens was seen in all pigs infected with T. suis.

MASSIVE EXPERIMENTAL INFECTION OF PIGS WITH STRONGYLOIDES RANSOMI. N. TAIRA1, S. URA2, N. NAKANISHI2 and T. MISHIBA2. NAT. INST. OF ANIM. HLTH. TSUKUBA, IBARAKI 305, JAPAN; KYOTO ANIM. SCI. R. & D. CENTER, SHIMOITABASHI, FUSHIMI, KYOTO 612, JAPAN.

Twelve pigs, 5.8 ~ 20.8 kg (body weight) were used in two experiments for determining the pathogenicity of Strongyloides ransomi (SRM). [Exp.I: Single infection] Eight pigs were exposed once at the rate of 100,000 (pig No.405), 320,000 (No.406), 1 million (No.407), 3.2 million (No.408), 10 million (No.409), 10 million on (No.410), 32 million (No.411) and 100 million (no.412) SRM larvae per 100 kg of body weight, respectively. Maximum EPG counts were 3,200, 88,000, 222,000, 312,000, 605,000, 1.2 million, 2.02 million and 2.32 million, respectively. Five pigs (Nos.405 ~ 409) survived, however 3 pigs (Nos.410, 411, 412) died at 15, 58, and 22 days after exposure, respectively. Diarrhoea did not occur in the survivors, however it occurred in the pigs that died form 8 or 12 days before death. [Exp.II: Double infection] Four pigs were exposed twice at the rate of 100,000, 320,000, 1 million and 3.2 million SRM larvae per 100 kg of body weight at a 60 day interval, respectively. Egg counts after the secondary infection were lower compared those following the primary infection; the maximum SRM-EPG values were 2,200, 2,800, 6,900 and 500 respectively. Diarrhoea or death did not occur in this experiment. Transient dermatitis associated with Sarcoptes scabiei was observed in all 12 pigs during SRM patency. Sudden death associated with SRM infection did not occur in these experiments.
DEVELOPMENT OF IMPROVED SERODIAGNOSTIC TESTS FOR SWINE EPERYTHROZOOONOSIS.
N. NONAKA*, R. W. BULL and B. J. THACKER. MICHIGAN STATE UNIVERSITY. EAST LANSING, MI 48824.

Enzyme-linked immunosorbent assay (ELISA) using horse radish peroxidase (HRP) conjugated rabbit anti-pig IgG (IgG-ELISA) and HRP conjugated protein A (ProA-ELISA), or a dot blot (DB) using ¹²⁵I-protein A were performed on sera obtained from experimentally infected pigs (EXP sera) and from naturally exposed pigs (field sera), using Eperythrozoon suis positive (Es⁺) and E. suis negative (Es⁻) antigen preparations. Serum antibody levels were determined by two methods: 1) actual optical density (OD) in ELISA or percent binding of protein A in DB, using Es⁺ antigen only (CrudeEs-Test); or 2) adjusted OD or percent binding calculated by subtracting the value obtained with Es⁻ antigen from Es⁺ antigen value (NetEs-Test). ELISA and DB results were compared to the indirect hemagglutination assay (IHA). EXP sera, starting at 2 weeks post-infection (WPI), were positive by ELISA and DB as determined by CrudeEs-Test and NetEs-Test, and by IHA. However, IHA failed to detect antibodies in all of the 1 WPI sera, while, ProA- and IgG-ELISA's and the DB detected antibodies in 71, 63 and 88% of the 1 WPI sera by CrudeEs-Test, and 57, 75 and 88% by NetEs-Test, respectively. The time course of IHA titer development following experimental infection did not correlate with the development of E. suis specific antibodies detected by the other assays, using CrudeEs-Test or NetEs-Test. However, the IHA titers did correlate with the results obtained with the ELISA's and DB using Es⁺ antigen, indicating that the IHA titer was not specific for E. suis antibodies. Apparently, IHA was detecting erythrocytic autoantibodies induced by E. suis infection. The results obtained with the field sera using CrudeEs-Test or NetEs-Test were significantly associated between the two ELISA's and the DB. However, neither the two ELISA's nor DB results were associated with the IHA results. Western blot analysis using E. suis antigen preparation revealed that the reaction of 37, 47, 71, 81 and 101 kd E. suis antigens with field sera in IgG western blots was closely associated with the results of the two ELISA's and the DB. The results of the two ELISA's NetEs-Test were significantly associated with the detection of all 5 antigens. However, there was no association between IHA results and the appearance of E. suis specific antigen bands in the western blots. It was concluded that NetEs-Test of either ELISA could provide a better serodiagnostic assay for swine eperythrozoonosis compared to the standard IHA.


For many years veterinary practitioners have been attempting to control mange and lice in swine herds with a myriad of chemicals. Beginning with unsophisticated approaches such as coating the animals with used petroleum products, progressing to topical applications of dusts and sprays from chemical classes known to be toxic to these external parasites, moving toward the use of systemic organophosphate sprays, and then the development of ivermectin for systemic control of both clinical and subclinical mange as well as lice. Complete elimination or eradication of these two parasites can be accomplished by starting a new herd from caesarean derived pigs; however, that approach may not be financially feasible for most swine producers. Many swine producers are not satisfied with just controlling disease but want to eliminate or eradicate the disease from their existing herd if possible. A unique program known as HERD MANAGE/LICE ELIMINATION (HM/LE) has been developed to eliminate sarcoptic mange and lice from a specific locale (a herd). The program describes specific treatments with ivermectin, combined with quarantine procedures which, when put into effect, can provide long-term elimination of these disease entities.
ANTHELMINTIC POTENTIAL OF POLYMERS AGAINST SWINE PARASITES. T.B. STEWART*1, S.H. ZUKOWSKI1, S. WILES1, K.D. DRAKE2 AND W.E. PUCKETT2. LOUISIANA STATE UNIVERSITY1, BATON ROUGE, LA 70803 AND BUCKMAN LABORATORIES INTERNATIONAL2, MEMPHIS, TN 38108.

Three polymers were shown to have some effects against the rat pinworm and several parasites of pigs when fed to pigs for 3 days at a dose of 500 mg/day/kg of bodyweight. We report results of a trial with the same three compounds against Ascaris suum, Trichuris suis, Oesophagostomum spp., Hyostrongylus rubidus, and Strongyloides ransomi, when fed at 600 mg/day/kg bodyweight for 7 days. Forty-four crossbred pigs were infected sequentially with the above parasites. Once the infections became patent, pigs were assigned by sex and weight to 5 groups of 8 each: 1) Polymer A; 2) Polymer B 3) Polymer C; 4) Fenbendazole (1.5 mg/day/kg bodyweight); and 5) Untreated control (including the extra 4 pigs). Necropsies and parasite recoveries were done 11-18 days after completion of treatment. Efficacies of the three Polymers and fenbendazole were higher than 99% against T. suis and Oesophagostomum spp.; that of Polymers A and B against S. ransomi were 99.6% and 91.4%, respectively. Polymer A had the greatest effect of the three Polymers on A. suum, 65.2% compared to 99.9% for fenbendazole. No H. rubidus were recovered from any pig in the drug treated groups, however, small numbers of worms only were found and in only 5 of 12 control pigs.

MAJOR PARASITES OF DAIRY AND BEEF CATTLE. C.R. REINEMEYER*1. UNIVERSITY OF TENNESSEE, KNOXVILLE, TN 37996-4500.

Numerous internal and external parasites compromise the health and productivity of dairy and beef cattle in North America. The parasites which consistently have the greatest economic impact include lice, numerous types of flies, trichostrongylid nematodes, liver flukes, and various protozoans including coccidia, Cryptosporidium and Neospora spp.

This presentation will review the basic biology and pathogenic mechanisms of the bovine parasites of economic importance, estimate their monetary impact, and describe the most appropriate diagnostic methods for each. A variety of therapeutic and preventive alternatives will be suggested, depending on types of management and the regional seasonality of parasite transmission. Problems which currently confound successful control of the various parasitic agents will be discussed.
PARASITE PROBLEMS IN SWINE PRODUCTION. J.D. MCKEAN.

Swine parasite infestations present pork producers and veterinarians with prophylactic and treatment challenges. Infestations that do not rise to clinically significant levels may cause economic losses difficult to quantitate by standard performance measurements. The introduction of intensive confinement facilities and management systems and the increased emphasis in food safety concerns have moderated the importance of several parasites and increased the importance of others. The direct and indirect production costs of swine parasitism, parasite ecology within production systems, and food safety issues are important considerations when evaluating appropriateness of medication regimens. The intensity of swine production management necessitates continued efforts to refine prophylactic and elimination programs for internal and external parasitism.

PARASITES OF POULTRY. M.D. RUFF AND L.D. SCHWARTZ. USDA/ARS, LIVESTOCK AND POULTRY SCIENCES INSTITUTE, BARC-EAST, BELTSVILLE, MD 20715 AND AVICON INC., OKEMOS, MI 48864.

Coccidiosis continues to be the major parasitic problem in commercially raised chickens and turkeys because 1) drug resistance is increasing and 2) even low levels of coccidia interact with other entities, such as bacteria, viruses, and mycotoxins, to cause significant adverse effects on production. In game birds, anticoccidials that are effective in chickens and turkeys are often ineffective or even toxic. Histomoniasis (blackhead) is a potential problem in turkeys and chukar partridges because the only two effective drugs have been removed from the market. Cryptosporidiosis can cause losses in chickens and turkeys, and especially in quail. Other protozoan diseases that may be encountered include trichomoniasis, hexamitiasis, toxoplasmosis, sarcosporidiosis, leucocytozoonosis, and hemoproteus. Common helminth parasites of farm raised poultry are ascaridia, capillaria, heterakis, syngamus, and cestodes. Confinement rearing has reduced helminth infections to species with direct life cycles, or a few with indirect life cycles. Most nematodes have direct life cycles, cestodes and trematodes have an indirect life cycles. Each parasite species requires a specific intermediate host and only species with access to the appropriate intermediate host occur in the confined environment. Diagnostic skills of practitioners should include parasite identification, knowledge of the life cycle in order to develop a control program, and knowledge of which drugs are efficacious, as well as which are FDA approved anthelmintic treatments.
Parasites costs Americans millions of dollars annually and affect all sizes of farm-raised fish from fingerlings to market fish. The lack of scientific information is hampering the control of fish parasites. Little information is available on the host immunity, vaccinology, host-parasite relationships, transmission, pathogenicity and environmental interactions. Protozoans cause the most significant parasitic problems in fish farming, especially warm water species. *Trichodina, Ambiphyrya*, proliferative gill myxosporean parasite and *Ichthyophthiritius* are the most frequently diagnosed protozoans. None of these parasites are infectious to man nor affect the food safety of the farm-raised fish. Searching for alternatives to chemicals in the control of farm-raised parasites is critical because of the approved usage of effective chemicals are becoming more restricted. A crucial step towards improving parasite control in farm-raised fish will be the development of preventative methods, such as vaccines, improved diagnosis, food and environmentally safe chemicals and integrated management programs.
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