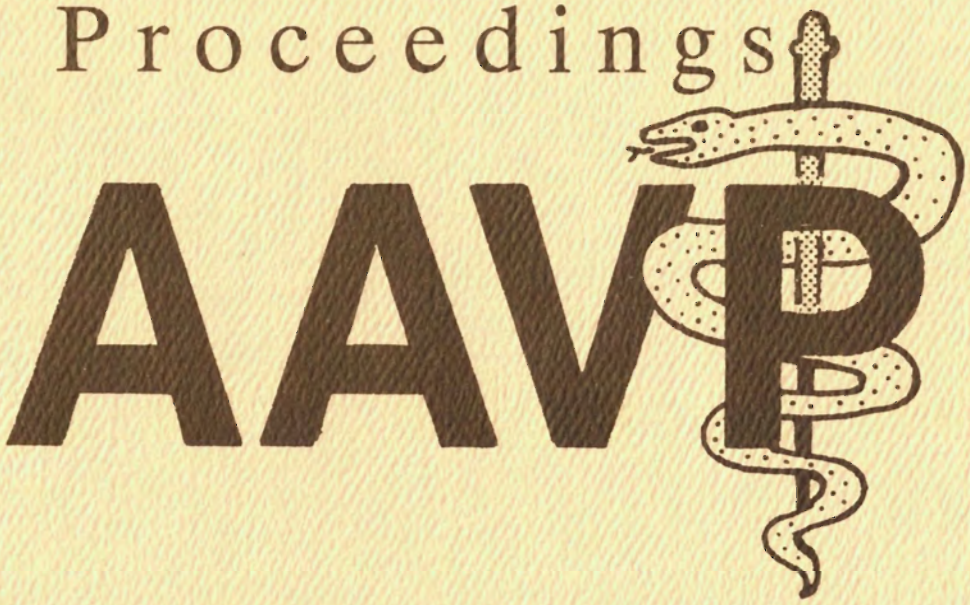
Proceedings



American Association of Veterinary Parasitologists

36th Annual Meeting

July 28-30
1991

Seattle, Washington

American Association of Veterinary Parasitologists
Founded 1956
Affiliated with the American Veterinary Medical Association

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The American Association of Veterinary Parasitologists gratefully acknowledges the above Corporations for their loyal support of the 1991 AAVP Conference.

Registration - 36th Annual Meeting
Stouffer Madison Hotel, Seattle, Washington
Corridor next to Municipal-Superior Rooms
Sunday 8:00 AM
Monday 8:00 AM

Social Program

Saturday, July 27, 1991

Stouffer Madison Hotel, Visions Room
Pre-Meeting Mixer, Cash Bar
7:30 - 9:00 PM

Sunday, July 28, 1991

The Spirit of Puget Sound Dinner-Cruise
AAVP Sponsored Social
6:30 - 8:30 PM

Monday, July 29, 1991

Stouffer Madison Hotel, North-West Room
Fort Dodge Laboratories Sponsored Social
6:30 - 8:30 PM

SCIENTIFIC PROGRAM OVERVIEW

36th ANNUAL MEETING
AAVP Seattle, Washington

SUNDAY Stouffer Madison Hotel Federal Superior Rooms SESSION A	Municipal- Room SESSION B	MONDAY Stouffer Madison Hotel Federal - Superior Rooms SESSION C	Municipal Room SESSION D	TUESDAY Washington State Convention + Trade Center Rooms 606\607 SESSION E
8:00 AM Registration 8:30 Opening Remarks 9:00 Invited Presentations Animal Rights		8:00 Epidemology	8:00 Protozoa	8:00 President's Symposium AAVP\AVMA Cosponsored
9:50 Coffee		10:00 Coffee	10:00 Coffee	10:00 Coffee
10:05 Mini Symposium Hemoparasites		10:15 Invited Presentation Hydatid Disease 10:45 Chemotherapy-3	10:50 Physiology\Bioch. Molecular Biology	Continued
12.00 LUNCH		LUNCH		12:00 LUNCH
1.00 Invited Presentation Marine Mammal Parasites		1:00 Chemotherapy-4	1:00 Continued 1:50 Immunity	
1:35 Chemotherapy-1	1:35 Genetics		2:30 Sheep,Goats,Swine	
2 10 Invited Presentation History of Dirofilaria				
2.40 Coffee				
3:00 Chemotherapy-2	3:00 Diagnostics	3:00 Coffee	Coffee	
3:55 Hemoparasites		3:20 Clinical\Pathology \Histology	3:15 Continued	
4:30 Presidential Address Awards Business Meeting		4:00 Mini Symposium Lyme Disease		
6:30 AAVP Social		6:30 Fort Dodge Labs Social		

PROGRAM 36TH ANNUAL MEETING

Saturday, July 27, 1991
Stouffer Madison Hotel, Visions Room

7:30 PM AAVP Pre-meeting Mixer; Cash Bar

Sunday, July 28, 1991
Stouffer Madison Hotel, Federal-Superior Rooms

8:00 AM Registration

8:30 Opening Remarks
President Roger Prichard
Vice-President and Program Chairman Ron Fayer

Session A1 - Parasitology Research; Animal Welfare and Animal Rights Issues.
Moderator: Bert Stromberg

9:00 Invited Presentation
1. Conducting research in an era of the changing moral status of animals.
Franklin M. Loew

9:25 Invited Presentation
2. Animals rights versus research.
Kay Holcomb

9:50 **COFFEE**

Session A2 - Hemoparasitic Diseases of Domestic Animals
Moderators: Richard B. Wescott and Mark C. Healy

10:05 3. Problems caused by persistence of rickettsial and protozoal hemoparasites.
T. C. McGuire

10:35 4. Immune responses and persistent rickettsemia in Anaplasma infected cattle.
T. C. McGuire

11:05 5. Antigenic variation and conservation of Babesia merozoite surface proteins.
T. F. McElwain

11:35 6. Immunodominant immune responses to defined epitopes for diagnosis of hemoparasitic infections.
D. P. Knowles

12:00 **LUNCH**

Sunday, July 28, 1991
Stouffer Madison Hotel, Federal-Superior Rooms

Invited Presentation

1:00 7. Important parasites of marine mammals.
Murray Dailey
Moderator: Peter M. Schantz

Session A3 - Chemotherapy 1
Moderator: Jorge Guerrero

1:35 8. Efficacy of ivermectin in an in-feed formulation against natural infestations of Sarcoptes scabiei var suis and Hematopinus suis.
J. E. Holste, D. H. Wallace, A. G. Foster, G. F. Ericsson and S. J. Gross

1:45 9. Efficacy of fluphenacur (Proposed INN), a benzoylphenyl urea insecticide for control of Ctenocepahlides felis infestations on dogs.
B. L. Blagburn, C. M. Hendrix, J. L. Vaughan, D. S. Lindsay and S. Barnett

1:55 10. Safety of permethrin-containing flea and tick control products on cats.
T. A. Miller, M. Macdonald and J. Spano

Stouffer Madison Hotel, Federal-Superior Rooms

Invited Presentation

2:10 11. The history of canine filariasis.
R. A. Roncalli
Moderator: J. P. Dubey

2:40 **COFFEE**

Session A4 - Chemotherapy 2
Moderators: Dwight D. Bowman and Mary Doscher

3:00 12. Dose response of RM 340 against adult heartworms in experimentally infected beagles.
M. T. Dzimianski, T. L. McTier, J. W. McCall, J. Brown and M. Keister

3:10 13. Assessment of effectiveness of RM 340 against Dirofilaria immitis in dogs by monitoring adult heartworm antigen: clinical vs. controlled trial.
J. W. McCall, T. L. McTier, M. T. Dzimianski, K. E. Acre, R. E. Holmes and J. P. Raynaud

Stouffer Madison Hotel, Federal-Superior Rooms

Session A4

3:20 14. Filaricidal activity of the new endectocide, moxidectin, against 1-, 2- and 3-month-old heartworm (Dirofilaria immitis) infections in dogs.
T. L. McTier, M. E. Doscher, I. B. Wood, M. T. Dzimianski and J. W. McCall

3:30 15. Treatment of trichinosis in the dog and cat.
R. A. Darrigrand, D. D. Bowman, M. Frongillo, S. C. Barr, J. E. Flanders and L. G. Carbone

3:40 16. Effects of milbemycin oxime on adult ascarids (Toxocara cati) in cats with naturally acquired infections.
D. D. Bowman, R. B. Johnson, M. Fogelson and D. I. Hepler

3:50 **BREAK**

Session A5 - Companion Animal Hemoparasites
Moderator: Stephen C. Barr

3:55 17. Dogs as long term carriers of Ehrlichia canis and a canine granulocytic agent (CGE).
S. A. Ewing, J. C. Fox, K. M. Kocan and R. W. Barker

4:05 18. New canine babesiosis in California.
P. A. Conrad, J. W. Thomford, I. Yamane, R. Houston and I. Gardner

4:15 19. Radiographic and angiographic findings in early experimental Dirofilaria immitis infections in cats.
R. A. Holmes, J. N. Clark, R. Roberts and R. E. Lewis

4:30 Presidential Address: **Roger Prichard**

4:45 Awards: **K. D. Murrell**, Awards Committee Chairman

5:00 Business Meeting

6:30 AAVP Social - Location to be Announced

Stouffer Madison Hotel, Municipal Room

Session B1 - Host and Parasite Genetics
Moderator: Michael Fleming

1:35 20. Sex-related susceptibility of bulls to gastrointestinal parasites.
R. P. Herd, W. G. Queen and G. A. Majewski

Stouffer Madison Hotel, Municipal Room

Session B1

1:45 21. Selection of beef cattle for high and low fecal EPG values.
L. C. Gasbarre, E. A. Leighton, C. J. Davies and R. B. Brinsfield

1:55 22. Genetic diversity within and among populations of Osertagia ostertagi vs timing of developmental arrest.
M. S. Blouin, J. B. Dame and C. Courtney

Stouffer Madison Hotel, Federal-Superior Rooms

Invited Presentation

2:10 11. The History of Canine Filariasis.
R. A. Roncalli

Moderator: J. P. Dubey

2:40 **COFFEE**

Stouffer Madison Hotel, Municipal Room

Session B2 - Diagnostics, Probes, Antigens
Moderators: Kevin R. Kazacos and Linda M. Aikens

3:00 23. Ten years of testing; trends in diagnostic veterinary parasitology.
A. R. Donoghue, A. J. Murphy, J. L. Gauthier and T. W. Schillhorn Van Veen

3:10 24. Development of ribosomal RNA-based probes for the detection and identification of coccidia.
A. A. Gajadhar and W. C. Marquardt

3:20 25. The cloning and characterization of a repetititve DNA sequence for differentiating sylvatic genotypes of Trichinella spiralis.
D. S. Zarlenga, F. Al-Yaman, D. J. Minchella, G. Larosa and D. Snyder

3:30 26. Phylogenetic relationships among the subfamilies of the trichostrongylidae.
E. P. Hoberg and J. R. Lichtenfels

Stouffer Madison Hotel, Municipal Room

Session B2

3:40 27. Antigenic cross-reactions of Fasciola hepatica with other helminth parasites of sheep in Morocco.
K. Khallaayoune and B. E. Stromberg

3:50 28. Detection of a stable diagnostic antigen from bile and feces of Fasciola hepatica infected cattle.
M. M. El Bahi, J. B. Malone, W. J. Todd, K. L. Schnorr and N. Morris

4:00 29. Differentiating of fasciolid (liver fluke) species by isoelectric focusing.
C. G. Lee, G. L. Zimmerman, D. M. Mulrooney and J. K. Bishop

4:10 30. Lack of specificity of crude antigen preparations in the diagnosis of swine eperythrozoonosis.
N. Nonaka, R. W. Bull and T. W. Schillhorn Van Veen

Stouffer Madison Hotel, Federal-Superior Rooms

4:30 Presidential Address, Awards, Business

Monday, July 29, 1991

Stouffer Madison Hotel/Federal-Superior Rooms

Session C1 - Epidemiology
Moderators: Owen Slocombe and Dan E. Snyder

8:00 AM 31. Cystic hydatid disease in the Xinjiang Uygur autonomous region, PRC.
F.L. Andersen, H.D. Tolley and P.M. Schantz

8:15 32. Overwintering of strongyles from ponies on pasture in Ontario.
J.O.D. Slocombe

8:30 33. The effect of anthelmintic treatment of cows at housing on the epidemiology of nematode infections in a cow-calf herd in Quebec.
S. Ranjan, C. Trudeau, R. Prichard, C. Piche and S. Bauck

8:45 34. Epidemiology of gastrointestinal nematodes in suckling calves in a spring-calving herd in northeast Mississippi.
C.E. Couvillion and R.R. Evans

9:00 35. Prevalence of gastrointestinal nematodes of cattle in south Texas.
T. Jiffar, T. Qurehsi and T.M. Craig

9:15 36. Extrapolation of a soil-based geographic information system (GIS) model for distribution of Fossaria bulimoides habitat on southwest Louisiana coastal marsh.
S.H. Zukowski and J.B. Malone

9:30 37. Use of Landsat MSS imagery and soil type in a geographic information system to assess site-specific fascioliasis risk on Red River Basin farms in Louisiana.
J.B. Malone, D.P. Fehler and A.F. Loyacano

9:45 38. The degradation of dung pats from ivermectin-treated cattle under field conditions.
D.H. Wallace, J.E. Holste, R. Roncalli and S.J. Gross

10:00 **COFFEE**

Stouffer Madison Hotel/Federal-Superior Rooms

10:15 Invited Presentation

39. Role of wildlife in the transmission of hydatid disease sensu lato to indigenous peoples.
Robert Rausch

Moderator: Ferron L. Andersen

10:45 *Session C2 - Equine helminths - Chemotherapy 3*
Moderators: Joseph A. DiPietro and Thomas R. Bello

10:50 40. A technique to measure lawns and roughs in pastures grazed by horses.
K. M. Ewert, J. A. DiPietro and C. S. Danner

11:00 41. Comparison of an interval deworming program using only ivermectin with a rotational interval deworming program in controlling equine endoparasites.
K. M. Ewert, J. A. DiPietro, L. M. Lawrence, D. F. Parrett and K. S. Todd

11:10 42. A comparison of equine parasite control programs utilizing fast rotation or exclusive ivermectin treatment.
D. D. French, T. R. Klei and M. R. Chapman

11:20 43. Alternative antiparasitic treatment of horses with pyrantel and ivermectin oral solutions compared with horses treated only with ivermectin oral solution.
T. R. Bello

11:30 44. Comparison of periodic treatments with ivermectin versus oxibendazole as parasite control programs for horses in southern Texas.
M. G. Scroggs, J. Hawkins, P. Vaden and J. Reid

11:40 45. Anthelmintic resistance on pleasure horse farms in Florida.
D. L. Repeta, N. Birnbaum and C. H. Courtney

11:50 46. Evidence for the lack of effect of ivermectin on encysted cyathastome larvae.
T. R. Klei, M. R. Chapman, D. D. French and H. W. Taylor

Stouffer Madison Hotel/Federal-Superior Rooms

12:00 **LUNCH**

Session C3 - Bovine helminths - Chemotherapy 4
Moderators: James C. Williams and Gerald W. Benz

1:00 PM 47. Response of large-animal veterinarians to a survey of cow-calf endoparasite control in Georgia.
J. A. Stuedemann, H. Ciordia, D. M. Blackmon, E. L. Roberson and M. Mehrban

1:10 48. The effect of parasite control on fertility in beef heifers.
A. M. Zajac, J. W. Hansen, W. D. Whittier and D. E. Eversole

1:20 49. Four years of treating beef cows in North Dakota with ivermectin improved weaning weights of calves from the treated cows.
J. J. Melancon and K. Wohlgemuth

1:30 50. Alternative treatment strategies with ivermectin (IVM) for control of gastrointestinal nematodes of cattle in Louisiana.
J. C. Williams, C. B. Nault and R. T. Ramsey

1:40 51. Effect of grazing systems and deworming programs with ivermectin on parasitemia of stocker calves.
S. E. Marley and R. M. Corwin

1:50 52. Controlled efficacy of ivermectin/clorsulon and other treatments against G.I. nematodes and liver flukes in cattle.
K. M. Newcomb, J. Guerrero and R. Najera

2:00 53. Evaluation of enproal blocks containing Bovatec and Safe-Guard for the improvement of liveweight gains and controlling nematode parasites in grazing cattle.
D. E. Snyder and D. I. Bransby

Stouffer Madison Hotel/Federal-Superior Rooms

Session C3

2:10 54. Efficacy of abamectin against gastrointestinal nematodes and lungworms of cattle.
R. M. Kaplan, C. H. Courtney, Q. Y. Zeng and A. D. Jernigan

2:20 55. Efficacy of morantel tartrate administered in a free choice trace mineral mix.
J. K. Bishop, G. L. Zimmerman, D. M. Mulrooney, D. W. Weber and R. M. Miller

2:30 56. Production of steers and heifers after treatment with oxfendazole.
J. E. Miller and S. Barras

2:40 57. Interaction of oxfendazole and closantel against induced Fasciola hepatica infection superimposed on natural gastrointestinal nematode infections in cattle.
G. L. Zimmerman, L. A. Britt, A. M. Decker, J. A. Whitaker, D. M. Mulrooney, J. K. Bishop and S. E. Knapp

2:50 58. Comparative evaluation of fenbendazole (5 mg/kg) administered in a 1 to 6-day feeding andivermectin (100 to 200 mcg/kg) administered sq.
D. H. Bliss, R. Muser, W. Kvasnicka, L. Krysl, L. Laurence and R. Lastovica

3:00 **COFFEE**

Session C4 - Clinical/Pathology/Histology
Moderator: Robert K. Ridley

3:20 59. Parasitic gastritis in a llama (Lama glama) associated with Teladorsagia spp.
L. G. Rickard

3:30 60. Light and scanning electron microscopy studies on the extrahepatic bile duct of sheep experimentally infected with Fasciola hepatica.
C. G. Lee, G. L. Zimmerman and J. R. Duimstra

3:40 61. Experimental bovine nematodiriasis: development of a model for clinical, pathological and chemotherapeutic studies.
D. E. Worley and F. M. Seesee

Stouffer Madison Hotel/Federal-Superior Rooms

Session C4

3:50 62. Histological and histochemical anlaysis of intestinal epithelium of Oesophagostomum columbianum (nematoda).
M. Johal

Session C5 - Lyme Disease - Mini Symposium
Moderators: B. A. Lissman and R. C. Johnson

4:00 63. Case study: identification of Lyme borreliosis in the dog and its clinical description.
B. A. Lissman

4:30 64. Development of an experimental canine borreliosis model.
L. G. Chavez, H-J. Chu, B. M. Carlson, R. W. Sebring and W. M. Acree

5:00 65. Passive and active immunization of hamsters.
R. C. Johnson

5:30 66. Efficacy study of a Borrelia burgdorferi bacterin for the prevention of Lyme disease in dogs.
H-J. Chu, L. G. Chavez, B. M. Carlson, R. W. Sebring and W. M. Acree

6:00 67. Safety study of a Borrelia burgdorferi bacterin for the prevention of Lyme disease in dogs.
D. R. Hustead, L. G. Chavez, H-J. Chu, B. M. Carlson, R. W. Sebring and W. M. Acree

Stouffer Madison Hotel/North-West Room

6:30 Fort Dodge Laboratories Social

Monday, July 29, 1991
Stouffer Madison Hotel/Municipal Room

Session D1 - Protozoa
Moderators: David S. Lindsay and Kenneth S. Todd

8:00 AM 68. National seroprevalence of Toxoplasma gondii in pigs.
J. P. Dubey, J. C. Leighty, V. C. Beal, W. R. Anderson, C. D. Andrews and Ph. Thulliez

Stouffer Madison Hotel/Municipal Rooms

Session D1

8:10 69. Survival of Neospora caninum cysts in murine tissues.
D. S. Lindsay, B. L. Blagburn and J. P. Dubey

8:20 70. Natural outbreaks of bovine coccidiosis in northern Colorado.
J. M. Vetterling

8:30 71. The role of humoral immunity in age related resistance to Cryptosporidium baileyi.
J. Hatkin, J. J. Giambrone and B. L. Blagburn

8:40 72. Occurrence of Giardia in llamas (Lama glama).
J. A. Jarvinen

8:50 73. Construction of a genomic library of Sarcocystis cruzi sporozoite DNA.
F. S. B. Kibenge, R. J. Cawthorn, D. Despres, P. McKenna and R. J. F. Markham

9:00 74. Antigen analysis of Sarcocystis neurona merozoites cultured from lesions of equine protozoal myeloencephalitis.
D. E. Granstrom, S. W. Davis, J. P. Dubey and P. F. Comer

9:10 75. In vitro cultivation of Sarcocystis neurona, the etiological agent of equine protozoal myeloencephalitis.
S. W. Davis and J. P. Dubey

9:20 76. An update on the control of coccidiosis in game birds.
M. D. Ruff

10:00 **COFFEE**

Stouffer Madison Hotel/Federal-Superior Rooms

10:15 Invited Presentation
39. Role of wildlife in the transmission of hydatid disease sensu lato to indigenous peoples.
Robert Rausch
Moderator: Ferron L. Andersen

Stouffer Madison Hotel/Municipal Room

Session D2 - Physiology, Biochemistry, Molecular Biology
Moderators: Timothy G. Geary and Byron L. Blagburn

10:50 77. Cloning of the gene for the major exsheathment protein from the second molt cuticle of infective Haemonchus contortus larvae.
L. M. Aikens, H. R. Gamble and R. D. Klein

11:00 78. Characterization of cDNA clones encoding putative malic enzymes from Haemonchus contortus.
R. D. Klein, L. Vanover, R. S. Graham, C. A. Winterrowd, M. A. Favreau, S. C. Nulf, S. J. Alexander, N. T. Hatzenbuhler, M. H. Shea and T. G. Geary

11:10 79. Cloning and characterization of phosphoenolpyruvate carboxykinase from the parasitic nematode Haemonchus contortus.
R. D. Klein, C. A. Winterrowd, N. T. Hatzenbuhler, M. H. Shea, M. A. Favreau, S. C. Nulf and T. G. Geary

11:20 80. Development of an in vitro physiological assay to assess effects of experimental agents on adult Haemonchus contortus.
E. M. Thomas, J. P. Davis, T. G. Geary and D. P. Thompson

11:30 81. Interaction of benzimidazole anthelmintics with Haemonchus contortus tubulin: binding affinity and anthelmintic efficacy.
G. W. Lubega and R. K. Prichard

11:40 82. Characterization of plasma-gastrointestinal exchange for albendazole metabolites after administration of netobimin to cattle.
C. Lanusse, L. Gascon, C. Trudeau and R. Prichard

12:00 **LUNCH**

1:00 83. Metabolic labeling of Dirofilaria immitis third- and fourth-stage larvae and the identification of two proteins associated with the molt.
G. R. Frank and R. B. Grieve

1:10 84. Partial characterization of the egg shell of Haemonchus contortus.
H. R. Gamble, L. M. Aikens and R. H. Fetterer

Stouffer Madison Hotel/Municipal Room

Session D2

1:20 85. Synthesis of tyrosine derived cuticular cross-links in nematode larvae.
R. H. Fetterer, M. L. Rhoads and J. F. Urban, Jr.

1:30 86. Mechanistic studies in the transcuticular delivery of antiparastic drugs: Ex vivo/in vitro correlation of solute transport by Ascaris suum.
D. P. Thompson, N. F. H. Ho, S. M. Sims, C. L. Barsuhn and T. G. Geary

1:40 87. Chromatograpic analyses of the lipids of adult Onchocerca gibsoni.
M. D. Maloney and L. H. Semprevivo

Session D3 - Immunity
Moderator: K. Darwin Murrell

1:50 88. In vivo injection of antibodies to interleukin-4 receptor and interferon gamma have opposing effects on immunity to Heligmosomoides polygyrus.
J. F. Urban, Jr., I. M. Katona and F. D. Finkelman

2:00 89. Anti-heart tissue antibodies in Trypanosoma cruzi infected dogs.
S. C. Barr and N. Norcross

2:10 90. A lectin-like eosinophil chemotactic factor in the secretory/excretory substances of a parasitic nematode.
P. Klesius

2:20 91. Further characterization of live subcutaneous postinfective Heligmosomoides polygyrus larval vaccine.
J. P. Tritschler II, L. H. Semprevivo and M. D. Maloney

Session D4 - Parasitology of Sheep, Goats and Swine - Treatment and Control
Moderators: William J. Foreyt and Charles H. Courtney

2:30 92. A survey of ovine parasite control practices in Tennessee.
C. R. Reinemeyer, B. W. Rohrbach, V. M. Grant and G. Radde

Stouffer Madison Hotel/Municipal Room

Session D4

2:40 93. Continual or episodic administration of prolactin alters population indices in ovine infections of Haemonchus contortus.
M. W. Fleming

2:50 94. The effect of diet on the kinetic disposition of oxfendazole in sheep.
D. A. Ali, D. R. Hennessy and J. W. Steel

3:00 **COFFEE**

3:15 95. The efficacy of moxidectin against an invermectin resistant strain of Haemonchus contortus in sheep.
T. M. Craig and T. A. Hatfield

3:25 96. Pharmacokinetics of closantel in goats and sheep.
N. C. Sangster, D. R. Hennessy, J. W. Steel and G. H. Collins

3:35 97. Efficacy of ivermectin against adult and fourth stage Stephanurus dentatus in swine.
R. Alva, A. G. Foster, D. H. Wallace, S. J. Gross and J. W. Wooden

3:45 98. Evaluation of levamisole hydrochloride and two analogs (U-81772 and U-84884A) for activity against parasitic flatworms.
S. S. Johnson, P. J. Diroma, P. M. Guimond, B. H. Lee and G. A. Conder

3:55 99. Efficacy of albendazole in ovine species.
K. L. Wohlgemuth and C. R. Miller

Tuesday, July 30, 1991

Washington State Convention and Trade Center, Rooms 606/607

Session E - AAVP-AVMA Joint Symposium
Diagnosis of Parasitic Infections Using State-of-the-Art Technology.
Moderator: Roger K. Prichard, President
American Association of Veterinary Parasitologists

9:30		Chairman's introductory remarks
9:35	100.	An overview of classic and state-of-the-art methods for diagnosing parasitic infections of veterinary importance. **D. S. Zarlenga**
10:05	101.	Comparative advantages of the new diagnostic technologies for the practitioner. **J. F. Williams and T. W. Schillhorn Van Veen**
10:35	102.	Applications of ribosomal RNA sequences to the diagnosis and phylogenetic analysis of parasites. **J. B. Dame**
11:05		**COFFEE**
11:25	103.	Repetitive DNA sequences: dot blots, RFLPs, probes and others. **B. Dale**
11:55	104.	Cloning and application of genes and gene products coding for diagnostic antigens **M. C. Jenkins**
12:25		Summary **R. K. Prichard**

Abstracts - 36th Annual Meeting

1

CONDUCTING RESEARCH IN AN ERA OF THE CHANGING MORAL STATUS OF ANIMALS. FRANKLIN M. LOEW, TUFTS UNIVERSITY SCHOOL OF VETERINARY MEDICINE. BOSTON AND NORTH GRAFTON, MA. 01536

In the 25 years since the Animal Welfare Act came into being in 1966, public concerns having to do with animals in research have moved from a focus on the acquisition of dogs and cats by research institutions to the types of research actually proposed or conducted. This movement has been accompanied by an elevated moral status of animals in post-rural, urban America. For scientists studying animals in their research, greater care in planning and carrying out their research is required, and they must be prepared to justify their research if challenged.

2

ANIMALS RIGHTS VERSUS RESEARCH. Kay Holcomb, Foundation for Biomedical Research, Washington, D.C. 20006

The animal rights movement is having a significant impact on the conduct of and public perceptions about medical research. The movement's philosophy continues to turn in the direction of antivivisection and the belief that human use of animals - for any purpose - is unnecessary and immoral. Traditional animal welfare concerns are translating into arguments that humans and animals have equivalent rights, with humans having no right to knowledge gained from studies with animals. Assessment of animal rights activities of the last 10 years indicates that the movement has been extremely successful in reaching the public with mixed messages about the necessity and appropriateness of animal research. This success is convincing many in the research and medical communities that educating the public about science and research is imperative.

3

PROBLEMS CAUSED BY PERSISTENCE OF RICKETTSIAL AND PROTOZOAL HEMOPARASITES. TRAVIS C. MCGUIRE*. DEPARTMENT OF VETERINARY MICROBIOLOGY AND PATHOLOGY, WASHINGTON STATE UNIVERSITY. PULLMAN, WA 99164

Several hemoparasitic diseases of animals continue to constrain efficient livestock production in most areas of the world. These diseases are caused by rickettsia (anaplasmosis and cowdriosis) and protozoa (trypanosomiasis, babesiosis and theileriosis). Animals infected with organisms causing these diseases become carriers, a condition that lasts for several years in some cases. Obviously, the causative organisms have mechanisms of evading elimination by host immune responses. In the case of African trypanosomiasis, it is known that antigenic variation of the surface coat protein is the major mechanism of evasion. For organisms in the genera *Anaplasma*, *Cowdria*, *Babesia*, and *Theileria*, mechanisms of persistence have not been clearly defined. However, it is clear that the ability of these organisms to persist in infected animals complicates vaccine development and results in reservoirs of infection for vector transmission.

4

IMMUNE RESPONSES AND PERSISTENT RICKETTSEMIA IN *ANAPLASMA* INFECTED CATTLE. TRAVIS C. MCGUIRE*. DEPARTMENT OF VETERINARY MICROBIOLOGY AND PATHOLOGY, WASHINGTON STATE UNIVERSITY. PULLMAN, WA 99164

Cattle infected with *A. marginale* become chronic carriers, perhaps for life. Blood from six cattle infected with the Florida strain for 5 years transmitted anaplasmosis to splenectomized calves and *A. marginale* DNA was demonstrated in erythrocytes 7 years after infection. These cattle had high antibody titers to several initial body surface proteins including the MSP1 complex and MSP2. Both the native MSP1 complex and native MSP2 induce partially protective immune responses against virulent challenge when used as subunit immunogens. Persistence of rickettsemia, albeit in low levels, demonstrates that the immune response in infected animals cannot eliminate the organism. This observation raises the question of whether it is possible to completely eliminate challenge infections by an immune response induced by vaccination. However, it is known that killed vaccines can induce immunity that will prevent clinical disease and it may be possible to induce defined immune responses that will keep rickettsemia levels below that required for tick transmission.

5

ANTIGENIC VARIATION AND CONSERVATION OF BABESIA MEROZOITE SURFACE PROTEINS. T.F. MCELWAIN*. DEPT. OF VETERINARY MICROBIOLOGY AND PATHOLOGY. WASHINGTON STATE UNIVERSITY. PULLMAN, WA 99164-7040.

Infection of cattle with Babesia bigemina and Babesia bovis induces a persistent carrier state during which carrier animals are protected against clinical disease after virulent challenge. One of the potential targets of the protective immune response is the surface of extracellular merozoites. A total of five polypeptides each have been identified on the surface of Mexico isolate B. bigemina (72, 58, 55, 45, and 36 kDa) and B. bovis (225, 60, 44, 42, and 16 kDa) merozoites with monoclonal antibodies. Each of these molecules contains epitopes exposed on the surface of intact, infectious merozoites. The 42 and 44 kDa B. bovis, and 55 and 45 kDa B. bigemina surface polypeptides are neutralization sensitive glycoproteins that are diffusely distributed in the merozoite surface coat. Extensive antigenic diversity of all four of these merozoite surface proteins has been detected among different geographic isolates. For example, four independent epitopes on the 42/44 kDa major merozoite surface protein of Mexico isolate B. bovis are not detectable in Australia or Israel isolates. Southern hybridization and DNA sequence comparisons indicate a potential mechanism of antigenic variation that is unique in these parasites. In contrast to these variant polypeptides, the 58 kDa B. bigemina protein and the 225, 60, and 16 kDa B. bovis proteins are expressed in a polar, punctate fashion on the surface of the merozoite, and are antigenically invariant in all isolates tested to date. The potential role of antigenic variation in the persistence of these parasites in their host will be discussed.

6

IMMUNODOMINANT IMMUNE RESPONSES TO DEFINED EPITOPES FOR DIAGNOSIS OF HEMOPARASITIC INFECTIONS

DONALD P. KNOWLES, JR., USDA-ARS, WASHINGTON STATE UNIVERSITY, PULLMAN, WASHINGTON 99164-7040

Rapid and accurate detection of animals infected with hemoparasites would enhance control procedures and evaluation of vaccines. Current tests based on the detection of serum antibodies are not widely used for several reasons, including the occurrence of either false positive or false negative results. Through examination of the antibody response to the erythrocytic stages of hemoparasitic diseases such as Anaplasma marginale and Babesia equi, proteins have been identified to which the infected hosts react with high antibody titers. Purification of these proteins and their use as subunit antigens in diagnostic tests will potentially improve the accuracy of these tests.

7

PARASITIC DISEASE PROBLEMS IN MARINE MAMMALS. M. D. Dailey, California State University, Long Beach, CA 90840-0101.

Marine mammals are heavily parasitized. Over 200 species of helminths have been recorded from these animals with very little information known about all but a few. The nematodes of both pinnipeds and cetaceans not only create severe problems for the marine host but certain species also involve man. Trematodes are known to play a role in single strandings of cetaceans along the California coast. Members of other parasitic groups such as cestodes, acanthocephala and anthropods also affect both wild and captive populations.

8

EFFICACY OF IVERMECTIN IN AN IN-FEED FORMULATION AGAINST NATURAL INFESTATIONS OF *SARCOPTES SCABIEI VAR SUIS* AND *HEMATOPINUS SUIS*. J.E. HOLSTE*, D.H. WALLACE, A.G. FOSTER, G.F. ERICSSON AND S.J. GROSS, ANIMAL SCIENCE RESEARCH, MERCK SHARP AND DOHME RESEARCH LABORATORIES, P.O. BOX 2000, RAHWAY, NJ 07065.

The efficacy of ivermectin as an in-feed formulation was evaluated in pigs naturally infested with the two most common ectoparasites of swine.

One study was conducted as a dose titration trial against *Sarcoptes scabiei var suis*, the dose-limiting ectoparasite of swine for ivermectin injectable. Treated animals were given 0.6% w/w premix in the basal ration at a level designed to deliver ivermectin at 0, 50, 100 or 200 mcg/kg body weight per day. These levels approximated 0, 1, 2 and 4 ppm ivermectin in the feed. Pigs were treated in the feed for seven days, after which time they had free access to nonmedicated basal ration until day 42, the end of the study. Except for one pig in the 50 mcg/kg/day group, all ivermectin-treated pigs were free of mange seven days after the end of the treatment period, and all treated pigs were free of mange on day 42.

A second study was conducted with 56 pigs housed 4 pigs per pen in 7 replicates of pens. Treated pigs were given ivermectin in the feed at 2 ppm for 7 days. No live mange mites were found on treated pigs from day 14 through trial termination on day 42.

Natural louse populations of *H. suis* were used in two studies in which treated pigs were fed ivermectin at 2 ppm in the feed *ad libitum* for seven days. Numbers of live lice on treated animals were reduced to zero by the end of the treatment period and remained at zero for at least seven days after treatment. By 21 days after the end of treatment, a few lice were seen on treated animals.

9

EFFICACY OF FLUPHENACUR (PROPOSED INN), A BENZOYLPHENYL UREA INSECTICIDE FOR CONTROL OF *CTENOCEPHALIDES FELIS* INFESTATIONS ON DOGS.

B. L. BLAGBURN*, C. M. HENDRIX, J. L. VAUGHAN, D.S. LINDSAY, AND S. BARNETT. AUBURN UNIVERSITY, AL 36849 AND CIBA-GEIGY CORPORATION, GREENSBORO, NC 27410.

The benzoylphenyl ureas are ovicidal and larvicidal compounds with demonstrated activity against a variety of insects. They are known to interfere with either the formation or deposition of chitin within developing insect eggs or larvae. We herein describe the efficacy of fluphenacur against the cat flea *Ctenocephalides felis* in dogs. Twenty-four, mature, purpose-bred, female Beagle dogs were divided into 8 groups of 3 dogs each. Each group of 3 dogs was housed in an outside sand and gravel run that was attached to a building providing an indoor habitat similar to owner residences. Dogs in all groups were placed on indoor/outdoor cycles of 12 hours each and provided food and water *ad libitum*. Dogs in groups 2,4,6 and 8 were treated orally with fluphenacur tablets at the rate of 10 mg/kg BWT on days 7, 37, 68, and 98 of the study. Dogs in groups 1,3,5 and 7 served as controls and as such were given placebo tablets on the same days as treated dogs. All dogs were infested with 100 newly emerged, unfed, insectary-reared adult *C. felis* on days 0, 2, and 6. The numbers of adult *C. 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. After enumeration, fleas were returned to their respective hosts. 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. Fluphenacur was safe and demonstrably effective in controlling *C. felis* infestations on dogs when administered at monthly intervals at a dosage of 10 mg/kg BWT.

10

SAFETY OF PERMETHRIN-CONTAINING FLEA AND TICK CONTROL PRODUCTS ON CATS.

T.A.MILLER*, VIRBAC-ALLERDERM, FORT WORTH, TX. 76118, J.MACDONALD & J.SPANO, COLLEGE OF VETERINARY MEDICINE, AUBURN UNIVERSITY, AL. 36849.

Permethrin has become a common active ingredient in flea and tick control products labelled for application to companion animals. All permethrin-containing products are labelled for dogs but there is clearly disagreement over treating cats with this active ingredient. Some product labels specifically contraindicate cats, some claim dogs and are silent on cats and some claim both dogs and cats without reservations.

The results of three studies, in which cats were treated topically with alcohol or water-based, multiple active ingredient flea and tick sprays, repeated frequently over several weeks or months, provided no evidence of adverse effects, either systemic or local. These findings do not, however, preclude the possibility of idiosyncratic adverse reactions in individual cats, especially in excessively fastidious self-grooming cats and in the more exotic breeds of cats that have reputations for adverse reactions.

11

THE HISTORY OF CANINE FILARIASIS.

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In all the ancient veterinary literature there is no description of the presence of worms in the heart of dogs. The first account of this occurrence was given by Panthot, a French physician, in 1679. In the U.S.A. the first report on heartworms in dogs was published in 1847 by Osborne. Between 1850 and 1880, Leidy, a physician from Philadelphia, published several reports on the occurrence of heartworms in dogs in the U.S.A.; also, he coined the name of Filaria immitis for this species of worms. The elucidation of the life cycle of heartworms took about 100 years. It was started by Gruby and Delafond in 1842 and completed by Kume and Itagaki in 1955. Otto and Maren were responsible for the development of the arsenical therapy for the treatment of adult heartworms (1947). Kume et al. developed a method to prevent heartworm disease in dogs by using daily dosages of D.E.C. (1962). Twenty-five years later with the introduction of ivermectin into the market, a significant advance was achieved with the reduction of the prophylactic treatment from once a day to once a month.

12

DOSE RESPONSE OF RM 340 AGAINST ADULT HEARTWORMS IN EXPERIMENTALLY INFECTED BEAGLES.

M.T. DZIMIANSKI,*[1] T.L. MCTIER,[1] J.W. MCCALL,[1] J. BROWN,[1] AND M. KEISTER.[2] [1]UNIVERSITY OF GEORGIA, ATHENS, GA. [2]RHONE MERIEUX, ATHENS, GA.

RM 340 has shown high efficacy against 4-month-old immatures and 7 to 12-month-old adults of Dirofilaria immitis when given as two deep IM injections to experimentally infected dogs in controlled trials. In the present experiment, a dose titration was conducted to determine the efficacy of various doses of RM 340 administered as 2 IM injections 24 hours apart. Thirty heartworm-naive beagles were each infected with 20 adult heartworms (10 males, 10 females) by IV transplantation. The dogs were randomly allocated to 5 groups of 6 dogs each. One group served as a sham treated control (sterile 0.9% saline), while the remaining 4 groups were given 2 injections of RM 340 at 2.8, 2.5, 2.2, or 1.9 mg/kg. Treatment was initiated when the approximate age of the transplanted heartworms was 8 months. The dogs were necropsied 8 weeks posttreatment. At necropsy, all of the control dogs had heartworms, with an average of 19.7 and a range of 19 to 20. In the dogs treated with RM 340, all of the male heartworms were killed at all dose levels. The percent reduction in female heartworms was 100, 81.4, 69.5, and 74.6 for 2 injections of 2.8, 2.5, 2.2, and 1.9 mg/kg, respectively. The number of dogs cleared of heartworms in each group of 6 dogs treated with RM 340 at 2.8, 2.5, 2.2, and 1.9 mg/kg were 6, 3, 3, and 2, respectively. A dose of RM 340 (x 2) that would kill 100% of the adult heartworms (95% confidence interval) was estimated by regression to be 2.64 ± 0.91 mg/kg. Using the probit method, the effective dose was calculated to be 2.77 ± 0.37 mg/kg.

13

ASSESSMENT OF EFFECTIVENESS OF RM 340 AGAINST DIROFILARIA IMMITIS IN DOGS BY MONITORING ADULT HEARTWORM ANTIGEN: CLINICAL VS. CONTROLLED TRIAL. J.W. MCCALL,*[1] T.L. MCTIER,[1] M.T. DZIMIANSKI,[1] K.E. ACRE,[2] R.E. HOLMES,[3] AND J.P. RAYNAUD.[4] [1]UNIVERSITY OF GEORGIA, ATHENS, GA. [2]ACRE FARM, EUSTIS, FL. [3]LOUISIANA STATE UNIVERSITY, BATON ROUGE, LA. [4]RHONE MERIEUX FRANCE.

A highly reliable and convenient immunodiagnostic test for use in assessing the effectiveness of adulticidal treatment for heartworms (HW) is needed by research scientists and veterinarians. In view of this, the effectiveness of 2 deep IM (lumbar muscles) injections of RM 340, melarsomine, given 3 hr apart against HW in dogs was evaluated by monitoring adult HW antigen (Ag) in serum using an ELISA test (Unit-Tec™ CHW, Pitman-Moore Co., Terre Haute, IN). In a clinical trial with 7 HW Ag-positive (+) dogs, 5 were Ag-negative (-) 3 months (mos.) after treatment. In a field study with 10 HW Ag+ dogs exposed to vector mosquitoes in GA and treated at the middle of the mosquito season (MS) (= Aug.) and again after the end of the MS (= Dec., 4 mos. later), only 1 was Ag+ 4 mos. after the first treatment, and none was Ag+ 4 mos. after the second treatment. In a large controlled trial with HW-naive beagles exposed to vector mosquitoes in GA, FL, and LA and treated tactically (i.e., mid-MS = Aug. and again after the end of the MS = Dec.), none of 14 treated dogs was Ag+ 4 to 5 mos. after the second treatment, and none of these 14 dogs had worms at necropsy, whereas, 8 of 8 control dogs with adult worms (ave., 19.1; range, 5-45) were Ag+ at this time. The use of RM 340 given as 2 IM injections of 2.2 mg/kg 3 hrs apart gave good results in terms of (1) curing the infection and (2) controlling the effects of seasonal contamination by tactical treatment (mid-MS = Aug. and again after the end of the MS = Dec.). These data lend further support for the use of ELISA HW diagnostic tests for more objective assessment of HW infection and cure by an adulticide, in experiments and in clinical evaluations.

14

FILARICIDAL ACTIVITY OF THE NEW ENDECTOCIDE, MOXIDECTIN, AGAINST 1-, 2-, AND 3-MONTH-OLD HEARTWORM (DIROFILARIA IMMITIS) INFECTIONS IN DOGS. T.L. MCTIER,*[1] M.E. DOSCHER,[2] I.B. WOOD,[2] M.T. DZIMIANSKI,[1] AND J.W. MCCALL.[1] [1]UNIVERSITY OF GEORGIA, ATHENS, GA. [2]AMERICAN CYANAMID COMPANY, PRINCETON, NJ.

Moxidectin, a derivative of the new broad-spectrum endectocide, nemadectin, was examined for prophylactic activity against 1-, 2-, and 3-month-old D. immitis in dogs. In a series of 4 controlled trials involving a total of 120 beagles, the dogs were experimentally infected with 50 L_3 each in the inguinal area. Dogs were randomly allocated into groups of 4 or 5 animals. Moxidectin was 100% effective against D. immitis when given as a single oral treatment at (1) 1.25, 2.5, 5.0, 10.0, and 50.0 mcg/kg body weight at 1 month postinoculation (PI) and (2) 0.5, 0.625, 1.25, 2.5, 5.0, 10.0, and 50.0 mcg/kg at 2 months PI. Efficacy at all dose levels, except 0.5, 0.625, and 50.0 mcg/kg, was confirmed in two or more experiments. When moxidectin was given at 3 months PI at 1.0 and 3.0 mcg/kg, it gave substantial, but not significant (i.e., $P > 0.05$, one-way ANOVA), reductions of 47.8% and 64.2%, respectively. Average recoveries of adult heartworms from the control dogs in the 4 trials ranged from 13.4 to 25.2. In conclusion, moxidectin appears to be the most potent prophylactic filaricide against D. immitis yet reported.

15

TREATMENT OF TRICHINOSIS IN THE DOG AND CAT. R.A. DARRIGRAND*, D.D. BOWMAN, M. FRONGILLO, S.C. BARR, J.E. FLANDERS, AND L.G. CARBONE. CORNELL UNIVERSITY. ITHACA, NY 14853.

Specific-pathogen-free Beagle puppies (6) and shorthair kittens (6) were inoculated with 7,500 first-stage larvae of Trichinella spiralis. Physical examinations (including the collection of blood and fecal samples) were performed weekly. Shortly post-inoculation (PI), all animals showed mild gastrointestinal disturbances, but no stages of T. spiralis were ever noted in the feces. Ten days PI, 3 puppies and 3 kittens were treated with 1.25 mg/kg milbemycin oxime twice a day for 10 days. Muscle biopsies were taken from the dogs and cats on days 26 and 29 PI. The mean numbers of larvae per gram of muscle were 30.3 in the control and 37.7 in the treated dogs. The mean numbers of larvae per gram of muscle in the control and treated cats were 318.7 and 89.3, respectively. Two dogs and two cats were removed from the study at this time. The remaining animals, 2 each of the control and milbemycin oxime treated animals, were given albendazole (50 mg/kg, per os, twice a day, for 7 days starting at 31 and 34 days PI in dogs and cats, resp.). Muscle biopsies were again taken at 46 and 49 days PI, for the dogs and cats, resp.; the mean number of larvae recovered from muscle was 0.56 for the dogs and 13.46 for the cats. These results indicate that albendazole is capable of significantly reducing the number of larvae of T. spiralis present within the muscles of the canine or feline host.

16

EFFECTS OF MILBEMYCIN OXIME ON ADULT ASCARIDS (TOXOCARA CATI) IN CATS WITH NATURALLY ACQUIRED INFECTIONS. D.D. BOWMAN*, R.B. JOHNSON, M. FOGELSON, AND D.I. HEPLER. CORNELL UNIVERSITY, ITHACA, NY 14853 AND CIBA-GEIGY CORPORATION, GREENSBORO, NC, 27419.

Milbemycin oxime is licensed for use in dogs for the prevention of heartworm (Dirofilaria immitis) infection and for the control of hookworms (Ancylostoma caninum). Other studies have shown it to be highly efficacious in the dog for the removal of adult Toxocara canis, Toxascaris leonina, and Trichuris vulpis. The purpose of this study was to determine the efficacy of milbemycin oxime in removing adult Toxocara cati from cats with naturally acquired infections. There were 17 cats with naturally acquired infections with ascarids and other parasites in the study. Egg counts made on the feces of each cat were used to rank the cats from highest to lowest based on the number of T. cati eggs in the feces. Nearest rank pairs were then randomly assigned to one of two groups. One group was treated with milbemycin oxime at a minimum dose of 0.5 mg/kg. Milbemycin oxime at this dosage was shown to be 100% efficacious in the removal of adult ascarids (T. cati). It was also found to be 90.0% efficacious in the removal of adult hookworms, Ancylostoma tubaeforme.

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DOGS AS LONG TERM CARRIERS OF EHRLICHIA CANIS AND A CANINE GRANULOCYTIC AGENT (CGE). S. A. EWING*, J. C. FOX, K. M. KOCAN AND R. W. BARKER. OKLAHOMA STATE UNIVERSITY. STILLWATER, OK 74078.

Classic canine ehrlichiosis, caused by Ehrlichia canis, is a tick-borne rickettsial disease characterized by an acute febrile phase with thrombocytopenia and a chronic phase that may involve spontaneous hemorrhage. Canine granulocytic Ehrlichia (CGE) has been associated with a milder acute disease and sometimes with a polyarthritic syndrome that may accompany a chronic phase. Transstadial, but not transovarial, transmission of E. canis by Rhipicephalus sanguineus has been recognized for decades. More recently, Dermacentor variabilis has been shown to transmit E. canis under experimental conditions; and transstadial transmission of CGE by Amblyomma americanum has been demonstrated in our laboratory. Difference of opinion exists concerning whether dogs or ticks are more important as reservoir hosts for canine ehrlichiosis. We report a series of experiments involving E. canis and CGE in which dogs have remained carriers for several months to a few years. Blood from dogs that remained asymptomatic for months or years was almost uniformly infectious for susceptible pups. One exceptional dog whose blood was infectious for 15 other dogs over a 10-month period appeared to lose infectivity by 21 months although she maintained high antibody titer. Attempts to transmit ehrlichial infections via three-host ticks that fed on dogs in acute and convalescent stages of infection will be discussed.

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NEW CANINE BABESIOSIS IN CALIFORNIA. P.A. CONRAD*, J.W. THOMFORD, I. YAMANE, R. HOUSTON, AND I. GARDNER. UNIVERSITY OF CALIFORNIA. DAVIS, CA 95616-8738.

In 1988, a small babesial parasite, Babesia gibsoni, was identified for the first time in California where it is being naturally transmitted by ticks to dogs which have no history of travel outside of the state. This is the first report of natural transmission and multiple cases of B. gibsoni infections in the United States. B. gibsoni causes an acute, hemolytic anemia with splenomegaly, icterus and hemoglobinuria. Acute clinical cases are often misdiagnosed as autoimmune hemolytic anemia due to the fact that infected dogs may test Coomb's positive. Both acute and chronic cases of B. gibsoni-infected dogs will be presented. Based on these studies, recommendations on how to diagnose and treat this new disease will be discussed. In addition, the results of our recent epidemiological studies on the prevalence of canine babesiosis in California will be presented.

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RADIOGRAPHIC AND ANGIOGRAPHIC FINDINGS IN EARLY EXPERIMENTAL *DIROFILARIA IMMITIS* INFECTIONS IN CATS. R.A. HOLMES*, J.N. CLARK, R. ROBERTS, R.E. LEWIS. LOUISIANA STATE UNIVERSITY, BATON ROUGE, LA 70803, MERCK RESEARCH LABORATORIES, RAHWAY, NJ 77777, AND UNIVERSITY OF GEORGIA, ATHENS, GA 30602.

Radiographic and angiographic studies have been done in cats, but only after infections have been established for some period of time. This study was done to document the cardiovascular and pulmonary changes when the larvae first invade the heart and arteries.

Three male and 3 female mixed breed cats were obtained from a commercial laboratory. Digital subtraction angiograms and thoracic radiographs were made on days 0, 75, 90, 120, 150, 180, and 210 after the inoculation of 100 infective larvae (L3) of *Dirofilaria immitis*. One of the cats had severe blockage of the caudal lobar pulmonary arteries resulting in the lack of perfusion distal to the blockage. Right ventricular and pulmonary artery enlargements were obvious on radiographs. Four of the 6 cats had some degree of blockage of the caudal lobar arteries, but not to the degree as the first. Radiographic lesions also were not as severe as the first but were noticeable. One of the cats had very mild lesions both angiographically and radiographically. It is apparent that the lesions due to *D. immitis* in the cat can be severe when the larvae initially enter the heart and pulmonary vessels.

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SEX-RELATED SUSCEPTIBILITY OF BULLS TO GASTROINTESTINAL PARASITES. R.P. HERD*, W.G. QUEEN, AND G.A. MAJEWSKI. THE OHIO STATE UNIVERSITY. COLUMBUS, OH 43210.

Cattle sex is rarely taken into consideration in the design of parasite control programs, yet a sex-related susceptibility of young bulls to nematode parasites and a significantly greater weight gain response to anthelmintic treatment of bulls compared with steers or heifers has been demonstrated in Australia (Copeman & Hutchinson, 1979).

In a preliminary study in Ohio, the effect of sex and hormonal implants were examined in 77 naturally infected yearling bulls, steers, and heifers of the Aberdeen Angus, Charolois, Hereford, and Limousin breeds. Fecal egg counts were compared in 7 groups of 10-14 animals each, all grazing the same contaminated pasture in the spring of 1990. Comparisons were made between untreated bulls, steers, and heifers, and between steers with and without hormonal implants, and between heifers with and without hormonal implants.

Bulls showed significantly ($p < 0.01$) higher fecal egg counts than steers and heifers. The results confirmed the studies of Copeman & Hutchinson (1979) and indicated the need to pay special attention to parasite control programs for young bulls. These results have important implications for the design of cattle research projects with mixed sexes, and for the evaluation of worm population data. The use of hormonal implants had no effect on fecal egg counts.

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SELECTION OF BEEF CATTLE FOR HIGH AND LOW FECAL EPG VALUES. L.C. GASBARRE*, E.A. LEIGHTON, C.J. DAVIES AND R.B. BRINSFIELD. USDA, ARS, LPSI, HELMINTHIC DIS. LAB., BELTSVILLE, MD 20705, WASHINGTON CONSULTING GROUP, WASHINGTON, DC., AND MD AGRI. EXP. STATION, WYE RES. AND ED. CTR.

Previous studies had indicated that the fecal trichostrongyle eggs per gram (epg) values of calves at the end of their first grazing season are significantly affected by host genetics. To expand these observations and to assess the role of genes of the bovine lymphocyte antigen (BoLA) complex in resistance/susceptibility, 2 different selective breedings were done to produce calves with either "high" or "low" epg values at weaning. In the first breeding, semen from bulls expected to produce "high" or "low" epg calves was used to inseminate randomly chosen cows. Bulls were selected based upon the estimated breeding value (EBV) for fecal epg values. In the second breeding, bulls and cows were bred to produce calves of a specific BoLA genotype. At weaning, calves from "high" sires passed approximately 3 times as many eggs as calves from "low" sires (i.e., high = 703, low = 250). This difference appeared to be due to the "low" calves ability to regulate epg values as the summer progressed. In contrast, "high" epg calves continued to pass increasing numbers of eggs throughout the summer. Calves produced based on BoLA type showed little or no differences in epg values. These results demonstrate that host genetics play a major role in the dynamics of trichostrongyle parasite transmission. The BoLA complex may be a component of this control, but current results indicate that BoLA effects are relatively minor.

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GENETIC DIVERSITY WITHIN AND AMONG POPULATIONS OF OSTERTAGIA OSTERTAGI VS TIMING OF DEVELOPMENTAL ARREST. Michael S. Blouin, John B. Dame, and C. Courtney, Department of Infectious Diseases, Bldg. 471, Mowry Rd., Univ. Florida, Gainesville, FL, 32611-0633.

Northern and southern populations of Ostertagia ostertagi show a genetically-based difference in the timing of developmental arrest(winter vs summer). We sampled 10 individual adult female Ostertagia ostertagi from each of two northern populations (Minnesota and Maine) and three southern populations (Tennessee, Alabama, and Louisiana), and scored each worm for the presence/absence of 37 mtDNA restriction sites. Results: (1) The estimates of within-population differentiation are 5 to 10 times greater than typical estimates reported for species in other taxa. (2) Ninety eight percent of the total genetic diversity is distributed within populations (rather than between regions, or among populations within regions). Winter- and summer-arresting O. ostertagi are, therefore, not highly differentiated, and there is certainly no evidence that the two groups are reproductively isolated. Efforts to find unique genetic markers that will distinguish between summer- and winter-arresting forms of O. ostertagi will require identification of the genes involved in developmental arrest.

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TEN YEARS OF TESTING; TRENDS IN DIAGNOSTIC VETERINARY PARASITOLOGY. A.R. DONOGHUE*, A.J. MURPHY, J.L. GAUTHIER AND T.W. SCHILLHORN VAN VEEN. MICHIGAN STATE UNIVERSITY. EAST LANSING, MI 48824.

In 1980 Michigan State University's Animal Health Diagnostic Lab established a diagnostic service in veterinary parasitology. Its primary function was to provide diagnostic laboratory services to veterinarians and animal owners in Michigan and surrounding states. The initial tests offered included fecal examination of large animals, parasite identification and serological testing for toxoplasmosis and heartworm disease. Added later were tests for eperythrozoonosis, Potomac Horse Fever and Lyme borreliosis, The number of tests performed annually increased from 1600 in 1983 to 7000 in 1989.

During the decade there were major changes in the requests for diagnostic service. The changes were influenced by the following factors: a. public awareness of a certain disease (lyme borreliosis is an example), b. new diagnostic technologies (dirofilariasis for example), c. changes in the disease control methods (e.g. swine eperythrozoonosis) and d. awareness of the service offered.

Major challenges of the diagnostic service were the quality control of the tests performed and provision for telephone consultations on both test interpretation and further action (i.e. treatment). Compilation of the data, by state or county, provided useful epidemiological information regarding the incidence and geographical distribution of infected animals.

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DEVELOPMENT OF RIBOSOMAL RNA-BASED PROBES FOR THE DETECTION AND IDENTIFICATION OF COCCIDIA. A.A. GAJADHAR, HEALTH OF ANIMALS LABORATORY, AGRICULTURE CANADA, SASKATOON, CANADA S7N 2R3, AND W.C. MARQUARDT, COLORADO STATE UNIVERSITY, FORT COLLINS, CO 80523.

Two regions of the primary structure of the small subunit rRNA of Sarcocystis muris bradyzoites were compared with nucleotide sequences of S. gigantea, Toxoplasma gondii, Plasmodium berghei and Mus musculus and used to design taxonomically specific probes for the detection and identification of coccidia. Twenty-base oligomers were labelled with ^{32}P and used in hybridation procedures. Total cellular RNA of purified S. muris, S. cruzi, T. gondii and Eimeria nieschulzi and coccidia-infected tissues of mice, cattle, sheep and swine, were assayed. One probe detected only S. muris and another successfully hybridized to several members of coccidia, including S. muris, S. cruzi, T. gondii and E. nieschulzi. One ng of total cellular RNA was sufficient to yield detectable hybrids in slot blot assays. The high sensitivity suggests that rRNA-based probes are capable of detecting individual parasites, and can assay low levels of coccidial infections not detectable by other methods. The results of this study show that it is possible to customize the specificity of rRNA-based probes for diagnostic, epidemiologic or taxonomic purposes.

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THE CLONING AND CHARACTERIZATION OF A REPETITIVE DNA SEQUENCE FOR DIFFERENTIATING SYLVATIC GENOTYPES OF TRICHINELLA SPIRALIS. D.S. ZARLENGA*, F. AL-YAMAN, D.J. MINCHELLA, G. LAROSA AND D. SNYDER. USDA, BIOSYSTEMATIC PARASITOLOGY LAB, BELTSVILLE, MD 20705.

A partial genomic DNA library constructed in pUC 13 using DNA from a sylvatic isolate of Trichinella spiralis (T. spiralis T5) was differentially screened with radiolabeled homologous genomic DNA and with DNA from T. spiralis T1 (pig genotype). One clone was identified and designated pUPB 3.7 which, by slot blot and Southern blot analyses, reacted specifically with T. spiralis T5 DNA and did not cross-react with DNA from any other T. spiralis genotype. The 482 base pair repetitive sequence which is 70% rich in A and T residues, comprises at least 2.7% of the parasite genome and can detect as little as 0.4 ng of DNA. When used to assess the prevalence of T. spiralis T5 within Indiana wildlife, DNA from 19 of 20 independently obtained sylvatic isolates reacted positively with the pUPB 3.7 probe. Similarly, 5 isolates obtained from Illinois raccoon also hybridized to the pUPB-3.7 probe. None of those DNAs which reacted with the pUPB-3.7 probe hybridized to the T. spiralis T1 specific sequence, pBP-2. Results indicate that T. spiralis T5 is well disseminated within the more temporate zones of North America and that within these geographical localities, T. spiralis T5 maybe the predominating genotype in wild mammals.

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PHYLOGENETIC RELATIONSHIPS AMONG THE SUBFAMILIES OF THE TRICHOSTRONGYLIDAE. E.P. HOBERG* AND J.R. LICHTENFELS. AGRICULTURAL RESEARCH SERVICE, BIOSYSTEMATIC PARASITOLOGY LABORATORY, BELTSVILLE, MD., 20705.

Phylogenetic studies among the six subfamilies of the Trichostrongylidae were initiated to provide a basis for a natural classification. Such a classification, representing hierarchical genealogical relationships, is important as the precursor for enhancing our understanding of parasite behavior, host-parasite evolution, and parasite biogeography among these economically significant nematodes. Analyses of 23 series of homologous morphological characters, primarily attributes of the synlophe and bursa, representing 27 states (polarized by taxonomic outgroup - Strongyloidea) were conducted with PAUP 2.4. A single cladogram (CI= 77.1%) recognized two major clades sharing a sister-group relationship and included 1) Cooperiinae, Libyostrongylinae, and Trichostrongylinae and 2) Graphidiinae, Ostertagiinae, and Haemonchinae. Members of the Graphidiinae-clade are restricted to the stomach or abomasum, whereas those of the Cooperiinae-clade parasitize either the intestine or stomach/abomasum of their hosts. Host-distribution appears consistent with a history of colonization with subsequent coevolution.

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ANTIGENIC CROSS-REACTIONS OF FASCIOLA HEPATICA WITH OTHER HELMINTH PARASITES OF SHEEP IN MOROCCO. K. KHALLAAYOUNE AND B.E. STROMBERG*. INSTITUT AGRONOMIQUE ET VETERINAIRE HASSAN II. RABAT, MOROCCO AND UNIVERSITY OF MINNESOTA. ST. PAUL, MN 55108.

An ELISA was developed to be used to diagnose sheep infected with Fasciola hepatica in Morocco. A crude extract of adult F. hepatica was separated on G200 Sephadex and the four fractions obtained were evaluated for activity in naturally infected sheep. Fraction I demonstrated the strongest activity with the least reactivity with normal sheep sera. Sheep in Morocco are naturally infected with several other helminths that may cross react with the liver flukes. When an extract of F. hepatica was reacted with sheep experimentally infected with Echinococcus granulosus, Cysticercus tenuicollis or Haemonchus contortus there were cross reactions with E. granulosus and C. tenuicollis. No cross reactivity was observed with the sera from sheep infected with H. contortus. Sera from sheep experimentally infected with F. hepatica were evaluated for cross reactivity with antigenic preparations from E. granulosus (hydatid fluid), C. tenuicollis, Moniezia expansa, H. contortus and Oestrus ovis. The fluke positive sera reacted with E. granulosus, C. tenuicollis and M. expansa at titers greater that 100 in 2, 1 and 7 of 18 sheep, respectively. No cross reactivity was observed with the H. contortus or O. ovis. These studies demonstrate that positive serological results from naturally infected animals may be very misleading.

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DETECTION OF A STABLE DIAGNOSTIC ANTIGEN FROM BILE AND FECES OF FASCIOLA HEPATICA INFECTED CATTLE. M.M. EL BAHI, J.B. MALONE, W.J. TODD* K.L. SCHNORR AND N. MORRIS. LOUISIANA STATE UNIVERSITY, BATON ROUGE, LA 70803

Diagnostic antigens from bile and feces from F. hepatica infected cattle were detected and characterized by SDS-PAGE and enzyme-linked immunotransfer blot (EITB) techniques. Samples of bile and feces were collected from 5 uninfected calves and from 10 infected calves for which F. hepatica burdens were known. Samples of bile and feces (mixed in equal volumes of water) were dialyzed in saline and concentrated by absorbtion against polyvinyl pyrollidone. A band detected by EITB (using a densitometer) in the area corresponding to 26 KD reacted with rabbit anti-fresh fluke antigen and infected cattle sera but not with negative rabbit sera, rabbit anti-F. hepatica egg sera, Fascioloides magna positive cattle sera or negative cattle sera. This band was not detected by Coomassie blue in SDS-PAGE gels or by Ponceau-S stained nitrocellulose strips. Band groups located at 104-66, 66-42, 42-26 and 25-16 KD reacted inconsistently with the above sera. Sera from mice hyperimmunized with F. hepatica ES products detected only the 26 KD band by EITB, without cross-reactivity with bands in the other MW ranges. Results suggest the 26 KD antigen may consist of a stable component of ES products and/or tegument related worm antigen. Diagnosis of F. hepatica through detection of specific, stable antigens in feces of infected animals offers the potential advantages over serum-based tests of better sample accessibility, detection of current vs historical infections, and possible semi-quantitation of fluke burdens. Patent pending, Louisiana State University.

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DIFFERENTIATION OF FASCIOLID (LIVER FLUKE) SPECIES BY ISOELECTRIC FOCUSING. C.G. LEE, G.L. ZIMMERMAN, D.M. MULROONEY* AND J.K. BISHOP. COLLEGE OF VETERINARY MEDICINE, OREGON STATE UNIVERSITY, CORVALLIS, OR 97331.

Electrophoretic analysis of tissue proteins has been used as an aid to taxonomy in various organisms. Of the various electrophoretic techniques, isoelectric focusing (IEF) has been a very powerful method for the differentiation of unique banding profiles of soluble proteins for different species of helminthic parasites. Adult Fasciola hepatica were recovered from 5 calves infected with 500 metacercariae and necropsied 16 weeks later. Fasciola gigantica were collected from naturally infected cattle in Hawaii. Fascioloides magna adults were recovered from hunter-killed white-tailed deer at a hunter check station 16 km east of Opalousas, Louisiana. Flukes were homogenized and centrifuged; protein concentrations were determined and adjusted to be 1.7 - 2.1 mg/mL. The samples were then subjected to IEF and the resulting protein banding profiles were compared. Unique, reproducible banding patterns were characteristic of each species, although those of F. hepatica and F. gigantica were similar. This technique of soluble protein IEF has been proved to be valuable in differentiating the 3 species of liver fluke.

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LACK OF SPECIFICITY OF CRUDE ANTIGEN PREPARATIONS IN THE DIAGNOSIS OF SWINE EPERYTHROZOONOSIS. N. NONAKA*, R.W. BULL and T.W. SCHILLHORN VAN VEEN. MICHIGAN STATE UNIVERSITY. EAST LANSING, MI 48824.

Chronic swine eperythrozoonosis is routinely diagnosed by an indirect hemagglutination assay (IHA) in which a crude antigen is used. IHA Ag includes both E. suis and host derived proteins, which may impair the specificity and sensitivity of the test. This study was aimed to clarify the effect of crude antigens in the diagnosis of eperythrozoonosis.

Concentrated plasma of E. suis parasitemic (EPE+) and parasite free pigs (EPE-) were prepared after removal of albumin and globulin. They were used as antigens in IHA and ELISA tests aimed to detect E. suis antibody in serum of artificially inoculated (EPS+) and parasite free pigs (EPS-). Both EPE+ and EPE- plasma samples reacted with EPS+ serum. Titers of EPS+ serum were over 1:160 in IHA and 1:8 in ELISA; titers of EPS- serum were negative in both tests.

Western blots of EPE+ and EPE- samples were prepared with EPS+ and EPS- serum. Four bands were observed at 62, 68, 73 and 85 kd in any combination of EPE+/- samples and EPS+/- serum. The intensity of the above bands was higher in EPS+ serum than in EPS- serum. This indicates a stimulation of antibody production against host plasma proteins by E. suis infection. In addition, two more bands were observed, at 25 and 31 kd, only in a combination of EPE+ sample and EPS+ serum, indicating that these proteins are E. suis specific. We conclude that at least some antibodies detected by the IHA and ELISA tests are directed against plasma proteins and not only against E. suis proteins.

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CYSTIC HYDATID DISEASE IN THE XINJIANG UYGUR AUTONOMOUS REGION, PRC. F.L. ANDERSEN*, H.D. TOLLEY. BRIGHAM YOUNG UNIV., PROVO, UT., AND P.M. SCHANTZ, CENTERS FOR DISEASE CONTROL, ATLANTA, GA.

Cystic hydatid disease is endemic in the Xinjiang Uygur Autonomous Region (XUAR) in northwestern PRC. Through a cooperative project between Chinese and American scientists a comprehensive baseline survey was conducted in 85 villages in two communities to collect demographic data on the inhabitants and complete a census of all domestic animals which might serve as definitive or intermediate hosts for Echinococcus granulosus. A 10-month study was then implemented to compare the efficacy of 3 levels of a local preventive and control program in 16 randomly selected villages in those communities. Results showed that a monthly interval for (1) a visit from a village hydatid disease control officer to each household, (2) distribution of praziquantel-medicated tablets for all dogs in the villages, and (3) distribution of specifically designed educational materials was the most optimum protocol tested for a significant reduction of infected dogs, and change of life style of village inhabitants to decrease transmission risk factors. On-going efforts in XUAR now include design and usage of standardized computer entry forms for collection of all epidemiological data on hydatid disease, and use of computer analyses and graphics into the over-all management of control efforts in that region.

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OVERWINTERING OF STRONGYLES FROM PONIES ON PASTURE IN ONTARIO. J.O.D. SLOCOMBE*. ONTARIO VETERINARY COLLEGE, UNIVERSITY OF GUELPH, GUELPH, ON, CANADA, N1G 2W1.

From May 18 to November 8, 1989, up to 16 ponies were on each of 3 pastures (pA,pB,pC,) after which they were in one group (up to 38 ponies) in loose housing and with access to pC. On April 16, 1990 pC was closed to ponies, and from May 17, 1990 to November 12 up to 13 ponies were on pB. Ponies were not on pA and pC during the 1990 grazing season and were not given anthelmintics during the study. Fecal samples were taken from each pony for analysis of strongyle eggs every 2 wk in each pasture season and every 6 wk overwinter. Herbage samples were taken every 2 wk in the pasture season from pA and pB in 1989 and pA, pB, and pC in 1990 for analysis of infective larvae. In 1989, herbage samples were taken from pC on September 21 and November 16.

In May 1989, mean epgs for the 3 groups were 575, 806 and 883. Mean epgs increased to 1267, 1638 and 1685, respectively, during the grazing season before declining. Mean epgs in winter ranged from 382 to 533. The mean epg for ponies going to pB in May 1990 was 789 and this increased to 1814 in June before declining. In 1989, herbage larval counts for each pasture were the highest, over 20,000 larvae/kg dry herbage, in September. In 1990, herbage larval counts and their trends for pB resembled those for 1989. Few larvae were found on pA and pC in 1990. Ambient temperatures in winter 1989-1990 appeared to prevent successful maturation of infective larvae from strongyle eggs deposited on pA up to mid-November and on pC to mid-April.

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THE EFFECT OF ANTHELMINTIC TREATMENT OF COWS AT HOUSING ON THE EPIDEMIOLOGY OF NEMATODE INFECTIONS IN A COW-CALF HERD IN QUEBEC.

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Following a two year epidemiological study in a cow-calf herd, a group of previously untreated cows from this herd was treated with ivermectin at housing and another group remained untreated as a control group. During the following grazing season, both groups of cows and their spring-born calves grazed two separate but adjoining pastures. Fecal egg counts of cows and calves, pasture larval counts and tracer worm burdens were monitored for both groups until the end of the grazing season. The data obtained support the previous two years' observations and confirm that hypobiosis of Ostertagia ostertagi occurs in the fall. Treatment of the cow prevented the spring rise in cow fecal egg counts observed during the two previous years and in the control cows in the present study. Despite the much greater grazing density in the treated group, the fecal egg counts of both groups of calves were similar throughout the study period. This indicates treatment of the cow had an impact in reducing spring pasture contamination. Pasture larval counts and tracer calf parasite burdens will also be presented. Supported by NSERC and MSD AgVet.

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EPIDEMIOLOGY OF GASTROINTESTINAL NEMATODES IN SUCKLING CALVES IN A SPRING-CALVING HERD IN NORTHEAST MISSISSIPPI.

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Parasitologic parameters were monitored in a spring calving herd in Northeast Mississippi during 1987-89. A group of 30 beef cows and calves were grazed on a 20 ha fescue/bermudagrass pasture. Neither cows nor calves received anthelmintics during the study. Counts of eggs per gram of feces (EPG) for cows and calves were determined in January, April, July, and September. Two calves were removed from the pasture in June, July, September, and October for necropsy and gastrointestinal nematode counts. For adult cows, the overall mean EPG was <5 during all sampling periods. The overall mean EPG of calves was 8, 31, and 60 in April, July, and September, respectively. Ten nematode genera were identified from calves, but *Ostertagia ostertagi* and *Cooperia* spp. comprised 84-100% of mean worm burdens. Total worm burdens of calves necropsied in October ranged from 5,150 - 30,150 worms. For *O. ostertagi*, mean burdens of adult and inhibited early fourth-stage larvae were <4000 worms for all necropsy periods over 3 years. Inhibited early fourth-stage larvae were found in low numbers (≤3600 worms), primarily in June and July. The proportion of *O. ostertagi* declined from June (43-85%)(Mean = 63%) through October (6-24%)(Mean = 16%). *Cooperia* spp. increased from 9-57% (Mean = 35%) of the worm population in June to 60-84% (Mean = 75%) in October. Other worm species were minor components of the worm population. Low burdens of *O. ostertagi* in the calves was attributed to lack of coincidence of worm transmission and peak grazing activity, and indicate that *O. ostertagi* is of minor importance for suckling calves.

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PREVALENCE OF GASTROINTESTINAL NEMATODES OF CATTLE IN SOUTH TEXAS. T. JIFFAR*, T. QURESHI AND T.M. CRAIG. TEXAS A&M UNIVERSITY, COLLEGE STATION, TX 77843.

Twenty-three head of cattle of mixed sex ranging from 6-12 months of age were used to evaluate the prevalence of gastrointestinal nematodes at the King Ranch, Texas Gulf Coast. Four calves were brought to the ranch headquarters at Kingsville in Nov/88, Mar/89, May/89, Dec/89 and May/90. The cattle were euthanatized and the abdominal contents were divided into the abomasum, small intestine and large intestine. Aliquot samples of the contents were collected and processed for total worm counts and identification.

The mean number of worms recovered on each of the collection dates in decreasing order were: 5800 (May/90) ,5600 (Mar/89), 4500 (May/89), 1230(Dec/89) 1150 (Aug/89), and 670 (Nov/88). The predominant nematode species recovered were Haemonchus placei (36.6%), Cooperia pectinata (34.8%) and Cooperia punctata (22.2%). Haemonchus placei was more abundant in the spring months while Cooperia species were distributed evenly. Ostertagia ostertagi was more abundant in May/89 than at other times. There was no apparent pattern of distribution for Oesophagostomum radiatum and Trichostrongylus axei. The observations were correlated with precipitation on the ranch.

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EXTRAPOLATION OF A SOIL-BASED GEOGRAPHIC INFORMATION SYSTEM (GIS) MODEL FOR DISTRIBUTION OF FOSSARIA BULIMOIDES HABITAT ON SOUTHWEST LOUISIANA COASTAL MARSH. S.H. ZUKOWSKI* AND J.B. MALONE, LOUISIANA STATE UNIVERSITY, BATON ROUGE, LA 70803.

A GIS model was used to test the hypothesis that distribution of Fossaria bulimoides follows interfaces of chenier and marsh soils on the Chenier Plain of Louisiana. Snail surveys were based on transects over 3 strata on 12 farms: 1) waveface (former beachfront) and 2) backslope (BS) chenier-marsh interfaces, and 3) chenier (relict beaches, above their interfaces with marsh). Twenty three per cent of snail habitat was found on chenier-marsh interfaces, mostly along interfaces adjacent to relatively broad cheniers; 61% of snail habitat occurred within the Hackberry-Mermentau (Hm) complex of chenier soil series, above interfaces (almost entirely on broad chenier). An additional 14% of habitat occurred on chenier soils of the Mermentau series, again associated with broad chenier. The proportion of farm comprised of chenier-marsh interface did not regress significantly against the proportion of farm occupied by F. bulimoides habitat. A revised model incorporating broad chenier along with adjoining Mermentau and chenier-marsh interface soils reflected actual habitat with a sensitivity of 91.3% and a specificity of 80.1%; the proportion of farm comprised of soils of this model also regressed against the proportion of farm comprised of F. bulimoides habitat ($p = 0.01$, $r^2 = 0.49$). Results indicate that a successful soil-based model for estimation of snail habitat distribution on the chenier plain region should include the area of soils of the Hackberry-Mermentau complex on broad cheniers, associated Mermentau soils and chenier-marsh interfaces.

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USE OF LANDSAT MSS IMAGERY AND SOIL TYPE IN A GEOGRAPHIC INFORMATION SYSTEM TO ASSESS SITE-SPECIFIC FASCIOLIASIS RISK ON RED RIVER BASIN FARMS IN LOUISIANA. J.B. MALONE*, D.P. FEHLER AND A.F. LOYACANO. LOUISIANA STATE UNIVERSITY, BATON ROUGE, LA 70803.

A geographic information system (GIS) was constructed in an ERDAS environment using LANDSAT satellite multispectral scanner data (MSS) , soil type maps from the USDA Soil Conservation Service, and farm boundaries for 15 study farms in a 2 quadrangle (USGS, 7.5') study area in the Red River basin near Alexandria LA. Fecal sedimentation examinations were done in the fall of 1989 on 12-15 random samples from each herd. Fecal egg shedding rates for F. hepatica ranged from 10-100% prevalence and 0.3-21.7 (x=7.7) eggs per two grams of feces (EP2G). For Paramphistomum microbothrioides, a rumen fluke which is also transmitted by the snail Fossaria bulimoides but not affected by flukicides, egg counts ranged from 0.13-91% and 0.1-42.8 EP2G (x=6.2). Herd P. microbothrioides egg shedding rates increased with the proportion of low-lying, hydric clays vs sandy loams/clay loams on pastures. F. hepatica egg shedding rates followed a similar trend, but were complicated by different treatment practices. In combination with an existing climate forecast based on the Thornthwaite water budget, results suggest an MSS/soils GIS can be used to develop a second generation forecast that accounts for both climate variation and site-specific differences in fascioliasis risk (Factor I-III) based on the proportion of soils prone to snail habitat and local flooding events. Supported by NIH Grant AI28192-01.

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THE DEGRADATION OF DUNG PATS FROM IVERMECTIN-TREATED CATTLE UNDER FIELD CONDITIONS. D.H. WALLACE*, J.E. HOLSTE, R. RONCALLI AND S.J. GROSS. ANIMAL SCIENCE RESEARCH, MERCK SHARP & DOHME RESEARCH LABORATORIES, P.O. BOX 2000, RAHWAY, NJ 07065.

Sixteen steers were allocated by restricted randomization on Day -7 body weight to four treatments: 1) untreated controls; 2) ivermectin SR bolus designed to deliver 12 mg ivermectin/day; 3) ivermectin injectable at 200 mcg/kg, once subcutaneously; and 4) fenbendazole suspension at 10 mg/kg, once orally. The cattle were grazed by treatment group on four similar contiguous pastures.

Five fresh dung pats were identified in each paddock on Days 0, 7, 14, 21 and 28 and the surface area measured weekly for eight weeks. Fifty-six days after identification, four of the pats were collected intact for examination in the laboratory; the fifth pat remained exposed until the end of the trial and was measured weekly and photographed periodically. Five additional dung pats were identified in each paddock on Days 0, 7, 14, 21 and 28 and 1/8 segments were collected 0 or 3 and 7 days after deposition, and weekly through 49 days. Each 1/8 segment sample was weighed and examined for fauna activity. One additional dung pat from each paddock was identified on Days 0, 7, 14, 21 and 28 and photographed periodically. For Treatments 1 and 2, the same procedures were also performed on Days 56, 84 and 112.

The mean weights of dung pats among treatment groups over time were similar, as were the dung pat surface area measurements. The mean volumes of dung pats 56 days after deposition were generally similar for any given deposition day. There was no difference in the presence of earthworms in soil under pats from untreated or ivermectin-treated cattle. Although Diptera larvae were absent from pats deposited by cattle 14-84 days after treatment with the ivermectin SR bolus, their presence was again noted in pats deposited after cessation of the ivermectin delivery period. There were no visible differences in dung beetle activity among treatment groups. Photographic records show no marked difference between groups as to the degradation rate of pats.

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THE ROLE OF WILDLIFE IN THE TRANSMISSION OF HYDATID DISEASE SENSU LATO TO INDIGENOUS PEOPLE. R. L. Rausch, Dept. of Comp. Med., University of Washington, Seattle, WA 98195.

Four species are recognized in the genus Echinococcus (Cestoda): E. granulosus (Batsch, 1786), with two major biotypes (cystic hydatid disease); E. multilocularis Leuckart, 1863 (alveolar hydatid disease); E. oligarthrus (Diesing, 1863) (disease in man not yet characterized); and E. vogeli Rausch and Bernstein, 1972 (polycystic hydatid disease). The European biotype of E. granulosus has a nearly cosmopolitan distribution in livestock-raising countries, where the cyle mainly involves synanthropic hosts (dog and domestic ungulates). Otherwise, the natural cycles of these cestodes are completed by means of well defined predator-prey relationships existing between their respective final and intermediate hosts. The Northern biotype of E. granulosus occurs throughout the holarctic zones of tundra and boreal forest, its range corresponding to the distribution of its natural hosts, the wolf and large deer. E. multilocularis is a common cestode in foxes in the zone of tundra and, farther south, in the steppe-regions of Eurasia. Small rodents serve as intermediate host. The hosts of E. vogeli, occurring in Central- and South America, are the bush dog, Speothos venaticus, and a large rodent, the paca, Cuniculus paca. E. oligarthrus, a cestode of wild felids in Central and SOuth America, might have some potential as a cause of hydatid disease in man. For that species also, rodents serve as intermediate host. Of great epidemiological significance is that domestic dogs replace the natural final hosts E. granulosus (Northern biotype), E. multilocularis, and E. vogeli, thereby becoming a source of infection for man in the respective biocoenoses.

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A TECHNIQUE TO MEASURE LAWNS AND ROUGHS IN PASTURES GRAZED BY HORSES. K. M. EWERT, J. A. DIPIETRO*, UNIVERSITY OF ILLINOIS, URBANA, ILLINOIS 61801 and C. S. DANNER, Jr., DANNER AERIAL SURVEY, URBANA, ILLINOIS 61801.

Percentage of lawns (grazable area) and roughs (fecal-fouled area) in pastures grazed by horses was determined utilizing aerial survey photography. A Cessna 180 airplane specially designed to perform survey work equipped with a large format cartographic aerial camera (Model RC8, Wild Heerbrugg, Heerbrugg, Switzerland) was flown to obtain the photographs. The flight was flown at an altitude of 1200 feet resulting in a photo negative scale of 200 feet per inch. Stereo aerial photographs were utilized in a stereo plotter (Model B8S, Wild Heerbrugg, Heerbrugg, Switzerland) to create topographic and planimetric maps. The three-dimensional image is exaggerated in the vertical scale making slight differences in elevations (in this case grass heights) easily discernible to the stereo plotter. The final map was drawn at a scale of 40 feet per inch. The area of lawns and roughs was calculated in square feet and acres from the final map by digitizing the area using Autocad V.10 software (Autodesk, Inc., Sausalito, California). From these values, percentages of lawns and roughs in the pastures were calculated.

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COMPARISON OF AN INTERVAL DEWORMING PROGRAM USING ONLY IVERMECTIN WITH A ROTATIONAL INTERVAL DEWORMING PROGRAM IN CONTROLLING EQUINE ENDOPARASITES. K. M. EWERT*, J. A. DIPIETRO, L. M. LAWRENCE, D. F. PARRETT, K. S. TODD,JR, UNIVERSITY OF ILLINOIS, URBANA, IL 61801.

Two treatment groups, A and B, each containing a minimum of ten mares, were identically maintained for two years on pasture or dry lot according to treatment groups. Mares in group A were treated with ivermectin paste (IVM) (0.2 mg/kg) every 60 days. Mares in group B were treated every 60 days with an alternation of oxibendazole (OXB) (10 mg/kg), pyrantel pamoate (6.6 mg/kg), and IVM. Qualitative and quantitative fecal exams were performed biweekly. Fecal larval cultures were done on the day of anthelmintic treatment and 2 weeks thereafter. Larvae per gram of herbage were determined from the pastures. On a monthly basis, the mares were weighed, and rump fat to calculate % body fat and body condition scores were determined. Eighty percent of the mares measured were detected passing strongyle eggs at the beginning of the study. Mares treated with only IVM were detected passing strongyle eggs fewer times than those mares on the rotational program. OXB was the least effective in reducing the percentage of fecal floatations containing strongyle eggs. By the end of the study, minimal reduction in strongyle positive fecal floatations was seen after treatment with OXB. Strongyle egg per gram (EPG) counts decreased dramatically after the first treatment of the study and remained so for the duration of the study. However, strongyle EPG counts for group B horses were always higher than for group A horses. Culture of feces for 3rd-stage strongyle larvae showed increases 60 days post-treatment for horses in group B, but remained at 0 for group A with the exception of one horse on one occasion. Larvae per kg of dry herbage were similar for both pastures at the onset of the study, gradually decreased through out the first year, and by the beginning of the second year of the study were no longer detected from either pasture. Mean weights and condition scores of the mares in both treatment were similar. Percent body fat was consistently lower for group B mares over the 2-year period.

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A COMPARISON OF EQUINE PARASITE CONTROL PROGRAMS UTILIZING FAST ROTATION OR EXCLUSIVE IVERMECTIN TREATMENT. D.D. FRENCH*, T.R. KLEI, M.R. CHAPMAN. LOUISIANA STATE UNIVERSITY, BATON ROUGE, LA 70803.

Three foal crops from 1988-1990 have been used to compare the effects of fast rotation to exclusive ivermectin treatment on parasite burdens and foal condition. Three groups of 12 mixed breed pony mares and their foals were raised on separate pastures. Mares were allowed to foal on pasture from March through May. One group served as non-treated controls (CON), a second received ivermectin (200 µg/kg) at 2-month intervals (IVM) and a third was treated with a fast rotation of anthelmintics at 2-month intervals. The rotation included pyrantel pamoate, oxibendazole,thiabendazole-piperazine and ivermectin. During 1990 each group was further separated into three replicate pastures. Eggs per gram of feces (EPG), larvae per gram of feces (LPG), body weight, condition score (CS), ultrasonic backfat (BF) and height of foals were determined from all animals.

Data revealed that both treatment groups had lower mean strongyle spp. EPG for all three years than did controls. Differences between groups in mare weight, CS or BF levels were not seen. Fecal cultures demonstrated increased LPG in ivermectin treated mares in 1990 when compared to previous years and to other treatment groups. Strongyloides westeri transmission was reduced and delayed in IVM foals in the last two years of the study . Peak Parascaris equorum EPG of both treatment groups were very low (0-27) when compared to CON foals (1400). Foal weight, CS, and BF values were higher in treated foals than in controls for all years, but little difference could be determined between ROT and IVM foals. No differences in height were determined between groups. This study supported in part by grants from the LSU Equine Veterinary Research Program and MSD/AGVET.

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ALTERNATIVE ANTIPARASITIC TREATMENT OF HORSES WITH PYRANTEL AND IVERMECTIN ORAL SOLUTIONS COMPARED WITH HORSES TREATED ONLY WITH IVERMECTIN ORAL SOLUTION. T.R. BELLO. SANDHILL EQUINE CENTER. SOUTHERN PINES, NC 28387.

An evaluation was done of practical treatment program with pyrantel pamoate suspension (PY) and ivermectin oral solution (IVM) in a seasonal rotational, in comparison with use of IVM only given at 2-month intervals for 2 years. At least 15 horses in each of 2 treatment groups were in 8 locations. Treatment sequence in the alternation program was PY (Feb), IVM (Apr), PY (June, Aug), and IVM (Oct, Dec). In the IVM only program, the drug was given at the same time as either treatments on the alternation program. Course of strongyle infections was monitored by fecal EPG and larval culture analyses of treatment pairs. Anoplocephala infection was monitored by fecal EPG.

Strongyle EPG and LPG numbers were reduced to 0 by first IVM treatment, increased slightly at 2 months, then repeated the reduced pattern for 2 years. Strongyle control was better with IVM only than with alternation program. Some IVM-treated horses had tapeworm infections which persisted. In the alternation program, IVM had persistent effect and apparently enhanced strongyle control by PY. Anoplocephala eggs were eliminated by the alternation program.

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COMPARISON OF PERIODIC TREATMENTS WITH IVERMECTIN VERSUS OXIBENDAZOLE AS PARASITE CONTROL PROGRAMS FOR HORSES IN SOUTHERN TEXAS. M.G. SCROGGS 1*, J. HAWKINS 2, P. VADEN 3, J. REID 4. 1 MSD AGVET, AMARILLO, TX., 2 MSD AGVET, STARKVILLE, MS., 3 UVALDE, TX., 4 BARKSDALE, TX.

During 1988, forty Arabian horses, 32 bred mares and 8 yearlings, were used to evaluate the relative efficacy of ivermectin and oxibendazole in reducing fecal egg output in horses pastured on small paddocks in Southern Texas when treated every 8 weeks. Horses were assigned to paddocks by farm management according to disposition and ability of individuals to cohabit. Treatments were randomly allocated to groups. Fecal samples were collected from representatives every two weeks until the end of the trial.

Treatment with ivermectin every 8 weeks proved to be very efficacious in reducing the mean strongyle egg count. Treatment with oxibendazole reduced mean egg counts for shorter intervals and would require more frequent use.

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ANTHELMINTIC RESISTANCE ON PLEASURE HORSE FARMS IN FLORIDA. D. L. REPETA*, N. BIRNBAUM, AND C. H. COURTNEY. UNIVERSITY OF FLORIDA, GAINESVILLE, FL 32611.

Ninety seven horses on 6 pleasure horse farms in North Central Florida were tested for the presence of worms resistant to either fenbendazole or ivermectin. On each farm horses were divided into 3 groups of not more than 10 horses each and given either ivermectin (200 mcg/kg Eqvalan Oral Liquid®), fenbendazole (5 mg/kg Safe-Guard® suspension), or left untreated as controls. Fecal egg counts were performed on the day of treatment and at 2, 4, and 8 weeks following treatment using a modified McMaster technique with a minimum sensitivity of 25 eggs per gram of feces. Ivermectin was uniformly effective on all 6 farms. Mean fecal egg counts of ivermectin-treated horses on all farms combined were reduced from a pretreatment value of 362 eggs per gram by an average of 99, 94 and 98 percent at 2, 4 and 8 weeks after treatment, respectively. In contrast, fenbendazole was effective on only 1 of the 6 farms. Mean fecal egg counts of fenbendazole-treated horses on all farms combined were reduced from a pretreatment value of 489 eggs per gram by an average of 71, 77 and 48 percent at 2, 4 and 8 weeks after treatment, respectively.

The widespread occurrence of fenbendazole resistance was unexpected, since all farms dewormed either exclusively with ivermectin or used a regular rotation between benzimidazole and non-benzimidazole anthelmintics. It is hypothesized that worms resistant to benzimidazole anthelmintics now have become widely dispersed throughout the pleasure horse industry in Florida as a result of the frequent movement and mixing of pleasure horses.

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EVIDENCE FOR THE LACK OF EFFECT OF IVERMECTIN ON ENCYSTED CYATHASTOME LARVAE. T.R. KLEI*, M.R. CHAPMAN, D.D. FRENCH, AND H.W. TAYLOR. LOUISIANA STATE UNIVERSITY. BATON ROUGE, LA 70803.

The efficacy of high dose (1.0 mg/kg) Eqvalan liquid drench on encysted cyathastomes was tested in a controlled study using 12 adult ponies with naturally acquired cyathastome infections. Following treatment ponies were separated into treated and untreated control groups and held in stalls for a period of 5 weeks. Cyathastome burdens (lumenal larvae, adults and encysted larvae) were determined at necropsy. Viability of encysted larvae based on morphologic integrity was assessed on fresh samples by observation of transilluminated gut and by histologic appearance of 12 larvae per pony. No adult cyathastomes were found in treated ponies. Lumenal cyathastome larval numbers were reduced by 87%. Encysted cyathostome larvae, identified by transillumination of the gut were reduced by 32%. This reduction was not statistically significant ($P > 0.05$) and no difference in viability of encysted larvae was noted. The data strongly indicated that ivermectin has little demonstrable effect on encysted equine cyathastomes. Supported in part by a grant from MSD/AGVET.

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RESPONSE OF LARGE-ANIMAL VETERINARIANS TO A SURVEY OF COW-CALF ENDOPARASITE CONTROL IN GEORGIA. J.A. STUEDEMANN*, H. CIORDIA[1], D.M. BLACKMON[2], E.L. ROBERSON[3] AND M. MEHRBAN[4]. USDA, ARS, WATKINSVILLE*,[4], GA 30677 AND THE UNIVERSITY OF GEORGIA, EXPERIMENT[1] 30212 AND ATHENS[2,3] 30602

In early 1990, a one-page questionnaire comprising 10 questions was sent to 55 practicing large-animal veterinarians (VETS) in Georgia. The objectives of the survey were to better understand the following: the number of cattlemen that utilize cow-calf endoparasite control practices; the type of formulations preferred, e.g., bolus, injectable, paste, topical, etc.; the recommendations VETS have regarding deworming of cows and/or suckling calves; and the concerns that beef producers or VETS have regarding helminth control. Thirty-five completed questionnaires were returned. Twenty-six VETS indicated that 50% or fewer beef cattle producers in Georgia dewormed their cows and calves with more cows receiving treatment than suckling calves. Succeeding questions were directed toward VETS' clientele. Twenty-one reported that 20% or less of their clients dewormed more than once per year with the most common time of treatment being spring or autumn. The preferred formulation was the injectable type (27 ranked it as the first choice). The second choice was less distinct with a somewhat similar preference for paste, suspension, or topical formulations. The least preferred type was the bolus. The most frequent question VETS or their clientele had regarding helminth control was: when is it cost-effective to deworm? In summary, the survey revealed that comparatively few producers deworm their cows and/or suckling calves.

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THE EFFECT OF PARASITE CONTROL ON FERTILITY IN BEEF HEIFERS. A.M. ZAJAC*, J.W. HANSEN, W.D. WHITTIER, D.E. EVERSOLE, VIRGINIA TECH. BLACKSBURG, VA 24061.

A study was undertaken to examine the effects of parasite control on growth and conception rate in beef heifers in southwestern Virginia. Forty mixed-breed heifers were purchased at weaning (October) from a local producer. The animals were divided into 2 groups. Control heifers were untreated, while animals in the experimental group received ivermectin (200 mcg/kg, SC) before being placed on pasture for the remainder of the fall and winter. Supplemental feed was provided to ensure adequate growth to breeding weight. The treatment group received ivermectin again in April at the start of the grazing season. On May 3, heifers were removed from pastures and exposed to bulls for natural breeding for 42 days. Pregnancy rates were determined 6 weeks after the end of the breeding season. Weight gain, fecal egg counts, serum pepsinogen levels and pasture larva counts were monitored throughout the study. No difference was seen in conception rates between the 2 groups. However, ivermectin treated animals showed a significantly greater cumulative weight gain than untreated animals. Treated heifers also had lower pepsinogen levels than untreated animals during much of the study, although values remained in the normal range for both groups. The results suggest that parasite control may not significantly increase conception rate in well nourished heifers in a spring breeding program in this area, even though an effect on weight gain is observed.

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FOUR YEARS OF TREATING BEEF COWS IN NORTH DAKOTA WITH IVERMECTIN IMPROVED WEANING WEIGHTS OF CALVES FROM THE TREATED COWS. J. J. MELANCON 1*, K. WOHLGEMUTH 2, 1. MSD AGVET, MAPLE GROVE, MN; 2. LINCOLN, NE.

During 1985-1988, two productivity trials were conducted in North Dakota cow/calf operations. In the first two year trial, four privately owned herds were used. Cows in each herd were divided into four groups: (1) treated in the fall; (2) treated in the spring; (3) treated in the fall and spring; and (4) untreated control. Fecal samples indicated all herds had cows with nematode infection, but at subclinical levels. Cows treated with ivermectin at 200 mcg/kg body weight subcutaneously weaned calves with a mean 205-day adjusted weaning weight which was 15.5 lb. heavier than the control group ($p = 0.02$).

In the second two year study, three herds were used. These herds were also positive for nematode infection based on fecal examination from cows. Half of the cows in each herd were treated with ivermectin in the fall, and half of the group was an untreated control. About July 1, half of the calves of each cow group were treated with ivermectin and the other half were untreated. Weaning weights of calves in this study supported the results from the previous study. Actual mean weaning weights for all calves from treated cows was 20 lb. greater than controls ($p = 0.05$). The 205 day adjusted weight for calves from treated cows was 32 lb. greater than calves from untreated cows ($p = 0.003$). In this trial treating calves when cows had been treated previously did not add any significant extra weight at weaning.

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ALTERNATIVE TREATMENT STRATEGIES WITH IVERMECTIN (IVM) FOR CONTROL OF GASTROINTESTINAL NEMATODES OF CATTLE IN LOUISIANA. J.C. WILLIAMS*, C.B. NAULT, R.T. RAMSEY, LOUISIANA STATE UNIVERSITY, BATON ROUGE, LA 70803.

A 3X per year treatment strategy (approx. 11/15, 3,1, 7/1) in stocker beef cattle yielded excellent results in terms of cattle gains and parasite control in 4 successive yearly experiments. During 1986-87, however, both IVM and fenbendazole strategic treatments yielded unsatisfactory gains or parasite control. Persisting wet and cold winter weather, high infectivity of pasture, and the long interval between 1st and 2nd treatments, were considered responsible. Consequently, an experiment was conducted during 1989-90 to compare the 3X treatment with an alternative strategy in which 3 short interval (6 week) treatments were given beginning in Nov. with a 4th treatment in late spring. Three groups of 11 crossbred heifers averaging 154 kg were used. Each group grazed on separate 1.2 ha pastures. Treatments were: Gp1, IVM (200 μgkg) on 11/15, 12/28, 2/8, and 6/12; Gp2, IVM (200 23 μg/kg) on 11/15, 2/13, and 6/12; Gp3 untreated controls. Fecal egg counts of all groups remained low after initial treatment and a brief period of extreme cold in late Dec. Control counts were significantly higher ($P<0.05$) than in treated cattle in Dec., Mar., July, and Aug. Peak pasture larval counts were observed for all groups in Dec. and late Mar. Liveweights of untreated cattle were lowest from Feb. and significantly lowest in April and from July to Oct. Gp2 cattle maintained a small but consistent advantage over Gp1 cattle that was never significantly different. Final group mean liveweights of Gp2 and Gp1 were 295 and 291 kg, respectively.

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EFFECT OF GRAZING SYSTEMS AND DEWORMING PROGRAMS WITH IVERMECTIN ON PARASITEMIA OF STOCKER CALVES. S.E. MARLEY* AND R.M. CORWIN, UNIVERSITY OF MISSOURI, DEPT VET MICROBIOLOGY, COLUMBIA MO 65211

Intensive rotational grazing has been demonstrated to utilize forage more effectively than conventional grazing by beef cattle as judged by forage quality and calf performance, but the possibility of increased parasite transmission had not been determined. This study compares intensive rotational vs conventional grazing of stocker calves on 12 - 10A cool season grass pastures, one-half treated at 5 wk intervals (May-Sep) with injectable ivermectin and one-half treated in May and again in August. A sentry calf also was present on each pasture and was necropsied in November for total gastrointestinal nematode numbers. All calves were weighed and fecal samples taken for epg and speciation by culture each 5 weeks. Data show that rotational grazing and 5-week deworming provide improved performance.

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CONTROLLED EFFICACY OF IVERMECTIN/CLORSULON AND OTHER TREATMENTS AGAINST G.I. NEMATODES AND LIVER FLUKES IN CATTLE. K. M. NEWCOMB,* J. GUERRERO, AND R. NAJERA. MSD AGVET. RAHWAY, NJ 07065.

Controlled anthelmintic and flukicidal efficacy of various compounds, alone or in combination, was studied in naturally parasitized cattle. Twenty calves were blocked according to fecal strongyle egg counts and treatments were sequentially allocated to animals in each block: untreated control; netobimin (20 mg/kg) PO; triclabendazole (12 mg/kg) PO/levamisole (7.5 mg/kg) IM; ivermectin (200 mcg/kg)/clorsulon (2 mg/kg) SC. Immediately after treatment, the cattle were placed together in a paddock for 6 - 8 days, after which they were killed for recovery, enumeration, and identification of gastrointestinal parasites and liver flukes.

Controlled efficacy of netobimin against intestinal helminths ranged from 73% to 89%. Activity against adult *Fasciola hepatica* was 70.5%, with no observable effect on immatures. Controlled efficacy of concurrently administered levamisole and triclabendazole ranged from 97% to 100% against intestinal helminths, 95% against adult flukes, and 72% against immature *F. hepatica*. Concomitant treatment with ivermectin was >99 - 100% effective in removing intestinal worm burdens, 100% against adult *F. hepatica*, and 52.1% against immature flukes. The group treated with ivermectin/clorsulon had significantly ($P<0.05$) fewer liver flukes than the netobimin group, and had statistically similar numbers as the group treated with levamisole and triclabendazole.

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EVALUATION OF ENPROAL® BLOCKS CONTAINING BOVATEC® AND SAFE-GUARD® FOR THE IMPROVEMENT OF LIVEWEIGHT GAINS AND CONTROLLING NEMATODE PARASITES IN GRAZING CATTLE. D.E. SNYDER* AND D.I. BRANSBY. USDA, ARS, ANIMAL PARASITE RESEARCH LABORATORY, AND AUBURN UNIVERSITY, AUBURN, AL 36830.

This report describes a protein-mineral supplement block containing an anthelmintic used in a strategic deworming treatment program to control nematode parasites in grazing steers.
Two replicates of 3 treatments were applied to tall fescue pastures (endophyte infected [51%]). Treatments were 1) Control, with no supplement blocks, 2) Enproal blocks with Bovatec only, and 3) Enproal + Bovatec blocks with medicated Safeguard blocks provided after 3 and 6 weeks of grazing. Pastures had been recently contaminated by cows naturally infected with trichostrongylid parasites. Each replicate (stocking rate=2.5 animals/acre) was grazed by 10 steers ($\bar{X}$ wt=217 kg) of mixed breed starting on 4/10/90 and continued for 132 days. All animals were treated with Panacur 10% paste (5 mg/kg B.W.) prior to initiation of grazing. All steers were weighed and fecal samples collected every 28 days to determine ADG and fecal EPG counts. Animal gains were typically low for endophyte infected fescue grazed over the summer period and may have been exacerbated by low rainfall in July and August which limited forage availability. The Enproal + Bovatec blocks increased gains by 18% compared to the control, while the additional use of Safeguard blocks increased gain by 30% over the control. Worm EPG's increased with time and at the end of the trial were highest on the control and lowest on the Safeguard block treatment. For ADG, treatment differences were significant at P=0.20, and for EPG data P=0.54.

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EFFICACY OF ABAMECTIN AGAINST GASTROINTESTINAL NEMATODES AND LUNGWORMS OF CATTLE. R.M. KAPLAN*, C.H. COURTNEY, Q.Y. ZENG, AND A.D. JERNIGAN. UNIVERSITY OF FLORIDA, GAINESVILLE, FL 32611 AND MERCK AND CO., INC., RAHWAY, NJ 07065.

The efficacy of abamectin against lungworms and gastrointestinal nematodes of calves was determined for both experimentally administered (n=14) and naturally acquired (n=16) infections. In each experiment half the calves were treated with abamectin (200 mcg/kg of body weight, SC) and half were left as untreated controls. Abamectin was both safe and effective. Efficacy was >99.9% against *Dictyocaulus viviparus*, *Haemonchus placei*, *Ostertagia ostertagi*, *Trichostrongylus axei*, *Cooperia punctata*, *Trichuris discolor*; 98.1% against *Cooperia oncophora*, and 84.7% against *Nematodirus helvetianus*. Except for some minor swelling at the injection site in 5 of the 15 treated calves, no adverse reactions were observed.

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EFFICACY OF MORANTEL TARTRATE ADMINISTERED IN A FREE CHOICE TRACE MINERAL MIX. J.K. BISHOP*, G.L. ZIMMERMAN, D.M. MULROONEY, D.W. WEBER, AND R.M. MILLER. OREGON STATE UNIVERSITY, CORVALLIS, OR 97331.

The sustained release trilaminate matrix is a highly effective delivery system for morantel tartrate in grazing cattle (Rickard et al., **Vet Parasitol** 1989:33;125-133). The purpose of this study was to evaluate an alternate delivery system for morantel tartrate in cattle grazing on pasture. A group of 24 yearling beef calves was randomly allocated into two groups. Group 1 received a standard trace mineral formulation whereas group 2 was provided a trace mineral mix containing morantel tartrate. Based on the anticipated average trace mineral consumption of calves of this weight and class, the formulation of the medicated (morantel tartrate) trace mineral mix was adjusted to provide 152 mg of morantel tartrate per day. The respective groups were turned onto two separate pastures supplied with their respective mineral mixes **ad libitum** in weather vane-type covered feeders. Twice weekly the amount of mineral mix consumed was determined by weigh-back of remaining mix and then fresh mix was added to a pre-determined amount. Target consumption was 0.126 lb/animal/day. Control group calves consumed 0.128 lb mineral mix/animal/day whereas treatment group calves consumed only 0.1025 lb/animal/day (81% of target). The mean number of nematodes recovered in the control calves was 139,725 compared to 122,629 in the treated calves. Ostertagia was the major genus recovered in both groups; the percentages of early fourth stage Ostertagia larvae in the control and treated groups were 88% and 90 % respectively. This data clearly indicates larval inhibition occurred in June. Parasite recovery showed this delivery system reduced the overall burden of adult Ostertagia by approximately 27% whereas reduction of fourth stage Ostertagia was only 10%. Results of this study further indicate that free-choice delivery systems for anthelmintics cannot assure consumption of effective doses for parasite control. Ostertagia kolchida were recovered from 3 animals and O. leptospicularis were recovered from 17 animals.

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PRODUCTION OF STEERS AND HEIFERS AFTER TREATMENT WITH OXFENDAZOLE. J.E. MILLER*, S. BARRAS. LOUISIANA STATE UNIVERSITY, BATON ROUGE, LA 70803

Two studies were conducted to evaluate production (weight gain) of fall-weaned beef steer and heifer calves on ryegrass winter/spring pasture subsequent to deworming using oxfendazole administered by intraruminal injection. In December 1988, 42 crossbred steers were allocated to control and treatment groups of 21 animals each based on weight and both groups received an initial treatment. The treatment group received 2 additional treatments 4 and 8 weeks after the initial treatment. Fecal egg count data indicated all treatments were effective. Monthly weight gains were consistently in favor of the treated group. In May, at the end of ryegrass grazing the treated group averaged 47.8 lbs. heavier than the control group and the average daily gain was 1.60 lbs. and 1.28 lbs. for the treated and control groups, respectively. In December 1989, 60 crossbred heifer calves were allocated to 3 treatment groups of 20 animals each based on weight. The control group (Group 1) remained untreated and the other 2 groups received an initial treatment. Group 2 received an additional spring treatment, and Group 3 received 2 additional treatments 4 and 8 weeks after the initial treatment. Fecal egg count data indicated all treatments were effective. Monthly weight gains for Group 3 were consistently greater than Groups 1 and 2, and Group 2 gains were greater than Group 1. In May, at the end of ryegrass grazing Group 3 averaged 15.3 and 71.6 lbs. heavier than Group 2 and 1, respectively; and Group 2 was 56.3 lbs. heavier than Group 1. Average daily gains for the 3 groups were 0.82 lbs., 1.16 lbs., and 1.25 lbs., respectively.

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INTERACTION OF OXFENDAZOLE AND CLOSANTEL AGAINST INDUCED FASCIOLA HEPATICA INFECTION SUPERIMPOSED ON NATURAL GASTROINTESTINAL NEMATODE INFECTIONS IN CATTLE. G.L. ZIMMERMAN*, L.A. BRITT, A.M. DECKER, J.A. WHITAKER, D.M. MULROONEY, J.K. BISHOP, AND S.E. KNAPP. COLLEGE OF VETERINARY MEDICINE, OREGON STATE UNIVERSITY, CORVALLIS, OREGON 97331 AND DEPARTMENT OF VETERINARY MOLECULAR BIOLOGY, MONTANA STATE UNIVERSITY, BOZEMAN MONTANA.

In late January 1990, 58 yearling beef calves harboring natural gastrointestinal nematodes (confirmed by fecal examinations and parasite recoveries from two herd cohorts) were transported from the herd of origin (near Big Timber, MT) to university facilities at Bozeman, MT). Rectal fecal samples were taken from all animals and each was treated with clorsulon (7 mg/kg) and housed for a seven day acclimation period. Each calf was infected orally with approximately 500 F. hepatica metacercariae and then turned onto pasture for six weeks. In late March, 1990, 48 of the calves were then randomly allocated into 6 treatment groups, of 8 animals each, which received the following anthelmintics orally: group 1, unmedicated controls; group 2, closantel at 10 mg/kg; group 3, oxfendazole at 4.5 mg/kg; group 4, oxfendazole at 4.5 mg/kg and closantel at 5 mg/kg; group 5, oxfendazole at 4.5 mg/kg and closantel at 10 mg/kg; group 6, oxfendazole at 4.5 mg/kg and closantel at 15 mg/kg. Equal numbers of calves were necropsied for parasite recoveries at 7, 8, 9, and 10 days post-treatment. Fluke burdens were reduced by 31%, 51%, and 76% in groups 2, 5, and 6 respectively. Overall, the efficacy of oxfendazole was >99% to 100 % against all of the gastrointestinal nematodes encountered (Ostertagia, Cooperia, Trichostrongylus, Trichuris, and Oesophagostomum). In this study, populations of inhibited Ostertagia ostertagi were not found; both O. kolchida and O. leptospicularis were recovered.

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COMPARATIVE EVALUATION OF FENBENDAZOLE (5 MG/KG) ADMINISTERED IN A 1 TO 6-DAY FEEDING AND IVERMECTIN (100 to 200 MCG/KG) ADMINISTERED SQ. D. H. Bliss*, R. Muser, W. Kvasnicka, L. Krysl, L. Laurence, and R. Lastovica. Verona, WI 53593.

The comparative efficacy of fenbendazole was evaluated for the control of gastrointestinal parasites of cattle when administered at the rate of 5 mg/kg body weight over a one to six-day period compared with the administration of ivermectin at 50% and 100% (100 to 200 mcg/kg) of the recommended dose. Forty cattle all harboring a natural, patent gastrointestinal parasite infection were used in the study. Ten animals received fenbendazole in an oral suspension, ten animals received fenbendazole in a free-choice mineral fed over a 6-day period, five animals received 100 mcg/kg ivermectin, five animals received ivermectin 200 mcg/kg and ten animals remained as non treated control animals. All animals were necropsied except the five animals receiving 200 mcg/kg ivermectin.

Results demonstrated that fecal worm egg counts in both fenbendazole treatments were reduced to negative or low counts (>1.0 eggs) between 48 and 72 hours following treatment. Worm egg counts in the ivermectin treated groups were not consistently reduced in the 100 mcg/kg while egg counts in the 200 mcg/kg treated groups were reduced to low levels by the 10th day. Nematode genera found at necropsy included *Ostertagia, Haemonchus, Cooperia, Trichostrongylus, Nematodirus* and *Oesophagostomum*. Overall, fenbendazole was highly efficacious (>99%) both when given either in a single dose oral suspension or in a mineral administered free-choice over a six-day period. Ivermectin given at a reduced dose (100 mcg/kg) was not found to be efficacious with an overall efficacy evaluation of only 43%. Ivermectin's efficacy at this reduced dose was especially low against *Cooperia spp.* and *Nematodirus helvetianus*.

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PARASITIC GASTRITIS IN A LLAMA (LAMA GLAMA) ASSOCIATED WITH TELADORSAGIA SPP. L.G. RICKARD.* OREGON STATE UNIVERSITY. CORVALLIS, OR 97331

A 7-year-old female llama was experimentally infected with Fasciola hepatica then grazed pasture alongside three domestic sheep. The llama was necropsied in January, 1990 (22 weeks post-infection) as were the sheep. Routine examination of the llama's gastrointestinal tract revealed numerous lesions in the caudal one-fifth of compartment 3. Grossly, the lesions consisted of diffusely coalescing, raised, umbilicated nodules. Microscopically, the mucosa was irregularly thickened. Numerous nematode larvae were present in glandular lumens, often extending to the base of the glands. Where most numerous, decreased numbers of parietal cells, attenuation of glandular epithelium and increased collagen within the lamina propria were evident.

Total numbers of adult nematodes present in compartment 3 were 6,510 and included Camelostrongylus mentulatus (2%), Trichostrongylus axei (47%) and Teladorsagia spp. (51%). With the exception of C. mentulatus, the sheep harbored the same species. Larval numbers in the llama were 56,710. Over 97% were Ostertagia-like early-fourth-stage larvae. Similar larvae were also present in the sheep. Based on the composition of the adult populations in the llama and sheep, the larvae were considered to be species of Teladorsagia. Additionally, the overwhelming numbers of early-fourth-stage larvae may indicate Teladorsagia spp. will undergo arrested development in the llama.

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LIGHT AND SCANNING ELECTRON MICROSCOPY STUDIES ON THE EXTRAHEPATIC BILE DUCT OF SHEEP EXPERIMENTALLY INFECTED WITH FASCIOLA HEPATICA. C.G. LEE*, G.L. ZIMMERMAN AND J.R. DUIMSTRA. COLLEGE OF VETERINARY MEDICINE, OREGON STATE UNIVERSITY, CORVALLIS, OR 97331.

Changes in the proximal common bile duct (CBD) of sheep, which contained adult Fasciola hepatica, were evaluated by light (LM) and scanning electron microscope (SEM). Nine ewes were infected with F. hepatica metacercariae and necropsied 18 weeks later. The CBD, which contained adult flukes, was recovered and examined by LM and SEM. The CBDs from 2 noninfected ewes were used as controls. On gross examination, the adult flukes were free in the lumen of the CBDs which were greatly enlarged. No extensive hemorrhage was found either in intrahepatic or in extrahepatic bile ducts of any animal. Histological examination revealed striking changes such as villous hyperplasia and hypertrophy of the epithelium, cell infiltrations, and arterial intimal inflammation. By SEM, most of the epithelial surface of the CBDs appeared intact. Villous hyperplasia and hypertrophy of the epithelium noted by LM was clearly seen by SEM. The epithelial damage seen as small areas of denuded surface by both LM and SEM was confined to a few areas of the mucosa. Absence of extensive hemorrhage and very confined epithelial damage will be discussed relative to the mode of feeding of adult flukes.

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EXPERIMENTAL BOVINE NEMATODIRIASIS: DEVELOPMENT OF A MODEL FOR CLINICAL, PATHOLOGICAL AND CHEMOTHERAPEUTIC STUDIES. D.E. WORLEY* AND F.M. SEESEE, VETERINARY MOLECULAR BIOLOGY LAB., MONTANA STATE UNIVERSITY, BOZEMAN, MT 59717; J.R. EGERTON, 8100 PONDEROSA, SALIDA, CO 81201.

Pathogenesis and host responses elicited by monospecific Nematodirus helvetianus infections were studied using 2-4 month-old Holstein calves reared in isolation. Ova recovered from bovine feces by washing on 125- and 200-mesh screens were cultured in water for 18-21 days at 28-29° C. Cultures then were held at room temperature for 5-7 days to permit hatching of ova. Single oral doses of 5,000-50,000 infective larvae induced patent infections persisting for an average of > 90 days. Larger doses resulted in higher levels of egg production and were more prolific overall. Peak egg production usually occurred during weeks 4-8 postinoculation. 45% and 17% of the inoculum was recovered as adults from calves necropsied at 69 and 77 days p.i., respectively. Weight losses averaging 4.4% occurred in all calves between 20 and 34 days postinoculation. Hematological and serum biochemical values remained within normal ranges for bovine species.

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HISTOLOGICAL AND HISTOCHEMICAL ANALYSIS OF INTESTINAL EPITHELIUM OF OESOPHAGOSTOMUM COLUMBIAUM (NEMATODA)

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Histologically the intestinal epithelium is devoid of any discernable cell walls but contains large prominent nuclei-rounded in shape in the anterior region and tuboid in the posterior. The presence of a large pyrininophylic nucleolus and secretory granules in the perinuclear space indicates protein synthesis in this area. The cytoplasm of intestinal epithelium contains lipid, protein and glycogen granules. On Luminal side a compact thick microvillar border contains interfilamentous material in the form of mucopolysaccharides. The tips of microvilli though pointed are full of proteinaceous and lipoidal secretions which are also observed in the lumen of the gut, suggesting their microapocrine nature. At the base of brush border lies the terminal web which itself is a highly active area of metabolic significance. The intestinal epithelium functions as an absorptive, digestive, Secretory and storage area. There is evidence of membrane transport of nutrients from the intestinal wall to the growth zones of the reproductive organs.

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CASE STUDY: IDENTIFICATION OF LYME BORRELIOSIS IN THE DOG AND ITS CLINICAL DESCRIPTION. BARRY A. LISSMAN, DVM*. SACHEM ANIMAL HOSPITAL. HOLBROOK, N. Y. 11741

Lyme Borreliosis has been a well recognized disease in dogs since the first description in a dog from Long Island. N.Y. in 1983. The disease is worldwide in its distribution, and has been reported in 44 of the states in the U.S. It is caused by the spirochete Borrelia burgdorferi and is transmitted primarily by the Ixodes sp. of ticks. Clinical manifestations can be divided into early and late infections. In early infections, lameness is the most common finding. Arthralgia and arthritis are most commonly found in the carpal and stifle joints. Dogs may exhibit lethargy, anorexia, fever, and/or lymphadenopathy. Erythema migrans, the skin lesion commonly seen in humans, is rarely seen in dogs. Dogs have also been reported having generalized pain especially in the cervical region. Neurological signs may include seizures, aggression, behavior changes, facial palsy, and progressive paralysis. Other reported symptoms have included uveitis, hepatitis, renal failure including glomerulonephritis, and cardiac disease including conduction disturbances such as A-V block and VPC's. In late infection, dogs can exhibit a chronic lameness and arthritis, chronic renal failure, cardiomyopathy, or chronic neurological disease. Diagnosis should be based on history, clinical findings and positive serology. Culture of the organism from the blood, synovial fluid, urine, or CSF is diagnostic; however, difficulty in culturing Borrelia makes this very impractical. Treatment in dogs is best accomplished early in the course of the disease with antibiotics such as doxycycline, tetracycline, or amoxicillin.

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DEVELOPMENT OF AN EXPERIMENTAL CANINE BORRELIOSIS MODEL. LLOYD G. CHAVEZ, JR.*, HSIEN-JUE CHU, BARBARA M. CARLSON, RANDAL W. SEBRING, AND WILLIAM M. ACREE. BIOLOGICAL RESEARCH AND DEVELOPMENT, FORT DODGE LABORATORIES, P.O.BOX 518, FORT DODGE, IA 50501.

An experimental challenge model for canine borreliosis has been developed which accurately reproduces the major clinical signs of the disease as described in the field. The post-challenge symptoms include fever, lameness, spirochetemia, depression, anorexia, and aggression.

Optimal growth conditions allow for routine isolation of the tick-borne spirochete Borrelia burgdorferi from canine blood and tissues. Spirochetemia is associated with most of the dogs displaying lameness, and a febrile response generally accompanies this symptom. During the post-challenge period, the major recurrent symptoms -- fever, lameness, and spirochetemia -- are quite sporadic. These results emphasize the need for daily observations in the accurate diagnosis of disease. The serum-neutralization assay that was developed indicates that infection does not stimulate strong neutralizing antibody; however, indirect immunofluorescence does demonstrate the occurrence of high antibody titers to B. burgdorferi. This challenge model is important to the study of B. burgdorferi pathogenesis, and will be useful in the development and evaluation of effective therapeutics and prophylactics.

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PASSIVE AND ACTIVE IMMUNIZATION OF HAMSTERS. RUSSELL C. JOHNSON*. UNIVERSITY OF MINNESOTA. MINNEAPOLIS, MN. 55455.

Immunogenic properties of Borrelia burgdorferi strains from similar and diverse geographical locations were compared in passive and active immunization studies. Strains studied included 297 (human spinal fluid isolate, CT), P/Gau (human skin isolate, Germany), IPS (tick isolate, CA), MM1 (MN mouse isolate), MMT1 (MN mouse tick isolate) and CT1 (tick isolate, WI). Hamsters passively immunized subcutaneously with 0.5 to 1.0 ml rabbit antisera (IFA titers 1:2048-1:8192) to virulent strains were fully protected from high level intraperitoneal challenge ($1x10^8$ cells) with the homologous strain but possessed little or no protection to challenge with heterologous strains. In contrast, active immunization with killed whole cells plus an adjuvant provided a greater spectrum of cross protection. Hamsters vaccinated with strain 297 (50 ug dry wt) were provided 87 to 100% protection to challenge with 297, IPS and MM1. Immunogenicity of strain 297 could be correlated with virulence. Rabbit antisera to avirulent 297 and vaccination with these cells (80 ug dry wt) only provided 0 to 43% protection to challenge with virulent 297.

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EFFICACY STUDY OF A BORRELIA BURGDORFERI BACTERIN FOR THE PREVENTION OF LYME DISEASE IN DOGS. HSIEN-JUE CHU*, LLOYD G. CHAVEZ, JR., BARBARA M. CARLSON, RANDAL W. SEBRING, AND WILLIAM M. ACREE. BIOLOGICAL RESEARCH AND DEVELOPMENT, FORT DODGE LABORATORIES, P.O.BOX 518, FORT DODGE, IA 50501.

The efficacy of a commercially available Borrelia burgdorferi bacterin was evaluated in three vaccination and challenge studies for protection of vaccinated dogs against experimentally-induced Lyme borreliosis. Following the experimental challenge with a tick-derived, low-passage B. burgdorferi preparation, nonvaccinated control dogs developed typical clinical signs of canine Lyme disease. The symptoms included intermittent lameness, fever, behavior changes, as well as the appearance of spirochetemia. By comparison, Lyme disease clinical signs and spirochetemia were significantly ($p<0.05$) reduced or prevented following challenge in the vaccinated dogs. A high level of serum-neutralizing antibody response against B. burgdorferi was also observed in the vaccinates following the administration of the bacterin. These results demonstrated the satisfactory immunogenicity and efficacy of the B. burgdorferi bacterin.

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SAFETY STUDY OF A BORRELIA BURGDORFERI BACTERIN FOR THE PREVENTION OF LYME DISEASE IN DOGS. DAVID R. HUSTEAD*, LLOYD G. CHAVEZ, JR., HSIEN-JUE CHU, BARBARA M. CARLSON, RANDAL W. SEBRING, AND WILLIAM ACREE. BIOLOGICAL RESEARCH AND DEVELOPMENT, FORT DODGE LABORATORIES, P.O. BOX 518, FORT DODGE, IA, 50501.

The safety of a commercially available Borrelia burgdorferi bacterin was evaluated. The bacterin was administered at exaggerated doses to dogs both naive and seropositive to Borrelia burgdorferi. The bacterin was administered to dogs in exaggerated doses that had been previously vaccinated with the bacterin and then challenged with pathogenic Borrelia burgdorferi. These animals were evaluated clinically and pathologically. Under normal clinical conditions the bacterin was administered to dogs in areas considered nonendemic and endemic with Borrelia burgdorferi. These dogs were evaluated clinically. No significant adverse responses were seen in any test animals. These studies demonstrated a high degree of safety associated with the use of this bacterin.

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NATIONAL SEROPREVALENCE OF TOXOPLASMA GONDII IN PIGS. J. P. DUBEY*, J. C. LEIGHTY, V. C. BEAL, W. R. ANDERSON, C. D. ANDREWS, PH. THULLIEZ. USDA, ARS, LIVESTOCK AND POULTRY SCIENCES INSTITUTE, ZOONOTIC DISEASES LABORATORY. BELTSVILLE, MD 20705.

Serum samples from 11,842 commercial pigs killed in 1983-1984 throughout the United States were tested for anti-Toxoplasma gondii antibodies by the agglutination test in dilutions of 1:25, 1:50, and 1:500. Anti-T. gondii antibodies were found in 23.9% of pigs. At dilutions of 1:25, 1:50, and 1:500, 13.5%, 6.9%, and 3.5% were serologically positive, respectively. The prevalence of anti-T. gondii antibodies was higher in breeder pigs (42%) than in market pigs (23%). These results indicate that anti-T. gondii antibodies are widespread in the national swine herd because the rates were consistent by region.

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SURVIVAL OF *NEOSPORA CANINUM* CYSTS IN MURINE TISSUES. D.S. LINDSAY*, B.L. BLAGBURN AND J.P. DUBEY. DEPARTMENT OF PATHOBIOLOGY, AUBURN UNIVERSITY, AL 36849 AND USDA, ZOONOTIC DISEASES LABORATORY, BELTSVILLE, MD 20705.

Neospora caninum produces thick-walled tissue cysts in the central nervous system of canines and other hosts. Little is known about the biology of this stage of the parasite. In the present study, groups of mice treated with methylprednisolone acetate (MPA) were inoculated with tachyzoites of the NC-1, NC-2 or NC-3 isolates of *N. caninum* and treated with sulfadiazine in their drinking water (1 mg/ml) to induce chronic infections. Mice were killed 13 months after inoculation and portions of their brains subjected to acid-pepsin digestion. Digests were inoculated into additional groups of MPA-treated mice. All mice inoculated with brain digests developed *N. caninum* infections, indicating that tissue cysts/bradyzoites of all three isolates survived and remained infective for mice for at least 13 months *in vivo*. Portions of the brains from mice originally inoculated with the NC-2 isolate were homogenized in HBSS and stored at 4 C for 7 or 14 days prior to inoculation into groups of MPA treated mice. Mice in both groups developed *N. caninum* infections. Results of these studies indicate that bradyzoites within tissue cysts of *N. caninum* can survive for at least 13 months *in vivo* and for at least 14 days at 4 C.

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NATURAL OUTBREAKS OF BOVINE COCCIDIOSIS IN NORTHERN COLORADO. JOHN M. VETTERLING*. PARASITOLOGIC SERVICES. P.O. BOX 475, FORT COLLINS, COLORADO 80522.

Every year during the winter months, bovine coccidiosis erupts in cattle feed yards in the northern great plains. Most cattle feeders are aware that their cattle have coccidiosis, but to our knowledge little data has been collected to document the outbreaks. Fortunately, feeders have anticoccidials and symptomatic treatment with which to alleviate the problem somewhat, but at an additional expense to production.

In 1987 and 1988 outbreaks of coccidiosis were observed in calves at the Colorado Animal Research Enterprises (C.A.R.E.) facilities northeast of Fort Collins. However, in 1990, we were able to follow the sequelae of signs in a group of calves that were transported, held on an anticoccidial compound and then subsequent to that drugs removal.

I believe this is the first report available from naturally infected calves.

This research was in cooperation with C.A.R.E.

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THE ROLE OF HUMORAL IMMUNITY IN AGE RELATED RESISTANCE TO *CRYPTOSPORIDIUM BAILEYI*.

J. HATKIN*, J.J. GIAMBRONE AND B.L. BLAGBURN. AUBURN UNIVERSITY, AUBURN, AL 36849

Cryptosporidium baileyi, an important protozoan pathogen of domestic avian species, causes overt respiratory disease when oocysts are inoculated intratracheally (IT) into broiler chicks 2 or 14 days of age, but not in 28- or 42-day-old chicks.The purpose of this experiment was to clarify the role of humoral immune mechanisms in the mediation of age related resistance to *C. baileyi* infections or respiratory disease. Broiler chicks were bursectomized *in ovo* with testosterone and with cyclophosphamide after hatching. Chicks were divided into 6 groups to evaluate the effects of bursectomy on antibody responses and susceptibility to *C. baileyi.* Chicks were raised to either 7 or 21 days of age prior to IT inoculation with *C. baileyi.* All birds were necropsied 10 days following IT challenge with 2×10^6 *C. baileyi* oocysts. Anti- *C. baileyi* specific IgG was present in the intact (non-bursectomized) chicks, but was undetectable in the bursectomized chicks. Cell mediated immunity, determined by the cutaneous basophil reaction, was identical in both intact and bursectomized birds. Clinical respiratory disease began 6 days post-inoculation in both intact & bursectomized birds. Intact birds had more severe clinical disease. Airsacculitis was similarly present in both intact & bursectomized birds. Normal bursas were present in non-bursectomized birds, but absent in bursectomized birds. Intact birds had greater, more uniform body weights than did bursectomized birds. Microscopic examination verified 3 of 13 intact chicks and 10 of 32 bursectomized chicks were infected with *C. baileyi.* These results indicate that age related resistance to *C. baileyi* in broiler chicks is not mediated solely by circulating specific anti-cryptosporidial IgG.

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OCCURRENCE OF GIARDIA IN LLAMAS (LAMA GLAMA).

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Cysts of Giardia were detected in feces from 2 llamas by centrifugal flotation of samples in Sheather's sugar solution. Both llamas were patients at the Large Animal Clinic (LAC) of the College of Veterinary Medicine (CVM) at Iowa State University (ISU) in Ames, IA. Fecal samples collected daily from a yearling female that was hospitalized for 7 days contained Giardia cysts at concentrations of 183,333 to 520,000 per gram of soft, unpelleted feces for 5 days, and 70,000 per gram of fluid feces for 1 day. The llama succumbed to systemic salmonellosis on the 7th day. A 3-month-old female llama, presented with bilateral cloudy corneas and diarrhea of approximately 1 week in duration, had 750,000 cysts per gram of unformed feces.

In a preliminary study to estimate the prevalence of Giardia in lamoids, feces were obtained from 30 animals from 16 different midwestern sources. Of the animals, 22 were patients at the LAC CVM ISU and 8 were not. An indirect fluorescent antibody test (Merifluor-Giardia, Meridian Diagnostics, Cincinnati, OH) was used to detect cysts in the fecal samples. Giardia cysts were found in feces from 3 (10%) of the animals from 3 different sources. The pathogenicity and species identity of Giardia from lamoids are currently unknown.

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CONSTRUCTION OF A GENOMIC LIBRARY OF *SARCOCYSTIS CRUZI* SPOROZOITE DNA. F.S.B. KIBENGE, R.J. CAWTHORN*, D. DESPRES, P. MCKENNA AND R.J.F. MARKHAM. UNIVERSITY OF PRINCE EDWARD ISLAND, CHARLOTTETOWN, PRINCE EDWARD ISLAND, CANADA.

Sarcocystosis is increasingly being recognized as a disease causing significant economic losses to the cattle industry, and it is essential that better methods are developed to detect and control this organism in livestock. Toward this end, a genomic library of *Sarcocystis cruzi* sporozoite DNA was constructed in bacteriophage lambda gt10 and screened by hybridization to ^{32}P-labeled *S. cruzi* merozoite DNA. The DNA insert of one clone, SL41, when labeled with ^{32}P hybridized only with *S. cruzi* DNA but not with bovine cellular DNA in dot-blots, and may provide an improved means of diagnosis of acute bovine sarcocystosis.

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ANTIGEN ANALYSIS OF SARCOCYSTIS NEURONA MEROZOITES CULTURED FROM LESIONS OF EQUINE PROTOZOAL MYELOENCEPHALITIS. D.E. GRANSTROM*, S.W. DAVIS[1], J.P. DUBEY[1], P.F. COMER. UNIVERSITY OF KENTUCKY, LEXINGTON, KY 40546 AND [1]USDA,ARS, ZOONOTIC DISEASES LABORATORY, BELTSVILLE, MD 20705.

Members of the genus Sarcocystis share many antigens. Antibodies from animals exposed to a single Sarcocystis sp. cross-react with antigen preparations from many Sarcocystis spp. This interferes with accurate interpretation of Sarcocystis immunoassays. The objective of the present study was to identify Sarcocystis neurona specific proteins.

Sarcocystis neurona merozoites were harvested from bovine monocyte cell cultures and purified on a colloidal silica step gradient. Purified zoites were solubilized in SDS-PAGE sample buffer followed by electrophoresis and silver staining or transfer to nitrocellulose for immunoblot analysis. Blotted proteins were then probed with various sera, (1-3) Sarcocystis cruzi, Sarcocystis muris, and S. neurona antisera produced in rabbits, (4) serum from a pony experimentally infected with Sarcocystis fayeri, (5) serum from a horse injected with S. neurona, (6-10) sera from 5 confirmed cases of equine protozoal myeloencephalitis. Two apparent S. neurona specific proteins were detected.

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IN VITRO CULTIVATION OF SARCOCYSTIS NEURONA, THE ETIOLOGICAL AGENT OF EQUINE PROTOZOAL MYELOENCEPHALITIS. S. W. DAVIS* AND J. P. DUBEY. USDA, ARS, LIVESTOCK AND POULTRY SCIENCES INSTITUTE, ZOONOTIC DISEASES LABORATORY. BELTSVILLE, MD 20705.

Sarcocystis neurona, the etiological agent of equine protozoal myeloencephalitis (EPM) was isolated from a horse with naturally-acquired myelitis by inoculation onto a bovine monocyte cell culture. S. neurona divided by endopolygeny and formed schizonts in the cytoplasm of cultured cells. Extracellular merozoites in Giemsa stained preparations were approximately 6.7 x 1.5 um with one pole more tapered than the other and a subterminal or central nucleus. It has been possible to maintain S. neurona in continuous culture for over 150 days by transfer of infected bovine monocytes to fresh cell monolayers. Cultured organisms reacted with sera from horses with histologically confirmed EPM as well as sera from horses experimentally infected with S. fayeri.

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AN UPDATE ON THE CONTROL OF COCCIDIOSIS IN GAME BIRDS. M. D. RUFF. ARS/ USDA, LIVESTOCK AND POULTRY SCIENCE INSTITUTE, BARC-EAST, BELTSVILLE, MARYLAND 20705

The control of coccidiosis in game birds is different than control in chickens for several reasons. The species of Eimeria infecting game birds are unique species that differ in drug sensitivity from those coccidia infecting chickens. Many anticoccidials commonly used in chickens are ineffective against game bird coccidia. Furthermore, some of the compounds such as sulfaquinoxaline in bob white quail and halofuginone in chukar partridge are toxic at levels that are safe for use in chickens. The practice of raising multiple age birds on the same game bird farm also complicates control as game birds are slow to develop immunity to coccidiosis and older birds remain carriers that can infect younger birds. Effective compounds identified to date include monensin and salinomycin in bob white and Japanese quail, and sulfaquinoxaline, rofenaid (sulfadinethoxine and ormetoprim) and lasalocid in chukar partridge.

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CLONING OF THE GENE FOR THE MAJOR EXSHEATHMENT PROTEIN FROM THE SECOND MOLT CUTICLE OF INFECTIVE HAEMONCHUS CONTORTUS LARVAE. L. MANSFIELD AIKENS*, H. R. GAMBLE AND R. D. KLEIN. USDA, ARS, HELMINTHIC DISEASES LABORATORY, BELTSVILLE, MD 20705 AND THE UPJOHN COMPANY, KALAMAZOO, MI 49001.

The ecdysis of infective Haemonchus contortus larvae, and related ruminant trichostrongyles, is effected by the enzymatic degradation of a specialized region of the second molt cuticle. This region contains a biochemically unique polypeptide, of M_r 160 kDa, termed the major exsheathment protein. This polypeptide, which is synthesized at approximately 6 days of larval development, has an amino acid composition that differs from known cuticle collagens.

Monoclonal antibody prepared to the major exsheathment protein was used to screen a H. contortus genomic library constructed in lambda gt11. A positive clone of approximately 200 base pairs was plaque purified and subcloned into a pBluescript phagemid for sequencing. Sequencing by the dideoxy chain termination method revealed a complete open reading frame encoding a 59 amino acid polypeptide. This sequence had no homology to known collagen gene sequences. Radioactive probes were prepared by nick translation from cloned DNA and used to screen mRNA prepared from various stages of the parasite to demonstrate developmental expression of this gene.

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CHARACTERIZATION OF cDNA CLONES ENCODING PUTATIVE MALIC ENZYMES FROM *HAEMONCHUS CONTORTUS*. R.D. KLEIN, L. VANOVER*, R.S. GRAHAM, C.A. WINTERROWD, M.A. FAVREAU, S.C. NULF, S.J. ALEXANDER, N.T. HATZENBUHLER, M.H. SHEA AND T.G. GEARY. THE UPJOHN COMPANY, KALAMAZOO, MI 49001.

NAD-dependent malic enzyme has a critical function in the anaerobic mitochondrial metabolism of parasitic nematodes. A proportion of the mitochondrial malate pool is converted to pyruvate and CO_2 by this enzyme in a dismutation reaction which generates NADH. Malic enzyme is the sole source of reducing equivalents for the mitochondria. To characterize this enzyme more completely, we undertook its cloning from a parasitic nematode of ruminants, *Haemonchus contortus*. Clones encoding putative malic enzyme cDNAs were transformed into a malic enzyme$^-$ strain of *E. coli*. Those which allowed this strain to grow when malate was the only carbon source (mal + phenotype) were chosen for further analysis. Plasmids which repeatedly conferred a mal + phenotype generated either NAD- or NADP-dependent malic enzyme activity in bacterial supernatants, determined by spectrophotometric and NMR techniques. Neither the NADP- or NAD-dependent malic enzyme clones showed significant similarity to mammalian malic enzyme sequences. Purification work is underway to confirm that these cDNA clones encode malic enzymes from *H. contortus*.

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CLONING AND CHARACTERIZATION OF PHOSPHOENOLPYRUVATE CARBOXYKINASE FROM THE PARASITIC NEMATODE *HAEMONCHUS CONTORTUS*

R.D. KLEIN*, C.A. WINTERROWD, N.T. HATZENBUHLER, M.H. SHEA, M.A. FAVREAU, S.C. NULF AND T.G. GEARY. UPJOHN LABORATORIES, KALAMAZOO, MI 49001.

Biochemical and metabolic data have led to the conclusion that the enzyme phosphoenolpyruvate carboxykinase (PEPCK; EC 4.1.1.32) contributes to a critical point of divergence in energy conservation pathways between mammals and nematodes. We have cloned a cDNA encoding this enzyme from a parasitic nematode of ruminants, *Haemonchus contortus* by functional complementation of a PEPCK$^-$, malic enzyme$^-$ strain of *E. coli* (E1786) using an egg stage *H. contortus* cDNA library in λZAPII. Selection was for growth on malate as the sole carbon source (mal$^+$ phenotype). We isolated a plasmid, pPEPCK, which reproducibly confers a mal$^+$ phenotype in E1786. The sequence of the 2.0 kb *Eco*R1 insert of pPEPCK predicts a 612-amino acid protein which shows about 74% similarity to *Drosophila melanogaster* and chicken PEPCK. Extracts of E1786[pPEPCK], but not E1786, contain IDP- or GDP-dependent PEPCK enzyme activity. Sequence analysis revealed that the open reading frame (ORF) in pPEPCK lacked a 5' initiation codon and was probably expressed as an in-frame fusion protein with β-galactosidase. A strategy combining library screening with PCR analysis of positive clones led to the identification of a clone containing 6 additional NH_2-terminal amino acids, including a met which, by comparison with known PEPCK amino acid sequence, is likely to be the translation initiation site.

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DEVELOPMENT OF AN IN VITRO PHYSIOLOGICAL ASSAY TO ASSESS EFFECTS OF EXPERIMENTAL AGENTS ON ADULT HAEMONCHUS CONTORTUS.

E.M. THOMAS*, J.P. DAVIS, T.G. GEARY AND D.P. THOMPSON. ANIMAL HEALTH THERAPEUTICS RESEARCH, THE UPJOHN COMPANY, KALAMAZOO, MI 49001 USA

Previous efforts to screen or determine intrinsic potencies and kinetics of experimental agents against the adult stage of gastrointestinal nematodes have been hindered by the absence of in vitro culture systems and objective/quantitative assays to measure drug effects. We conducted a series of studies to determine culture conditions suitable for in vitro maintenance of adult H. contortus. The parasites were manually removed from the abomassa of lambs 6-12 weeks post-inoculation, washed 3X with HEPES-buffered RPMI-1640 (pH = 7.4) containing antibiotics, placed in borosilicate culture tubes containing 2.5 ml of the same medium, and maintained at 37°. Parasite viability and responsiveness to experimental compounds were objectively monitored using a Micromotility Meter®. Data acquisition and analysis steps were fully automated, with an IBM-PC AT computer providing data acquisition, timing, control, and mainframe link; while a Zymark® robotic system provided sample handling. The crucial factors for maintenance of adult H. contortus in RPMI-1640 were the number of parasites per culture tube, worm gender, and gas phase; the best combination being five females/tube and air as the gas phase. Using these culture conditions, we determined the concentration and time-dependent effects of several anthelmintic and non-anthelmintic agents on H. contortus motility during 48 hr incubations. Results of these tests suggest that the assay will detect the effects of compounds that act at neuroreceptors and some energy pathways in H. contortus but a number of compounds with other modes of action may not be detected. That is, the paralyzing actions of Ivermectin®, levamisole, and closantel were detected at concentrations as low as at 1 nM, 0.1 μM, and 1 μM respectively; while the effects of 10 μM thiabendazole, albendazole, fenbendazole, and stibophen were not detected in this assay, even following 48 hr incubations.

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INTERACTION OF BENZIMIDAZOLE ANTHELMINTICS WITH HAEMONCHUS CONTORTUS TUBULIN: BINDING AFFINITY AND ANTHELMINTIC EFFICACY. G.W. LUBEGA* AND R.K. PRICHARD. INSTITUTE OF PARASITOLOGY OF McGILL UNIVERSITY. MONTREAL, CANADA H9X 1C0.

The ability of various benzimidazoles (BZs) to bind tubulin was assessed by determining their IC_{50} values (the concentration of unlabelled drug required to inhibit 50% of the labelled drug binding), K_a (the apparent equilibrium association constant) and B_{max} (the maximum binding at infinite [drug] = [drug-receptor]). The ability of unlabelled benzimidazoles - fenbendazole (FBZ), mebendazole (MBZ), oxibendazole (OBZ), albendazole (ABZ), rycobendazole (albendazole sulphoxide, ABZSO), albendazole sulphone ($ABZSO_2$), oxfendazole (OFZ) and thiabendazole (TBZ) - to bind tubulin was determined from their ability to inhibit the binding of [^{3}H]MBZ or [^{3}H]OBZ to tubulin in supernatants derived from unembryonated eggs or adult worms of Haemonchus contortus. The binding constants (IC_{50}, K_a and B_{max}) correlated with the known anthelmintic potency (recommended therapeutic doses) of the BZ compounds except for OFZ and ABZSO whose K_a values were lower than could be expected from anthelmintic potency. Differences in drug metabolism are believed to account for the anomalous behaviour of OFZ and ABZSO. Nevertheless, [^{3}H]OFZ, like [^{3}H]OBZ, [^{3}H]MBZ or [^{3}H]ABZ, had a reduced ability to bind tubulin (lower B_{max}) from BZ-resistant H. contortus.

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CHARACTERIZATION OF PLASMA-GASTROINTESTINAL EXCHANGE FOR ALBENDAZOLE METABOLITES AFTER ADMINISTRATION OF NETOBIMIN TO CATTLE. C. LANUSSE*, L. GASCON, C. TRUDEAU AND R. PRICHARD. INST. PARASITOL., MCGILL UNIV., CANADA.

The antiparasite efficacy of Benzimidazole and pro-Benzimidazole anthelmintics not only depend on their affinity for parasite tubulin, but also on their ability to reach high and sustained concentrations on the site of parasite location; this in turn depends on host-related pharmacokinetic, metabolic and tissue distribution factors. The present work was carried out to establish the compartmental distribution of Netobimin (NTB) and its Albendazole (ABZ) metabolites in cattle. Eight parasite-free Holstein steers (140-150 kg) were surgically fitted with permanent cannulae in the rumen, abomasum and ileum. Animals were orally treated with a suspension of zwitterion Netobimin at 20 mg/kg. Samples of plasma and ruminal, abomasal and ileal fluids were analyzed for NTB, ABZ, ABZ sulfoxide (ABZSO), ABZ sulfone ($ABZSO_2$) and amino-ABZ-sulfone (NH_2ABZSO_2) by HPLC over 96 h post-treatment. NTB parent drug was only found in the GI tract and for only 12-18 h post-treatment. ABZSO and $ABZSO_2$ were the main metabolites found in plasma. They were reversibly exchanged between plasma and GI compartments in the first 30-36 h post-treatment. These metabolites were greatly concentrated in the abomasum, probably due to ion-trapping exchange between plasma and the GI compartments; this phenomenon probably accounts for the presence of ABZ and its metabolites in the GI tract for 72 h post-treatment. The presence of ABZ and ABZSO in abomasum and intestine for that period of time is relevant for anthelmintic efficacy against GI parasites. The kinetics of the NH_2ABZSO_2 metabolite was characterized for the first time.

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METABOLIC LABELING OF DIROFILARIA IMMITIS THIRD- AND FOURTH-STAGE LARVAE AND THE IDENTIFICATION OF TWO PROTEINS ASSOCIATED WITH THE MOLT. G.R. FRANK AND R.B. GRIEVE. COLORADO STATE UNIVERSITY. FORT COLLINS, CO 80523.

Due to the difficulties and expense in generating large numbers of third- and fourth-stage larvae, more sensitive means of identifying proteins were sought for future studies on their kinetics of production, biological roles and immunological relevance. The infective third-stage larvae of Dirofilaria immitis were collected from Aedes aegypti and cultured in vitro to the fourth-stage. Larval proteins were metabolically labeled using 35S-cysteine and methionine in different media and for different lengths of time. Labeled proteins in the excretory-secretory component (ES) and the larval homogenates were evaluated by SDS-PAGE under reducing and non-reducing conditions and by two dimensional gel electrophoresis. Numerous proteins ranging from 14 to greater than 200 kilodaltons (kd) were identified from both the ES and the larval homogenates. Both fractions demonstrated shared and unique molecules. Using timed labeling, age- and stage- specific proteins were identified, with at least two molecules of approximately 20.5 and 22 kd being associated with the molt from the third- to fourth-stage. Both molecules appear to be recognized by sera from dogs immune to infection, but not by sera from their infected non-immune cohorts. (Supported by The Edna McConnell Clark Foundation and Paravax, Inc.).

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PARTIAL CHARACTERIZATION OF THE EGG SHELL OF HAEMONCHUS CONTORTUS. H. R. GAMBLE*, L. MANSFIELD-AIKENS AND R. H. FETTERER. USDA, ARS, HELMINTHIC DISEASES LABORATORY, BELTSVILLE, MD 20705.

The nematode egg shell serves as the primary barrier for protection of the developing embryo. Following embryonation in ruminant trichostrongyles, the first stage larva hatches, by a mechanism which is not completely understood, but appears to be mediated in part by enzymes. To understand the action of enzymes on the egg shell it is important to have a clear understanding of the composition and organization of the egg shell.

At the EM level, the egg shell of Haemonchus contortus was similar in appearance to the general pattern described for many nematodes, including an outer vitelline layer bounded by a trilaminate membrane, a broad medial region, thought to contain chitin in some species, and an electron dense basal region, thought to contain lipid. Exposure of H. contortus eggs to proteases resulted in disruption of the outer region of the shell, including separation of the bounding membrane, and the apparent removal of some components of the medial and basal regions. Exposure to chitinase appeared to deplete components of the medial region of the shell, while collagenase was without effect. After normal hatching, shells appeared similar to those treated with protease and chitinase, but also lacked the basal, lipid layer. Biotinylation of intact egg shells resulted in limited labelling. Labelled, extracted components consisted of three major components on SDS gels. Labelled components were digested by proteases and chitinase, but not collagenase or lipase.

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SYNTHESIS OF TYROSINE DERIVED CUTICULAR CROSS-LINKS IN NEMATODE LARVAE
R.H. FETTERER*, M.L. RHOADS AND J.F. URBAN, JR. USDA, ARS, LPSI, HELMINTHIC DISEASES LABORATORY. BELTSVILLE, MD 20705.

Previous studies had demonstrated the presence of the cross-linking amino acids dityrosine and isotrityrosine in the cuticular proteins from Ascaris suum and Haemonchus contortus. The mechanism for the synthesis of these cross-links in parasitic nematodes is unknown. Experiments were carried out to assess the in vitro synthesis of tyrosine derived cross-links in larval nematodes. Third stage A. suum larvae (L3) were cultured in vitro in the presence of tritiated tyrosine. Cultured larvae were harvested after 3 or 6 days in culture (DIC). The presence of tritiated dityrosine or isotrityrosine was detected by chromatographic methods following acid hydrolysis of isolated cuticles. A. suum larvae synthesize radiolabeled dityrosine and isotrityrosine in cuticular proteins. The rate of synthesis appears higher in larvae labeled after 6 DIC compared to those larvae labeled after 3 DIC. Inhibitors of peroxidase activity reduced the synthesis of dityrosine and isotrityrosine suggesting the formation of these cross-links is mediated by a peroxidase type enzyme system. Cell free homogenates of A. suum larvae (in vitro derived L4) mediated the conversion of radiolabeled tyrosine to dityrosine suggesting the presence of an enzyme in A. suum responsible for formation of cuticular cross-links. The above results suggest that nematodes synthesize the cuticular cross-links dityrosine and isotrityrosine via a peroxidase enzyme system.

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MECHANISTIC STUDIES IN THE TRANSCUTICULAR DELIVERY OF ANTIPARASITIC DRUGS: *EX VIVO/IN VITRO* CORRELATION OF SOLUTE TRANSPORT BY *ASCARIS SUUM*.
D.P. THOMPSON[1], N.F.H.HO[2], S.M. SIMS[2], C.L. BARSUHN[2] AND T.G. GEARY[1]*. [1]PARASITOLOGY RESEARCH, [2]DRUG DELIVERY SYSTEMS RESEARCH, THE UPJOHN COMPANY, KALAMAZOO, MI 49001

Using live, intact *Ascaris suum* and a closed perfusion system (the *ex vivo* paradigm), the disappearance kinetics and tissue distribution of selected radiolabeled permeants were measured to determine the potential importance of the transcuticular pathway for drug absorption. The data support the conclusions established by previous *in vitro* transport studies which utilized excised cuticle-hypocuticle tissue preparations. The external surface of *Ascaris* can be breached by drugs and the rate-determining barrier is the lipoidal hypocuticle tissue, provided the permeant is sufficiently small to traverse the aqueous-filled, negatively charged collagen matrix of the cuticle. The *ex vivo* permeability coefficients of the model permeants for the cuticle-hypocuticle barrier were in good quantitative agreement with the *in vitro* permeability coefficients. The lipophilic permeants hydrocortisone and p-nitrophenol were preferentially distributed in the gut tissue, whereas the hydrophilic permeant urea was distributed evenly throughout the organism. Ligated and nonligated *Ascaris* showed no significant differences in either uptake kinetics or tissue distribution of the permeants. This suggests that the transcuticular pathway is the major route of drug absorption as compared to oral ingestion.

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CHROMATOGRAPHIC ANALYSES OF THE LIPIDS OF ADULT ONCHOCERCA GIBSONI. M. D. MALONEY*, AND L. H. SEMPREVIVO. UNIVERSITY OF MASSACHUSETTS, AMHERST,MA01003

Despite the possible effects of parasite lipids on the course of infection, little information was available on the lipid composition of parasitic helminths of the genus Onchocerca. Lipids were extracted from adult Onchocerca gibsoni with chloroform/methanol and the total lipid content characterized. Glycolipids were isolated from other lipid classes by Florisil column chromatography and fractionated by DEAE-Sephadex ion-exchange chromatography. HPTLC revealed the presence of 9 neutral glycolipid bands and 15 acidic glycolipid bands which stained for sialic acid with resorcinol. Non-carbohydrate-containing lipids were analyzed by a combination of TLC and amino column chromatography. Triacylglycerols, cholesterol, cholesterol esters, and free fatty acids were found to be major components of the neutral lipid fraction. Diacylglycerols and monoacylglycerols were minor components. Phosphatidylethanolamine and phosphatidylcholine were the predominant phospholipids. Phosphatidylserine, phosphatidylinositol, sphingomyelin, lysophosphatidylcholine, and lysophosphatidylethanolamine were also present in significant amounts while only traces of cardiolipin and phosphatidic acid were observed. Several minor lipids and phospholipids remained unidentified. These results indicate that adult O. gibsoni have a non-glycosylated lipid composition which resembles that of other parasitic nematodes as well as a substantial repertoire of glycolipids including many with the characteristics of gangliosides. This work was supported by a grant from the McConnell Clark Foundation.

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IN VIVO INJECTION OF ANTIBODIES TO INTERLEUKIN-4 RECEPTOR AND INTERFERON GAMMA HAVE OPPOSING EFFECTS ON IMMUNITY TO HELIGMOSOMOIDES POLYGYRUS. J.F. URBAN, JR.*, I.M. KATONA, AND F.D. FINKELMAN. USDA, ARS, LPSI, HELMINTHIC DISEASES LABORATORY, BELTSVILLE, MD 20705 AND UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES, BETHESDA, MD 20814.

Earlier studies in mice demonstrated that depletion of $CD4^+$ T cell activity in vivo increases the fecundity of H. polygyrus from a primary infection and blocks protective immunity to a challenge infection. These same effects resulted from treatment of mice with antibodies to interleukin 4 (IL-4) and/or the IL-4 receptor. Because IL-4 is a product of $CD4^+$ T cells of the Th2 phenotype and IL-4 expression is repressed by interferon gamma (IFN), a product of Th1 cells, the effect of neutralization of IFN activity in vivo was evaluated in this experimental system. Mice treated with anti-IFN during a primary infection with H. polygyrus showed a decrease in worm fecundity while agents that increased the production of IFN, like poly I:C and Brucella abortus, increased worm fecundity. These results suggest that modulation of the lympokines expressed prominently by either Th1 or Th2 cells can have dramatic effects on host responses to chronic gastrointestinal nematode infection.

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ANTI-HEART TISSUE ANTIBODIES IN TRYPANOSOMA CRUZI INFECTED DOGS. S.C. BARR*, N. NORCROSS. CORNELL UNIVERSITY. ITHACA, NY 14850.

We have previously reported the clinical, pathologic, parasitologic, and immunologic features of dogs infected with either pathogenic (Tc-O) or non-pathogenic (Tc-D) Trypanosoma cruzi isolates. Tc-O infected dogs develop dilational cardiomyopathy by day 240 post-infection (PI), while Tc-D infected dogs had no cardiac disease. Both Tc-O and Tc-D infected dogs develop similar anti-T. cruzi antibody titers. It has been postulated that cross reacting antibodies between T. cruzi and heart tissues, specifically adrenergic beta-receptors, are important in the development of Chagasic cardiomyopathy. In a preliminary step to test this hypothesis, we first developed an ELISA to determine if sera from Tc-O and Tc-D infected dogs contained anti-heart tissue antibodies. Anti-heart tissue antibody titers were increased in Tc-O infected dogs by day 30 PI, still increased by day 100 PI from pre-infection levels, but reduced from levels at day 30 PI. By day 240 PI, levels had declined from day 100 PI but were still elevated above pre-infection levels. At no time were anti-heart tissue antibody titers significantly elevated above those of uninfected control dogs. We then used western blot analysis to determine if common or different protein bands within heart tissues were identified by antibodies from Tc-O and Tc-D infected dogs. Protein bands with approximate molecular weights of 115, 90, 81, 62, and 55 kDa were consistently identified, although very faintly, by sera from individual dogs from both groups. Although the faintness of staining interfered with accurately reading of the blots, there did not appear to be many differences between paterns identified by sera from Tc-O and Tc-D infected dogs. To identify possible cross reacting antigens between T. cruzi and heart tissues, western blots prepared by reacting sera from infected dogs with the 2 antigen preparations were compared. 115 and 80 kDa bands were common to both antigen preparations reacted with sera from Tc-O infected dogs while 90 and 80 kDa bands were identified in both antigen preparations by sera from Tc-D infected dogs. Further work is needed to elucidate the role of these common protein bands in the pathogenesis of canine trypanosomiasis.

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A LECTIN-LIKE EOSINOPHIL CHEMOTACTIC FACTOR IN THE SECRETORY/EXCRETORY SUBSTANCES OF A PARASITIC NEMATODE. PHILLIP KLESIUS*, USDA, ARS, ANIMAL PARASITE RESEARCH LABORATORY. AUBURN, AL 36830.

We found that Lectin-like proteins of excretory/secretory substances from larvae of Ostertagia ostertagi had eosinophil chemotactic activity. A fucose-agarose affinity column iosolated the eosinophil chemotactic factor (ECF). L-fucose inhibited the chemotactic activity. The ECF had molecular sizes of about 66, 22, and 14 KDa by SDS-Page analysis. Neuraminidase treatment of eosinophils inhibited the chemotactic response of ECF. We found that the L-fucose binding lectin, Ulex europaeus was chemotactic for bovine eosinophils. This lectin competitively inhibited ECF activity. The ECF and U. europaeus lectin apparently recognize similar sugar sequences on glycoproteins. Bovine eosinophils have fucose-containing glycoprotein receptors that react with ECF lectin-like proteins. Evidence suggests that larvae secrete fucose-specific lectin-like proteins responsible for eosinophil chemotaxis.

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FURTHER CHARACTERIZATION OF LIVE SUBCUTANEOUS POSTINFECTIVE HELIGMOSOMOIDES POLYGYRUS LARVAL VACCINE. J. P. TRITSCHLER II*, L. H. SEMPREVIVO AND M. D. MALONEY. UNIVERSITY OF MASSACHUSETTS, AMHERST, MA 01003.

BALB/c mice vaccinated subcutaneously (s.c.) with living H. polygyrus L4 develop a high degree of immunity. Experiments were conducted with several inbred (BALB/c, C3H, C57BL/6) and one outbred strain (CD1) of mice to further characterize our subcutaneous vaccine model and to establish its duration in BALB/c mice. The treatments were subcutaneous L4 vaccine, oral L3 vaccine and control. The subcutaneous L4 groups were immunized s.c. with 10 L4 on day 0. The oral L3 groups were immunized orally with 50 L3 on days 0, 3, 5, 7, 10, and 12 and the infection truncated with levamisole (50 mg/kg body weight) on day 27. The control groups were not immunized. All mice were challenged per os with 40 L3 on day 32. The percentage of protection compared to the controls for the oral L3 groups were 81.0%, 41.8%, 18.2%, and 91.7%, and for the subcutaneous L4 groups were 84.7%, 14.0%, 0%, and 17.2% for BALB/c, C3H, C57BL/6, and CD1, respectively. While the hierarchy is similar within the inbred strains, the major difference in treatment response occurred within the outbred CD1 mice suggesting that immune responses of oral L3 and subcutaneous L4 may be similar but are probably not identical. In other trials, groups of 9-11 BALB/c mice were vaccinated s.c. with 10 L4 on day 0 and challenged with 40 L3 on day 34 or 70. The percentage of protection compared to naive controls was 95% for day 34 challenge and 98% for day 70 challenge. Results suggest that the capacity to respond to a subcutaneous L4 vaccine,like the oral vaccine, is influenced by host genetic background.

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A SURVEY OF OVINE PARASITE CONTROL PRACTICES IN TENNESSEE. C.R. REINEMEYER*, B.W. ROHRBACH, V.M. GRANT and G. RADDE. UNIVERSITY OF TENNESSEE, KNOXVILLE, TN 37996-4500.

A random sample of 126 sheep owners was selected from members of a state-wide sheep producers' organization. Owners were contacted by phone and asked to provide information about their farms, sheep, parasite control practices and sources of information. 125/126 owners (99%) completed a standard questionnaire.

The average owner grazed 20 lambs, 20 ewes and 2 rams on 3 pastures totalling 20 acres. In decreasing order, sheep were dewormed according to a regular schedule, to coincide with breeding management practices, or to treat clinical signs of parasitism. Proportions of producers deworming the various classes of sheep 0, 1, 2, 3, 4 or more than 4 times annually were as follows: *lambs* - 3, 28, 40, 16, 9 and 8%, respectively; *ewes* - 3, 8, 20, 16, 34 and 22%, respectively; and *rams* - 1, 9, 19, 15, 38 and 19%, respectively. Similar proportions of owners used a single drug class exclusively or alternated among classes within a farming year. The most common drugs used exclusively were ivermectin (39 to 66%), levamisole (19 to 33%) and benzimidazoles (13 to 24%). At least one ovine anthelmintic had been discontinued by 52% of producers due to poor clinical efficacy or inconvenience of administration. Over 82% of all producers planned to use the same drugs and/or identical regimens in the following year. Sheep-oriented publications, other sheep owners, and veterinarians were the most common sources of information about parasite control programs and choice of anthelmintics.

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CONTINUAL OR EPISODIC ADMINISTRATION OF PROLACTIN ALTERS POPULATION INDICES IN OVINE INFECTIONS OF HAEMONCHUS CONTORTUS. MICHAEL W. FLEMING*, USDA, ARS, LPSI, HELMINTHIC DISEASES LABORATORY. BELTSVILLE, MD 20705.

When prolactin was administered previously to lambs (days 28 to 40 P.I.) during patency of H. contortus infections (> 20 days P.I.), female worms had increased growth and fecundity, suggesting a mechanism for periparturient egg rise. The current studies were initiated to further define the temporal limits of these prolactin-induced effects on H. contortus. Experiment 1 was designed to determine the effects of prolactin on infections when hormonal exposure encompassed the entirety of in vivo development. Lambs were injected daily with saline or 25 I.U. of ovine prolactin from 5 days pre-inoculation with infective larvae until 38 days P.I. Fewer and larger worms were recovered from the prolactin-treated lambs; fecundity was enhanced also in this group. In experiment 2, lambs were injected daily with saline or prolactin during weeks 1, 2, or 3 of patency. Fecal egg production was monitored through each week. No differences occurred in the number of males, but the number of females decreased with treatment and with time. Egg concentrations and production increased in week 2 of prolactin treatment only. No differences in fecundity occurred with treatment or with time. Collectively, enhanced nematode fecundity and growth appears dependent on extended prolactin exposure of lambs during patency, not with the coincidence of early developmental stages.

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THE EFFECT OF DIET ON THE KINETIC DISPOSITION OF OXFENDAZOLE IN SHEEP. D.A. ALI, D.R. HENNESSY* AND J.W. STEEL. CSIRO DIVISION OF ANIMAL HEALTH, GLEBE, AUSTRALIA.

The dynamics of oxfendazole (OFZ) disposition in digesta fluid and particulate matter was compared in sheep maintained on two levels of a lucerne wheaten chaff diet. The flow rate of digesta fluid and particles from the rumen and abomasum, together with abomasal secretions, increased at the higher feed intake. OFZ and its metabolites were predominantly associated with the particulate phase of digesta and amounts in digesta fluid and the bloodstream were significantly lower in animals on the higher feed intake. Reduced systemic levels were possibly due to reduced residence time of drug at the site(s) of absorption. These results indicate that the level of feed intake plays an important role in drug availability.

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THE EFFICACY OF MOXIDECTIN AGAINST AN IVERMECTIN RESISTANT STRAIN OF HAEMONCHUS CONTORTUS IN SHEEP. T.M. CRAIG* AND T.A. HATFIELD. TEXAS A & M UNIVERSITY, COLLEGE STATION, TX 77843.

Eight groups of six lambs each were infected with an estimated 5,000 infective larvae of Haemonchus contortus. Three groups were administered a strain which had no evidence of anthelmintic resistance and the other five groups a strain resistant to benzimidazoles and ivermectin. Twenty-eight days post infection each lamb was administered either no drug, 0.2 or 0.4 mg/kg moxidectin, or 0.4 or 0.8 mg/kg ivermectin. Compared to controls the percent reduction of H contortus in the susceptible strain was 99.9% for ivermectin at 0.2 mg/kg and 100% for moxidectin at 0.2 mg/kg. In the resistant strain there were more worms recovered from the lambs treated at 0.4 mg/kg ivermectin than in controls and at 0.8 mg/kg there was an 8% reduction in worms. There was a 99.9% and 100% reduction for moxidectin at 0.2 and 0.4 mg/kg respectively.

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PHARMACOKINETICS OF CLOSANTEL IN GOATS AND SHEEP.
N.C. SANGSTER*, D.R. HENNESSY, J.W. STEEL AND G.H. COLLINS. DEPARTMENT OF VETERINARY PATHOLOGY, UNIVERSITY OF SYDNEY AND CSIRO McMASTER LABORATORY, SYDNEY, AUSTRALIA

Closantel, a salicylanilide anthelmintic which persists in plasma and prevents reinfection of sheep for about 28 days, has been used in Australia for strategic control of *H. contortus*, especially those strains resistant to benzimidazoles and levamisole. Farmers have also used closantel to control *H. contortus* in goat herds. Little is known of the behaviour of closantel in goats although there have been several reports of toxicity in kids.

When Angora goats and Merino sheep were given an oral dose of 7.5 mg closantel/kg the areas under the plasma concentration/time curves were 316 and 732 μg.h/ml, respectively. Plasma concentrations were similar until day 5, after which they declined more rapidly in goats. A similar trend was observed after intramuscular injection of closantel. In both species all of the drug detected in plasma was bound to plasma protein. Plasma levels in kids and adult goats were similar and little drug was transferred in milk from treated does. The reported toxicity is unlikely to be due to pharmacokinetic effects. If plasma concentrations are critical for killing establishing larvae, the protection period offered by closantel in goats could be no more about 10 days.

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EFFICACY OF IVERMECTIN AGAINST ADULT AND FOURTH STAGE STEPHANURUS DENTATUS IN SWINE. R. ALVA*, A.G. FOSTER, D.H. WALLACE, S.J. GROSS, J.W. WOODEN. MERCK SHARP & DOHME RESEARCH LABORATORIES, DIVISION OF MERCK & CO., INC., P.O. BOX 2000, WBC-125, RAHWAY, NJ 07065.

Sixty crossbred pigs were utilized to evaluate the efficacy of ivermectin against adult (N=30) and fourth-stage (N=30) Stephanurus dentatus. The pigs were allocated by restricted randomization on body weight. Each pig was given 5000 S. dentatus infective larvae orally on Days -227, -226, -225, and -168 (adult study) and 10 days before treatment in the immature study. Pigs were euthanatized on Days 215 or 216 (adult study) and on Days 38 or 39 (immature study). Treatments were 1) nonmedicated controls; 2) ivermectin injectable at 300 µg/kg/body weight once; 3) ivermectin 0.6% premix in-feed at 2 ppm daily for 7 consecutive days. Day 0 was the first day of treatment.

Both formulations of ivermectin were 100% effective ($p<0.01$) against adult and immature S. dentatus. There were no adverse reactions associated with treatment.

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EVALUATION OF LEVAMISOLE HYDROCHLORIDE AND TWO ANALOGS (U-81772 AND U-84884A) FOR ACTIVITY AGAINST PARASITIC FLATWORMS. S.S. JOHNSON*, P.J. DIROMA, P.M. GUIMOND, B.H. LEE AND G.A. CONDER. UPJOHN LABORATORIES, KALAMAZOO, MI 49001.

Recently, analogs of the anthelmintic drug levamisole were reported to have activity against parasitic flatworms. Since the parent compound has activity only against nematodes, this finding suggested the possibility other analogs of levamisole with an expanded spectrum might be found. As a levamisole analog with expanded anthelmintic spectrum would have important therapeutic and commercial advantages, we attempted to replicate the initial report. To determine if we could demonstrate activity for levamisole hydrochloride, the best literature compound (U-81772), and another potent nematocidal levamisole analog (U-84884A) against these flatworms, two experiments were conducted using a fluke model (*Fasciola hepatica*/mouse). A third experiment was run using a tapeworm model (*Hymenolepis diminuta*/rat). In the former two experiments, female CF-1 mice (six/treatment group) were inoculated with two metacercariae of *F. hepatica* each and then treated on day 14 or days 14-17 postinoculation (PI). In the first of these experiments, Groups 1-3 received a single oral treatment of vehicle only, levamisole hydrochloride (60 mg/kg), or U-81772 (60 mg/kg), respectively, while Groups 4-6 were treated orally on 4 consecutive days with levamisole hydrochloride (60 mg/kg/day), albendazole (a known flukicide; 385 mg/kg/day), or U-81772 (60 mg/kg/day), respectively. For the second experiment, Groups 1-5 received oral treatments for 4 consecutive days with albendazole (385 mg/kg/day), levamisole hydrochloride (60 mg/kg/day), U-81772 (60 mg/kg/day), U-84884A (60 mg/kg/day), or vehicle only, respectively, while Group 6 was not treated. On day 28 PI, all mice in both experiments were sacrificed and examined for flukes and for pathogenesis of the liver and peritoneal cavity. The third experiment used female Fischer 344 rats, which were inoculated *per os* with 4 cysticercoids of *Hymenolepis diminuta* and treated on day 21 PI. One group each received a single treatment of praziquantel (a known cesticide; 5 mg/kg, subcutaneously), levamisole hydrochloride (60 mg/kg, *per os*), U-81772 (60 mg/kg, *per os*), or vehicle only (*per os*), while one group each was treated orally on 4 consecutive days with levamisole hydrochloride (60 mg/kg/day) or U-81772 (60 mg/kg/day). There was no significant reduction for either parasite following treatment with levamisole, U-81772, or U-84884A in these studies. We conclude, in contrast to the initial report, that levamisole and its analogs have no utility against parasitic flatworms.

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EFFICACY OF ALBENDAZOLE IN OVINE SPECIES. K.WOHLGEMUTH, C.R.MILLER, SMITHKLINE BEECHAM ANIMAL HEALTH, EXTON, PA 19341.

A new drug application for the use of albendazole (trade name: Valbazen) in sheep has been submitted and clearance is expected in 1991. To evaluate the efficacy of albendazole in sheep, controlled studies (10-dose titration and 27-dose confirmation studies) were conducted in the U.S. and internationally. Doses of 2.5 to 15 milligrams per kilogram of body weight were studied, establishing an effective dose of 7.5 mpk. Efficacy against a wide range of parasites was evaluated. A claim is expected for Fasciola hepatica, Fascioloides magna, Monieza expansa, Thysanosoma actinoides, Dictyocaulus filaria, Haemonchus contortus, Ostertagia circumcincta, Trichostrongylus axei, Nematodirus spathiger, N. filicollis, Cooperia oncophora, Marshallagia marshalli, T. colubriformis, Oesophagostomum columbianum and Chabertia ovina.

Oral toxicity studies were conducted at doses of 0 - 500 mpk. Clinical pathologic or histologic changes were not observed as a result of using albendazole at 37.5 mg per kg or less, approximately a four-fold safety margin. Albendazole labeling may include a cautionary statement concerning use during the first 30 days of pregnancy. Doses of 10 mpk or higher have been shown to be teratogenic or affect conception rates in sheep. In summary, albendazole is a safe and effective anthelmintic for use in sheep at 7.5 mpk, has a broad spectrum of coverage and is expected to be a useful tool for the control of internal parasites in sheep.

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AN OVERVIEW OF CLASSIC AND STATE-OF-THE-ART METHODS FOR DIAGNOSING PARASITIC INFECTIONS OF VETERINARY IMPORTANCE. D.S.ZARLENGA. USDA, ARS, BIOSYSTEMATIC PARASITOLOGY LABORATORY. BELTSVILLE, MD 20705.

Diagnosing parasites of veterinary interest is a rapidly changing discipline. Though classic morphology as well as site and host specificities are still used today, the onslaught of immunochemical and molecular biological techniques has and continues to change the course of parasite identification. Currently, DNA based methodologies are being developed to better address problems related to specificity, subjectivity, sensitivity and the labor intensive effort needed for accurate sample analysis. This presentation will briefly review major historical methodologies as well as current state-of-the-art techniques available to veterinary practitioners for diagnosing parasitic infections with special emphasis on the application and future potential of molecular biological methods to this discipline.

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COMPARATIVE ADVANTAGES OF NEW DIAGNOSTIC TECHNOLOGIES IN PARASITOLOGY FOR THE PRACTITIONER. J.F. Williams and T.W. Schillhorn Van Veen, Depts. of Microbiology, and Large Animal Science, Michigan State University, E. Lansing, MI 48824.

Contemporary biotechnology has created means of determining the presence of parasites by detection of their DNA/RNA or gene products, rather than by conventional methods based on morphology or indirectly through antibody detection. Methods based on nucleotide or monoclonal antibody probes promise enhanced specificity and sensitivity, speed and, eventually convenience. However, we are a long way from seeing routine application of these techniques in daily practice. The practical comparative advantages that can accrue from new techniques have to emerge from studies where the confidence we have in the interpretation of conventional procedures and their clinical correlates, is surpassed by the ease, accuracy, reliability and cost effectiveness of the new methods. Moreover, an even better understanding will be required of the difference between infection and disease.

In the short run the practitioner will benefit from the ways in which the new techniques can enhance the precision of epidemiological studies in the research lab, and from improvement in the range of diagnostic approaches available to the lab-based diagnostician. Even her practitioners will have to be aware of different methods of sample collection and preservation and shipment in order to take advantage of these technologies. There are even at present important shortcomings, despite the relative simplicity of the sample range and type for conventional testing. The extraordinarily powerful amplification techniques which are already changing diagnostic virology and to a lesser extent bacteriology, are likely to remain in the realm of the research parasitologist. Nevertheless practitioners will be exposed to these technologies and some applications are predictable and will be discussed. The need for evaluation of the new techniques in the context of emerging vaccination technologies and drug resistance will also be emphasized.

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APPLICATION OF RIBOSOMAL RNA SEQUENCES TO THE DIAGNOSIS AND PHYLOGENETIC ANALYSIS OF PARASITES. John B. Dame, Department of Infectious Diseases, Bldg. 471 Mowry Rd., University of Florida, Gainesville, FL 32611-0633.

New methods of diagnosing infectious diseases are being developed using nucleic acid hybridization-based technologies to detect and identify small numbers of infectious agents in clinical samples. This new approach may replace many less specific tests and holds great promise for improved detection of parasites and other pathogens which are difficult to culture in vitro. Developing such tests requires the preparation of specialized nucleic acid probes complementary to an abundant, species-specific nucleic acid target sequence found in the pathogen. Ribosomal RNA molecules qualify for use as target sequences, since they : 1) are the most abundant nucleic acids in the cell constituting ~90% of the total nucleic acids; 2) contain species-specific regions; 3) are easy to obtain and analyze by nucleic acid sequencing; and 4) offer a unified basis for detection of a wide range of pathogens. In addition to the rapidly evolving regions useful for preparing species-specific probes, other regions of the rRNA molecule evolve more slowly providing conserved regions suitable for establishing phylogenetic relationships. The large number of phlyogenetically informative positions in the molecule and the availability of a large data base containing the sequences of small subunit rRNAs from hundreds of species, makes this sequence the one most commonly used for determining phylogeny.

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PARASITE REPETITIVE DNA SEQUENCES: THEIR POTENTIAL IN DEVELOPING DIAGNOSTIC DNA PROBES FOR PARASITIC DISEASE. B. DALE, PARAVAX, INC., MOUNTAIN VIEW, CA 94043.

The basis for the development of deoxyribonucleic acid (DNA) probes as instruments for clinical diagnosis of infectious disease lies in the fact that all organisms have sequences in their DNA unique to themselves. When such sequences are identified, a complement of the sequence is created and a reporter molecule ("tag") is attached to the complementary DNA. The tagged complement to the organism's (parasite's) DNA can then be hybridized to its natural counterpart generating a positive identification even in a rather complex clinical sample. One development issue associated with DNA probes is the enhancement of the sensitivity of the detection method. This must be accomplished either by development of significantly more sensitive detection systems (i.e., the "tag"), or by amplifying the copies of the target gene prior to initiating detection. In the case of eucaryotic DNAs (including parasite DNA), there exist families of DNA, "repetitive DNAs", which are multiple copies of the same unique sequence, thus representing a naturally amplified gene target. This presentation will describe groups of repetitive sequence DNAs found in parasites that are being exploited for generation of diagnostic DNA probes. In addition, artificial amplification of gene targets via PCR methodology will be discussed, as well as the test methods used to visualize the hybrids (RFLPs, dot blots, in situ, liquid phase, and solid phase).

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CLONING AND APPLICATION OF GENES AND GENE PRODUCTS CODING FOR DIAGNOSTIC ANTIGENS. M.C. JENKINS*. PROTOZOAN DISEASES LAB. AGRICULTURAL RESEARCH SERVICE. USDA. BELTSVILLE, MD 20705.

Diagnosis of parasitic infections is often difficult due to the wide-range of clinical symptoms presented and the prevalence of carriers with subclinical infections. Thus, a diagnostic test based on detection of either the parasite itself or antibodies specific for the parasite would be useful. The choice between these two detection methods depends on several factors, including the timing of the immune response during an infection and the ease of obtaining and processing infected host material. Recombinant DNA cloning and polymerase chain reaction technologies have been applied in diagnostic tests for several parasitic organisms. We have developed genus- and species-specific recombinant Eimeria antigens that allow for reliable diagnosis of coccidiosis based on detection of immunoglobulin in serum of infected chickens. Nucleic acid probes based on differences in ribosomal RNA sequences between species of Eimeria are also being developed to allow for detection of the parasite within infected tissue.

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