PROCEEDINGS

AAVP

American Association of Veterinary Parasitologists

35th Annual Meeting

July 21-24
San Antonio, Texas
1990
American Association of Veterinary Parasitologists

Founded 1956
Affiliated with the American Veterinary Medical Association

Officers 1989 - 1990

President: Bert E. Stromberg
University of Minnesota
St. Paul, MN 55108

President Elect: Roger K. Prichard
McGill University
Montreal, PQ H9X 1C0

Vice President: J. Owen D. Slocombe
University of Guelph
Guelph, ON N1G 2W1

Secretary/Treasurer: S.D. 'Bud' Folz
The Upjohn Company
Kalamazoo, MI 49901

Past-President: Harold C. Gibbs
University of Maine
Orono, ME 04469

Committee Chairpersons

Archives: John C. Schlotthauer
University of Minnesota
St. Paul, MN 55108

Awards: James C. Williams
Louisiana State University
Baton Rouge, LA 70803

Constitution/Bylaws: Joseph F. Urban
USDA, ARS, LPSI
Beltsville, MD 20705

Education: Ann M. Zajac
Virginia Tech
Blacksburg, VA 24061

Finance: Thomas J. Kennedy
Boehringer Ingelheim Inc.
St. Joseph, MO 64502

Newsletter: H. Ray Gamble
USDA, ARS, LPSI
Beltsville, MD 25705

Nominations: George A. Conder
The Upjohn Company
Kalamazoo, MI 49001

Outreach/Research: James E. Miller
Louisiana State University
Baton Rouge, LA 70803

Program: J. Owen D. Slocombe
University of Guelph
Guelph, Ontario N1G 2W1

Publications: Walter M. Boyce
University of California
Davis, CA 95616
**Former Presidents**

**of the**

**American Association of Veterinary Parasitologists**

<table>
<thead>
<tr>
<th>Years</th>
<th>President</th>
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<tbody>
<tr>
<td>1956-1958</td>
<td>L.E. Swanson</td>
</tr>
<tr>
<td>1958-1960</td>
<td>F.R. Koutz</td>
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<td>1960-1962</td>
<td>W.H. Krull</td>
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<td>1962-1964</td>
<td>S.M. Gaafar</td>
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<td>1964-1966</td>
<td>E.D. Besch</td>
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<td>1966-1968</td>
<td>G.C. Shelton</td>
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<td>1968-1970</td>
<td>J.H. Greve</td>
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<td>1970-1972</td>
<td>H.J. Griffiths</td>
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<td>1972-1973</td>
<td>D.E. Cooperrider</td>
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<td>1973-1975</td>
<td>D.L. Lyles</td>
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<td>1975-1977</td>
<td>H.J. Smith</td>
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<td>1977-1979</td>
<td>N.F. Baker</td>
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<td>1979-1981</td>
<td>E.L. Roberson</td>
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<tr>
<td>1981-1983</td>
<td>J.F. Williams</td>
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<td>1983-1985</td>
<td>J.B. Malone</td>
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<td>1985-1986</td>
<td>R.M. Corwin</td>
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<td>1986-1987</td>
<td>K.D. Murrell</td>
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<tr>
<td>1988-1989</td>
<td>H.C. Gibbs</td>
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</tbody>
</table>
Winners - AAVP Awards

Distinguished Veterinary Parasitologist

1985  J.P. Dubey
1986  N.D. Levine
1987  E.J.L. Soulsby
1988  J.F. Williams
1989  K.D. Murrell

Hoechst-Roussel Agri-Vet Company

Graduate Student Research Award

1987  L.G. Rickard
1988  D.A. Cross
1989  S.C. Barr

Distinguished Service

1976  R.R. Bell
1987  N.F. Baker
1988  D.E. Cooperrider
MINUTES
Meeting of Veterinary Parasitologists
held at
San Antonio, Texas, October 16, 1956

The meeting was called to order by Dr. Leonard E. Swanson, Chairman, A.V.M.A. Committee on Parasitology, at 2:08 p.m.

Those present:
Wilford S. Bailey
Norman F. Baker
Charlie N. Barron
E. G. Batte
George E. Cauthen
James R. Douglas
Charles C. Durbin
F. D. Enzie
Julius F. Frank
S. M. Gafar
T. J. Galbraith
W. G. Haberman
E. M. Jones
F. R. Koutz
Wendell H. Krull
James F. Landram
Mark C. Mottis, Jr.
George Shelton
B. T. Simms
Leonard E. Swanson
Clarence H. Thompson, Jr.
Richard D. Turk
C. A. Woodhouse

Dr. Bailey had much to do with laying the foundation for an organization of veterinary parasitologists during the time that he was Chairman of the A.V.M.A.

Doctor Swanson stated that his committee on Organization of Veterinary Parasitologists, under the chairmanship of Dr. F. R. Koutz, had prepared a constitution for the American Association of Veterinary Parasitologists and that he was ready to report.

The constitution of the American Association of Veterinary Parasitologists was read by Dr. Koutz. Then the articles of the constitution were read and considered separately. They were discussed and corrections were made so that they met with the approval of the majority present. A motion was made by Dr. Koutz to adopt the corrected Constitution of the American Association of Veterinary Parasitologists. The motion was seconded by Dr. Gafar. The motion carried.

The Society proceeded with the election of officers.

Dr. Turk made a motion nominating Dr. Swanson for President. The motion was seconded by Dr. Barron who asked that nominations be closed. The motion was carried. Dr. Swanson, in accepting the presidency, stated that he would do all in his power to further the interests of the Association, and asked for the help of the membership in carrying out the duties of his office.

Dr. Bailey made a motion nominating Dr. Enzie as Vice-president. The motion was seconded by Dr. Turk. The motion carried.
Dr. Barron made a motion nominating Dr. Krull as Secretary-Treasurer for three years. The motion was seconded by Dr. Koutz. Dr. Woodhouse asked that nominations be closed. The motion carried.

Dr. Swanson queried the membership concerning the type of report that the A.V.M.A. Committee on Parasitology should make in the future. He was of the opinion that time could be saved by the committee members if he asked individual members to abstract the articles in specific journals. Some discussion ensued in which several members took part and no specific recommendations were made. Dr. Swanson concluded that it would probably be best for the Chairman of the Committee to canvass the opinion of the Association by writing a letter to each individual, requesting information on the subject.

Dr. Koutz stated the next A.V.M.A. meeting would be at Cleveland, Ohio, and invited the membership of the Association to visit the Department of Parasitology of the Ohio State University on August 17, 1957. He indicated that a more formal invitation would be issued before the time of the meeting.

Dr. Simms discussed the dearth of papers on parasitology at the meetings of the A.V.M.A. for the last couple of years and informed the Association that there were only five papers on the program of the current meeting which had anything to do with this subject. He was of the opinion that we should take this seriously and consider methods of correcting the situation. He also emphasized the fact that he thought that we should be able to supply some exhibits in parasitology at future meetings. The President agreed that something should be done.

The exact status of our Association in relation to the A.V.M.A. was discussed briefly. It was agreed that the Association would have to be considered for acceptance by the A.V.M.A., and that the matter should be taken care of through correspondence between Dr. Swanson and Dr. Kingman.

The President asked for a motion for adjournment, and Dr. Turk moved adjournment.

[Signature]
Wendell Krull
Secretary-Treasurer
Registration - 35th Annual Meeting

Hilton Palacio del Rio Salon del Rey
Corridor next to Central/North Room

Sunday  8:00 a.m.
Monday  8:00 a.m.

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Social Program - 35th Annual Meeting

Saturday, July 21, 1990
Hilton Palacio del Rio, Salon del Rey South Room
7:30 pm Society pre-meeting mixer

Sunday, July 22, 1990
Hilton Palacio del Rio, El Mirado/Condesa Room
6:30 - 8:30 pm CIBA-GEIGY Social

Monday, July 23, 1990
Hilton Palacio del Rio, Corte Real Room
6:30 - 8:30 pm SMITHKLINE BEECHAM ANIMAL HEALTH Social
<table>
<thead>
<tr>
<th>Time</th>
<th>Sunday</th>
<th>Monday</th>
<th>Tuesday</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 am</td>
<td>Session A1</td>
<td>Opening Remarks</td>
<td>9:30 am</td>
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<tr>
<td>8:50 am</td>
<td>Veterinary Parasitology in the 90's</td>
<td>Epidemiology</td>
<td>Parasitic Zoonosis</td>
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<tr>
<td>10:00</td>
<td>8:00 am</td>
<td>Session C1</td>
<td>10:15</td>
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<tr>
<td>10:15</td>
<td>Session B2</td>
<td>Clinical Reports/Prevalence</td>
<td>10:00</td>
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<td>10:30</td>
<td>Session B3</td>
<td>Anthelmintic Resistance</td>
<td>10:15</td>
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<tr>
<td>11:35</td>
<td>Awards</td>
<td>Education in Veterinary Parasitology</td>
<td>11:05</td>
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<tr>
<td>12:00 noon</td>
<td>Lunch</td>
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<td>11:25</td>
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<tr>
<td>1:00 pm</td>
<td>Session A3</td>
<td>Immunity</td>
<td>Cont'd</td>
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<tr>
<td>2:15</td>
<td>Session A4</td>
<td>History</td>
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<tr>
<td>2:45</td>
<td>Session A5</td>
<td>Chemotherapy 1</td>
<td></td>
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<tr>
<td>3:00</td>
<td>3:00</td>
<td>COFFEE</td>
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<tr>
<td>3:15</td>
<td>Session B5</td>
<td>Chemotherapy 2</td>
<td></td>
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<tr>
<td>3:15</td>
<td>Session C4</td>
<td>Development/Public Health</td>
<td></td>
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<tr>
<td>4:05</td>
<td>Session A6</td>
<td>CIBA-GEIGY Corporate Presentation</td>
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<tr>
<td>4:45</td>
<td>Presidential Address</td>
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<tr>
<td>5:00</td>
<td>Business Meeting</td>
<td>SMITHKLINE BEECHAM Corporate Presentation</td>
<td></td>
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</tbody>
</table>
Program - 35th Annual Meeting

Saturday, July 21, 1990

Hilton Palacio del Rio, Salon del Rey South Room

7:30 PM  Society pre-meeting mixer

Sunday, July 22, 1990

Hilton Palacio del Rio, Salon del Rey Central/North Room

8:00 AM  Registration

8:50  Opening Remarks
President Bert Stromberg
Vice President and Program Chairman Owen Siocombe

Session A1 - Veterinary Parasitology in the 90's
Moderators: Jeffrey F. Williams and Thomas W. Craig

9:00  Invited Presentation
1. Parasitology at risk: the need for a commensal relationship between parasitological societies
   W.M. Kemp*

9:25  Invited Presentation
2. Shape of the animal industry in the next decade
   J.I.H. Phillip*

9:50  Invited Presentation
3. Veterinary medical education and veterinary parasitology in the 90's
   J.A. Shadduck*

10:15 Coffee

Session A2 - Protozoal Myeloencephalitis
Moderators: George A. Conder and Linda M. Pote

10:30 Invited Presentation
4. Equine protozoal myelitis in North America
   R. Fayer*

10:55 5. Immunohistochemical localization of protozoan parasites in lesions of equine protozoal myeloencephalitis

   J.P. Dubey*

11:20 7. Bovine fetal encephalitis and myocarditis associated with protozoal infections: A two year retrospective study of cases in California.

11:35 Awards
Awards Chairman: James C. Williams
Remarks by Distinguished Parasitologist Awardee

12:00 Lunch
### Session A3 - Immunity

**Moderators:** Thomas R. Klei and Raymond H. Fetterer

<table>
<thead>
<tr>
<th>Time</th>
<th>Presentation</th>
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<tbody>
<tr>
<td>1:00 PM</td>
<td>Invited Presentation</td>
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<tr>
<td>8.</td>
<td>Cellular immunity to the trematode infections <em>Fasciola hepatica</em> and <em>Schistosoma mansoni</em> in man and animals&lt;br&gt;    B.L. Doughty*</td>
</tr>
<tr>
<td>1:25</td>
<td>Production and characterization of monoclonal antibodies against excretory-secretory products of <em>Fasciola hepatica</em>&lt;br&gt;    M. Solana*, R.K. Ridley and H.C. Minocha</td>
</tr>
<tr>
<td>1:40</td>
<td>Bovine T-cell immunity to <em>Fasciola hepatica</em> antigens&lt;br&gt;    S.Q. Hasan* and B.L. Doughty</td>
</tr>
<tr>
<td>1:55</td>
<td>The use of monoclonal antibodies for vaccine development against fascioliasis&lt;br&gt;    M.T. Suderman*, C. Hicks, S. Hasan and B.L. Doughty</td>
</tr>
<tr>
<td>2:10</td>
<td>Identification and partial characterization of a CDNA clone from <em>Taenia crassiceps</em> cysticerci messenger RNA expressing a diagnostic antigen for bovine cysticercosis&lt;br&gt;    D.S. Zarlenga* and M.L. Rhoads</td>
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### Session A4 - History

**Moderator:** Owen Slocombe

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>2:25</td>
<td>Invited presentation</td>
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<tr>
<td>13.</td>
<td>Ribbons of history; the story of Cyclophyllidean tapeworms&lt;br&gt;    W.C. Campbell*</td>
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<tr>
<td>2:45</td>
<td>Coffee</td>
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### Session A5 - Chemotherapy 1

**Moderators:** H. Ray Gamble and Robert G. Arther

<table>
<thead>
<tr>
<th>Time</th>
<th>Presentation</th>
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<tbody>
<tr>
<td>3:00</td>
<td>Prevention of <em>Dirofilaria immitis</em> infection with 5-oxime derivatives of milbemycin.&lt;br&gt;    R.B. Grieve*, G.R. Frank, V.A. Stewart, J.C. Parsons, D. Abraham and P.S. MacWilliams</td>
</tr>
<tr>
<td>3:15</td>
<td>Milbemycin oxime: efficacies against intestinal parasites in the dog.&lt;br&gt;    C.M. Hendrix*, B.L. Blagburn, D.D. Bowman, R.B. Grieve, J.V. Bowles, D.S. Lindsay and D.I. Hepler</td>
</tr>
<tr>
<td>3:30</td>
<td>Target animal safety studies with milbemycin oxime.&lt;br&gt;    W.R. Campbell*, D.I. Hepler and J.R. Philip</td>
</tr>
<tr>
<td>4:00</td>
<td>Evaluation of milbemycin oxime in clinical trials in dogs in Canada&lt;br&gt;    J.O.D. Slocombe*, A. Braithwaite, B.E. Cameron, C.L. Dancho, I. Moore, M.C. Lake and D. Bushell</td>
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</table>

### Session A6 - CIBA-GEIGY Corporate Presentation

**Moderator:** Randy C. Lynn

<table>
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<tr>
<th>Time</th>
<th>Presentation</th>
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<tbody>
<tr>
<td>4:05</td>
<td>Presidential Address: Bert Stromberg</td>
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<tr>
<td>4:45</td>
<td>Business meeting</td>
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<tr>
<td>5:00</td>
<td>CIBA-GEIGY Social - Hilton Palacio del Rio, El Mirador/La Condesa Room</td>
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</tbody>
</table>
Monday, July 23, 1990

Hilton Palacio del Rio, Salon del Rey Central/North Room

Session B1 - Epidemiology
Moderators: Harold C. Gibbs and Julie Ann D. Jarvinen

8:00 AM
19. Seasonal transmission of gastrointestinal nematodes of sheep on St. Croix.
   C.H. Courtney* and S. Wildeus

8:15
   H.R. Gamble*

8:30
   C.S. Eddi and J.C. Williams*

8:45
22. Epizootiology of fascioliasis in Montana.
   S.E. Knapp* and L.A. Britt

9:00
23. Use of NOAA polar orbiting satellite data to modify a climate based fascioliasis forecast.
   J.B. Malone*, P. Wilson, O.K. Huh and A.F. Loyacano

9:15
   O.T. Diaw, A. Gueye, G. Vassiliades and T.W. Schillhorn van Veen*

9:30
25. Two year study of the epidemiology of nematode infections in a cow-calf herd in Quebec.
   R. Prichard*, S. Ranjan, C. Trudeau, C. Piche and S. Bauck

9:45
   M. Kennedy* and C. Piche

10:00 Coffee

Session B2 - Clinical Reports/Prevalence
Moderators: John C. Schlotthauer and Charles M. Hendrix

10:15
27. The occurrence of a clinical infection of Nematodirus helvetianus in young dairy cattle in Wisconsin.
   D.H. Bliss*, E. Dickinson, J.H. Schafer and D.A. Armstrong

10:30
28. Intestinal changes caused by infection by Nematodirus helvetianus in dairy calves.
   E.O. Dickinson*, D.A. Armstrong, J.H. Schafer and D.H. Bliss

10:45
   R.F. Taylor, L.G. Biehl, D.W. Miskimins and D.H. Bliss*

11:00
30. Relationships among adult cattle on Louisiana coastal marsh pasture between fecal sedimentation results for Fasciola hepatica and paramphistome.
   S.H. Zukowski* and J.B. Malone

11:15
31. Isolation of Toxoplasma and Isospora from the meat of some farm animals
   A.M. Abdel-Gawad*, A.M. Nassar and M. Hilali

11:30 Session B3 - Ad Hoc Committee's Report on Education in Veterinary Parasitology
Moderator: B.L. Blagburn

12:00 Lunch
Monday, July 23, 1990

Session B4 - Control/Production/Prevalence
Moderators: Thomas J. Kennedy and C. Ed Couvillion

1:00 PM
32. Performance of pigs sequentially infected with *Ascaris suum*, *Oesophagostomum* spp and *Strongyloides ransomi*
   T.B. Stewart*, J. Guerrero, M.C. Fox and L.L. Southern

1:15
33. Animal and forage responses to broiler litter-grain supplementation, parasite control and stocking rate on grazed Coastal Bermudagrass
   D.D. Kee, D.E. Snyder* and D.I. Bransby

1:30
34. Comparison of anthelmintic programs for grazing sheep in southwestern Virginia

1:45
35. A three-year study of parasitism in a beef cow-calf herd in North Central Minnesota
   B.E. Stromberg*, D.L. Haggard, J.C. Schlotthauer and J.W. Rust

2:00
36. Summary of reproductive improvements following parasite control using fenbendazole

2:15
37. Ecotoxicity of anthelmintics

2:30
38. Gastrointestinal parasites of exotic ruminants in Texas.
   T.M. Craig* and S.H. Mercer

2:40
   D.M. Mulrooney*, J.K. Bishop and G.L. Zimmerman

2:50
   S.A. Marley* and R.L. Byford

3:00
Coffee

Session B5 - Chemotherapy 2
Moderators: Walter M. Boyce and Alan A. Marchiondo

3:15
41. Study of the efficacy of ivermectin against early migratory stages of *Parascaris equorum* larvae in pony foals

3:30
42. Experimental infections with *Uncinaria stenocephala* in young dogs: Treatment with nitroscanate

3:45
43. Nematocidal activity of *Bacillus thuringiensis* toxins: a novel approach for controlling parasitic worms in livestock

4:00
44. Influence of formulation and route of administration on the pharmacokinetics of netobimin and its metabolites in cattle.
   C.E. Lanusse*, S. Ranjan, C. Trudeau and R.K. Prichard
4:15 45. Therapeutic efficacy of ivermectin in a sustained-release bolus against nematodes of cattle

4:30 46. Critical evaluation of the variable efficacy of oxfendazole against inhibited larvae of
   Ostertagia ostertagi
   J.E. Miller* and T. Olson

4:45 47. Efficacy of ivermectin delivered from a sustained-release bolus against natural infestations of five African tick species.
   M.D. Soll*, G.W. Benz, I.H. Carmichael and S.J. Gross

5:00 48. Comparative efficacy of albendazole and ivermectin/clorsulon against liver flukes and intestinal nematodes in cattle.
   H.C. Lloyd, D.A. Armstrong, C.R. Miller and J.H. Schafer*

5:15 Session B6 - SMITHKLINE BEECHAM ANIMAL HEALTH Corporate Presentation
Moderator: Robert Widerkehr

6:30 SMITHKLINE BEECHAM ANIMAL HEALTH Social - Hilton Palacio del Rio, Corte Real Room
Monday, July 23, 1990

Hilton Palacio del Rio, Salon del Ray South Room

Session C1 - Immunity/Biochemistry
Moderators: Joseph F. Urban and Dolores E. Hill

8:00 AM
49. Isolation of lamina propria eosinophils from the caprine duodenum and evaluation by chemiluminescence
   D.A. Cross*

8:15
50. Naturally occurring immunomodulation in canine lymphatic filariasis
   D.M. Schreuer*, K. Nakagaki, B. Hammerberg

8:30
51. Kinetics of liver trapping of infective larvae in murine toxocariasis
   J.C. Parsons* and R.B. Grieve

8:45
52. In vivo injection of antibodies to interleukin 4 or interleukin 4 receptor blocks the protective responses to chronic parasitism with *Heligmosomoides polygyrus*
   J.F. Urban, Jr.*, I.M. Katona and F.D. Finkelman

9:00
53. The sensitivity of a monoclonal antibody-based antigen-detection enzyme immunoassay for diagnosis of *Trypanosoma congolense* infection in goats.
   R.A. Masake* and V.M. Nantulya

9:15
54. Lymphocyte activation *in vitro* by *Ascaris suum* cuticular antigens
   D.E. Hill*, R.H. Fetterer and J.F. Urban

9:30
55. The occurrence of tyrosine derived cross-links in *Haemonchus contortus* cuticle
   R.H. Fetterer* and M.L. Rhoads

9:45
56. Protein content of the body wall of *Oesophagostomum columbianum* (Nematoda)
   M. Johal*

10:00 Coffee

Session C2 - Anthelmintic Resistance
Moderators: Roger K. Prichard and Patricia A. Conrad

10:15
57. Molecular understanding of levamisole resistance
   J.A. Lewis* and J.T. Fleming

10:45
58. Utility of a *Haemonchus contortus/JIRD* (Meriones unguiculatus) model for studying resistance to levamisole

11:00
59. Anthelmintic pharmacokinetics in goats and sheep
   N.C. Sangster*, D.R. Hennessy, J.W. Steel and G.H. Collins

11:15
60. Cloning and characterization of two *β*-tubulin cDNAs from *Haemonchus contortus*

11:30
61. Benzimidazole resistance in *Haemonchus contortus*, genetic analysis and benzimidazole binding to *in vitro* expressed *β*-tubulin

11:45
62. Codon usage among nematodes
   T.G. Geary*, B.L. Lee and R.D. Klein

12:00 Lunch
Monday, July 23, 1990

Session C3 - Heartworm
Moderators: Robert B. Grieve and John A. Pankavich

1:00 PM
63. Heartworm in Minnesota - A two year update
   J.C. Schlotthauer* and B.E. Stromberg

1:15
64. An update on the factors that affect the radiographic diagnosis of heartworms in cats
   R.A. Holmes* and P. Dacosta

1:30
65. Milbemycin oxime: efficacy against precardiac stages of *Dirofilaria immitis* in cats
   B.L. Blagburn*, C.M. Hendrix, J.V. Bowles, D.S. Lindsay and D.I. Hepler

1:45
66. Efficacy of RM 340 compared with thiacetarsamide judged by objective criteria.
   1. Controlled laboratory tests in canine models
      M.T. Dzimianski*, J.W. McCall, T.L. McTier and J.P. Raynaud

2:00
67. Efficacy of RM 340 compared with thiacetarsamide judged by objective criteria.
   2. Controlled or critical tests with naturally infected dogs
      J.P. Raynaud* and J.W. McCall

2:15
68. Preliminary controlled experiments to prevent heartworm disease by seasonal IM
    injections of RM 340
    J.W. McCall, M.T. Dzimianski, T.L. McTier, R.A. Holmes* and J.P. Raynaud

2:30
69. Preliminary results on the epidemiology of heartworm in "tracer" beagles seasonally
    exposed to natural infection in three southern states (USA)
    J.W. McCall*, M.T. Dzimianski, T.L. McTier, R.A. Holmes and J.P. Raynaud

2:40
70. Adult heartworm antigen in beagles exposed to natural infection and given seasonal
    IM injections of RM 340 in controlled tests in three southern states (USA)
    T.L. McTier*, M.T. Dzimianski, R.A. Holmes, J.W. McCall and J.P. Raynaud

2:50
71. Immunodiagnosis and chemoprophylaxis of *Dirofilaria immitis* infection in ferrets
    (*Mustela putorius furo*)
    P. Supakornrdej*, A.E. Mansour, S.J. Rowan and J.W. McCall

3:00
Coffee

Session C4 - Development/Public Health
Moderators: Ann M. Zajac and John W. McCall

3:15
72. Tissue cyst formation by *Toxoplasma gondii* in cell cultures.
    D.S. Lindsay*, J.P. Dubey and B.L. Blagburn

3:30
73. *In vitro* investigations on the action of robenidine against *Toxoplasma gondii*.
    X. Daliae*

3:45
74. Susceptibility of three species of Kansas snails to infection by *Fasciola hepatica*
    *Linnaeus, 1758* and *Fascioloides magna* (Bassi, 1875) Ward, 1917.
    R.D. McKown* and R.K. Ridley

4:00
75. Banding patterns in isoelectric focusing gels as a means for identification of *Fasciola hepatica*
    infected snails
    Decker and J.A. Whitaker
**Monday, July 23, 1990**

4:15  76. *In vitro* culture of equine strongyles to the fourth stage  
      T.R. Klei*, M.R. Chapman, G.W. Hutchinson and M.J. Cenac

4:30  77. Early development of *Strongylus vulgaris* in pony foals  
      B.M. McCraw* and J.O.D. Slocombe

4:45  78. Veterinary practitioner recommendations for treatment and prevention of intestinal  
      nematodes in dogs: Implications for public health  
      J.B. Harvey* and P.M. Schantz

5:00  79. Incidence of *B. divergens* bovine babesiosis on human health in Europe  
      A. Gorenflot* and P. Brasseur

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**Tuesday, July 24, 1990**

Convention Centre, Plaza B & C Rooms

*Session D - Joint AAVP-AVMA Symposium  
Parasitic Zoonosis  
Moderator: Dr. Bert E. Stromberg, President,  
American Association of Veterinary Parasitologists*

9:30 AM  Chairman's opening remarks

9:35  80. Larva migrans caused by roundworms and hookworms of dogs and cats  
      P.M. Schantz*

10:05  81. Epizootiology of Lyme disease  
      J. Piesman* and G. Maupin

10:35  82. Is trichinellosis still a problem in the U.S.?  
      K.D. Murrell*

11:05  Coffee

11:25  83. Giardiasis - A zoonosis?  
      S.L. Erlandsen* and W.J. Bemrick

11:55  84. *Cryptosporidium*: a protozoan pathogen infectious for animals and man  
      R. Fayer*

12:25  Summary
      B.E. Stromberg
1. PARASITOLOGY AT RISK: THE NEED FOR A COMMENSAL RELATIONSHIP BETWEEN PARASITOLOGICAL SOCIETIES. W.M. KEMP*, DEPARTMENT OF BIOLOGY, TEXAS A & M UNIVERSITY, COLLEGE STATION, TEXAS

Parasitology is a diverse discipline with parasitologists involved in veterinary medicine, medicine, wildlife research and all aspects of basic research. Newly developing problems in funding, plus newly developing opportunities in interdisciplinary research and clinical application of new knowledge require enhanced communication and mutual support between these different areas of parasitology. Several societies already exist whose primary purpose is to serve the needs of their membership. It is proposed that these different societies join in a Confederation of Parasitological Societies so that the discipline of parasitology may be fully represented as a unified whole and that communication between the different disciplinary groups may be facilitated. Each society would retain its own individual identity and membership would be maintained as each member society. Societies which would be invited to join the Confederation would be the American Association of Veterinary Parasitologists, American Society of Parasitologists, American Society of Tropical Medicine and Hygiene, American Wildlife Parasitologists, American Society of Protozoologists, and the American Fisheries Society Parasitology Division). Advantages and disadvantages will be discussed.

2. SHAPE OF THE ANIMAL INDUSTRY IN THE NEXT DECADE. J.I.H. PHILLIP*, SMITHKLINE BEECHAM ANIMAL HEALTH, WEST CHESTER PA 19380.

The rate of change in our industry has been accelerating in recent years. Application of new biotechnologies, which promised much, yielded little until the late '80's. But, the prospect of radical departures from conventional scientific methods appears to have provoked vigorous debate both inside and outside the agriscience industry.

The Delaney clause was but a forerunner of several political incursions into science. The objectives so often are laudable but the control mechanisms lack selectivity. Is that the fault of academia and industry rather than politicians?

Governments worldwide respond by increasing the height of regulatory hurdles. Consequent R&D expense inflation is often cited as the major reason for industrial consolidation.

Against this background, the need for better parasite control measures seems inexhaustible. The animal antiparasitic drug market has burgeoned to $1.6 billion. Maintenance of the current agricultural structure into the next century demands that the levels of parasite control increase rather than decrease. How will it be achieved?
Parasitology offers some of the best and worst in veterinary medical education. At its best, it's exciting biology which offers clear guidance to the prevention and treatment of animal diseases. At its worst, it's an agonizing compendium of complex life cycles and long meaningless names. The 90's will bring, at the very least, new and exciting data on molecular biology of parasites; better understanding of host-parasite relationships; effective anti-parasite vaccines, and new diagnostic tests. The information explosion will continue but become more manageable by use of computer-aided instruction and electronic communication devices. Problem-solving, self-teaching and greater emphasis on small-group learning environments will characterize many veterinary curricula. Parasitology is well-positioned to lead effective, innovative curricular modification linked to outcome assessment of student learning.

EQUINE PROTOZOAL MYELOENCEPHALITIS (EPM) IN NORTH AMERICA.

EPM, first diagnosed in North America in the early 1960's, has recently been confirmed in nearly 400 horses examined in 9 states and 1 Canadian province by the presence of organisms or indicative histopathology. In neurons and a variety of cells associated with the central nervous system, organisms are found directly in the cytoplasm. Individuals resemble motile stages of coccidia and groups resemble immature or mature schizonts. Lesions from a few micrometers to several centimeters in size consist of necrosis and inflammation of neural tissue as well as adjacent perivascular tissue in the CNS, usually the spinal cord. Neurologic dysfunction resulting from such lesions include lameness and other gait problems, ataxia, paresis, various types of facial paralysis, and muscle atrophy. If untreated, the course is progressive and fatal. However, successful treatment has been reported with certain anti-protozoal drugs. No pattern is apparent with regard to seasonal occurrence, geographic location, sex or breed. Despite an extensive age range over half the affected horses are four years old or younger. Specific noninvasive diagnostic tests are needed.

Sections of brain and spinal cord from horses affected with equine protozoal myeloencephalitis were stained using the Avidin/Biotin immunoperoxidase method. Protozoal parasites in lesions stained positively with polyclonal rabbit antiserum, prepared with soluble Sarcocystis cruzi cystozoite antigen of bovine origin. Organisms were detected only in cases previously confirmed by H&E staining. Tests using Toxoplasma gondii antiserum and normal rabbit serum as primary antibody were negative. Sections of Sarcocystis cruzi-infected bovine muscle and Toxoplasma gondii-infected feline tissues were used as positive controls. Sarcocysts stained positively only with Sarcocystis antiserum. Toxoplasma cysts stained positively with both Toxoplasma and Sarcocystis antisera.

6. FATAL PROTOZOA CANINE ENCEPHALITIS. J.P. DUBEY*, ZOONOTIC DISEASES LABORATORY, LPSI, ARS, USDA, BELTSVILLE, MD 20705 AND L.N. SLIFE, ANIMAL DISEASE LABORATORY, ILLINOIS DEPARTMENT OF AGRICULTURE, GALESBURG, IL 61402.

Toxoplasma gondii and Neospora caninum are 2 apicomplexan parasites known to cause encephalitis in dogs. We report fatal encephalitis in a 4-month-old male Rottweiller dog associated with an unidentified coccidium. The dog died after a 5-day illness following entrapment in icy pond water. The main microscopic lesions were in the brain and consisted of chronic meningeal encephalitis characterized by malacia, vasculitis, neovascularization, perivascular cuffs, and infiltrations of mononuclear cells and neutrophils. Developmental stages of a coccidium were found in neural cells. The parasites were located in a parasitophorous vacuole and divided by schizogony. Merozoites budded peripherally leaving a central residual body. The organism did not stain with anti-T. gondii and anti N. caninum.
7. BOVINE FETAL ENCEPHALITIS AND MYOCARDITIS ASSOCIATED WITH PROTOZOAL INFECTIONS: A TWO YEAR RETROSPECTIVE STUDY OF CASES IN CALIFORNIA. B.C. BARR, M.L. ANDERSON, P.C. BLANCHARD, B.M. DAFT, N. KINDE, P.A. CONRAD* UNIVERSITY OF CALIFORNIA, DAVIS CA 95616

Over a period of several years, numerous bovine fetuses submitted to the California Veterinary Diagnostic Laboratory System had been examined which had a multifocal nonsuppurative necrotizing encephalitis, nonsuppurative myocarditis, and other inflammatory changes resembling lesions seen in Toxoplasma infected ovine fetuses. Tests to rule out viral and bacterial etiologies were negative. In a few cases, protozoal clusters were seen in the brain. A 2 year retrospective study was conducted to evaluate these cases which were identified by specific histologic criteria and the absence of other etiologic diagnoses. Of 445 total bovine fetuses submitted, 82 or 18%, representing 56 herds, met the histologic criteria. All but 2 cases were from dairies. In 17 (21%) of these cases, clusters of protozoa were found. Multiple abortions were recorded at several establishments. No salient clinical signs were noted in the dams. Abortions occurred year-round but were slightly more frequent in winter months. Fetuses ranged from 3 to 9 months gestation with the majority between 5 and 7 months gestation. Protozoal cysts were found in the brain parenchyma in 10 fetuses; primarily in endothelial cells in 4 cardiac myofibers in 1 fetus. Sarcocystis species was considered the etiology in 2 cases, but the etiology in the remaining cases is uncertain. These findings suggest that many if not all of these cases represent protozoal fetal infections, and that protozoal fetal infections are a significant cause for abortion in California dairies.

8. CELLULAR IMMUNITY TO THE TREMATODE INFECTIONS FASCIOLA HEPATICA AND SCHISTOSOMA MANSONI IN MAN AND ANIMALS. B.L. DOUGHTY*, DEPARTMENT OF VETERINARY MICROBIOLOGY AND PARASITOLOGY, COLLEGE OF VETERINARY MEDICINE, TEXAS A & M UNIVERSITY, COLLEGE STATION, TEXAS 77843

The field of cellular immunology has increased our understanding of the host-parasite relationship with regard to the induction of protective immunity and the elucidation of protective effector mechanisms in immune animals and man. Continued analysis of parasite antigens and the host cellular immune responses has rapidly progressed to the molecular level with the use of purified native protein antigens and/or recombinant antigens in combination with analysis of T cell responses aided by T cell cloning technologies. Our increased understanding of the host immune response to complex helminth infections provides us with new insights which we can maximize through vaccination to provide the host with a competitive edge to reduce or eliminate parasite infections. Alternatively, as in the case of schistosomiasis, immunopathology associated with granulomatous hypersensitivity to egg antigens has come under intense scrutiny with modern cellular-immunological techniques. As the immunological events associated with schistosome egg granulomas become molecularly defined, so will our ability to reduce the harmful immunological events which lead to the morbidity associated with this disease. Current knowledge of immunoparasitology can be used to develop rational approaches to either protective vaccines or anti-pathology vaccines. This work was supported from grants by the Texas Agricultural Experiment Station, Texas Higher Education Coordinating Board, NIH # AI 21776 and UNDP/World Bank/WHO.
9.

PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBodies AGAINST EXCRETORY-SECRETORY PRODUCTS OF FASCIOlA HEPATICA. M. SOLANO*, R.K.
RiDLEY AND H.C. MINOCHA. KANSAS STATE UNIVERSITY, MANHATTAN, KS 66506.

Balb/c mice approximately 8 weeks of age were injected with Fasciola hepatica excretory-secretory products. Spleen cells from the immunized mice were fused with P3X63-Ag8.653 myeloma cell line using 50% polyethylene glycol. Those wells exhibiting cell growth were screened for antibody production using ELISA. Four hybridomas designated mAb 589.9.49.62.55, S89.24.27.17.33, S89.46.71.44.66 and S89.179.57.39.70 were cloned three times by limiting dilution. Isotype analysis of these mAbs showed them to be IgM, IgG3, IgG1, and IgM. By immunoblot, the mAbs recognized different antigenic polypeptides migrating between 29 and 56 kilodaltons. We evaluated the specificity of the mAbs by ELISA with antigens of Fascioloides magna, Anoplocephala magna, bovine serum albumin (BSA), bovine viral diarrhea virus (BVDV) and Madin-Darby Bovine Kidney (MDBK) cells. Monoclonals 589.9.49.62.55. and S89.24.27.17.33. appeared to have good specificity as they did not recognize any of those five antigens; mAbs 89.46.71.44.66. and S89.179.57.39.70 cross-reacted with antigens of F. magna and A. magna but not with BSA, BVDV and MDBK cells.

10.

BOVINE T-CELL IMMUNITY TO FASCIOlA HEPATICA ANTIGENS. S.Q.
HASAN* AND B.L. DOUGHTY. DEPARTMENT OF VETERINARY MICROBiology & PARASITOLOGY, COLLEGE OF VETERINARY MEDICINE,TEXAS A & M UNIVERSITY. COLLEGE STATION, TX 77843.

Adult Fasciola hepatica antigens were homogenized without inhibitors and solubilized. The crude solubilized antigen was fractionated by SDS-PAGE (non-reducing, 12.5%) and transferred onto immobilon-P membranes. Peripheral blood mononuclear cells from F. hepatica infected hyperimmune cattle were isolated and analyzed for T-cell responsiveness using standard T-cell Western assays. Our results indicate that 1) cellular immune responses correlate to increased resistance, 2) bovine T cells respond to antigens presented both in a soluble and solid phase form, 3) and twelve antigens with molecular weights ranging from 14 to 125 KD have been identified as T cell immunogens.

In a parallel study, analyses of antibody westerns confirmed that some groups of proteins elicit a stronger B cell response. Thus we conclude that F. hepatica antigenic homogenates contain immunogens that elicit a predominant T-cell response and / or B cell response.

These studies were supported by grants from the TEXAS AGRICULTURAL EXPERIMENT STATION and the TEXAS HIGHER EDUCATION COORDINATING BOARD ADVANCED TECHNOLOGY PROGRAM.

The production of mouse monoclonal antibodies against Fasciola hepatica membrane proteins has produced two protective monoclonal antibodies. One (mabD4) afforded 45-50% protection against a challenge infection in mice while recognizing the protective antigen of molecular weights 228 and 140 kDa from adult worm tegument preparations. The second (mabEI1) afforded 62% protection against challenge in the rodent model and recognized two glycoproteins in the adult tegument of 228 and 163 kDa.

Light level immuno-gold labelling of adult worms with these two monoclonals demonstrated differences in epitope localization. MabD4 recognizes tegument, tegument spines and vitelline gland antigens, whereas, mabEI1 appears to be restricted to tegument surface antigens, excluding spines. Results of the passive protection experiments suggests that the antigen(s) recognized by these two monoclonals are expressed in the juvenile stage. Immuno-gold labelling of antigenic epitopes and visualization at the light and transmission electron microscopy levels in the developmental stages will confirm these observations.

These studies were supported by grants from the Texas Agricultural Experiment Station and the Texas Higher Education Coordinating Board Advanced Technology Program.

12. IDENTIFICATION AND PARTIAL CHARACTERIZATION OF A CDNA CLONE FROM TAENIA CRASSICEPS CYSTICERCI MESSENGER RNA EXPRESSING A DIAGNOSTIC ANTIGEN FOR BOVINE CYSTICERCOSIS. D.S. ZARLENGA* AND M.L. RHOADS. USDA, ARS, LIVESTOCK & POULTRY SCIENCES INSTITUTE. BELTSVILLE, MD 20705.

We previously identified and purified an antigenic fraction from the cyst fluid of Taenia hydatigena (designated THFAS) that demonstrates applicability as a sensitive and specific reagent for the diagnosis of bovine cysticercosis. Rabbit antiserum to THFAS identified a homologous antigenic protein in the cyst fluid of the cestode Taenia crassiceps. Consequently, a cDNA expression library was constructed using poly A mRNA purified from T. crassiceps cysticerci and screened with rabbit antisera to THFAS or with bovine antisera to Taenia saginata to identify a recombinant THFAS homologous epitope. Positive clones were identified which reacted with both antisera. One strongly reactive clone, designated ATCA-2, generated a fusion protein 130 kDa in size and contained a cDNA insert 0.45 kb in length. The antigen from ATCA-2 interacts strongly with sera from cattle experimentally infected with T. saginata, and does not cross-react with sera from cattle infected with Fasciola hepatica or with other common gastrointestinal cattle parasites. Preliminary results further demonstrate that T. saginata antibodies from naturally-infected cattle can also be detected with this cloned antigen.
13.

RIBBONS OF HISTORY: THE STORY OF CYCLOPHYLLIDEAN TAPEWORMS. W. C. CAMPBELL*, DREW UNIVERSITY, MADISON, NJ 07940

The cystic larvae of cyclophyllidean tapeworms warrant our attention because of their importance to the meat and livestock industries and to the veterinary aspects of public health. Cestode cysts are big enough to have been seen by the naked eye since ancient times, but the recorded descriptions were gross and the connection between the cysts and tapeworms was unknown. The connection was suggested in the late 17th Century, and again in the late 18th Century, and led to controversy as to whether the cystic forms were species in their own right, or embryonic forms, or worms that had degenerated as a result of straying into the wrong host. In the mid-19th Century, introduction of the concept of alternation of generations, coupled with the emerging popularity of experimentation led to the conclusion that the parenteral cysts are larval forms of the enteral tapeworms. Induction of strobilar infections by the oral administration of cysts was carried out in domestic animals and man. The induction of cystic infections by the administration of eggs or proglottids followed, and the experiments were prudently confined to non-human animals. Within a quarter century (1850-1875) the essential life-cycle of the major cyclophyllidean tapeworms was worked out, but many aspects of cyst biology remained to be addressed in the present century.

14.

PREVENTION OF DIROFILARIA IMMITIS INFECTION WITH 5-OXIME DERIVATIVES OF MILBEMYCN. R.B. GRIEVE*, G.R. FRANK, V.A. STEWART, J.C. PARSONS, D. ABRAHAM, AND P.S. MACWILLIAMS. COLORADO STATE UNIVERSITY, FT. COLLINS, CO 80523, THOMAS JEFFERSON UNIVERSITY, PHILADELPHIA, PA 19107, UNIVERSITY OF WISCONSIN, MADISON, WI 53706

The 5-oxime derivatives of milbemycin (80% ethyl + 20% methyl) (milbemycin) were studied for their efficacy in preventing experimentally induced Dirofilaria immitis infections. Milbemycin was 100% effective in preventing infection when administered at monthly intervals beginning one month after infection and at dosages ranging from 0.05-1.0 mg/kg. Milbemycin was also totally effective in preventing infection when it was administered (0.5-0.99 mg/kg) 30 or 45 days after infection. Less than complete efficacy was observed when a single treatment occurred 60 or 90 days after infection. When dogs were treated at both 60 and 90 days after infection complete efficacy was restored. Similar results were obtained in dogs which received multiple infections as well as multiple treatments. These data, and the known efficacy of milbemycin against intestinal nematodes at the target dosage (0.5-0.99 mg/kg), suggest that milbemycin has considerable potential as an anthelmintic.

The milbemycins are a complex group of natural fermentation antibiotics produced by *Streptomyces hygroscopicus aureolacrimosus*. Chemically, they are 16-membered macrocyclic lactones containing a spiroketal system of two six-membered rings. The milbemycins are closely related to the avermectins (produced by *Streptomyces avermitilis*), differing only in the substituent at the 13 position. Two analogues have been selected for development, Milbemycin D (Japan) and Milbemycin A4-Oxime (MO; CIBA-GEIGY Corp., United States). In the present series of studies we report the results of efficacies of MO against mature *Ancylostoma* spp. in naturally infected dogs, immature and mature *A. caninum* in experimentally infected dogs, mature *Toxocara canis* in experimentally infected dogs, and mature *Trichuris vulpis* in naturally infected dogs. MO possessed the following efficacies: > 95% against mature *Ancylostoma* spp. in naturally infected dogs at dosages of 0.5 mg/kg and above; 49%, 81%, 83% and 91% against 36-, 120-, 216-, and 360-hour-old experimentally induced *A. caninum*, respectively, at 0.5 mg/kg; 100% against experimentally induced mature *T. canis* at low (5.68 mg/dog) and high 34.08 mg/dog) dosages, and > 97% against adult *T. vulpis* in naturally infected dogs using the dosage regimen effective in *D. immitis* prophylaxis.


Chronic-, reproduction-, and puppy-safety studies have been conducted in the beagle dog with milbemycin oxime. The chronic study evaluated safety at 1X, 3X, and 5X the use rate given over three successive days each month for 10 months. The reproduction study evaluated standard reproductive parameters during premating, mating, gestation, parturition, and weaning in animals given 3X the use rate daily through the study; the premating period was a minimum of three months. A separate acute study was conducted in 8-, 10-, and 12-week-old puppies at 1X, 5X and 15X, and 25X the monthly use rate. Safety has also been evaluated in collies at up to 25X the use rate.

Results have indicated that milbemycin oxime does not have any adverse chronic or reproductive effects in the dog. A slight temporary ataxic reaction was noted in young puppies at multiple doses of 5X the use rate; ataxia increased in severity at 15X and 25X the use rate. No effects were noted in collies at any tested rates.
17.

A multi-location, well-controlled clinical field trial employing essentially identical study protocols was conducted during 1987-88. The overall objective of the study was to evaluate the prevention of heartworm disease and control of hookworm infection when used under typical veterinary practice conditions. The study employed a total of 24 individual veterinary hospitals and clinics in nine states. Milbemycin oxime was administered monthly utilizing various tablet sizes based on the weight of the dog. The tablets provided a minimum monthly dose of 0.5 mg/kg of body weight. Individual dogs received 10 months of treatment. Critical evaluation end-points were (a) efficacy in preventing heartworm and controlling hookworm, (b) safety of the product as characterized by the professional investigator, and (c) overall acceptability as perceived by the pet owner.

Milbemycin oxime was completely effective in preventing heartworm disease (100%) and highly effective in controlling hookworm infections (97.5%) during the extended field trial period.

18.
EVALUATION OF MILBEMYCIN OXIME IN CLINICAL TRIALS IN DOGS IN ONTARIO. J.O.D. SLOCOMBE*, A. BRAITHWAITE, B.E. CAMERON, C.L. DANCHO, I. MOORE, M.C. LAKE, AND D.A. BUSHELL. 1*UNIVERSITY OF GUELPH, GUELPH; 2*VETERINARY CLINICS IN KINGSVILLE, TILLSONBURG, MORPETH; 4*CIBA-GEIGY, MISSISSAUGA; ONTARIO, CANADA.

Heartworm Trial - In May 1988 and at 3 clinics, 83 client-owned asymptomatic and microfilaremic dogs were assigned, using a randomization table, to be treated with milbemycin (58 dogs) or Heartgard 30 (25 dogs). Treatments were given monthly from June to November. A blood sample from each dog was examined for microfilariae by a concentration procedure before the first treatment and 120-150 and 300-365 days later. At the end of the trial in May 1989, all dogs were amicrofilaremic. One dog given milbemycin had mild diarrhea. Intestinal Nematodes Trial - From February-June 1989, 98 naturally infected dogs were used at 7 locations; a research facility and 6 privately owned kennels. At each location, dogs with similar infections were paired on fecal nematode eggs/gram (epg) and members of a pair were randomly assigned to treatment with milbemycin or a placebo. A fecal sample was taken from each dog before treatment and 7-10 days later. The number of dogs with Toxocara canis was 28, Ancylostoma caninum 40, Uncinaria stenocephala 12 and Trichurs vulpis 30. The mean % reduction in epg for dogs treated with milbemycin and with T. canis was 96.0, T. leonina 100, A. caninum 98.1, U. stenocephala 78.0 and T. vulpis 99.4. One dog vomited within 4 hours of treatment.
19.

SEASONAL TRANSMISSION OF GASTROINTESTINAL NEMATODES OF SHEEP ON ST. CROIX.

C. H. COURTNEY* AND S. WILDEUS. UNIVERSITY OF FLORIDA, GAINESVILLE, FL 32611

AND UNIVERSITY OF THE VIRGIN ISLANDS, KINGSHILL, ST. CROIX, VI 00850.

Monthly acquisition of gastrointestinal nematodes by tracer lambs on St. Croix was followed for 1 year in two flocks of sheep. One (AES) was an intensively managed flock, and the other (CIF), on an similar adjacent pasture, was less intensively managed. Each month 3 tracer lambs were grazed with the ewe flock at each site, then necropsied and gastrointestinal nematodes counted. Fecal egg counts were determined at approximately 2 week intervals for both lambs and ewes.

*Haemonchus contortus* and *Trichostrongylus colubriformis* were the most abundant nematodes acquired by tracer lambs. Significantly (*P*=0.0006) greater numbers of adult *H. contortus* were acquired during the 4 month period of July through October than at other times. Significantly (*P*=0.0003) greater numbers of adult *T. colubriformis* were acquired from October through December than at other times and significantly (*P*=0.039) fewer *T. colubriformis* were acquired from May through August than at other times. There was no significant difference (*P*=0.85) in acquisition of *H. contortus* by tracer lambs at AES and CIF, but tracer at CIF acquired significantly (*P*=0.017) more *T. colubriformis* than at AES. *Cooperia curvica*, *Strongyloides papillosus* and *Oesophagostomum columbianum* were occasionally present, although in insufficient numbers for meaningful analysis. Ewes at both CIF and AES showed a significant (*P*<0.02) lactation rise in fecal egg counts. Total monthly rainfall was significantly correlated (*r*=0.708, *P*=0.01) with the number of *H. contortus* acquired by tracer lambs, marginally correlated with fecal egg counts of ewes (*r*=0.513; *P*=0.088), and not correlated (*r*=-0.169; *P*=0.599) with the number of *T. colubriformis* acquired by tracer lambs. Monthly fecal egg counts of ewes was only marginally (*r*=0.555; *P*=0.061) correlated with the acquisition of *H. contortus* by tracer lambs and not correlated (*r*=-0.259; *P*=0.415) with acquisition of *T. colubriformis*.

20.

RESISTANCE OF ST. CROIX LAMBS TO *HAEMONCHUS CONTORTUS* IN EXPERIMENTALLY AND NATURALLY-AQUIRED INFECTIONS. H.R. GAMBLE*, USDA, ARS, LPSI, HELMINTHIC DISEASES LABORATORY, BELTSVILLE, MARYLAND 20705.

Several studies have demonstrated that certain tropical and subtropical breeds of sheep have increased resistance to nematode infection when compared to temperate, domestic breeds. This resistance has been demonstrated as reduced parasitism resulting from challenge infection under experimental conditions and as a reduced periparturient rise in lactating ewes of these breeds. In the present study we have examined parasitological and immunological parameters of experimental or naturally acquired infections with *Haemonchus contortus* in St. Croix and Dorset lambs. Following experimental infections, St. Croix lambs developed significantly greater levels of resistance to infection with *Haemonchus contortus*, as compared with Dorset lambs; this resistance was influenced both by age and prior exposure to parasites. In grazing experiments on infected pasture, St. Croix lambs had significantly lower egg counts as early as five weeks following initial exposure. Further, St. Croix lambs had >99% fewer worms in the abomasum upon necropsy, as compared with age matched Dorset lambs. Lymphoproliferative assays using peripheral cells or mesenteric lymph node cells demonstrated no differences between the two breeds in mitogen or antigen reactivity.
Primary objectives were to more precisely define time sequences in induction, duration and maturation of larvae. Three groups of 18 Holstein calves (raised free of nematode infection) were placed successively on a contaminated pasture in March (Gp1), April (Gp2), and May (Gp3) of 1988. Each group grazed for 3 weeks, after which 2 calves were slaughtered for worm recovery. The remaining 16 calves of each group were then divided into 2 subgroups, 8 remaining on pasture and 8 being confined in pens. Two calves of each of the subgroups were slaughtered sequentially in June, July, August, and September. Pairs of parasite free tracer calves were also grazed monthly on the pasture for 3 week periods in June through September. Greatest numbers of inhibited larvae (EL4Y and the % inhibition were found in calves of Gp1; smallest numbers were found in Gp2. Levels of inhibition in continuously grazed and confined calves were similar. Population density-dependence and immunity appeared to be of much less importance than environment in induction of inhibition. Concerted maturation was not observed until September, when marked quantitative changes in numbers of adults and EL4 occurred in both pastured and confined calves.

Snail collections from suspected or known liver fluke locations have been made since August 1988. Each collection has been cataloged and submitted for identification to the U.S. National Museum of the Smithsonian Institution. Of these, the snail identified as the principal vector for Fasciola hepatica is Fossaria modicella. This snail has been collected from fourteen different sites ranging from the Centennial Valley in south western Montana to the Flathead Lake area and from sites on both the western and eastern sides of the Continental Divide. Two other snail species that have been collected and are known vectors for this parasite are Lymnaea stagnicola and Stagnicola montanensis.
USE OF NOAA POLAR ORBITING SATELLITE DATA TO MODIFY A CLIMATE BASED FASCIOLIASIS FORECAST. J.B. MALONE, P. WILSON, O.K. HUH AND A.F. LOYACANO, LOUISIANA STATE UNIVERSITY, BATON ROUGE, LA 70803.

NOAA polar orbiting satellite data (AVHRR) were collected and analyzed for the period of February-June, 1989 to determine whether satellite climate data could be used in lieu of regional climate station data in a fascioliasis risk forecast used in Louisiana. Satellite soil surface temperature values (Channel 4 Thermal AVHRR; resolution 1Km²) were used to define 3 moisture domains (wet, intermediate, dry) in a 2 quadrangle (7.5') study area of the Red River Basin near Alexandria, LA. based on the known buffering effect of moisture on daily fluctuation in minimum and maximum temperature. Fifteen commercial cattle operations were identified, 5 from each moisture domain, to determine whether Fasciola hepatica infection prevalence and intensity was associated with moisture domain. Fall, 1989 pretreatment fecal sedimentation results ranged from 28.5 to 100% prevalence and 0.6 to 21.7 EP2G. Initial analysis indicates a positive association between high infection rates and moisture domain wetness. The influence of soil type within domains, water table, stocking rates and treatment history in modifying this association is currently being assessed.

DROUGHT, DAMS AND DISEASE. EXAMPLES OF A DYNAMIC PARASITE ECOLOGY IN SENEGAL. O.T. DIAW, A.GUEYE, G.VASSILIADES AND T.W.SCHILLHORN VAN VEEN*. INSTITUTE OF AGRICULTURAL RESEARCH, DAKAR, SENEGAL AND MICHIGAN STATE UNIVERSITY, EAST LANSING, MI.

Senegal is a country in the West African savanna with a vegetational stratification from desert to tsetse infested tree savanna over a distance of less than 200 miles. Major changes in the environment occurred during the last decades: droughts in the mid seventies and eighties, construction of a dam in the Senegal river and increased rainfall in the late eighties.

The droughts and subsequent loss of vegetation diminished the spread of the tsetse fly, and allowed livestock to move southward. The droughts also reduced the habitat of ticks, mainly Amblyomma and Boophilus, and caused a change in the enzootic stability of tickborne diseases such as anaplasmosis and cowdriosis. Indirectly the drought led to the construction of a dam in the Senegal river (to prevent salt infiltration). The changes in water management caused an increase in mosquito population and a subsequent outbreak of Rift Valley Fever. They also resulted in an increase of snail habitats, and in 1989 Biomphalaria spp., common in the upstream region of the river, were for the first time found in the irrigation canals of the lower valley. The relatively high rainfall in 1989 re-created streams and caused flooding for the first time since the early seventies. An increase in mosquito-borne diseases including malaria occurred.

These changes, some of which were predicted, demonstrate the dynamic nature of the interaction between environment, host, intermediate host and parasite.
A two year epidemiological study was conducted on a previously untreated cow-calf herd in Quebec, as part of a study to define optimal timing for strategic nematode control. During the two grazing seasons (May-October/November 1988 and 1989), the cow-calf herd and replacement heifers were on separate pastures. The mean fecal egg counts of cows and heifers were high at turnout, but declined steadily as the grazing season progressed. The mean fecal egg counts of calves started to rise soon after birth, increased steadily during the grazing season and remained high during housing. The numbers of infective larvae on pasture steadily increased as the grazing season progressed and peaked during September-October. Ostertagia, Cooperia and Nematodirus were the most prevalent genera found at necropsy and on pasture. There is evidence for overwintering of these larvae on pasture. Ostertagia may overwinter in the host as L₄ larvae. The data obtained indicate that the calves did not develop resistance during their first grazing season and may suffer from subclinical parasitism. The high nematode egg output of cows during the early grazing season may serve as a source of infection for their calves and may contribute to the late season rise in pasture larval counts. Supported by Agriculture Canada/NSERC/MSD AgVET

A two year study was conducted on a previously untreated cow-calf herd in southern Alberta to determine the pattern of acquisition and transmission of intestinal nematodes. The number of nematode eggs (epg) shed in the feces of cows and calves, pasture larval numbers and tracer calf burdens were monitored on a regular basis during the grazing seasons of 1988 and 1989. In both years, maximum epg output from cows occurred in the spring and decreased progressively during the grazing season. Calf fecal egg output increased soon after turnout to summer pasture and continued to increase during the grazing season reaching peak numbers at housing in the fall. The number of infective larvae on pasture peaked in August-September and decreased thereafter. Ostertagia and Cooperia were the predominant species recovered from the tracers and on pasture. Although there is some evidence that some larvae survive overwinter on pasture, the data suggest the high nematode egg output of cows in the spring contributes to pasture infectivity and subsequent infection in calves.
27.

THE OCCURRENCE OF A CLINICAL INFECTION OF NEMATODIRUS HELVETIANUS IN YOUNG DAIRY CATTLE IN WISCONSIN. D.H. BLISS*, E. DICKINSON, J. H. SCHAFER, and D. A. ARMSTRONG.

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Routine fecal check of pastured dairy calves from a large commercial dairy operation in southern Wisconsin indicated high level of Nematodirus eggs. No other worm eggs were found in the feces. The calves were reportedly gaining slowly and exhibited rough hair coats (for midsummer conditions) with severe diarrhea seen in a number of animals. Calves with the highest egg counts were purchased and moved to a study location for observation and treatment. Fourteen of the purchased cattle were randomly allotted equally into two treatment groups based on fecal worm egg counts. Treatment (albendazole -10 mg/kg body weight) was administered immediately following allotment to one group of seven calves while a second group of seven calves remained as nontreated controls. An efficacy evaluation was conducted following treatment.

Clinical conditions of the calves indicated an outbreak of clinical disease caused by Nematodirus. No adverse reactions were observed from the use of albendazole. Differential worm counts revealed that only Nematodirus helvetianus was present in the trial animals. Overall, albendazole was highly efficacious (97.4% against L4 stage, and 98.2% against early L5 and adult worms). Clinical signs, histopathological, and morphological observations on this Nematodirus infection will be presented.

28.

INTESTINAL CHANGES CAUSED BY INFECTION BY NEMATODIRUS HELVETIANUS IN DAIRY CALVES. E.O. DICKINSON*, D.A. ARMSTRONG, J.H. SCHAFER AND D.H. BLISS. NORDEN LABORATORIES, 601 W. CORNHUSKER HWY., LINCOLN NE 68501

Natural infection by Nematodirus helvetianus in a group of Wisconsin dairy calves was studied. Fecal worm egg counts and differential worm studies identified N. helvetianus as the only parasite in the study group. Samples of upper small intestine from calves harboring only larval stages and others harboring predominately adult parasites were collected for histopathological and scanning electron microscopy (SEM) studies. Also, specimens of L4, early L5 and adult parasites were prepared for SEM studies.

Histopathological examination of samples from calves infected by the L4 stage revealed marked thickening of the mucosa caused by diffuse infiltration of inflammatory cells, predominately lymphoplasmacytic cells, into the submucosa propria. There was no evidence in these specimens that the larvae had penetrated the mucosa. Instead, the nature of the lesion suggested inflammation due to diffusion of irritating chemicals and/or antigenic stimulation. Samples from calves harboring adult forms had less severe inflammation of the submucosa propria. Adult parasites were firmly intertwined around intestinal villi but there was no indication of tissue invasion. There was evidence that the adults caused atrophy, degeneration, and necrosis of surface enterocytes. This damage and subsequent inflammation was evidently caused by pressure of the intertwined parasites plus physical irritation by longitudinal ridges on the parasite surfaces. Light microscopy and SEM revealed morphologic features that elucidated these mechanisms of damage.
29.


A swine farm in west-central Illinois had a dirt pasture pen of pigs weighing 80-90 lbs which broke with a severe diarrhea in January, 1989. The pigs had been housed in a slotted floor nursery-grower building and dewormed with fenbendazole (9 mg/kg B.W.) approximately one week prior to being placed in a dirt pen for final growing and finishing. Twenty-three days after being moved to the dirt pen environment, the pigs began to develop clinical signs of scouring. During a three week period, 65 of 540 pigs (12 percent) died and another 77 (14 percent) were mildly to severely stunted.

Three pigs from this farm were taken to the Illinois State Diagnostic Laboratory at Galesburg for necropsy. No adult whipworms (Trichuris suis) were found on gross post mortem examination, but numerous cross-sections of T. suis larvae were seen on histopathological examination. No other causative agent was isolated. Subsequent necropsies produced adult T. suis. Efforts to control the outbreak was initiated immediately. The results of an intensive investigation on the clinical, epidemiological, diagnostic and treatment aspects of this outbreak are reviewed.

30.

RELATIONSHIPS AMONG ADULT CATTLE ON LOUISIANA COASTAL MARSH PASTURE BETWEEN FECAL SEDIMENTATION RESULTS FOR FASCIOLA HEPATICA AND PARAMPHISTOME. S.H. ZUKOWSKI AND J.B. MALONE. LOUISIANA STATE UNIVERSITY, BATON ROUGE, LA 70803

Adult cattle on Louisiana coastal marsh pastures were sampled for ova of Fasciola hepatica and the paramphistome Cotylphoron ctylophorum, both using the snail Fossaria bulimoides as intermediate host. Collections from pasture of 15 - 18 samples per herd were made in June 1988 (4 herds treated for fascioliasis/6 untreated herds), August 1988 (5 treated/12 untreated herds) and March 1989 (6 treated/7 untreated herds). In untreated herds there were correlations of 0.55 between mean F. hepatica ep2g and prevalence, 0.63 between paramphistome ep2g and prevalence, and 0.49 between prevalence of F. hepatica and paramphistome (all p < 0.05). In treated herds there were correlations of 0.57 between F. hepatica ep2g and prevalence and 0.74 between paramphistome ep2g and prevalence (both p < 0.05), but no significant correlation between prevalence of F. hepatica and the paramphistome.
31.

ISOLATION OF TOXOPLASMA AND ISOSPOR A FROM THE MEAT OF SOME FARM ANIMALS,
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The serological tests carried out in Egypt introduced many cases of Toxoplasmosis among farm animals. Regarding the isolation of Toxoplasma, the new experiment attempted to feed the infected meat of buffaloes, cattle, camels, pigs and donkeys to cats. Cats were 2-4 weeks old, coccidia-free. It has been concluded that the cats fed on the infected meat of camels or donkeys or pigs shedded Toxoplasma oocysts after 7, 7 and 6 days post infection respectively. The patent period ranged between 3-4 days. However, cats fed on the meat of buffaloes and cattle did not shed Toxoplasma oocysts. The oocysts were sporulated after 3 days at 27°C in 2.5% potassium dichromate. Isospora oocysts were excreted from the cats fed on the meat of camels, or donkeys or buffaloes. The prepatent period ranged between 5-7 days and the patent period was 8-15 days. Two type of Isospora were detected: the largest type, Isospora felis of 40 x 30 μm and the smaller one, Isospora revolta which is ovoid in shape and of 20-30 x 15-20 μm.

Using the same technique, another complementary study investigated the Toxoplasma and Isospora among other farm animals, namely sheep and goats. The study proved that the meat of these animals could be infected by Toxoplasma and Isospora (unpublished data).

32.


Low-level sequential infections of pigs averaging 38 kg (Trial I) or 15.3 kg (Trial II) in weight were made with with 2,000 Ascaris suum, 10,000 Oesophagostomum spp and 10,000 Strongyloides ransomi. Each individually housed pig was weighed every two weeks and the feed consumed monitored until the groups reached market weights of 106.6 kg and 97.3 kg, respectively. Results indicated that infections were deleterious to pig performance. Heavier infected pigs gained weight slower (P<.01) and consumed more feed (P<.03) than control pigs, although the feed to gain ratios were not different. Lighter infected pigs gained weight and consumed feed at the same rate as control pigs (P>.10), however the gain to feed ratio for infected pigs was higher (P<.04) than that for control pigs. Differences in performance were greatest during the first 35 days of the trial. Of particular interest was the greater effect of lower infection rates relative to body weight on heavier pigs than higher infection rates on lighter pigs.
33.


Information on the effects of parasite control under various management schemes is tenuous for steers grazing Coastal Bermudagrass (cbg). Our aim was to address this situation during a 140-day grazing trial.

Treatments were: broiler litter-grain supplementation (lgs) at stocking rates (sr) 9.9, 19.8, 29.7, 39.7 or 49.6 steers/ha with (w/) or without (w/o) parasite control (pc); or w/o lgs at sr of 4.9, 9.9 or 13.8 steers/ha w/ or w/o pc, all on continuously grazed cbg. Animal weight & forage height (fh) data were collected every 28 days. Fecal egg counts (epg), plasma pepsinogen and pasture larval counts were collected and deworming of pc groups occurred every 56 days. Backfat (bf) was measured at the beginning and end of the study. Data were analyzed by regression analysis using pooled deviations from regression as the error term. ADG and fh decreased as sr increased. The regression ADG vs. sr was positively affected by pc (P<0.001), lgs (P<0.005) and had a significant lgs*sr (P<0.005) interaction. The regression of fh vs. sr in the treatments w/o lgs was not affected by pc, but in the treatments w/ lgs, pc reduced fh (P<0.02). Change in bf and epg were not affected by sr, thus the error terms were pooled and ANOVA's performed. Change in bf was positively affected by pc (P<0.05) and lgs (P<0.001). EPG was reduced by pc (P<0.01).

34.

COMPARISON OF ANTHELMINTIC PROGRAMS FOR GRAZING SHEEP IN SOUTHWESTERN VIRGINIA. A.M. ZAJAC*, C. THATCHER, D. NOTTER, J.W. HANSEN AND S. UMBERGER. VIRGINIA TECH, BLACKSBURG, VA 24061

Gastrointestinal parasitism is recognized as one of the most serious problems facing sheep producers in Virginia. Lambs in this area traditionally receive anthelmintic treatments monthly throughout the grazing season. This program is expensive, would be expected to contribute to the development of resistance and does not prevent economic loss in the face of heavy parasite challenge. In an effort to design a rational anthelmintic program for grazing lambs, three treatment regimes were compared. In a strategic program, Group 1 lambs received ivermectin (200micrograms/kg) 0, 3, 6, 9, and 12 weeks after pasture turnout in the spring and Group 2 lambs received levamisole at the same intervals. Group 3 lambs were treated with ivermectin at 0, 8, 16, 20 and 24 weeks after turnout. Group 4 lambs received ivermectin monthly throughout the grazing season. Fecal samples were collected at approximately 2 week intervals and weights were determined at approximately the same intervals. Two tracer lambs each were placed on the pastures of Groups 2 and 4 at 4 intervals during the grazing season. Group 3 lambs became clinically parasitized by early August, requiring anthelmintic treatment, and Group 1 and 2 animals required additional treatments in mid-September to prevent losses. These results suggest that use of a strategic anthelmintic program in this area will not protect lambs throughout the grazing season unless additional fall treatments are given.
A THREE YEAR STUDY OF PARASITISM IN A BEEF COW-CALF HERD IN NORTH CENTRAL MINNESOTA. B.E. STROMBERG*, D.L. HAGGARD, J.C. SCHLOTTHAUER, AND J.W. RUST. UNIVERSITY OF MINNESOTA, ST. PAUL, MN 55108

A herd of cross-bred cows (Hereford, Angus, Charalais) was divided into two groups based on age and sire of the dam. One group was designated as the untreated control while the other was treated with Ivermectin in a strategic deworming program. Cows and their calves were followed over the next three years, 1987 to 1989. The weather for this period was hotter and drier than the 30 year average. The fecal egg count from the cows showed an increase in the spring and a drop in the fall in the control group, while the treated group remained low throughout the study. The EPG's of the untreated calves increased throughout the grazing season, peaking in October or November. The treated calves EPG's remained low. Tracer calves grazed for one month periods and showed different populations and population dynamics in each of the 3 years. Likewise, productivity as measured by calf weaning weights also varied. The greatest productivity was in the 1988 grazing season, with a 205 day adjusted weaning weight advantage of 34.01 lbs. for the treated calves. There was also an advantage to deworming the calves at weaning, even when the calves would be held only for an additional 30 days.

SUMMARY OF REPRODUCTIVE IMPROVEMENTS FOLLOWING PARASITE CONTROL USING FENBENDAZOLE

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Five studies were designed to evaluate the effect of strategic parasite control programs on the productivity of cow/calf herds. In each study reproductive performance was evaluated by rectal palpation and/or actual calving rates. The five studies involved a total of 735 beef cows and were conducted from 1984 to 1988 on 3 experiment stations in Minnesota (3), Florida (1), and Georgia (1 study). Using a split pasture design, productivity of untreated control herds was compared to herds on a preventive (strategic) anthelmintic treatment program. The treatment program included a single dose of fenbendazole suspension (5 mg/kg) prior to the start of the breeding season. Improvements in pregnancy rate or actual calving rate ranged from 4 to 22.5%. Results document an average improvement of 11.5% (P <.03). Pretreatment cow infection levels (herd average) generally did not exceed 10 EPG.

The effects of anthelmintics on ecosystem processes after excretion in the feces or urine of treated animals has received scant attention in the past. Anthelmintics may be registered for use without full assessment of their potential environmental consequences. The residues of phenothiazine, dichlorvos, coumaphos, ruelene, piperazine, and the avermectins have been reported to have deleterious effects on invertebrate species that play an important role in maintaining the pasture ecosystem. Ivermectin is a unique drug in that it is almost totally excreted in the feces of treated animals, it is a potent insecticide, and it has an environmental persistence as long as, or longer than some commonly used insecticides. It is also toxic at very low concentrations: the LC50 value of ivermectin for rainbow trout is .003 ppm. Studies with 8 horses in Ohio in 1988 showed that concentrations as high as 8.5 ppm of ivermectin were excreted in horse feces in the first 24-48 hours after treatment at the recommended dosage of 0.2 mg/kg. It affected a large range of invertebrate species inhabiting dung and caused a significant delay (P < .05) in the rate of dung degradation during autumn. In a comparison of the ecological effects of ivermectin and oxibendazole with 21 horses under natural grazing conditions in 1989, there was a significantly (P = .001) greater rate of disappearance of dung pats from oxibendazole-treated horses than from ivermectin-treated horses. After 6 months, the grazing area lost to feces was 5.0 meters²/horse/week for the ivermectin-treated horses, 1.7 meters²/horse/week for the oxibendazole-treated horses, and 1.8 meters²/horse/week for control horses. These results do not include the larger area of pasture rejection surrounding the fecal pats. Further studies are needed to assess the effects of avermectin dung residues on the grazing area, nutrient recycling, pasture productivity, and species composition.

GASTROINTESTINAL PARASITES OF EXOTIC RUMINANTS IN TEXAS. T.M. CRAIG, AND S.H. MERCER. TEXAS A & M UNIVERSITY. COLLEGE STATION, TX 77843 AND P.J. SCHOLL. U.S. LIVESTOCK INSECTS LABORATORY. KERRVILLE, TX 78029.

The worms in the abomasum and first meter of small intestine were collected from 57 Aoudad, Blackbuck and Nilgai antelope and Axis, Fallow and Sika deer slaughtered for commercial venison production during the summer of 1988. Other samples were received through the Texas Veterinary Medical Diagnostic Laboratory. In general the bovids were much more heavily parasitized than the cervids and in some instances the level of parasitism indicated clinical parasitism. Haemonchus contortus was the most abundant species of parasite throughout the study. Other parasites include Trichostrongylus colubriformis, T. axei, T. probolurus, Camelostongylus mentulatus, Spiculoptraqia asymetrical, Nematodirus sp., Ostertaqia sp. and Thysanosoma actinoides. The interactions of various hosts and parasites will be discussed.
During a routine anthelmintic trial conducted during the summer of 1988 at Oregon State University, numerous specimens of *Ostertagia leptospicularis* Asadov, 1953 were recovered from the abomasal samples from 17 or 23 beef calves (mixed breed). The calves, harboring naturally acquired gastrointestinal nematodes, had been transported from the ranch of origin in Mollala, Oregon to the Oregon State University Beef ranch. No *O. leptospicularis* had ever been recovered from any calves having previously grazed the University Beef ranch. Identifications were based on the measurement and morphology of the spicules, gubernaculum, genital cone, and esophagus. The synlophe pattern was as described by Lichtenfels, et al. Additional species recovered included *O. kolchida*, *O. ostertagi* and *O. lyrata*. This represents the first report of *O. leptospicularis* and the second report of *O. kolchida* from North America.

Horn fly, *Haematobia irritans* (L.), dispersal between cattle herds was studied during the spring, summer and fall seasons to determine if climatological factors and/or seasonality were significantly affecting horn fly movement.

To examine horn fly dispersal, four fly-free herds of cattle were placed in uncontaminated pastures north, east, northeast and west of a herd of cattle with an established population of horn flies present. Horn fly counts and climatological data were collected daily for a 20-day period during each season. These data suggested horn fly dispersal was lowest during the spring and that climatological data did not significantly influence dispersal.
41.


Fourteen pony foals were inoculated with 1809 ± 169.8 infective Parascaris equorum eggs on day -4.5. The foals were allocated to replicates of 2 and treatments were assigned to each replicate randomly. Treatments administered on day 0 included untreated control and 0.2 mg of ivermectin /kg. The foals were euthanatized 25 days after inoculation (day 20.5) and examined for the presence of $P$ equorum larvae in the small intestine. The mean number of 4th-stage $P$ equorum larvae recovered from control and ivermectin treated foals was 519.7 (112-860) and 0.17 (0-1) respectively. Treatment with ivermectin was 99.97% effective against early migratory larvae of $P$ equorum when compared to controls. Adverse reactions due to treatment were not observed. Two untreated tracer foals necropsied 4.5 days after inoculation with infective $P$ equorum eggs had a mean of 120 larvae recovered from liver tissue. Larvae were not recovered from lung tissue or small intestinal contents of the tracer foals.

42.


This study evaluated the clinical presentation of infections with $U$. stenocephala and treatment with nitroscanate. Five of 7, specific-pathogen free, 8-week-old Beagles were fed 1,000 infective larvae. Routine physical exams, complete blood counts, blood chemistries, and fecal examinations were performed weekly. The 5 pups receiving larvae developed patent infections within two weeks. At four weeks postinfection, the dogs were treated with nitroscanate (50 mg/kg). No hookworm eggs were detected in the feces during the next two weeks. However, 3 weeks after treatment, the 5 pups were again shedding eggs. The nitroscanate dosage was repeated, and eggs were no longer detected in fecal samples. The dogs were placed in foster homes. Three weeks later, feces from 2 of these 5 dogs were reexamined, patent infections were again present, and the dogs were treated with pyrantel pamoate. Follow-up of these dogs revealed eggs again a month later. The dogs were treated with fenbendazole, and again the dogs were shedding eggs a month after this treatment. The pups remained healthy and without signs throughout the experiment. There was a comparative increase in peripheral eosinophils in the infected dogs, but these values were within standard normal ranges. All other blood parameters were normal. From these trials it appears that nitroscanate removed the adult $U$. stenocephala and that arrested larvae are reestablishing infections within the intestine after treatment.

The strains of the bacterium Bacillus thuringiensis (BT) produce various proteinaceous crystalline inclusion bodies termed delta-endotoxins. These endotoxins are commonly studied and commercialized as biological insecticides. Mycogen has isolated and tested selected BTs on free living and parasitic nematodes at various stages of development both inside and outside of the host animal.

Several BT isolates were tested on species of Haemonchus, Ostertagia, Trichostrongylus, Nippostrongylus, and Nematospiricoides using in vitro fecal assays and rodent-model assays. The data indicate that the toxins are specific for nematodes and significantly reduce the larval development (>90% reduction) when applied at rates of <20 µg/g feces. Oral doses of <100 µg/mouse/day show reduction in egg counts from 80-100% depending on species and isolate. Large animal studies in progress indicate that the same phenomena is present. These observations reveal the potential of a feed-through for larval (free-living stages) control. Reductions in the infected animal worm burdens require very high doses which still give unsuitable control. The cause for the "ovicidal" effect is unknown. This gives rise to the possibility of developing a natural product having a novel mode of action with no mammalian toxicity. Possible delivery systems could include pasture sprays, feed/mineral supplements, or controlled release devices.

INFLUENCE OF FORMULATION AND ROUTE OF ADMINISTRATION ON THE PHARMACOKINETICS OF NETOBIMIN AND ITS METABOLITES IN CATTLE. C.E. LANUSSE*, S. RANJAN, C. TRUDEAU AND R.K. PRICHARD. INST. PARASITOL., MCGILL UNIV., CANADA H9X 1C0

Netobimin (NTB) is an anthelmintic that exerts its broad-spectrum activity by cyclisation to albendazole (ABZ) metabolites. NTB can be formulated as a solution or a suspension for either oral or parenteral administration. The pharmacokinetics and comparative bioavailability of NTB and its metabolites were studied in cattle, after administration SC (12.5 mg/kg) and oral (20 mg/kg) of both NTB trisamine solution and NTB zwitterion suspension, in two 4 x 4 cross-over design experiments. Plasma was analysed for NTB, ABZ, albendazole sulphone (ABZSO) and albendazole sulphoxide (ABZSO2) by HPLC over 72 hours post-treatment. While NTB was detected only after SC treatments, ABZ was not found in plasma at any time after either SC or oral administrations. After both SC treatments (trisamine and zwitterion formulations), NTB parent compound showed a fast absorption reaching an early Cmax (0.75 h) and was rapidly eliminated (T ½ from 2.59 to 3.57 h). ABZSO and ABZSO2 were the principal metabolites detected after both SC and oral treatments. Both parenteral formulations were bioequivalent in terms of ABZSO and ABZSO2 pharmacokinetic profiles. However, after oral treatment, the zwitterion suspension resulted in a two-fold higher pharmacokinetic profile (AUC, Cmax) than the trisamine solution. Both oral treatments resulted in longer T ½ and mean residence time and a higher AUC for ABZSO when compared with SC treatments. We conclude that both formulation and route of administration may affect the rate of NTB conversion and the kinetics of active metabolites.
45.


The efficacy of ivermectin in a sustained-release bolus was evaluated in twelve cattle (197-255 kg) harboring induced infections of adult nematodes. Cattle were randomly allocated to groups: 1) untreated control; 2) ivermectin bolus delivering 8 mg ivermectin/day. Each calf received approximately the following numbers of L3 larvae: 1,000 Bunostomum phlebotomum on Day -56; 1,000 Oesophagostomum radiatum on Day -49; 2,500 Dictyocaulus viviparus on Day -31; 20,000 Cooperia spp., 15,000 Ostertagia ostertagi and 7,500 Haemonchus placei on Day -27; 10,000 Trichostrongylus axei, 10,000 Trichostrongylus colubriformis and 3,000 Nematodirus helvetianus on Day -25. The calves were euthanatized 28 or 29 days after treatment (Day 0). Ivermectin was 100% effective against all nematodes tested (P<0.05). No T. colubriformis were recovered from control or treated calves. There were no adverse reactions associated with treatment. All boluses were recovered from either reticulum or rumen.

46.

**CRITICAL EVALUATION OF THE VARIABLE EFFICACY OF OXFENDAZOLE AGAINST INHIBITED LARVAE OF OSTERTAGIA OSTERTAGI.** J.E. MILLER* AND T. OLSON. LOUISIANA STATE UNIVERSITY, BATON ROUGE, LA 70803

One of the hypotheses for variable efficacy of benzimidazole anthelmintics against inhibited *Ostertagia ostertagi* larvae is differences in larval metabolic activity during the inhibition season. In southern temperate regions, metabolic activity is thought to be high during induction (February-April) of inhibition and emergence (August/September) of inhibited larvae. Metabolic activity is thought to be low during the quiescent period (May-July). Forty-eight steer calves commenced grazing 10 acres of pasture at Idlewild Research Station in November, 1988. In each of the months of March, May, July, and September, 1989, 12 animals were removed and placed on concrete. After 3 weeks, 6 animals were treated intraruminally with oxfendazole (4.5 mg/kg) and 6 were left as untreated controls. Seven days after treatment the steers were slaughtered and gastrointestinal nematodes were enumerated and identified. Oxfendazole had excellent efficacy (>98.1%) against adult nematodes, high efficacy against inhibited *O. ostertagi* in March (89.4%) and September (94.3%), low efficacy in May (41.5%), and moderate efficacy in July (68.5%). The results indicate that the efficacy of oxfendazole is variable and correlates nicely with expected larval metabolic activity, in that, increased efficacy was observed during periods of expected high larval metabolic activity and reduced efficacy was observed during periods of expected low larval metabolic activity.
47.


Twenty cattle were allocated to treatment with a prototype bolus designed to deliver 12 mg of ivermectin/day intraruminally or designated as unmedicated controls. Cattle grazed a single natural pasture. At various intervals after treatment, 4 replicates were confined to individual tick collection stanchions for 4- or 5-day periods. Ticks recovered were counted by species, sex, stage and degree of repletion; engorged females were weighed and incubated to determine the number that oviposited. For the other 6 replicates, half-body counts of adult ticks (classified by species, sex and degree of repletion of females) were made at 1- and 2-week intervals through Day 90. Among replicates periodically confined to stanchions, significantly fewer (P<0.05) engorged female Boophilus decoloratus, Hyalomma spp., Rhipicephalus appendiculatus and Rhipicephalus evertsi were collected from bolus-treated cattle than from controls. Numbers of engorged adult female Amblyomma hebraeum were reduced but differences were not statistically significant (P>0.10). Among cattle maintained continuously on pasture, a significant (P<0.05) overall treatment effect was seen for A. hebraeum, B. decoloratus and R. evertsi. A significant (P<0.05) treatment by linear time effect was seen for all species counted except R. appendiculatus, indicating that differences between treatment groups increased with time. Except for Boophilus, reductions in tick numbers on treated animals were not visually apparent.

48.


The efficacy of albendazole was compared with that of ivermectin and clorsulon in cattle naturally infected with liver flukes and intestinal nematodes. One hundred three (103) Brahman or Brahman cross cows 5 to 6 years of age located on a ranch in the Okeechobee, Florida, area were used on study. The cows were assigned to one of two treatment groups. Group I (51 cows) received 10 mg/kg albendazole suspension as a drench. Group II (52 cows) received 0.2 mg/kg ivermectin subcutaneously and 7 mg/kg clorsulon suspension as a drench. All cows were infected with Fasciola hepatica. In addition, 23 Group I cows and 19 Group II cows were infected with intestinal nematodes. Fecal eggs per gram were determined pre- and post-treatment. In the albendazole group, 96.1% of the cows exhibited no fluke eggs post-treatment. In the ivermectin/clorsulon group, 92.3% of the cows were cleared of fluke eggs. In both groups post-treatment fecal samples were negative for nematodes.
ISOLATION OF LAMINA PROPIA EOSINOPHILS FROM THE CAPRINE DUODENUM AND EVALUATION BY CHEMILUMINESCENCE. D.A. CROSS. USDA, ARS, ANIMAL PARASITE RESEARCH LABORATORY, P.O. BOX 952, AUBURN, AL 36831-0952.

Isolation and purification of cells derived from the lamina propria of the duodenum will lead to a better understanding of the mucosal immune interactions which occur during host responses to invading parasites. Duodenal segments obtained from goats which were infected with the nematode Trichostongylus colubriformis were used as a source for the isolation of eosinophils.

Following collagenase treatment of epithelium-free segments of duodenum, cell suspensions were centrifuged on discontinuous Percoll gradients. Eosinophils were consistently recovered at greater than 85% purity. The optimal parameters for chemiluminescence were established using 5 x 10⁵ cells with a 1:640 dilution of luminol and 250 μg of pre-opsonized zymosan.

Results indicated that cells derived using this procedure were viable and responded to appropriate stimuli by increased chemiluminescence. Further research is necessary to evaluate the eosinophil response to parasite- and host-produced factors which are present in the gastrointestinal tract during parasitic infection.

NATURALLY OCCURRING IMMUNOMODULATION IN CANINE LYMPHATIC FILARIASIS. D.M. SCHREUER*, K. NAKAGAKI, B. HAMMERBERG. COLLEGE OF VETERINARY MEDICINE, NORTH CAROLINA STATE UNIVERSITY, RALEIGH, NORTH CAROLINA 27606.

Brugia pahangi infected beagle dogs were used as a model for the study of the pathogenesis of lymphatic filariasis. Previous studies indicated that selected breeding pairs produced litters of predominantly high or low responder dogs. New litters of these two types of dogs were raised. At three months of age the puppies were infected in both rear paws with 15 infective larvae. Peripheral blood lymphocytes (PBL) and lymphocytes from lymphnode biopsies from Brugia infected and uninfected limbs were collected at 4, 7 and 11 weeks post infection and at 3 occasions during chronic infection. The in vitro blastogenic responsiveness to mitogen and parasite antigen and the in vitro production of total and antigen specific IgG and IgE were measured as well as in vitro production of IL-2. During early infection (from 4 to 11 weeks post infection) both types of dogs showed strong infected node lymphocyte blastogenic responses(SI 20) that were seldom equaled by PBL and uninfected node lymphocytes. Both high and low responder dogs showed loss of blastogenic responsiveness at a later stage of infection. High responder dogs regained and maintained blastogenic responses to parasite antigens while low responders failed to regain parasite specific blastogenic responsiveness.
51.

KINETICS OF LIVER TRAPPING OF INFECTIVE LARVAE IN MURINE TOXOCARIASIS. J.C. PARSONS* and R.B. GRIEVE. COLORADO STATE UNIVERSITY. FORT COLLINS, CO 80523.

We have previously demonstrated that sensitized C57BL/6 (B6) mice trap infective T. canis larvae in the liver at 1, 2, and 3 weeks postchallenge (PC). A plateau in the level of trapping occurred with a sensitization dose of 75-125 eggs. In addition, we determined that B6 mice trap significantly more larvae than DBA/2 mice. In this study the distribution of challenge larvae in sensitized mice was examined to determine the earliest onset of liver trapping and the duration of this phenomenon. In all experiments, B6 mice were given a sensitization dose of 125 eggs on day 0 PI and challenged with 500 infective eggs on day 28 PI. Initially, larval numbers were determined within various tissues of each mouse on days 0.5, 1, 2, 3, 5, and 6 PC. Migration patterns were similar among the test and control groups except the peak of larval numbers in the liver, seen at 1 day PC in control mice, was delayed until 3 days PC in the test group. Larval trapping occurred within the liver of test mice at least by day 5 PC. Subsequently, larval numbers were determined within the liver, skeletal muscle, brain, and eyes of mice at 4, 8, 12, and 16 wk PC. Larval numbers within the liver of test mice were similar both at 5 days PC and 16 wk PC implying that larvae were trapped in this organ rather than delayed in their migration to other body sites. Liver trapping did not protect the eyes or brain of sensitized mice from larval migration, nor did it result in larval killing. (Supported by NIH Grant EY-05677)

52.

IN VIVO INJECTION OF ANTIBODIES TO INTERLEUKIN 4 OR INTERLEUKIN 4 RECEPTOR BLOCKS THE PROTECTIVE RESPONSES TO CHRONIC PARASITISM WITH 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 20814-4799.

Mice inoculated with third-stage larvae of H. polygyrus become chronically infected with adults that persist in the intestines and pass eggs for several months. A partial immunity to re-exposure develops in BALB/c mice that are inoculated with H. polygyrus and anthelmintic treated 3 weeks after inoculation. The infection is characterized by a marked increase in circulating IgE and eosinophilia. Injection of H. polygyrus exposed mice with monoclonal antibody to IL4 or IL4R results in a decrease in IgE production, an increase eosinophilia, and an increase in the fecundity of adult worms derived from a challenge exposure. In addition, anti-IL4 and anti-IL4R antibodies block protective immunity to a challenge exposure when given together or separately. Antibodies against IL5 block eosinophilia, but have no apparent effect on the infection in mice. These results suggest that IL4, and/or the cells that produce it, are crucial to the development of immunity to gastrointestinal nematode parasites.
53.
THE SENSITIVITY OF A MONOCOLONAL ANTIBODY-BASED ANTIGEN-DETECTION ENZYME IMMUNOASSAY FOR DIAGNOSIS OF TRYPANOSOMA CONGOLENSIS INFECTION IN GOATS. R.A. MASAKE* AND V.M. NANTULYA. INTERNATIONAL LABORATORY FOR RESEARCH ON ANIMAL DISEASES. P.O. BOX 30709, NAIROBI, KENYA.

The sensitivity of a monoclonal antibody-based antigen-detection enzyme immunoassay (Antigen-ELISA) for the diagnosis of *T. congolense* was evaluated using sera from experimentally infected goats as well as field sera from goats in a trypanosomiasis endemic area. Ten goats (Galla X East African Maasai) were infected with different clones of *T. congolense* and left to run a chronic course for 46 months. During this period, monthly blood samples were collected and analysed for the presence of trypanosomes and antigens in peripheral blood circulation. The total number of blood samples tested was 383. Out of these, 361 (94.3%) were positive for circulating antigens while only 42 (10.9%) had demonstrable trypanosomes as revealed by the microhaematocrit centrifugation technique. In an analysis of serum samples from goats in an area known to be endemic for trypanosomiasis, 106 out of 131 (80.9%) were positive for *T. congolense* antigens while none of the corresponding blood samples was positive for trypanosomes. Control sera from 40 goats in a trypanosomiasis-free area were all negative for antigens. Hence, the Antigen-ELISA technique was 8 times more sensitive than the microhaematocrit centrifugation technique in monitoring *T. congolense* infections in goats.

54.
LYMPHOCYTE ACTIVATION IN VITRO BY ASCARIS SUUM CUTICULAR ANTIGENS. D.E. HILL*, R.H. FETTERER, and J.F. URBAN. USDA, ARS, LIVESTOCK & POULTRY SCIENCES INSTITUTE, HELMINTHIC DISEASES LABORATORY. BELTSVILLE, MD 20705.

Ascariasis in swine is a major economic problem worldwide. The role of the parasite's cuticle in the pathogenesis of infection is not well understood. The effect of isolated cuticular preparations from distinct developmental stages of *A. suum* on swine lymphocyte biostogenesis was studied. Mononuclear cells were isolated from swine blood from both non-infected and *A. suum* infected swine and were placed in 96-well culture plates at $2 \times 10^5$ cells/well. Cuticular preparations from 2nd, 3rd, and 4th stage larvae were prepared by grinding the larvae in reducing agents (urea, 2-ME) and boiling for 10 min. at 100°C. The solution was centrifuged and the pellet (insoluble cuticles) was digested with trypsin for 24 hrs. Cuticles were dissected from young and mature adult *Ascaris* and treated as above. Cuticular digests were added to wells with cells at 1, 10, and 30 ug protein per well. On day 5 of culture, 0.5 uCi of 3H-thymidine was added to each well. Cells were lysed 6 hrs. later and the nuclei collected on glass fiber filters. The nuclear incorporation of 3H-thymidine was determined by liquid scintillation spectrophotometry.

Results indicated that cuticular preparations from 3rd stage *A. suum* specifically stimulated lymphocytes from infected swine but not from non-infected swine. Cuticular preparations from the other stages tested had no stimulatory effect. Cuticular antigens from 3rd stage *A. suum* may be particularly important in the development of the cellular immune response in *Ascaris* infections.
55.

**THE OCCURRENCE OF TYROSINE DERIVED CROSS-LINKS IN HAEMONCHUS CONTORTUS CUTICLE.** R.H. FETTERER* AND M.L. RHoads. USDA, ARS, LIVESTOCK & POULTRY SCIENCES INSTITUTE, HELMINTHIC DISEASES LABORATORY. BELTSVILLE, MD 20705.

The cuticle was isolated from in vitro exsheathed *Haemonchus contortus* infective larvae (2M), in vitro derived third stage larvae (L3) and in vivo derived adults. Acid hydrolysates of 2-mercaptoethanol (2ME)-soluble and 2ME-insoluble cuticular proteins were analyzed by HPLC for tyrosine derived cross-linking amino acids. Dityrosine and isotrityrosine were identified by their chromatographic behavior, absorbance spectra, and other chemical characteristics in both the 2ME-soluble and 2ME-insoluble fractions from all the stages examined. Dityrosine and isotrityrosine were found in greater amounts in the 2ME-soluble proteins. When intact 2M cuticles were labeled with I-125 prior to acid hydrolysis, radiolabel was recovered in tyrosine but not dityrosine or isotrityrosine indicating that the tyrosine cross-links are not susceptible to iodination in the intact cuticle. The above results support a hypothesis that tyrosine derived cross-links are important components of *H. contortus* cuticular proteins and suggest that tyrosine derived cross-links may be targets for novel control strategies.

56.

**PROTEIN CONTENT OF THE BODY WALL OF OESOPHAGOSTOMUM COLUMBIANUM (NEMATODA).**

MANJEET JOHAL, DEPTT. OF ZOOLOGY,
PUNJABI UNIVERSITY PATIALA-147002. INDIA

The body wall of *Oesophagostomum columbianum*, contains 10.52% of protein distributed in outer and inner cortical layers, basal layer, hypodermis and contractile region of the muscle cells. Besides the general proteins, there is also the presence of proteins linked with disulphide bonds and amino groups. The covalent linked disulphide bonds are responsible for stability and resistance of the cortical layers. The nature of protein forming the basal layer is unique in having collagen closely associated with acid mucopolysaccharides and glycogen. The synthesis of proteins takes place partly in the inner cortical layer but mostly in the hypodermis. An intense RNA activity too, is observed in these areas. The proteins synthesized in the hypodermis are conveyed to the cuticle through a system of pore canals extending between the hypodermis and the cortex and also through large pores present at the base of each lateral ala.
Levamisole is a nicotine analog. It kills nematodes by overstimulating muscle acetylcholine receptors. Mutations in receptor genes allow a mutant nematode to survive extremely high concentrations of levamisole. A binding assay using a tritiated derivative of levamisole detects a receptor with a nicotinic pharmacological profile in wild-type extracts. Mutants in 7 resistance genes are all altered or deficient in receptor binding activity. Several of these genes encode structural subunits of a nicotinic acetylcholine receptor. Other genes needed to make this receptor may be important because they control the appearance of the receptor during development or affect the maturation and processing of the receptor. Transposon tagging allows receptor genes to be readily cloned whether or not they encode receptor structural subunits. The first two receptor genes cloned by transposon tagging do encode structural subunits of the receptor and have strong homology to subunits of Drosophila and vertebrate nicotinic acetylcholine receptors. Study of receptor mutants in the nematode C. elegans should help in understanding levamisole resistance in natural populations of parasitic nematodes and should aid in the design of better anthelmintics.

Since levamisole was introduced in the early 1970's, it has been a major tool in controlling helminth infections in animals and man. Its usefulness is threatened by a growing incidence of resistance in the field. Trichostrongylid nematodes of sheep, especially Haemonchus contortus, are most commonly identified as exhibiting resistance. A number of in vitro assays have been developed to identify and study resistance to levamisole for H. contortus, but no in vivo models have been available to study resistance for this parasite. The studies reported herein were conducted to determine the utility of a recently described H. contortus/jird (Meriones unguiculatus) model for examining levamisole resistance for H. contortus. Immunosuppressed jirds (0.02% hydrocortisone in feed) were inoculated with ~1,000 exsheathed infective larvae of H. contortus (resistant or susceptible to levamisole), treated per os on day 10 postinoculation (PI) with levamisole hydrochloride or analogs of the drug, and necropsied on day 13 PI. Each stomach was removed, opened longitudinally, incubated in distilled water at 37°C for 5 hr, fixed in formaldehyde solution, and stored for subsequent examination. Stomach contents were examined using a stereomicroscope (15-45X). Doses of levamisole hydrochloride and its analogs which elicited percentage clearances of ≥93 for the susceptible strain cleared ≤53% of the resistant worms. These data are consistent with observations on the activity for each of the drugs against wild type and levamisole-resistant strains of Caenorhabditis elegans. Thus, the H. contortus/jird model does provide a useful in vivo tool to study resistance to levamisole and possibly other classes of anthelmintics.
ANTHELMINTIC PHARMACOKINETICS 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

The pharmacokinetics of several anthelmintics were compared in goats and sheep in order to investigate the cause of poor performance of these drugs in goats in the field and the rapid development of anthelmintic resistance. Profiles of oxfendazole (OFZ), albendazole (ABZ) and levamisole (LEV) have been completed.

The Cmax, Tmax and area under the curve (AUC) for OFZ, but not its metabolites, were reduced in goats compared with sheep, and clearance from plasma was faster. With ABZ similar differences between species were obtained except that clearance was equivalent. Dose rates above 10 mg of OFZ/kg did not significantly increase AUC. It is recommended that goats are given double the recommended dose for sheep of these benzimidazoles or that drug be given over 2-3 days. No differences were found in the behaviour of LEV between species after both intraruminal and subcutaneous administration.

CLONING AND CHARACTERIZATION OF TWO B-TUBULIN cDNAs FROM HAEMONCHUS CONTORTUS.
S.C. NULF1, R.D. KLEIN1*, N.T. HATZENBUHLER1, R.K. PRICHARD2, M.H. SHEA1, L. TANG2, M.A. FAVREAU1 AND T.G. GEARY1. 1THE UPJOHN COMPANY, KALAMAZOO, MI 49001 AND MC GILL UNIVERSITY, STE. ANNE DE BELLEVUE, CANADA.

Considerable evidence suggests that the primary target of the anthelmintic benzimidazoles is microtubule dynamics, particularly the β-tubulin protein. To better characterize the complement of β-tubulin genes in the ruminant nematode parasite Haemonchus contortus, we cloned the genes for two isoforms of this protein. One, clone 8-9, was pulled from a λgt11 larval library supplied by Drs. D. Zarlenge and R. Gamble (USDA). The other (12-16) was pulled from an immature adult H. contortus library in lambdaZAPII. For each, library probing was initially performed with the cDNA for the mouse β-5 isoform (a gift from Dr. N. Cowan). Both genes code for proteins of 448 amino acids. The clones are 80% identical at the nucleotide level and 92% identical at the amino acid level. The greatest divergence in sequence occurs at the extreme COOH terminal, as expected for true isoforms. Both genes are 90% identical to the mouse β-5 isoform, but neither is a recognizable number of the known vertebrate isoform classes. Regions which differentiate the nematode β-tubulins from vertebrate β-tubulins include, in addition to the COOH terminus, amino acid residues 270-300 and the region around residues 126 and 165. Southern hybridization analyses show that the 3' and 5' halves of both genes recognize discrete bands in restriction maps. We suspect that at least one additional isoform exists. We have also cloned a 12-16 homolog which differs extensively at the 5' and 3' untranslated regions from the prototype.
61.

BENZIMIDAZOLE RESISTANCE IN HAEMONCHUS CONTORTUS, GENETIC ANALYSIS AND BENZIMIDAZOLE BINDING TO IN VITRO EXPRESSED β-TUBULIN. L. TANG1, R. PRICHARD1*, G. MATLASHEWSKI1, S. NULF2, R. KLEIN2 AND T. GEARY2.

1INSTITUTE OF PARASITOLOGY, MCGILL UNIVERSITY, MONTREAL, QC, CANADA H9X 1CO, and 2THE UPJOHN COMPANY, KALAMAZOO, MI 49001.

Two distinct β-tubulin genes have been isolated and sequenced from a benzimidazole (BZ)-susceptible Haemonchus contortus egg cDNA library. The predicted amino acid sequences of both β-tubulins are 448 residues long. These β-tubulin cDNAs have been used to probe genomic DNA from BZ-susceptible (S) and resistant (R) H. contortus strains. DNA from S and R H. contortus eggs was digested with EcoRI, SphI, SpeI and Hind III restriction enzymes and subjected to Southern blot analysis. The restriction enzyme patterns between S and R strains were different for each β-tubulin probe. The results indicate that there is at least one β-tubulin gene that is different between the S and R strains and are consistent with resistance being associated with a loss of one β-tubulin gene. Northern blot analysis of mRNA from S and R H. contortus eggs indicated that the levels of mRNA are similar between these strains. β-tubulin was expressed in vitro by transcription and translation of a cDNA tubulin gene from S H. contortus. The in vitro produced protein was characterized by two-dimensional electrophoresis and Western blot using a β-tubulin monoclonal antibody. The in vitro expressed β-tubulin was shown to bind [3H]oxibendazole.

Supported by NSERC/UpJohn

62.

CODON USAGE AMONG NEMATODES. T.G. GEARY*, B.L. LEE AND R.D. KLEIN. THE UPJOHN COMPANY, KALAMAZOO, MI 49001.

A potentially useful strategy for probing cDNA or genomic libraries from parasitic nematodes for specific proteins of interest is to use DNA sequences which code for the desired protein in other organisms. However, among metazoans, the majority of cloned genes have been obtained from mammals, and their use in cross-phylum probing of nematode gene libraries is problematic. Similarly, the free-living nematodes (such as C. elegans) are thought to have diverged from parasitic species as much as 5 x 10^8 years ago. Differences in the frequency with which various codons are used among species (the codon bias) may impede attempts to detect homologous genes with DNA probes. To determine if a common codon bias exists among nematodes, we compared codon frequency among species of the order Rhabditida (Caenorhabditis elegans), Strongylida (Haemonchus contortus), Ascaridida (Ascaris suum) and Spirurida (Brugia malayi, B. pahangi and Onchocerca volvulus). Comparison by simple correlation among these organisms showed that the three filariae were quite similar (r≥0.8), that H. contortus was similar to these three species and to A. suum (r≥0.69), and that A. suum was distinct from the filariae (r<0.6). There was little similarity between the filariae and C. elegans (r≤0.4).

These data demonstrate that not all nematodes share a common codon bias and that genes obtained from one may not necessarily be useful as probes for the homologous genes in others.
63.

HEARTWORM IN MINNESOTA - A TWO YEAR UPDATE. J.C. SCHLOTTHAUER* AND B.E. STROMBERG. UNIVERSITY OF MINNESOTA, ST. PAUL, MN 55108

Heartworm, *Dirofilaria immitis*, infection has been enzootic in dogs in the state of Minnesota since 1939. It became epizootic in dogs in western Hennepin County in 1955. The spread of the infection in Minnesota has been closely monitored by periodic, statewide, mail surveys of practising veterinarians. The last semi-annual survey was conducted in January 1989 and related to the events of calendar year 1988.

*D. immitis* is now considered to be enzootic in 67 of the 87 counties of Minnesota. It is primarily a parasite of dogs but has also been found in domestic cats and in feral coyotes and timber wolves. Recent surveys have shown a continued, progressive extension of the infection into new counties but a decline in the number of new cases - particularly in the older areas of intense heartworm activity immediately west and north of the cities of Minneapolis and St. Paul. The decline in new infections in the older "hotbeds" is thought to be due to widespread acceptance and use of preventive medication among dog owners and breeders.

64.

AN UPDATE ON THE FACTORS THAT AFFECT THE RADIOGRAPHIC DIAGNOSIS OF HEARTWORMS IN CATS. R.A. HOLMES* AND P. DACOSTA. LOUISIANA STATE UNIVERSITY. BATON ROUGE, LA 70816

Heartworm (*Dirofilaria immitis*) infection is difficult to diagnose in cats. As with dogs, one of the diagnostic mainstays is thoracic radiography. Radiographic findings of heartworm in cats is similar to dogs. While the lesions in dogs and cats are somewhat similar, cats and dogs are morphologically different, and if dog radiographic cardiovascular enlargement criteria are used for the cat, diagnostic errors may be made.

Digital subtraction contrast angiography was done on 11 healthy cats to determine the effects of systolic and diastolic cardiac cycles on the appearance of cardiovascular structures. Normal criteria for pulmonary artery size and cardiac shape for the cat will be presented and compared with those of the dog. Some of the radiographic features of heartworm in cats will be compared to those of the dog.
MILBEMYCIN OXIME: EFFICACY AGAINST PRECARDIAC STAGES OF Dirofilaria immitis IN CATS. B.L. BLAGBURN*, C.M. HENDRIX, J.V. BOWLES, D.S. LINDSAY AND D.I. HEPLER. AUBURN UNIVERSITY, AL 36849 AND CIBA-GEIGY CORPORATION, GREENSBORO, NC 27410

Twenty-four, mature, purpose-bred, domestic short hair cats (12 males and 12 females) ranging in age from 6 months to one year were each infected (Day 0) with 100 infective third stage larvae of Dirofilaria immitis. Cats were allocated into 2 treatment groups designated A & B (n=6 males, 6 females/group). Cats in group A received milbemycin oxime at a rate of 2.8 mg/cat (0.68 - 1.22 mg/kg). Cats in group B received placebo tablets at a similar rate. Cats in each group were treated 9, 37, 65, 93, 121, 149, and 177 days postinfection. All cats underwent thoracic radiography on days 35 or 36, and gain on days 202 - 206. Blood was collected from each cat on selected treatment days and at necropsy. One cat in group B died as a result of an innate cardiomyopathy on day 66. All remaining cats were euthanatized and subjected to necropsy on days 209 - 213. A sample of blood taken at necropsy from each cat was examined for microfilariae using the modified Knott's procedure. The cardiopulmonary tracts, eyes and central nervous system were examined for the presence of D. immitis. Heartworms were recovered from 7 of 11 cats in the placebo group (Mean [positive cats only] = 7.4; range = 1-14). Heartworms were not recovered from any of the milbemycin treated cats. Microfilariae were not observed in blood collected at necropsy from any of the cats.

EFFICACY OF RM 340 COMPARED WITH THIACETARSAMIDE JUDGED BY OBJECTIVE CRITERIA. 1. CONTROLLED LABORATORY TESTS IN CANINE MODELS. M.T. DZIMIANSKI,*, J.W. MCCALL, T.L. MCSTIER, AND J.P. RAYNAUD. 1COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF GEORGIA, ATHENS, GA 30602. 2RHONE MERIEUX FRANCE.

Thiacetarsamide is the only heartworm adulticide to have been developed since 1950. Recent reduction in confidence in its effectiveness points to the need for both alternative drugs and more objective criteria by which to evaluate them. RM 340 is now being developed following our proposed new scheme for overall assessment of efficacy (OAE) of filaricidal drugs based on a combination of the percentage of worms killed and the percentage of dogs cleared, with specified ranges in percentages for very high, high, average, low or nil and overall categories with OAEs of "Highly Effective" (HE), "Partially Effective" (PE) and "Non-Effective" (NE). Data presented here on RM 340 was collected from controlled experiments using 82 beagles infected by IV transplantation of 10 pairs of Dirofilaria immitis and treated at 8 to 12 months of age (adults) and from controlled experiments using 63 beagles given 50 L3 (SC) and treated at 4 months of age (immatures). Two dosages were selected for further development based on effectiveness against both adults and immatures and on flexibility in treatment schedule, with negligible reaction at the injection site. A dosage of 2.2 mg/kg x 2 given 3 hr apart was 96.0% effective against adults with 9 of 12 dogs cleared (OAE,HE) and 99.3% effective against immatures with 5 of 6 dogs cleared (OAE,HE). A dosage of 2.5 mg/kg x 2 given 24 hr apart was 97.8% effective against adults with 8 of 12 dogs cleared (OAE,HE) and 95.8% effective against immatures with 3 of 6 dogs cleared (OAE,HE). In similar studies, the standard dosage of thiacetarsamide was 57.3% effective against 7 to 8-month-old adults with none of 7 dogs cleared (OAE,NE) and 29.8% effective against 4-month-old immatures with none of 5 dogs cleared (Blair et al., Proc. Heartworm Symp. '83) (OAE,NE).
EFFICACY OF RM 340 COMPARED WITH THIACETARSAMIDE JUDGED BY OBJECTIVE CRITERIA.

2. CONTROLLED OR CRITICAL TESTS WITH NATURALLY INFECTED DOGS. J.P. RAYNAUD* and J.W. MCCALL. 2 RHONE MERIEUX FRANCE. 3 COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF GEORGIA, ATHENS, GA 30602.

Jackson (JAVMA 142: 23-26, 1963) reported nearly 100% efficacy against adult *Dirofilaria immitis* with thiacetarsamide, but recent studies sponsored by the American Heartworm Society (McCall et al., Palumbo et al., Todd et al., Proc. Heartworm Symp. '80) showed an overall average worm kill of 62.9% with 12 of 30 dogs cleared of worms. A new adulticide (macrofilaricide), RM 340, is now being developed for deep IM (lumbar) injection. For controlled and critical tests reported here, the dogs were grouped by the degree of clinical disease (asymptomatic, moderate, severe) and the optimum therapeutic programs were determined. Combined results of selected posologies from experiments in Australia, Italy, Japan, Morocco, and France are presented.

<table>
<thead>
<tr>
<th>Clinical Class</th>
<th>Dosage Median (range)</th>
<th>Worm Burden % Worm Kill</th>
<th>Dogs Cleared Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>2.5 x 2 / 24 hr 5 (1-50) 100 (75-100)</td>
<td>13/17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.2 x 2 / 3 hr 12 (4-38) 100 (66-100)</td>
<td>17/18</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>2.5 x 2 / 24 hr 15 (3-35) 100 (70-100)</td>
<td>8/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.2 x 2 / 3 hr 12 (5-31) 100 (80-100)</td>
<td>13/15</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>2.5 mg/kg once - (5-&gt;30) 55 (15-100)</td>
<td>10/18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 x 2 / 24 hr 73 (20-85) 100 (72-100)</td>
<td>6/8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.2 x 2 / 3 hr 27 (9-88) 100 (80-100)</td>
<td>13/19</td>
<td></td>
</tr>
</tbody>
</table>

A therapeutic program for each clinical situation is proposed: asymptomatic, 2.2 mg/kg x 2 / 3 hr or 2.5 mg/kg x 2 / 24 hr; moderate, 2.5 mg/kg x 2 / 24 hr; severe, 2.5 mg/kg once, then full treatment 1 month later.

PRELIMINARY CONTROLLED EXPERIMENTS TO PREVENT HEARTWORM DISEASE BY SEASONAL IM INJECTIONS OF RM 340. J.W. MCCALL, 1 M.T. DZIMIANSKI, 1 T.L. MCTIER, 1 R.A. HOLMES,* 2 and J.P. RAYNAUD. 3 1 UNIVERSITY OF GEORGIA, ATHENS, GA. 2 LOUISIANA STATE UNIVERSITY, BATON ROUGE, LA. 3 RHONE MERIEUX FRANCE.

In view of the high level of activity of RM 340 against adult and 4-month-old immatures of *Dirofilaria immitis*, 3 similar field studies were conducted in states with moderate (GA,FL) or high (LA) enzootic potential to determine the effectiveness of RM 340. In each study, 30 naive beagles, allocated to groups of 5 each, were placed under field conditions for various intervals from April 1988 to April 1989. Dogs on the "Strategic Program" were exposed to infection for 12 months and treated on 3 occasions, which were not necessarily related to the mosquito season (MS). Dogs on the "Tactical Program" were exposed for 8 months and treated on 2 occasions (i.e., mid-MS and after MS). Treatments were initiated in August 1988. Each treatment consisted of 2 deep IM (lumbar) injections of 2.2 mg/kg 3 hr apart every 4 months. At each site, one group of non-treated control dogs was exposed for 12 months and each of 3 groups of "Tracer" dogs was exposed for 3 consecutive, 4-month periods (Apr-Aug, Aug-Dec, Dec-Apr). All dogs were bled at specified times for collection of serum and examination for microfilariae. All dogs were brought indoors at the last treatment (and/or after designated exposure) and held for 5 months before necropsy. With the exception of 1 dog at the GA site which had a live female heartworm, all of the dogs on the "Strategic Program" were free of heartworms, whereas most of the "Tracer" dogs exposed during this 8-month period (Apr-Aug, Aug-Dec) at the 3 sites had worms.
69.
PRELIMINARY RESULTS ON THE EPIDEMIOLOGY OF HEARTWORM IN "TRACER" BEAGLES SEASONALLY EXPOSED TO NATURAL INFECTION IN THREE SOUTHERN STATES (USA).

Although general assumptions exist regarding the regional transmission of Dirofilaria immitis in the USA, little controlled data is available to verify or refute these claims. Parallel to field studies in GA, FL, and LA in which a new heartworm adulticide (RM 340) was evaluated, 3 groups of 5 "Tracer" beagles at each site were exposed to potential infection during 3 consecutive, 4-month periods, respectively; an additional group of 5 dogs exposed for 12 months was included. Exposure for the 12-month group and the first set of "Tracer" dogs was initiated in April 1988. After exposure, all dogs were held indoors for 5 months. Transmission occurred at all of the 3 sites during the periods of April to August and August to December, but no transmission was evident at any of the 3 sites during the period of December to April. Based on worm measurements and microfilaremia data, most of the transmission occurred late in the April-August period and early in the August-December period in all of the 3 sites. Heavier worm burdens in LA, compared with those in GA and FL, indicated a higher level of transmission at this site. Worm recoveries in the dogs exposed for 12 months reflected the cumulative transmission in "Tracer" dogs: In LA, 4 of the 5 dogs had worms, with a group average of 25.2 (range, 0-45); in GA, 4 of the 4 dogs had worms, with an average of 6.75 worms (range, 5-8); and in FL, 5 of the 5 dogs had worms, with an average of 5.4 worms (range, 1-13). Follow-up studies to confirm this unexpected and surprising lack of transmission from December to April are underway.

70.

Semi-quantitative determination of adult heartworm antigen levels (UNI-TEC CHW, Pitman-Moore, Inc.) in the serum of naive beagles exposed to natural infection with Dirofilaria immitis and treated every 4 months for 12 months ("Strategic" Program) with RM 340 was found to be useful in monitoring the acquisition of infection and predicting the effectiveness of treatment. This study was complementary to a study on chemoprophylaxis by seasonal injection, which was conducted in GA, FL, and LA. The data presented here is from 2 groups (1 treated, 1 control) of 5 dogs each which were placed under field conditions from April 1988 to April 1989 at each of the 3 sites. The treated dogs were given 2 deep IM (lumbar) injections of RM 340 at 2.2 mg/kg 3 hr apart beginning in August 1988. All dogs were bled for collection of serum and examined for microfilariae prior to exposure, at 4-month intervals during exposure, and again at necropsy 5 months after the exposure period. In regard to the 14 (total) control dogs at the 3 sites, 1 of 4 from GA and 1 of 5 from FL had positive reactions (weak) as early as August 1988, but none of the control dogs from LA had antigen until December 1988, when 2 of the 5 were positive. In general, antigen levels and percentages of antigen-positive dogs increased with time in the control groups. At necropsy, 13 of the 14 control dogs had heartworms and were antigen-positive. Antigen was detected on at least one occasion in only 1 of the treated dogs from LA, 1 from FL, and 2 from GA, but at necropsy, only 1 of the treated dogs (GA) was positive and it had a single live female worm. A similar pattern was seen with the CITE® Semi-Quant™ Test (IDEXX Corp.), but this test was somewhat less sensitive.

Twenty-four (12 male and 12 female) ferrets were each given 20 third stage infective larvae of *D. immitis* by subcutaneous (SC) inoculation. A group of 6 ferrets was selected as nontreated controls. The remaining 18 ferrets were allocated to 4 groups of 4 or 5 each and one month later were given ivermectin as single oral doses of 0.006, 0.0125, 0.025, and 0.05 mg/kg of body weight, respectively. One control animal died at 170 days postinoculation (PI), and adult worms were found in the heart and associated vessels at necropsy. The remaining animals were euthanatized at 219-227 days PI. At necropsy, no worms were recovered from any of the treated ferrets, while all of the control ferrets had heartworms (mean recovery, 54%; range, 45-65%). Three of the 5 control ferrets had a few microfilariae at 217 days PI.

Serum samples collected monthly were examined for adult heartworm antigen using the UNI-TEC™ CHW test kit. None of the control ferrets were positive at 120 days PI, while 2 of 5 were positive at 150 days, and all were positive at 182 days PI. Sera from all ivermectin-treated ferrets were negative for the antigen throughout the experiment.

TISSUE CYST FORMATION BY *Toxoplasma gondii* IN CELL CULTURES. D.S. LINDSAY*, J.P. DUBEY, AND B.L. BLAGBURN. AUBURN UNIVERSITY, AL 36849 AND ZOONOTIC DISEASES LABORATORY, USDA, ARS, BELTSVILLE, MD 20705

*Toxoplasma gondii* is a serious pathogen causing disease in many animals. Toxoplasmic encephalitis is a fatal condition that occurs in many patients with AIDS. This encephalitis is thought to occur because the latent tissue cyst stage is reactivated due to viral induced immunosuppression. Bradyzoites present in tissue cysts are also resistant to commonly used chemotherapeutic agents, which complicates treatment. Clearly, a cell culture system for studying bradyzoite biology would be advantageous. We have found that an isolate of *T. gondii* from the goat (GT-I isolate) will form tissue cysts in bovine monocyte and human fetal lung cell cultures. When tissue cultures were fed to cats, the cats shed oocysts in their feces 5 to 7 days postinoculation. Acid-pepsin digestion and transmission electron microscopy of infected cell cultures also indicated that tissue cysts were present. This in vitro culture system may now be optimized and used to detect compounds that inhibit bradyzoite development.
73.

**IN VITRO INVESTIGATIONS ON THE ACTION OF ROBENIDINE AGAINST TOXOPLASMA GONDII.**

XIAO DALIAE*. JIANGXI AGRICULTURAL UNIVERSITY, NANCHANG, CHINA.

The effect of robenidine on *Toxoplasma gondii* was studied *in vitro* by means of light and electron microscopy. The penetration of *T. gondii* into cultured cells was significantly inhibited by 0.1 ppm robenidine which was added with the parasite inoculum. It was also found that robenidine (0.1 ppm) could inhibit multiplication of *T. gondii*. It inhibited the first, second, third, etc. multiplication of the parasites, when robenidine was added to the culture at varying times. The experiment demonstrated that robenidine had no effect on non-proliferating toxoplasmas. These results revealed the mode of action of robenidine against *T. gondii*. When compared with the control under electron microscopy, it was observed that the fine structure of the parasites was markedly changed 24 hours after 0.1 ppm robenidine had been added to the media of the parasites. The fine structural changes in the parasites were amyllopectins and enlarged vacuoles in the cytoplasm of the parasites. Distensions of the perinuclear space inside of the parasites continued to occur with increased time of exposure to the drug. After 48-72 hours, the changes in the fine structure of the parasites were even more remarkable. There was a heavy accumulation of amyllopectins, and the vacuoles were heavily swollen. There was variation in the swelling from slight to gross distension of the perinuclear space, of the Golgi apparatus, and of the endoplasmic reticulum in the parasites. The mitochondria did not appear to be affected by the drug until much later. The membrane of the parasite had misshapen outlines, and was present in isolated segments rather than a continuous structure. These changes of the fine structure of the parasite revealed the mechanism of action of the robenidine which was discussed and analysed in the present study. In addition to the present study, the effect of sulfamethoxine (SMM) on *T. gondii* was also observed in cell cultures, and compared with the effect of robenidine.

74.

**SUSCEPTIBILITY OF THREE SPECIES OF KANSAS SNAILS TO INFECTION BY FASCIOLA HEPATICA LINNAEUS, 1758 AND FASCILOIDES MAGNA (BASSI, 1875) WARD, 1917.**

R.D. MCKOWN* AND R.K. RIDLEY. KANSAS STATE UNIVERSITY, MANHATTAN, KS 66506

Fluke eggs were collected at slaughter from the gall bladders of cattle with monospecific infections of *Fasciola hepatica* or *Fascioloides magna*. The eggs were incubated at room temperature (22°C) until miracidia hatched 17-21 days later. Three species of lymnaeid snails were collected from different areas in Kansas and reared in the laboratory. Individual 2nd generation snails, tentatively identified as *Pseudosuccinea columella* and *Stagnicola sp.* (Dr. Stuart Knapp, pers. comm.) were placed in a microtiter plate well and exposed for 12 hours to 10 *F. hepatica* or *F. magna* miracidia. Of the three species tested for susceptibility to *F. hepatica*, *Pseudosuccinea columella* was susceptible, *Stagnicola sp.* was refractory, and all specimens of the third, unidentified species died without evidence of infection. Both *P. columella* and *Stagnicola sp.* when exposed to miracidia of *F. magna* were refractory to infection. Two hundred-fifty (250) metacercariae from *Pseudosuccinea columella* were fed to a helminth-free calf; the infection became patent in 92 days, and 7 *F. hepatica* adults were recovered at necropsy.
75.


Banding patterns obtained by soft laser densitometer scans of isoelectric focusing (IEF) gels were used as a means of identifying snails infected with Fasciola hepatica. Laboratory reared Pseudosuccinea columella were exposed to freshly hatched F. hepatica miracidia. When infections were mature (>45 days), the infected as well as parasite naive snails were dissected and homogenates (4 molar urea-phosphate buffered saline) were prepared from hepatopancreas, foot, and mantle tissues of the snails and cercariae. Actual IEF was performed on a micro-processor controlled automated electrophoresis and staining (coomassie brilliant blue R) system (Pharmacia Phast System) using prepared IEF gels (pH 3-9). Isoelectric point (pI) standards were included on each gel. Gels were then scanned by an LKB Ultrascan laser densitometer. The pI's of actual peaks were assigned by the LKB GelScan software. Comparisons were achieved by computer overlays, providing distinctly different patterns of homogenates obtained from infected versus non-infected snails.

76.


Previous attempts to culture Strongylus spp. L3 to the L4 stage have only been successful when feeder cells were used as part of the culture system (Farrar and Klei, 1985, J. Parasitol 71,489.) S. vulgaris, S. edentatus and mixed cyathostome spp. L3 were successfully cultured (routinely >50% molt to the L4) by using a modification of methods developed by Douvres et al. without the inclusion of feeder cells. Larvae were unsheathed and placed in leighton tubes containing KW-2 media containing fetal calf serum (Douvres, F.W. 1970, 56, Sect II, Pt I, 83), gassed gently with a mixture of 10% CO2, 5% O2, 85% N2 and incubated in CO2 incubator at 37-38°C. Concentrations of 1000 - 2000 L3 per ml of media produced ideal results. Molting of S. vulgaris, S. edentatus and mixed cyathostome species began at 5, 9 and 19 days respectively. This culture method has been successfully used to measure viability of stored L3, irradiation effects on L3 and surface antigen characteristics of L3 and L4.
EARLY DEVELOPMENT OF STRONGYLUS VULGARIS IN PONY FOALS. B.M. MCCRAW* AND J.O.D. SLOCOMBE. ONTARIO VETERINARY COLLEGE, UNIVERSITY OF GUELPH, GUELPH, ONTARIO, CANADA. N1G 2W1.

Fifteen foals were inoculated with 5000 to 100,000 S. vulgaris infective larvae and examined at necropsy 2-8 days post-inoculation (PI). Between 2 and 3 days PI, gradually increasing numbers of mononuclear cells were observed in the mucosa and submucosa of the ileum, cecum and right ventral colon. By day 3 PI, many larvae were found in the submucosa of the ileum as well as one in an arteriole of the cecum. A few neutrophils and thrombosis of arterioles were observed in the ileum. On day 4 PI, a larva undergoing a molt to the 4th stage was present in the submucosa of the ileum. All larvae at this time ranged from 33.6 to 38.4 µm in diameter. Dose dependent hemorrhagic foci and congestion along with edema and a heavy infiltration of neutrophils were observed in the submucosa of the ileum, cecum and right ventral colon. The walls of many arterioles were now undergoing disintegration and there was an abundance of collagen especially in the cecum and right ventral colon. The muscularis of the intestine was also variously infiltrated. By day 5 PI, hemorrhagic congestion was very extensive and thrombosis was present in small arteries. Plasma cells were observed for the first time and larvae were now rarely observed. After day 5 PI no larvae were observed in sections. Eosinophils were uncommon at all times. It is concluded that the molt to the 4th stage occurs mainly during day 4 PI.

VETERINARY PRACTITIONER RECOMMENDATIONS FOR TREATMENT AND PREVENTION OF INTESTINAL NEMATODES IN DOGS: IMPLICATIONS FOR PUBLIC HEALTH. J.B. HARVEY* AND P.M. SCHANTZ. CENTERS FOR DISEASE CONTROL. ATLANTA, GA 30333.

A telephone survey was conducted of a systematic random sample of 450 veterinarians to assess current veterinary practices regarding intestinal parasite control in dogs. We analyzed the results to determine whether current practices concerning prophylaxis and treatment of canine roundworm (Toxocara canis) infection are adequate to prevent the potential public health risks of toxocaral larval migrans.

Over half (53%) of veterinarians surveyed indicated they had "little" or "no" concern regarding the potential zoonotic hazards of canine roundworms. Twenty-nine percent (29%) of veterinarians "do not" discuss the potential human health risks of roundworm with their clients or discuss these risks "only when asked". Less than a third (31%) of those surveyed recommend that pups receive their first exam/treatment for intestinal parasites before 4 weeks of age. Less than half (46%) place pups on prophylactic drug therapy for roundworm infection. Sixty-nine percent of respondents recommend routine testing/treatment of nursing bitches for roundworms. We conclude that current veterinary practices concerning roundworm treatment and control are inadequate for maximum prevention of environmental contamination with eggs of T. canis and ask "why not?" since veterinary parasitology textbooks emphasize this concern.
INCIDENCE OF *B. DIVERGENS* BOVINE BABESIOSIS ON HUMAN HEALTH IN EUROPE. A. GORENFLOT AND P. BRASSEUR. UNIVERSITE PARIS V, 4 AVENUE DE L'OBSERVATOIRE, 75270 PARIS CEDEX 06, FRANCE.

Babesiosis is widely spread in Europe in domesticated animals: oxen, dogs, horses. *B. divergens*, a bovine species different from *B. bovis* by its morphology and its tick vector (Ixodes ricinus) is responsible of bovine babesiosis in Europe. Mortality rate is limited by chemotherapy, but the disease aftermath (poor weight gain and reduced milk production) is associated with significant economical losses specially in Ireland, Great Britain and France.

Frequent in animals, babesiosis is rare but severe in man in Europe where 17 clinical cases were reported since 1957 (9 cases in France); *B. divergens* was implicated in most cases. Among these patients 82 p. cent had been splenectomized since various times (4 weeks to 36 years) and for different reasons: traffic or surgical injury, therapeutic procedure (_leukemia, Hodgkin disease, immune thrombocytopenia_). Babesiosis was contracted by farmers, foresters, campers, walkers between June and October. In splenectomized patients, *B. divergens* induced a severe intravascular hemolysis, rapidly associated with an acute renal failure and often with an acute pulmonary oedema. Hemoglobin fell to 70-80 g/l sometimes less than 40 g/l. Parasitemia was variable (1 to 80 p. cent). The cytological identification of the implicated _Babesia_ species was always difficult for _B. divergens_ lost its typical peripheral position in human erythrocytes. Such an identification required to study patient's immune response against different _babesia_ antigens and to inoculate Gerbils and splenectomized calves with patient's blood.

*B. divergens* acute human babesiosis behaving like drug resistant malaria, 63 p. cent of the splenectomized patients died before 1987. We proposed to perform in severe cases a blood exchange transfusion followed by a chemotherapy by Clindamycine + Quinine. This therapeutic procedure allowed a rapid clinical and biological improvement without any recurrence in the three splenectomized patients who developed acute babesiosis in Europe since 1987.

LARVA MIGRANS CAUSED BY ROUNDWORMS AND HOOKWORMS OF DOGS AND CATS.

P. M. SCHANTZ* CENTERS FOR DISEASE CONTROL, ATLANTA, GEORGIA 30333

Visceral and cutaneous larva migrans are the terms most commonly given to disease syndromes in humans caused by the common roundworms, _Toxocara_ spp. and hookworms, _Ancylostoma_ spp. of dogs and cats. Zoonotic transmission of roundworms are the most common because of the high infection prevalence in pups and kittens in all parts of the country, the frequency of pet ownership and the persistence of the infective stages of _Toxocara_ spp. in the soil. Cutaneous larva migrans caused by animal hookworms is more regional in its distribution and sporadic in its occurrence. Practicing veterinarians can play vital roles in preventing zoonotic transmission of these parasites by recommending appropriately-timed preventive anthelminthic therapy of pets and educating clients about the potential health hazards and how to prevent them. This presentation will review the clinical and epidemiologic characteristics of these diseases and emphasize the veterinarians' role in prevention.
81.

EPIZOOTIOLOGY OF LYME DISEASE/ THE IMPORTANCE OF SPIROCHETE ISOLATION IN DETERMINING RESERVOIR POTENTIAL FOR LYME DISEASE. J. PIESMAN*, AND G.O. MAUPIN. CENTERS FOR DISEASE CONTROL. FORT COLLINS, CO 80522

Several methods have been utilized to identify the importance of wildlife and domestic animals as reservoirs of the Lyme disease spirochete (Borrelia burgdorferi). Serological identification of antibodies to spirochetes can be a useful tool. The potential for cross reaction with spirochetes other than B. burgdorferi necessitates careful development of serological tests on a species by species basis. Xenodiagnosis can be used to determine how infectious a potential reservoir population is to vector ticks. The logistical difficulties of holding wildlife and domestic animals while larval ticks feed, and maintaining a large tick colony render xenodiagnosis as strictly a research tool. Lyme disease spirochetes have been isolated from various tissues, including blood, skin, kidney, spleen, liver, eye, and urinary bladder. Blood and skin can be easily obtained without sacrificing the test animal. An ear biopsy method has been developed to allow routine sampling of rodent populations for Lyme disease spirochete infection. The method also works for lagomorphs. The main difficulty encountered with ear punch biopsy isolation of spirochetes is contamination. Various disinfectant regimes are under evaluation for reducing contamination problems.

82.

IS TRICHINELLOSIS STILL A PROBLEM IN THE UNITED STATES? K. D. MURRELL*, AGRICULTURAL RESEARCH SERVICE, USDA. PEORIA, IL 61604

Over the past century, interest in the control of trichinellosis has waxed and waned, influenced more by economic concerns than by public health considerations. Over this period, research on the epidemiology, diagnosis, and control of swine trichinellosis has yielded effective tools and management strategies that would, if applied in a comprehensive manner, produce a level of control equivalent to that achieved by Europe. In the absence of regulatory efforts, other forces have provided a measure of control, resulting in a low level of infection, as reflected in recent prevalence studies and human morbidity and mortality reports.

Trichinellosis in the U.S., then, cannot be given high priority in comparison to other foodborne parasites such as toxoplasmosis and cysticercosis. The story of trichinellosis research, can however, serve as a paradigm for research on other foodborne parasites.
The intestinal protozoon, Giardia, has been shown to be a common parasite in both domestic and wild animals, but its zoonotic potential has remained controversial. Studies on cross-species transmission have demonstrated that experimental animal hosts (i.e. gerbils, mice) can be infected with Giardia from selected mammalian hosts. However, these hosts cannot be infected with avian derived Giardia (parakeets or herons), nor can mammalian Giardia infect avian hosts such as chicks, ducklings, or parakeets. The high levels of Giardia cysts in human sewage (up to $10^5$/gallon) would suggest the improper disposal of raw human waste as a major potential source for waterborne outbreaks of giardiasis.

The development of new immunological techniques for diagnosis of Giardia has provided important information on mechanisms involved in the dietary regulation of Giardia cyst excretion. This, together with new molecular methods for the speciation of Giardia, may lead to a clarification of the zoonotic controversy. (Supported by Environmental Protection Agency cooperative agreement CR-814622)

Cryptosporidiosis is primarily an enteric disease of young, immunologically naive, or immunocompromised animals. Transmission is by fecal-oral route of the oocyst. Isolates from mammals generally infect other mammalian species and those from avians infect other avian species but transmission to mammals from avians, fish or reptiles is not known to occur. Human infection is often associated with close personal contact, poor sanitation and certain occupations. Waterborne transmission is well documented. The life cycle is unique with regard to location within host cells and ability to recycle internally. Immunocompetency greatly affects site of infection and clinical features of disease. Infections can range from asymptomatic to severe; the most prominent sign is usually watery diarrhea associated with anorexia, weight loss, dehydration and abdominal discomfort. No chemotherapeutic modalities are known to be effective.
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