PROCEEDINGS

AAVP

American Association of Veterinary Parasitologists

34th Annual Meeting
July 16 — 18
Orlando, Florida
1989
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OF THE

AMERICAN ASSOCIATION OF
VETERINARY PARASITOLOGISTS

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AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS

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Program and Abstracts
34th Annual Meeting
American Association of Veterinary Parasitologists
Orlando Marriott Hotel, 8001 International Drive,
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Sunday, July 16, 1989, Orlando Marriott - Tangerine Room (A&B)

7:30 a.m. Registration

8:45 - 9:00 Opening Remarks
President: Harold C. Gibbs
Vice-President & Program Organizer: Roger K. Prichard

Session A I - Public Health
Orlando Marriott - Tangerine Room (A&B)
Moderators: Jeffrey F. Williams and H. Ray Gamble

Invited Presentation
9:00 - 9:30 1. Neosporosis - An update. J.P. Dubey*

9:30 - 9:45 2. Immunohistochemical diagnosis of Neospora caninum and Toxoplasma gondii in tissue sections. D.S. Lindsay* and J.P. Dubey


10:00 - 10:30 Coffee

Session A II - Nematode Cuticle
Orlando Marriott - Tangerine Room (A&B)
Moderators: Robert B. Grieve and Charles Mackenzie

Invited Presentation
10:30 - 11:00 4. Recent developments in cuticular biochemistry of parasitic nematodes. R.H. Fetterer*


11:45 - 12:00 8. Observations on oogenesis and egg shell formation in Oesophagostomum columbianum, a nematode parasite of sheep and goat in India. M. Johal*

12:00 - 1:30 Lunch
Sunday, July 16, 1989

Session A III—Population Biology—Orlando Marriott, Tangerine Room (A&B)

Moderators: Ann M. Zajac and John B. Malone

1:30 – 2:00
Invited Presentation

9. The population biology of trichostrongyloid parasites in ruminants. G. Smith*

2:00 – 2:15

10. Moderate anthelmintic resistance reduces general fitness, but further anthelmintic selection improves fitness in Haemonchus contortus. M.E. Scott*, N. Maingi and R.K. Prichard

2:15 – 2:30
11. Exogenous and endogenous prolactin enhances fecundity of Haemonchus contortus in sheep: evidence for a mechanism that regulates periparturient egg rise. M.W. Fleming*

2:30 – 2:45


2:45 – 3:00

3:00 – 3:15
Coffee

Session A IV — Equine

Orlando Marriott – Tangerine Room (A&B)

Moderators: Thomas R. Klei and Bert E. Stromberg

3:15 – 3:30

3:30 – 3:45

3:45 – 4:00

4:00 – 4:15

4:15 – 4:45
Awards
Remarks by Distinguished Parasitologist Awardee

4:45 – 5:00
Presidential Address: Harold C. Gibbs

5:00
Business Meeting

7:30 – 9:00
AAVP Society Social, Courtyard Terrace of the Mardi Gras and Royal Orleans Restaurants, Mercado Festival Center, 8445 International Drive
Monday, July 17, 1989

Session B I - Epidemiology, Orlando Marriott, Tangerine Room A
Moderators: James C. Williams and Joseph A. DiPietro


8:45 - 9:00  19. Epizootiology of gastrointestinal nematodes of cattle in selected areas of Oregon with particular reference to Ostertagia spp. L.G. Rickard* and G.L. Zimmerman


9:30 - 9:45  22. Seasonal transmission patterns of cattle nematodes in the middle Atlantic region: Role of spring and fall hypobiosis. K.D. Murrell*, E. Leighton, B. Boswell and L. Gasbarre

9:45 - 10:00 23. Epidemiologic information on the transmission of gastrointestinal nematode populations in cattle in central Alabama. D.E. Snyder*

10:00 - 10:30 Coffee

Session B II - Equine
Orlando Marriott, Tangerine Room A
Moderators: Craig Reinemeyer and Gary Zimmerman


10:45 - 11:00 25. Strategic control of strongyles with ivermectin in mature horses in Ontario. O. Slocombe*

Monday, July 17, 1989

Session B II (Continued) - Tangerine Room A

11:15 - 11:30  27. Efficacy ofEqvalan oral liquid as a feed
topdress for control of equine cyathostomes.
C.H. Courtney*, Q.Y. Zeng and J.B. Bogdansky

11:30 - 11:45  28. The anthelmintic efficacy of continuous feeding
of pyrantel tartrate to horses. R.F. Daniels, G.D.
Hackett, Jr., S.J. Wickler and R.M. McCormick

11:45 - 12:00  29. Observation of Anoplocephala eggs in the feces of
horses and ponies in Marion County, Florida.
R.L. Asquith*, R.E. Bradley, T.J. Lane and
J. Kivipelto

12:00 - 1:15  Lunch

Session B III - Control and Production
Orlando Marriott, Tangerine Room A
Moderators: Owen Slocombe and Joseph P. Tritschler II

1:15 - 1:30  30. Efficacy of albendazole against nematode,
cestode, and trematode parasites of sheep. R.S. Rew
and J.P. Freeman*

1:30 - 1:45  31. Efficacy of the natural product, Paraherquamide,
against 6-day-old Trichostrongylus colubriformis in
the gerbil (Meriones unguiculatus). D.A. Ostlind*,
W.G. Mickle, D.V. Ewanciw and F.J. Andriuli

1:45 - 2:00  32. Efficacy of nemadectin against gastrointestinal
nematodes in cattle. G.L. Zimmerman*, E.P. Hoberg
and J.A. Pankavich

2:00 - 2:15  33. Effect of oxfendazole against inhibited 4th-stage
larvae of Ostertagia ostertagi in feedlot cattle.
J.C. Williams*, D.T. Bechtol, A. Waite and
R.C. Herschler

2:15 - 2:30  34. Productivity of calves in a cow-calf herd in respon­
se to anthelmintic treatment. C.E. Couvillion*,
R.R. Evans, J.A. Hawkins, C. Seifker and
J.R. Jackson

2:30 - 2:45  35. Efficacy of ivermectin in-feed against parasites
of swine. R. Alva-Valdes, D.H. Wallace, A.G. Foster,
G.F. Ericsson and J.W. Wooden

2:45 - 3:00  36. Amprolium (CoridR) for prevention of porcine
neonatal coccidiosis. R.L. Blagburn*, T.R. Boosinger,
T.A. Powe and C.M. Hendrix

3:00 - 3:15  37. Efficacy of albendazole vs. cattle parasites.
A.L. Shor*

3:15 - 3:30  Coffee
Monday, July 17, 1989

Session B IV - Small animals
Orlando Marriott, Tangerine Room A
Moderators: Charles Courtney and Gil H. Myers

3:30 - 3:45

3:45 - 4:00

4:00 - 4:15
40. Evaluation of two adult heartworm antigen diagnostic test kits using well defined dog and cat sera. M.T. Dzimianski*, T.L. McTier and J.W. McCall

4:15 - 4:30
41. Dose confirmation of pyrantel pamoate as an anthelmintic in cats. R.K. Ridley*, K.S. Terhune and D.E. Granstrom

4:30 - 4:45
42. Field experience with a new ivermectin formulation for dogs. R.S. Blakely and B. Shofstall

4:45 - 5:00

5:00 - 5:15

5:15 - 5:30
45. A clinical double blind study to test the effectiveness of the Elexis Ultrasonic Flea Collar. R.P. Knowles, R. Stone, J. Szust* and A. Mauck

5:30 - 5:50
Session B V - Elexis Corporation Presentation
Orlando Marriott - Tangerine Room A
Moderator: Ronald W. Stone

6:00 - 8:00
Elexis Corporation Social
Orange Room, Orlando Marriott, 8001 International Drive
Monday, July 17, 1989

Session C I - Biochemistry, Orlando Marriott, Tangerine Room B
Moderators: George A. Conder and J.P. Dubey

8:30 - 8:45  46. Effects of nutritional factors on reproductive function of adult Schistosoma japonicum cultured in vitro. X. Hua* and S. Zhou

8:45 - 9:00  47. Characterization of 5'-methylthioadenosine metabolism in Fasciola hepatica. C.T. Ayer, M.K. Riscoe and D.T. Clark


9:30 - 9:45  50. Intestinal cell granules identified as zinc sulfide in Ancylostoma caninum during the adult form of the nematode. A.J. Gianotti and D.T. Clark

9:45 - 10:00 51. A study of possible interference between anthelmintic treatment superimposed on low dose supplementation with antibiotic when fed to pigs. A.R. Donoghue*, N. Nonaka, B. Thacker, and T.W. Schillhorn Van Veen

10:00 - 10:30 Coffee

Session C II - Toxicology and Pharmacology
Orlando Marriott, Tangerine Room B
Moderators: Timothy Geary and Ann R. Donoghue

10:30 - 10:45 52. Detection of ivermectin in plasma and tissue samples of treated animals by radioimmunoassay. M. Elkassaby, C. Marschke*, T. Geary, T.W. Schillhorn Van Veen and J.F. Williams

10:45 - 11:00 53. Pharmacokinetic profiles of albendazole metabolites after intraruminal and subcutaneous administration of netobimin in sheep. C.E. Lanusse* and R.K. Prichard

11:00 - 11:15 54. Target animal safety studies with albendazole. C.R. Miller*, V.J. Theodorides, H.H. Birkhead and I.W. Daly
Monday, July 17, 1989

Session C II (Continued) - Tangerine Room B


12:00 - 1:30  Lunch

Session C III - Immunogenetics and Immunity
Orlando Marriott, Tangerine Room B
Moderators: K. Darwin Murrell and Robert M. Corwin

1:30 - 1:45  58. Antigenic diversity among 13 geographic isolates of Eimeria maxima. W.L. Current*

1:45 - 2:00  59. Investigations of the role of host genetics in the regulation of cattle gastrointestinal nematode infections. L.C. Gasbarre*, E.A. Leighton, C.J. Davies and K.D. Murrell

2:00 - 2:15  60. SLAa miniature swine react against Trichinella spiralis encysted muscle larvae. K.B. Madden*, J.K. Lunney and K.D. Murrell


2:30 - 2:45  62. Subcutaneous vaccination of mice with fourth stage Heligmosomoides polygyrus (Nematospiroides dubius) larvae. K.S. Larrick, L.H. Semprevivo and J.P. Tritschler II

2:45 - 3:00  63. A preliminary study on vaccination of Rhesus monkeys against amebiasis. H. Kumar* and S. Ahmad

3:00 - 3:30  Coffee
Monday, July 17, 1989

Session C IV - Immunity and Immunopathology
Orlando Marriott, Tangerine Room B

Moderators: Lou C. Gasbarre and Joseph F. Urban

3:30 - 3:45  64. The activities of eosinophils in tissues: A parasitological model. C.D. Mackenzie and F. Douglas

3:45 - 4:00  65. Pathogenesis of filarial lymphadenopathy in dogs. S. Miller*, K. Nakagaki and B. Hammerberg


4:45 - 5:00  69. Ultrastructural observation of antibody- and complement-dependent neutrophil-mediated damage to schistosomula of Schistosoma japonicum in vitro. X. Hua*, J. Cheng and Y. Li

Tuesday, July 18, 1989

Session D  - JOINT AAVP - AVMA SYMPOSIUM
"ARTHROPOD TRANSMITTED PARASITIC DISEASES"
"A COMMEMORATION OF THE DISCOVERY OF THE EPIDEMIOLOGY OF TEXAS FEVER"

Orange County Convention and Civic Center, Room 7AB
9800 International Drive, Orlando, Florida

Moderator: Dr. Harold C. Gibbs
President, American Association of Veterinary Parasitologists

8:30 - 8:35  Chairman's Opening Remarks

8:35 - 9:10  71. A tribute to the discoverers of cattle Texas fever. R.A. Roncalli*


9:40 - 10:10 73. Canine Erlichiosis (synonym: tropical canine pancytopenia-TCP). M. Ristic*

10:10 - 10:30 Coffee

10:30 - 11:00 74. Clinical approach to Feline Dirofilariasis. E.C. Hawkins

11:00 - 11:30 75. Anaplasmosis, current research towards vaccination. A.F. Barbet*, D. Allred, T. McElwain, T.C. McGuire and G. Palmer

11:30 - 12:00 76. Lyme Borreliosis. S.W. Nielsen*, J.E. Post, S.D. Wright and E.E. Shaw
Neosporosis is a recently recognized fatal protozoan infection of dogs caused by *Neospora caninum*. Experimentally, it can also infect cats and rodents. *Neospora caninum* is an obligate intracellular parasite that has been misdiagnosed as *Toxoplasma gondii*. Only asexual stages are known and they resemble *T. gondii*. Although the complete life cycle of *N. caninum* is unknown, the parasite can be transmitted transplacentally in dogs and cats. Tachyzoites are 5 to 7 x 1 to 5 μm. Tissue cysts are up to 100 μm in diameter. Predominant clinical findings are hepatitis and neuromuscular disorders. Antibodies can be detected using cell-culture derived *N. caninum* tachyzoites as antigen. An immunoperoxidase test has been developed to distinguish *N. caninum* from *T. gondii*.

Because *Neospora caninum* and *Toxoplasma gondii* are structurally related protozoan parasites that can cause disease in dogs and other mammals a test is needed to differentiate the two. An avidin-biotin-peroxidase complex (ABPC) immunoperoxidase staining method was developed to detect *N. caninum* in paraffin embedded tissue sections. Cell culture grown tachyzoites of *N. caninum* were used to make specific anti-serum in rabbits. The anti-serum was used to probe tissues from dogs naturally infected with *N. caninum* or *T. gondii* and from dogs and other mammals experimentally inoculated with *N. caninum* tachyzoites. The ABPC test detected both tachyzoites and bradyzoites of *N. caninum*. No reaction was observed to *T. gondii*, *Hammondia hammondi*, *Sarcocystis cruzi*, *S. tenella*, *S. capracaenis*, *Besnota jellisoni* or *Caryospora bigenetica*. When rabbit anti-*T. gondii* serum was used in the ABPC test no reaction to *N. caninum* was observed. Hematoxylin and eosin stained tissue sections containing *N. caninum* or *T. gondii* could be destained and used in the ABPC test without loss of reactivity.

Normal human milk (NHM) was reported to contain components that have antimicrobial and antiprotozoal activities. Protozoa sensitive and susceptible to in vitro treatment with NHM included Giardia lamblia, Entameoba histolcutica and Trichomonas vaginalis. The killing activity in NHM was not antibody dependent and was specific to human milk, as neither cow nor goat milk possessed the activity. Initially, it was hypothesized that a bile salt-stimulated lipase (BSSL) found in the milk of humans, but not lower mammals, may be the active component. BSSL is stimulated by bile salts both in vitro and in vivo, and cleaves fatty acids from their natural substrates, milk triglycerides and monoglycerides.

To further identify the range of antiprotozoal activity of NHM, we evaluated NHM, milk from other lower mammals, and purified BSSL, for effects on Eimeria tenella sporozoite infectivity (anticoccidial activity). Sporozoites of Eimeria tenella were killed in vitro in a time- and concentration-dependent manner by exposure to diluted concentrations of normal human milk. Sodium cholate (bile salt-stimulator) potentiated the anticoccidial activity. The anticoccidial activity was not found in milk of lower mammals (cow, sheep, goat, dog). The component in human milk showing the activity was initially hypothesized to be a bile salt-stimulated lipase. However, testing of purified lipase (with or without sodium cholates) indicated no anticoccidial activity. Consequently, we theorize the active component may be a free fatty acid.

DEVELOPMENTS IN CUTICULAR BIOCHEMISTRY OF PARASITIC NEMATODES.
R.H. FETTERER*. HELMINTHIC DISEASES LABORATORY, USDA, ARS, LIVESTOCK AND POULTRY SCIENCES INSTITUTE. BELTSVILLE, MD 20705.

The cuticle of parasitic nematodes has been the subject of recent studies directed at understanding its molecular and biochemical nature with the ultimate goal of applying this knowledge to the development of novel parasite control schemes. Three basic areas of cuticle biology have been investigated: (1) structural biochemistry, (2) dynamic properties of the cuticle including synthesis and ecdysis and, (3) cuticular permeability. Cuticular proteins consist of two general classes: (1) Collagen-like proteins that are extracted from the cuticle with reducing agents, and (2) proteins that are insoluble in reducing agents. Stage specific difference in soluble cuticular proteins have been observed in both Ascaris suum and Haemonchus contortus. The insoluble cuticular proteins from the 2nd molt cuticle of H. contortus may contain both dityrosine crosslinks as well as a tanning mechanism similar to that of insects, thus imparting to the cuticle an increased resilience and resistance to environmental degradation. Investigations of the cuticular permeability of the isolated A. suum cuticle demonstrate that the passive cuticular transport is related to the octanol partition coefficient of a nonpolar solute. This model can be used to predict passive solute movement into intact parasites and thus optimize anthelmintic delivery.
CHARACTERIZATION OF PROTEINS FROM THE SECOND MOL'T CUTICLE OF HAEMONCHUS CONTOR TUS WITH REFERENCE TO ECDYSIS. H.R. GAMBLE, J.P. PURCELL AND R.H. FETTERER, USDA, AGRICULTURAL RESEARCH SERVICE, BELTSVILLE, MARYLAND 20705

Infected larvae of Haemonchus contortus and other ruminant trichostrongyles cast the second molt (2M) sheath through a series of events including the enzymatic digestion of a circular (ring) region of the sheath (approximately 20 µm from the anterior end in H. contortus). The enzyme involved is a 44kDa metalloprotease produced by the parasite. Substrate proteins found in the ring region are biochemically unique from the remainder of the cuticle. Monoclonal antibody probes have been used to demonstrate that the ring region is composed of proteins of 160, 120 and 105kDa; upon exposure to the 44kDa metalloprotease these proteins are digested to a major 98kDa product with additional lower molecular weight components. These substrate proteins are unique to the 2M cuticle of H. contortus; immunoblots demonstrate that these proteins first occur at 5-6 days of development of the free-living larvae and are absent from subsequent stages. Related proteins are found in other trichostrongyles, including Ostertagia, Cooperia, and Trichostrongylus, however, structural differences in these proteins exist among species. Second molt cuticle proteins which are not susceptible to digestion by the 44kDa enzyme differ from digestible proteins in amino acid composition and size. Three "families" of soluble cuticle proteins have been identified using monoclonal antibody probes. These families are differentially expressed during parasite development.


It is accepted that the cuticle of nematodes is an important site of interaction with the host and plays a role in the uptake of nutrient and other molecules; however, the significance of these structures, and their component constituents, in these activities in different species remains far from clear.

This current study describes some immunological, histochemical and morphological approaches to defining the functional characteristics of the cuticular structures of Ascaris, Onchocerca and Brugia sp. The surface of nematodes carries epitopes recognized by specific polyclonal antibodies and certain monoclonal antibodies. These antibodies are potential probes for stage specific antigens of nematode species. The enzyme content of the cuticle and the underlying structures varies with stage and developmental stage. It is apparent that the interface between the cuticle and associated hypodermis is a highly specialized zone, and is high in concentration of phosphatase enzymes and in bombesin-like peptides; the filamentous projections extending from the cuticle into the hypodermal cells are centers of enzyme and peptide concentration.

This evidence suggests that the cuticle contains structures that are in all likelihood important in active transport of substances.

Transport barriers of the collagen matrix and the lipoidal component of Ascaris suum cuticle were studied using model solutes varying in molecular size, lipophilicity and charge. Studies were carried out in a two-chamber diffusion cell system wherein the preparations were mounted. The neutral compounds (water, urea, glucose, sucrose, hydrocortisone, testosterone and ivermectin) ranged in molecular radius from 1.96 to 6.0 \(\text{Å}\), the cationic solutes (methylamine, chloroquine and erythromycin) from 2.65 to 6.22 \(\text{Å}\) and the anionic permeants (acetate, benzoate, taurocholate) from 2.88 to 5.68 \(\text{Å}\). The permeability coefficient of the collagen matrix versus molecular radius relationship showed the interdependence of size and charge of the permeants. The permeability of neutral solutes decreased monotonically with size. While protonated amines permeated the aqueous pores faster than neutral solutes of comparable size, anions of the weak acids showed a slower velocity. A biophysical model which accounted not only for the diffusion of molecules within a fixed electrostatic field, but also for molecular sieving by pore channels, was used in the mechanistic interpretation of the data. The average pore size was estimated to be 15 \(\text{Å}\) in radius. In transport studies employing lipid-containing cuticular tissues, the effective permeability coefficient was delineated into the permeability coefficients of the collagen matrix and lipid component to determine the rate-controlling barrier. While each solute penetrated the water-filled collagen matrix, the rate-determining step generally was passive diffusion across the lipid component. The exception was water, in which the transport kinetics was 75% matrix-controlled. In general, permeation across the lipid layer was more favorable for lipophilic compounds.

8. OBSERVATIONS ON OOGENESIS AND EGG SHELL FORMATION IN OESOTHAGOSTOMUM COLUMBIANUM, A NEMATODE PARASITE OF SHEEP AND GOAT IN INDIA. M. JOHAL, DEPARTMENT OF ZOOLOGY, PUNJABI UNIVERSITY PATIALA 147002 INDIA.

The oogonia proliferated in the germinal zone of ovary, divide mitotically into oocytes which accumulate nutritive products in the growth zone and show tremendous increase in size. Maturation starts in the last part of the ovary but is completed in the uterus after fertilization. Fertilization also initiates egg shell formation.

In developing oocytes protein is accumulated in the form of rough granules which are subsequently broken down and arranged around the periphery. Its quantity remains static and it forms the major bulk of the egg yolk. There is a progressive increase in the amount of carbohydrate in oocytes, it is later used for the formation of chitinous layer of the egg shell, leaving little traces in cytoplasm of mature ova. An intense activity of RNA and DNA is observed in dividing stages. Lipid is restricted to oolemma only in early stages but is incorporated in great amount in mature ova in the form of egg yolk. Egg shell is composed of three layers: a thin lipoidal vitelline layer, a chitinous layer formed endogenously from glycogen reserves of ovum and the outer uterine layer, secreted in the form of lipoprotein strands by the uterine wall, forming a thick resistant coat around the egg.
THE POPULATION BIOLOGY OF TRICHOSTRONGYLID PARASITES IN RUMINANTS. G. SMITH*.
NEW BOLTON CENTER, UNIVERSITY OF PENNSYLVANIA SCHOOL OF VETERINARY MEDICINE, 382 W. STREET ROAD, KENNETT SQUARE, PA 19348.

It will be argued that a single model may satisfactorily describe the population biology of trichostrongylid parasites in cattle and sheep. The model accounts for observed changes in parasite abundance within the host in terms of two processes: a progressive decline in the proportion of parasites that become established (i.e. survive to the fourth larval stage), and changes in the mortality of established worms that are related to the host's experience of infection. In order to illustrate the generality of the model, examples will be drawn from many of the important trichostrongylid genera and will include parasites whose predilection site is the small intestine.

MODERATE ANTHELMINTIC RESISTANCE REDUCES GENERAL FITNESS, BUT FURTHER ANTHELMINTIC SELECTION IMPROVES FITNESS IN HAEMONCHUS CONTORTUS.
M.E. SCOTT*, N. MAINGI AND R.K. PRICHARD. INSTITUTE OF PARASITOLOGY OF McGINL UNIVERSITY, QC, CANADA H9X 1C0.

The objective of the study was to determine whether selection pressure on a moderately resistant strain of Haemonchus contortus for increased resistance to thiabendazole (TBZ) would lead to changes in the establishment, survival or reproduction of the parasite in the absence of the drug. A strain of H. contortus showing moderate resistance to TBZ was selected through a series of 5 in vitro and 4 in vivo steps. At stages during the selection process, four strains (R₁ through R₄) were used in the experimental protocol, and compared with the original strain (R₀). The LD₅₀ in the egg hatch assay increased from 2.0 (R₀) to 2.7 mM (R₄). Significant increases in establishment, in net daily egg output, in worm recoveries after three months of infection, and in degree of pathology were associated with increased selection. The strains were also compared with a fully susceptible strain (S) with an LD₅₀ in the egg hatch assay of 1.0 µM. The susceptible strain was more fit in terms of establishment and egg production than strains R₀ through R₃. However, strain R₄ appeared to be quite similar to the susceptible strain. These results help to explain apparent discrepancies in the literature concerning the relationship between drug resistance and "fitness", and have important implications for the spread and the management of the problem of anthelmintic resistance. Supported by NSERC and FCAR. R₀ and S strains provided by SmithKline, PA.

Periparturient egg rise in sheep is associated with lactation; lactogenesis is correlated with increased nematode egg production, and, conversely, weaning of lambs decreases egg production. Prolactin, a proteinaceous hormone from the adenohypophysis, is a regulator of milk production and is a potential physiological link between milk production and egg production of these hemophagous abomasal parasites. Daily injections of partially purified ovine prolactin in lambs with patent infections of H. contortus increased total daily nematode egg production as well as the lengths of female worms. However, fewer female worms were recovered after 12 days of prolactin treatment when compared to saline-treated control lambs. Likewise, injection of the opioid peptide, met-enkephalin, which stimulates endogenous secretion of prolactin, had similar sex-specific effects as exogenously administered prolactin on the female populations of H. contortus experimental infections. Apparently, prolactin selectively enhanced female nematode metabolism, thereby increasing egg production but decreasing their life span.


The fecundity of Haemonchus contortus in naturally infected sheep on pasture and experimentally infected sheep reared indoors was found not to vary with the current density of infection.

This study was carried out in two parts. In the first part we measured the fecundity of H. contortus in 22 lambs on pasture between April - November 1987. Fecal egg counts were carried out every three weeks for the duration of the study period. Changes in fecal output were also monitored. Pairs of randomly selected lambs were removed from pasture every three weeks and slaughtered for post mortem worm counts. In the following year, 48 lambs housed indoors were infected once only with various numbers of third stage H. contortus larvae. Fecal egg counts were carried out twice weekly, and changes in fecal output were monitored as before. Eight weeks post infection the lambs were slaughtered for post mortem worm counts. Results from both experiments were analyzed by comparing different mathematical models for changes in fecal egg output over the period of study. A model in which fecundity was held constant irrespective of the current intensity of infection was found to provide as good a fit to the data as models which represented fecundity as some function of the host’s experience of infection.
A SURVEY OF HELMINTH PARASITES IN BACKYARD FLOCKS IN EASTERN MICHIGAN, AND INFECTION OF TURKEYS BY CONTROLLED EXPOSURE. N. NONAKA*, A.R. DONOHUE AND T.W. SCHILLHORN VAN VEEEN, CLINICAL PARASITOLOGY LABORATORY, MICHIGAN STATE UNIVERSITY, E LANSING, MI 48823.

Intestinal parasitism, except coccidiosis, has declined considerably in domestic birds, with the introduction of confined production systems. Parasitism, however, is still a major problem in backyard flocks. This study was aimed at identifying the occurrence and severity of intestinal parasitism in backyard flocks and to test a method of inoculation of experimental animals by controlled exposure.

Survey: Seventy-two samples 1-2 lbs of fresh litter were collected from 14 backyard flocks. The samples were mixed and a sucrose flotation was performed on a 5 gram aliquot. This was examined for the presence of helminth eggs, oocysts or other evidence of parasites. The following parasites eggs/oocysts were observed (% of samples positive): Ascaridia spp. (47%), Capillaria spp. (41%), Syngamus spp. (7%), Strongylus spp. (12%), soil nematodes (42%), Coccidia spp. (54%), and mites (70%).

Inoculation: Litter from turkey pens known to contain Ascaridia and Capillaria eggs and oocysts was placed on the floor of a pen and 20 one-day-old turkeys were introduced. After 8 weeks, the turkeys were removed and sacrificed. The intestinal system was removed and examined for the presence of intestinal parasites. Ascaridia, Capillaria and Heterakis were found in 100%, 100% and 88% of turkeys, respectively. No other helminth parasites were found. It can be concluded from these results that inoculation by controlled exposure with contaminated litter may be an alternative to individual inoculation.

EFFICACY OFIVERMECTIN IN A STRATEGIC EQUINE PARASITE CONTROL PROGRAM. D.D. French, T.R. Klei, M.R. Chapman, and J.C. French. Louisiana State University Agricultural Center and School of Veterinary Medicine, Baton Rouge, La.

Ten pregnant shetland pony mares were allowed to graze a contaminated pasture. These mares were treated with ivermectin at strategic periods of the year (STR) based on previously identified peak larval burdens on pasture (March, May and September). All mares foaled during late March and April. Foals were treated with ivermectin in May and September. This control program followed a preceding study in which spring treatment of mares only was ineffective in controlling foal parasites. Parameters used to assess efficacy of treatment included body weight, condition score, backfat values and eggs per gram of feces (EPG) of Strongyle spp., Parascaris equorum and Strongyloides westeri. These data were then compared to that recovered from 2 similar broodmare bands that received treatment with ivermectin at regular 8 week intervals (MAX) and no anthelmintic treatment (CON).

The results indicate that strategic treatment of mares delays, but does not inhibit transmission of Strongyle spp. to foals after parturition. There was little difference in P. equorum EPG values between the foals in the STR group and CON group. Strongyloides westeri transmission was reduced (nearly eliminated) in both the STR and MAX groups when compared to controls. Condition scores and backfat values for the STR foals were intermediate between MAX and CON foals. Supported in part by MSD AgVet, Rahway, NJ and the LSU Equine Veterinary Research Program.
15.
A SURVEY OF EQUINE PARASITE CONTROL PRACTICES IN TENNESSEE. C.R. REINEMEYER* AND B.W. ROHRBACH. UNIVERSITY OF TENNESSEE, COLLEGE OF VETERINARY MEDICINE. KNOXVILLE, TN 37901-1071.

A random sample of 130 horse owners was selected from subscribers to an equine extension publication in Tennessee. Owners were contacted by phone and asked to provide data about their farms, horses, parasite control practices and information sources. 128/130 owners (98.5%) completed a survey questionnaire.

The average owner grazed 8.5 horses on 2 pastures totalling 29.4 acres. All owners treated foals, weanlings and yearlings at least once annually; a second treatment was given to 60% of foals, 91% of weanlings, and 100% of yearlings. Although 110 owners (85.9%) reported following a regular deworming schedule, only 55.5% treated adult horses at least 3 times per year. Ivermectin and "tube wormers" were the 2 most commonly employed products. Little awareness of anthelmintic resistance was exhibited; only 14.3% of respondents had discontinued the use of an anthelmintic for any reason, and over 90% did not plan to change current regimens or drugs. Equine magazines and veterinarians were the major sources of information about anthelmintics and treatment regimens. An interest in obtaining additional information about equine parasite control was expressed by 63.3% of respondents.

16.

Benzimidazole resistance in cyathostomes has been documented throughout the U.S. However, studies identifying species involved have been limited and only reported from the states of Kentucky and Washington. A critical study was conducted using 5 pony foals which were allowed to graze for 6 months on a quarter horse farm previously shown by fecal strongyle egg count methods to harbor benzimidazole resistant cyathostomes. Thiabendazole at its recommended dosage was the drug tested. Eleven species of cyathostomes were identified from these ponies. Varying degrees of resistance (16 to 72% efficacy) to TBZ were identified in 7 species. (Cyathostomum coronatum, C. catinatum, Cyclicostephanus minutus, C. calicatus, C. longibursatus, C. goldi, and Cyclicoclyclus nassatus). Six of these species have previously been shown to be resistant in Kentucky and the additional species was identified in Washington. Three additional species shown in other studies to be resistant, Cyathostomum labratum, Cyclicoclyclus insignis, and C. leptostomus, although present in this study were not resistant.
Sixteen pony foals were moved to helminth-free facilities within 12 hr after birth. Once the foals were at least 7 days-old they were inoculated by nasogastric tube with 2000 ± 545.5 infective Parascaris equorum eggs on day -28. The foals were allocated to replicates of 4 and treatments were assigned to each replicate randomly. Treatments administered on day 0 included untreated control, 0.2 mg of ivermectin /kg, 10 mg of oxibendazole/kg, and 6.6 mg of pyrantel salt (pamoate) /kg. All anthelmintics were formulated as pastes and administered orally. The foals were euthanatized 42 days after inoculation (day 14) and examined for the presence of P. equorum larvae in the small intestine. The mean number of 4th-stage P. equorum larvae recovered from foals treated as controls was 1603.8 ± 513.3 (305-2480). The mean number of larvae recovered from foals treated with ivermectin, oxibendazole, and pyrantel was 29.2 ± 28.0 (0-113), 889.5 ± 561.5 (1-2345), and 413.0 ± 284.0 (0-1204) respectively. Treatment with ivermectin, oxibendazole, and pyrantel was 98.2%, 44.5%, and 74.2% effective respectively against 28 day-old P. equorum when compared to controls. Adverse reactions due to treatment were not observed.

Fluke transmission was highly seasonal. No transmission occurred during the months of July, August and September in all 3 years. Transmission began as early as October of one unusually wet year (1986) but usually began in December (1987) or January (1985). February, March, and April were the months of peak transmission. The last month of transmission before the summer hiatus was either May (1986 & 1987) or June (1984 & 1985).
19.

EPIZOOTIOLOGY OF GASTROINTESTINAL NEMATODES OF CATTLE IN SELECTED AREAS OF OREGON WITH PARTICULAR REFERENCE TO OSTERTAGIA SPP. L.G. RICKARD* AND G.L. ZIMMERMAN. COLLEGE OF VETERINARY MEDICINE, OREGON STATE UNIVERSITY, CORVALLIS, OREGON 97331.

During 1985-1987 tracer calves were used to establish the general seasonal patterns of transmission of gastrointestinal nematodes in the Willamette Valley, the southern Oregon coast, high range east of the Cascades and the Klamath Basin (Oregon-California border). Eight sets of 3-4 tracer calves each were allowed to graze 2-3 weeks on pasture at times corresponding to spring, summer, fall and winter in each area. Four genera of nematodes were commonly recovered from each area: Ostertagia, Trichostrongylus, Cooperia and Nematodirus. Variations in the species composition and the transmission patterns of each occurred between all areas. Larval inhibition was most clearly defined for Ostertagia and Cooperia. Although winter inhibition of Ostertagia was the typical pattern in most areas the onset of hypobiosis was variable; on the southern Oregon coast, hypobiotic populations were apparently absent. Inhibition of Cooperia followed the same general pattern seen for Ostertagia.

20.


Twelve calves (mean weight = 176 kg) were used to confirm the efficacy of ivermectin delivered from a prototype sustained-release bolus against natural infections of gastrointestinal nematodes, including hypobiotic early fourth-stage larvae of Ostertagia ostertagi. Control calves (n=6) were given a placebo bolus; calves of the treatment group (n=6) were given an active bolus designed to release ivermectin at 8 mg/day. Following treatment, the animals in each group were housed in a separate pen with concrete flooring. All 12 calves were necropsied 28 days posttreatment for recovery, identification, and enumeration of parasites. Compared to control calves, the ivermectin-treated calves had significantly (P<0.01) fewer hypobiotic and developing fourth-stage larvae and adults of Ostertagia (O. ostertagi and O. lyrata), and adults of Trichostrongylus axei and Cooperia (C. oncophora, C. punctata and C. surinabada). Anthelmintic efficacy was >99% for Cooperia spp. and 100% for the other parasites listed. Populations of Nematodirus helvetianus and Capillaria spp. were inadequate for statistical analysis. No adverse reactions were observed.

The patterns of gastrointestinal and lung nematode infections in an untreated grazing cow-calf herd were studied on the island of Montreal to define optimum timing for strategic parasite control and treatments. During the grazing season, May to December 1988, the cow-calf herd and the replacement heifers were on separate pastures. At monthly intervals, fecal egg counts were taken from cows, calves and heifers and fecal cultures were made to identify the nematode spp. present. Pasture larval counts and worm-free tracer calves were used during the grazing period to determine nematode parasite larvae available. Mean fecal epg for the cows and heifers were relatively high before turnout to pasture. As the grazing season progressed, there was a rapid decline in their epgs. The fecal epg of the calves started to rise soon after birth and remained at substantial levels throughout. Pasture infectivity remained high from July to October. Ostertagia spp., Cooperia spp. and Nematodirus spp. were the most prevalent nematodes found. The tracer worm burdens and fecal egg counts of calves indicate that the young calves may suffer from subclinical parasitism and that they do not develop significant resistance to nematode infestation during their first grazing season. The high epg output by the cows during the early grazing period may serve as a source of infection for their calves.

22. **Seasonal Transmission Patterns of Cattle Nematodes in the Middle Atlantic Region: Role of Spring and Fall Hypobiosis.** K.D. Murrell¹; E. Leighton², B. Boswell¹, and L. Gasbarre¹. ¹Beltsville Agricultural Research Center, USDA/ARS, Beltsville, MD 20705; ²Wye Research and Education Center, University of Maryland, Agricultural Experiment Station, Queenstown, MD.

A longitudinal study (3 years) was carried out in a cattle herd in eastern Maryland to determine the seasonal transmission patterns of the common nematode parasites. Tracer calves and pasture herbage sampling served as the primary monitors of transmission. Detailed soil and climatic data were gathered to permit assessment of these variables as modulators of transmission. The results reveal that: (1) the basic transmission pattern is the "northern" type (spring-summer-fall); (2) the transmission dynamics of individual species of parasites often varied greatly; (3) overwintering of larvae from fall contamination of pasture is an important feature for some species, while summer conditions favored a different species complex; and (4) hypobiosis occurs in both spring (Ostertagia ostertagia) and fall (primarily Haemonchus placei): The role of hypobiosis in stabilizing parasitic populations will be discussed as will the influence of different climatic variables on these transmission patterns. Strategies for optimizing control for parasites in herds in this climate zone will be suggested.
23.

**EPIDEMIOLOGIC INFORMATION ON THE TRANSMISSION OF GASTROINTESTINAL NEMATODE POPULATIONS IN CATTLE IN CENTRAL ALABAMA. D.E. SNYDER*. USDA-ARS, ANIMAL PARASITE RESEARCH LABORATORY. AUBURN, AL 36831.**

Epidemiologic information on parasite population numbers, types and changes occurring throughout the year (12/87-12/88) and their effects on cattle were evaluated at a test site located in Auburn, Alabama.

To determine seasonal parasite transmission patterns, nematode-free holstein tracer calves were grazed with the site located resident cattle population. Two tracer calves were placed at the test site and allowed to graze for 30 day intervals, then held in drylot for 14 days prior to necropsy for total gastrointestinal worm recovery. Additional information obtained included estimations of pasture larval counts at bimonthly intervals, serum pepsinogens, weight gains, fecal egg counts, mean monthly rainfall and temperature extremities, and abomasal pH and gross lesions at necropsy. Total worm counts were lowest at the beginning of the study, rising during the months of February through June and the highest total numbers of worms being recovered during July through September. Relationships between parasitic types, numbers and stages of development found throughout the year will be presented.

24.

**Seasonal transmission of equine parasites in South Louisiana. D.D. FRENCH, T.R. KLEI, M.R. CHAPMAN, LOUISIANA STATE UNIVERSITY, AGRICULTURAL CENTER, and School of Veterinary Medicine, BATON ROUGE, LA.**

Parasite free tracer foals were allowed to graze with 10 untreated pony mares to define the seasonal transmission of equine parasites. Tracer foals were placed on the pasture during 4 seasons. They remained on the pasture for 8 weeks, were held for 4 weeks, euthanized and complete parasite burdens determined. Pasture strongyle L₃ burdens were determined bimonthly.

Results indicate that transmission of Strongylus spp occurred predominantly in the winter and spring months. Cyathostome transmission occurs year around, however adult cyathostomes were found only in tracers grazed during the spring. Mean peak number of adult cyathostomes recovered was 7640. These recoveries correlate well with pasture larval burdens which peak in early March. Encysted cyathostome larvae were enumerated in the mucosa of the cecum and colon using a transmural illumination technique. The majority of encysted larvae were found in tracers during the fall and winter seasons. These results suggest that a fall inhibition of some cyathostome spp. may occur. Anoplocephala perfoliata were recovered during all seasons of the year with peak numbers found during the spring. Gastrophilus spp. were recovered only in tracers grazed during the fall and winter seasons. Parasite burdens were absent or markedly reduced in tracers grazed at the same time intervals with mares that received ivermectin treatments every 8 weeks.

Supported in part by grants from MSD/AGVET, a division of Merk and Co., Inc. and by the Louisiana Equine Veterinary Research Program.
25.

STRATEGIC CONTROL OF STRONGYLES WITH IVERMECTIN IN MATURE HORSES IN ONTARIO.
OWEN SLOCOMBE*, DEPARTMENT OF PATHOLOGY, ONTARIO VETERINARY COLLEGE,
UNIVERSITY OF GUELPH, GUELPH, ONTARIO N1G 2N1

At a riding stable, 30-35 mature horses were treated with ivermectin (Eqvalan paste) on April 26 1988, July 8 and November 15. A fecal sample from each horse was examined for strongyle eggs prior to the first treatment, every two weeks thereafter until two weeks after the last treatment and then monthly until April 1989. From late April to November, herbage samples, from one pasture on which the horses were kept, were examined every two weeks for strongyle larvae. About 15 kilometers directly from the stable, 15 ponies were placed from May 15 to November 10 on a pasture, on which they had been in previous summers, and then housed for the winter. At this site, fecal and herbage samples were analysed in similar fashion to that for the stable.

Prior to treatment, the mean strongyle eggs per gram (epg) for the horses was 284. After treatment, the mean epg was zero and for the remainder of the study at zero or as high as 48.5 (October 24). Larvae were recovered from pasture on August 15, September 26, October 24 and November 7, with the highest number, 3120 larvae per kg of dry herbage (1pkg), in September. The mean strongyle epg for the ponies was 562.5 at turnout, rose to 1357.1 on June 28, with another peak of 1231 on September 6 and low over the winter. Larvae were recovered from pasture prior to turnout, were not found in June, but in several thousand 1pkg thereafter the highest being 70,760 on July 25.

26.


A clinical trial was done to determine if treatment of mares with ivermectin (IVM), within 12 hrs of parturition, was effective in preventing Strongyloides westeri infections in their foals. The mares (n=15) were treated, within 12 hr of parturition, on day 0 with 1 of 3 treatments; 0.02 ml IVM vehicle /kg, 0.2 mg of IVM paste /kg, or 0.2 mg of IVM liquid /kg. Milk samples (>100 ml/sample) from the mares were examined for S. westeri larvae on days 10-12. Fecal samples from foals were examined for parasite eggs on days 3, 7, 11, 13, 15, 17, 19, and weekly from day 21-63.

S. westeri larvae were detected once in milk from 1 mare treated with IVM paste, 1 mare treated with IVM liquid, and none were observed from mares treated with IVM vehicle. S. westeri eggs could not be detected in any of the foals until day 13. S. westeri egg per gram (SWEPG) counts were higher in foals whose dams were treated with IVM vehicle (0-1500) than those whose dams were treated with IVM paste (0-1000) or IVM liquid (0). MeanSWEPG counts ranged from 0-103.0, 0-4.6, and 0 in foals whose dams were treated with IVM vehicle, IVM paste, and IVM liquid respectively. Foals whose dams were treated with IVM vehicle were more consistently detected passing S. westeri eggs after day 11; from 1-4, 0-1, and 9 foals whose dams were treated with IVM vehicle, IVM paste, and IVM liquid were detected passing eggs on days 13-63 respectively. Foals whose dams were treated with IVM and detected passing S. westeri larvae in their milk did not develop patent S. westeri infections.

After attempting to drench a particularly fractious horse with Eqvalan Oral Liquid® (MSD AgVet) the senior investigator discovered that the product, containing 10 mg/ml ivermectin and labelled only for use as an oral drench or for administration via nasogastric tube, was apparently quite palatable and efficacious if simply mixed with the animal's feed. In a follow up study, the product was administered as a topdress (10 ml/500 kg bodyweight) on the grain ration of 33 horses while the grain ration of 13 additional horses was left untreated as a control.

There was no evidence of reluctance to consume grain topdressed with this product. Both treated and untreated grain was consumed by all horses within 20 minutes of feeding. Mean fecal egg counts of ivermectin-treated horses were reduced by 98.5% from 300.7 eggs per gram (EPG) on the day of treatment to 4.5 EPG 2-4 weeks after treatment. Mean fecal egg counts of control horses were reduced by only 3.4% from 323.1 EPG to 305.8 EPG. Fecal egg counts remained at or near zero for up to six weeks following ivermectin treatment, but egg counts began to increase by the eighth week following treatment.

The anthelmintic efficacy of continuous feeding of Pyrantel tartrate to horses.


The present study evaluated the antiparasitic efficacy of continuous feeding of pyrantel tartrate (PT) to horses. PT (Benminth) is an anthelmintic agent that has been used for 15 years in the swine industry. It is a therapeutic and prophylactic anthelmintic against endoparasites representing 3 nematode suborders (Strongylata, Ascaridata and Trichurata). PT was fed daily, as a top dressing, to 7 Arabian horses (1-3 years of age) for 7 weeks. 7 similar horses were also maintained as untreated control animals. All 14 horses were maintained on irrigated pastures and were sampled weekly to determine eggs per gram (EPG) of fecal matter. Fecal cultures and larvae identification established only small strongyles (cyathostomes) present. PT significantly reduced (p<0.05) the EPG in all test horses when compared to the controls. This reduction occurred with the first sampling period. Treated animals gained significantly more weight (p<0.005) than the controls.
29.


The frequency of Anoplocephala ssp. eggs in the feces of both ponies and horses in Marion County, FL was studied. Approximately one-third of all animals tested during a 12-month period were found to be passing Anoplocephala ssp. eggs in the feces. The numbers of eggs passed were low, ranging from 1 to 24 EPG's. These EPG's were determined using a double centrifugation method following filtration of the samples through a 200 micron sieve. Treatment with pyrantel pamoate at twice the therapeutic dose rate was effective in reducing the tapeworm EPG's to 0 within 2 weeks post-dosing.

The epidemiology of this parasite was observed using 4 adult horses housed in separate non-connecting 1 acre paddocks arranged linearly east to west. Initially, only the horse in the eastern-most paddock was passing Anoplocephala ssp. eggs. Within a 12 month period the infection had spread systematically from the eastern-most paddock to western-most paddock and all 4 horses were passing tapeworm eggs.

30.

EFFICACY OF ALBENDAZOLE AGAINST NEMATODE, CESTODE, AND TREVETODE PARASITES OF SHEEP. R.S. REW and J.F. FREEMAN*. SMITHKLINE BECKMAN ANIMAL HEALTH PRODUCTS, WEST CHESTER, PA 19380

Albendazole was evaluated against parasitic nematodes, cestodes, and trematodes in sheep. Thirty-two (32) controlled efficacy trials were conducted with an oral suspension of 2.5-15 mg/kg body weight against mature worms and fifteen (15) trials were conducted against immature stages of parasitic infection. Groups of sheep were treated and post-mortem worm counts done seven or more days after treatment and compared to those of vehicle-treated or untreated controls. The sheep were either experimentally or naturally infected with one or more species of parasite.

Albendazole at 7.5 mg/kg was shown to have 97% efficacy against adult liver fluke, Fasciola hepatica; >98% efficacy against adult tapeworms, Moniezia expansa and Thysanosoma actinoides; >96% efficacy against L3, L4 and adult lungworm Dictyocaulus filaria; >99% efficacy against L3, L4 and adult stomach worms Haemonchus contortus, Trichostrongylus axei, and Ostertagia circumcincta and intestinal worms Nematodirus spathiger, N. filicollis, Cooperia oncophora, Marshallagia marshalli, Trichostrongylus colubriformis, Oesophagostomum columbianum and Chabertia ovina, except no tests were conducted on L4 L. axei or C. ovina; and, 57% efficacy against the deer fluke Fascioloides magna.

No toxicity was seen in any trial as a result of albendazole treatment.
31.
EFFICACY OF THE NATURAL PRODUCT, PARAHERQUAMIDE, AGAINST 6-DAY-OLD TRICHOSTRONGYLUS COLUBRIFORMIS IN THE GERBILL (MERIONES UNGUICULATUS).

The discovery of the anthelmintic activity of paraherquamide, a product of Penicillium paraherquei, will be reported elsewhere. We here report its efficacy against 6-day-old T. colubriformis in gerbils. Single oral dosages of 6.25, 3.125, 1.56 and 0.78 mg/kg were 100, 99, 98, and 96% effective, respectively. A dosage of 0.39 mg/kg was 66% effective. No gross toxic manifestations were observed following a single oral dosage of 200 mg/kg.

32.
EFFICACY OF NEMADECTIN AGAINST GASTROINTESTINAL NEMATODES IN CATTLE.
G.L. ZIMMERMAN*, E.P. HOBBERG, AND J.A. PANKAVICH. COLLEGE OF VETERINARY MEDICINE, OREGON STATE UNIVERSITY, CORVALLIS, OR 97331 AND AMERICAN CYANAMID, PRINCETON, NJ 08540.

The anthelmintic activity of the experimental antiparasitic macrocyclic lactone, nemadectin was evaluated using calves with naturally acquired gastrointestinal nematodes. Three groups of 5 calves each were used as controls or principals treated with oral nemadectin at either 0.2 or 0.4 mg/kg body weight. Following necropsy of calves 10-11 days later, the intestinal nematodes were recovered and enumerated. At the respective dose levels, reductions were significant, with efficacies as follows: larval (including inhibited 4th stage) and adult Ostertagia ostertagi, 99.8 and 99.9%; larval (including inhibited 4th stage) and adult Cooperia spp, 100 and 99.6-100%; Trichostrongylus spp, >99.9%; and Oesophagostomum radiatum, 100%. Although efficacies against Nematodirus helvetianus, Trichuris discolor, and Capillaria spp were 100%, numbers were inadequate for statistical analysis.
EFFECT OF OXFENDAZOLE AGAINST INHIBITED 4th-STAGE LARVAE OF OSTERTAGIA OSTERTAG! IN FEEDLOT CATTLE. J.C. WILLIAMS*, D.T. BECHTOL, A. WAITE, and R.C. HERSCHLER. LOUISIANA STATE UNIVERSITY, BATON ROUGE, LA, AGRI RESEARCH DIVISION, CANYON, TX, SYNTEx RESEARCH, PALO ALTO, CA.

Oxfendazole (Synanthic®, intrarum. inj., 4.5 mg/kg) was compared with levamisole (6.0 mg/kg, s. c., inj.), ivermectin (200 µg/kg, s. c., inj.) and untreated control cattle in evaluating removal of O. ostertagi inhibited larvae (EL) and effect on feedlot performance. Yearling beef cattle (407) averaging 296 kg were acquired from auction barn sales in Mississippi (early July) and trucked to Texas. Following a 7-day acclimation, 3-4 cattle (total 18) from each of 6 sources were randomly selected for slaughter and assessment of extent of infection with EL4. Abomasal digest yielded a range of 180-79,800 worms (avg. 20,123); all were positive and Ostertagia EL4 were predominant. 360 cattle were randomly allocated into 4 groups of 90 animals on July 13 based on body weights and source. Each group consisted of 9 replicates of 10 cattle per individual pen. One animal from each pen was killed at 12 or 13 days (July 25-26) after treatment and also at the end of the feeding period (Dec. 15). Only July worm count data are given. Average numbers of O. ostertagi recovered from untreated controls were: adults-1,602, DL4-1,146, and EL4-4,285 (56.5%). Numbers of EL4 ranged from 0 to 13,038. Reductions (%) against respective stages of O. ostertagi were OXF-99.8, 98.5, 97.4; LEV-74.0, 0, 0; and IVM-100.0, 99.4, 99.4. Average total gains (kg), daily gains, and conversion ratios for controls, OXF, LEV, and IVM groups were: 494, 3.23, 7.96; 518, 3.39, 7.30; 510, 3.34, 7.55; 515, 3.37, 7.56.

PRODUCTIVITY OF CALVES IN A COW-CALF HERD IN RESPONSE TO ANTHelmINTIC TREATMENT. C. E. COUVILLON* AND R. R. EVANS, COLLEGE OF VETERINARY MEDICINE AND MISSISSIPPI AGRICULTURAL & FORESTRY EXPERIMENT STATION, MISSISSIPPI STATE UNIVERSITY; J. A. HAWKINS, MSD AG VET, MEMPHIS, TN; C. SEIFKER AND J. R. JACKSON, COLLEGE OF VETERINARY MEDICINE, MISSISSIPPI STATE UNIVERSITY.

The effect of anthelmintic treatment was studied in a spring calving herd in Northeast Mississippi. Cows were randomly divided into 5 groups (30 cows/group) based on age. In both years, *cows and †calves were treated with ivermectin as follows: Group 1 *(Jan Apr Jul) †Jul; Group 2 *(Apr Sep) †Sep; Group 3 *(Jan Jul) †Jul; Group 4 *(No treatment) †Jul; and; Group 5 *(No treatment) †No treatment. Cows were continuously grazed on separate, 20 ha fescue/bermudagrass pastures from January, 1987 through October, 1988. Calves were weighed at birth and again at weaning in October. In June, July, September and October of 1987, two calves from each group were necropsied and gastrointestinal nematodes were counted. In groups 1-4, mean total worm counts did not exceed 850 during any of the four necropsy periods. In the untreated group, the mean total worm count increased from June (700 worms) through October (1,650 worms). The mean weaning weights in groups where cows and calves both were treated (groups 1-3) were 14.5, 9.1 and 15.5 kg, respectively, greater than the untreated control. The mean weaning weight in group 4, where only calves were treated, was 3.9 kg less than the untreated control. The data suggest that treatment of both cows and calves, as opposed to calves only, improves productivity. This improvement apparently occurs due to the reduction of already low burdens of gastrointestinal nematodes.
35. EFFICACY OF IVERMECTIN IN-FEED AGAINST PARASITES OF SWINE.
MERCK & CO., INC., ROUTE 2, BOX 136, FULTON, MO 65251

The efficacy of ivermectin as an in-feed formulation was evaluated in pigs against naturally acquired gastrointestinal, pulmonary and cutaneous parasites (experiment 1, n=24), and induced infections of intestinal nematodes (experiment 2, n=24). Treatments consisted of ivermectin in-feed at 100 or 200 μg/kg of body weight per day for 7 days or non-medicated feed for 7 days.

At 100 μg/kg/day the efficacy against naturally acquired infections was 97.7% for Ascaris suum, 97.8% for Metastrongylus spp, >99% for Oesophagostomum spp, 100% for Macracanthorhynchus hirudinaceus and 89.7% for Ascarops strongylinia. Against induced infections (4th-stage larvae) the efficacy was 100% for A suum and 96.9% for Oesophagostomum spp.

At 200 μg/kg/day, the efficacy against naturally acquired infections was 100% for A suum, H rubidus, Metastrongylus spp, A strongylinia, >99% for Oesophagostomum spp and 85.9% for M hirudinaceus. Against induced infections (4th-stage larvae) the efficacy was 100% for A suum and 95% for Oesophagostomum spp.

At 100 and 200 μg/kg/day, ivermectin eliminated the mite Sarcoptes scabiei var suis by posttreatment day 14.


Amprolium (Corid®, 9.6% solution, MSD Agvet, Rahway, NJ) was given per os at the following dosages to the numbers of suckling pigs indicated: 10 mg/kg (n=8); 20 mg/kg (n=8); 30 mg/kg (n=8); 40 mg/kg (n=8). A similar group (n=32) were infected with Isospora suis but not treated. Another group (n=27) were neither treated with amprolium nor infected with I. suis. The study was replicated within litters (excepting noninfected-nontreated pigs) to eliminate sow effects. Pigs received amprolium twice daily on days -1 through 21. Pigs were weighed twice weekly and the amprolium dosages adjusted appropriately. Pigs (2 to 4 days old) were infected with 300,000 sporulated I. suis oocysts on day 0. Fecal specimens were collected on the following days postinfection. 5, 7, 9, 11, 13, 15, 18, 21, 23, 25 and 28. Specimens were graded either 1, 2 or 3 with the higher numbers to indicate severe diarrhea. Oocysts per gram of feces was determined for each specimen. Pigs treated with amprolium at all dosages gained more weight and had lower fecal consistency indices than infected-nontreated pigs. Weights of treated pigs compared favorably to weights of noninfected-nontreated pigs. Fourteen of 32 infected-nontreated pigs died from coccidiosis. Treated pigs continued to excrete oocysts in their stools. Results indicate that amprolium, when used as reported in this study, exerts an observable effect on experimentally-induced I. suis infections in suckling pigs. This project was supported by USDA IR-4 Grant No. 82-CSRS-2-1012.
37.

EFFICACY OF ALBENDAZOLE VS. CATTLE PARASITES. A. L. SHOR, SMITHKLINE ANIMAL HEALTH PRODUCTS, WEST CHESTER, PA 19380

Albendazole is effective against a broad spectrum of internal parasites of cattle. Studies were conducted with both naturally and artificially-infected cattle, ranging in weight from 46-560 kg. Many different breeds and both sexes were included in the studies. A single oral dose of 10mg/kg in a suspension resulted in a greater than 95% reduction in Fasciola hepatica liver fluke infection and a similar reduction in Dictyocaulus viviparus lungworm infection. Tapeworms (Moniezia spp.) were also reduced by a similar extent. Greater than 93 per cent removal of the following gastrointestinal roundworms was also accomplished: Hemonchus contortus, Ostertagia ostertagi, Trichostrongylus axei, Strongyloides sp., Nematodirus sp., Cooperia, Trichostrongylus colubriformis, Ostertagia radiatum. Where relevant, albendazole was effective against adults and fourth stage larvae.

A second series of studies was conducted with a 40% albendazole paste formulation. This was 99% effective at 10mg/kg, single oral dose, for adults and 91% effective against immature flukes. When direct comparisons of the suspension and paste formulations were made in the same studies they were found to be equally effective.

38.

DIROFILARIA IMMITIS INFECTIONS IN OREGON DOGS: PERCEPTIONS OF VETERINARIANS AS DETERMINED BY A WRITTEN SURVEY. L.S. RICHARDS* AND G.L. ZIMMERMAN, COLLEGE OF VETERINARY MEDICINE, OREGON STATE UNIVERSITY, CORVALLIS, OR 97331.

A survey of veterinarians in Oregon was conducted in order to determine their perception of the range of Dirofilaria immitis, the actual number of heartworm disease (HWD) cases they diagnosed during the last 2 years, as well as their diagnostic and potential treatment protocols. A questionnaire was distributed to all members of the Oregon Veterinary Medical Association. Responses by over 120 practitioners throughout the state were summarized. The 142 cases reported last year represented an increase of 45 cases compared to the 97 cases of two years ago. Infections of D. immitis have been diagnosed in nearly all regions of Oregon. History of the dogs confirmed that at least 56% of these cases were acquired in Oregon. Seventy seven percent of the veterinarians did not routinely screen dogs for HWD. Of those that did, most utilized only one diagnostic test, usually the modified Knotts technique. The vast majority of the veterinarians did not believe HWD was a problem in Oregon, nor did they believe it would become a significant risk to dogs in the state. Most did not believe HWD was present in their local area, and although they were unsure where, they thought it might be found in other areas of the state. This survey suggested that the actual range and incidence of HWD in Oregon is unknown.
39.

PRESENCE OF HEARTWORMS IN OREGON DOGS AS DEMONSTRATED BY A SEROLOGIC SURVEY
J.K. BISHOP*, G.L. ZIMMERMAN, AND D.M. MULROONEY, COLLEGE OF VETERINARY
MEDICINE, OREGON STATE UNIVERSITY, CORVALLIS, OR 97331.

A statewide survey for heartworms in dogs was conducted using serum samples
provided by veterinary practitioners. Blood samples were taken during the
fall of 1988 and winter of 1989. Criteria for selection of the individual
dogs included: > 2 years of age, native to Oregon, and at risk for exposure
to mosquitoes. Serology was conducted using a commercial test kit that
detected antigens to Dirofilaria immitis. Test results were read directly and
confirmed by OD measurements using an ELISA reader. Of more than 500
serum samples, approximately 8% were positive for antigens of D. immitis. This
study demonstrated the presence of heartworms in all regions of Oregon.

40.

EVALUATION OF TWO ADULT HEARTWORM ANTIGEN DIAGNOSTIC TEST KITS USING WELL
DEFINED DOG AND CAT SERA. M.T. DZIMIANSKI*, T.L. MCTIER, AND J.W. MCGALL,
COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF GEORGIA, ATHENS, GA 30602.

Uni-Tec™D (membrane type) and Assure/CH™ (dipstick type) were evaluated
with 89 dog and 46 cat serum samples. In dogs, Uni-Tec™D detected antigen
as early as six months postinfection (PI) in 70% of this sample type and
consistently (96%) in samples collected seven months PI or later. Antigen
was rarely detected in five month PI samples (22%), and prepatent samples
collected prior to this were negative. Samples from amicrofilaremics having
a single female heartworm or a pair of heartworms (1 male, 1 female)
were negative. Ninety-six percent of the samples from dogs with two or more
female worms were positive, but it should be noted that there was a limited
number of samples from dogs with two to five female worms. No apparent
cross-reactivity with Dipetalonema reconditum, Toxocara canis, Toxascaris
leonina, Ancylostoma caninum, or Uncinaria stenocephala was detected. In
cats, antigen was detected as early as six months PI. Seventy-seven percent
of the samples collected seven months PI or later were positive. The test
was positive when only one adult female heartworm was present.

Using the above samples from dogs and cats, the Assure/CH™ kit gave
similar results with the dog samples. In regard to cats, forty-two percent
of the samples taken seven months or later PI were positive.
DOSE CONFIRMATION OF PYRANTEL PAMOATE AS AN ANTHELMINTIC IN CATS. R.K. RIDLEY*, K.S. TERHUNE AND D.E. GRANSTROM. DEPT. OF LABORATORY MEDICINE, COLLEGE OF VETERINARY MEDICINE. MANHATTAN, KS 66506

Thirty cats of mixed breeding and various ages with natural and/or induced infections of Ancylostoma spp. and T. cati were assigned to one of three treatment groups: 1) non-medicated controls, 2) Paste Formulation at 20 mg base/kg, or 3) Granules Formulation at 20 mg base/kg. Study parameters measured included body weights, clinical observations, physical examinations, fecal egg counts, and worm counts at necropsy.

Pyrantel pamoate when administered at 20mg/kg in paste form was 99.5% effective in removing adult Ancylostoma spp., and 100.0% effective against adult Toxocara cati. When administered in granule form, pyrantel pamoate was 97.9% effective against Ancylostoma and 100% effective against Toxocara.

At 20 mg/kg body weight, the paste formulation was 99.3% effective in reducing egg counts of Ancylostoma spp.; the granule formulation was 99.7% effective. At the same dose level, the granule formulation was 97.7% and 99.9% effective in reducing fecal egg counts in Ancylostoma spp. and Toxocara cati respectively.

FIELD EXPERIENCE WITH A NEW IVERMECTIN FORMULATION FOR DOGS. R. S. BLAKELY, B. SHOFSTALL. CENTRAL HOSPITAL FOR ANIMALS, ROUTE 3, BOX 32 A, CARTERVILLE, ILLINOIS

One hundred-two microfilariae-free dogs were utilized to evaluate the acceptability of ivermectin chewables as a heartworm preventive in dogs under field conditions in southern Illinois. The dogs were on chewable diethylcarbamazine for 30 days before the start of the study. All dogs were tested for blood circulating microfilariae and Dirofilaria immitis antigen prior to treatment, at 4 to 5 months and at 11 to 16 months after initiating treatment. Treatment groups consisted of: A) Daily chewable Filaribits® (DEC) at approximately 3 mg per 1 lb of body weight plus monthly vehicle for ivermectin chewable, B) Monthly HEARTGARD-30® tablets, and C, D, E, F) Monthly ivermectin chewables. All dogs were on preventive medication for at least 11 months. The HEARTGARD-30® tablets and ivermectin chewables were given as follows: Dogs up to 25 lb one 68 µg dose; dogs 26-50 lb one 136 µg dose; dogs 51-100 lb one 272 µg dose. Drug acceptability was evaluated by the owners of the dogs using a numerical system.

All dogs receiving HEARTGARD-30® or ivermectin chewables tested negative for blood circulating microfilariae and antigens to Dirofilaria immitis before and after treatment. One dog receiving Filaribits® tested positive twice for blood circulating microfilariae 13 months after receiving the first treatment.
43. EFFICACY OF NEW IVERMECTIN FORMULATIONS AGAINST 30 AND 45 DAY OLD INDUCED DIOROFILARIA IMMITIS INFECTIONS AND FIELD SAFETY OF A CHEWABLE FORMULATION ADMINISTERED TO COLLIES. A.J. PAUL, K.S. TOBD, JR.*, W.J. TRANQUILLI, J.A. DIPIETRO AND M.A. WALLIG. UNIVERSITY OF ILLINOIS. URBANA, IL 61801

The efficacy of ivermectin in a beef based chewable and in two modified tablet formulations was evaluated against induced heartworm infections in a study involving 52 beagle dogs. All formulations were given orally at 6 μg/kg, against 30-day-old infections of Dirofilaria immitis. Infections were induced with 50 infective larvae. In addition, the ivermectin chewables were given orally at 2 μg/kg 30 days postinfection (dpi) and at 6 μg/kg 45 dpi. Replicates of six or eight dogs were formed based on sex and weight and, within replicates, randomly allocated to treatment group. In replicates of eight dogs, the additional animals were assigned to the control group and to the group receiving ivermectin chewables at 6 μg/kg 30 dpi. Dogs were housed individually. Necropsies were conducted approximately six months postinfection. All ten controls had male and female adult heartworms at necropsy (geometric mean total worm count = 35.0). Six of eight dogs that received ivermectin chewables at 2 μg/kg had adult heartworms (geometric mean = 2.25, efficacy = 93.6%, p < .01 compared to controls). All dogs that received ivermectin at 6 μg/kg at 30 or 45 dpi were free of heartworms at necropsy (100% efficacy, p < .01). The field safety of ivermectin in the chewable formulation, administered monthly at 3x the use level to 44 collies over a period of one year, was demonstrated under field conditions.

44. EFFECT OF ULTRASOUND ON FLEA ACTIVITY. R.W. STONE*, R.P. KNOWLES (POSTHUMOUSLY), R.A. GARCIA, A. MAUCK AND J. SZUST.

Original data from Elexis Corp., claimed that certain high frequency compressional wave energies were effective in altering flea behavior and killing fleas. Clinical validation of company data was confirmed in an independent veterinary hospital. Pulsed Modulated Burst Circuit (PMBC) ultrasound in the range of 130DB significantly affects flea behavior. The effect of exposure to PMBC sound on flea activity (jumping) was studied and compared to activity (jumping) in control groups. Over a series of 8 trials, flea jumping was inhibited between 63-93% as compared to controls after 5 minutes of close exposure to PMBC Sound. Removal of PMBC Sound caused the fleas to resume normal jumping activity in 5 minutes. Results show a statistically significant (99% confidence level) decrease in flea activity when the fleas are exposed to PMBCS. Four experiments including a series of 7 trials on virgin fleas in 2 ounce vials were conducted to determine the killing effectiveness of PMBC Sound on fleas. When fleas were exposed to PMBCS for between 49.5 and 80 hours, the flea kill rate ranged from 17%-48% more than control groups. PMBC Ultrasound has a definite killing effect on fleas.
A CLINICAL DOUBLE BLIND STUDY TO TEST THE EFFECTIVENESS OF THE ELEXIS ULTRASONIC FLEA COLLAR. R.P. KNOWLES (POSTHUM.), R. STONE, J. SZUST*, A. MAUCK.

A study was designed and undertaken to test the ability of the electronic flea collar (Elexis Corp.) to repel fleas in clinical patients. The study was designed to measure by actual flea count the precise effect of ultrasound on fleas on dogs in normal household environments.

There are multiple variables affecting flea infestation levels in every living environment of dogs and cats and it is difficult to evaluate any flea control program. As clinicians, a product that produces consistently lower flea counts with high client satisfaction would be considered desirable. Since clinical impressions can be misleading this study attempted to scientifically quantify and validate our experience. Active and placebo collars were placed on dogs in their normal home environments after their flea counts were taken. Counts were uniformly taken for over two months. Neither the owner nor the technician making the counts was aware of the status of the collar on the animals tested. Test results showed that active collars effectively repelled fleas in a significant majority of dogs, while placebo collars had no demonstrable effect. When placebo collars were replaced by active collars the counts were reduced identically to the active collars.

EFFECTS OF NUTRITIONAL FACTORS ON REPRODUCTIVE FUNCTION OF ADULT SCHISTOSOMA JAPONICUM CULTURED IN VITRO. X. HUA* AND S. ZHOU. HUBEI MEDICAL COLLEGE, WHUAN, P.R. CHINA.

The effects of various basic media, sera and chemical compounds on the oviposition of the adult worms cultured in vitro were compared. The results indicate that RPMI/1640 medium, rabbit and human sera are favorable for the oviposition, and ATP, 5-HT, hypoxanthine, caseine hydrolysate and vitamin C promote the oviposition. On the contrary, norepinephrine inhibits the oviposition. 851 medium was made up by adding the ingredients promoting oviposition to 841 medium. The oviposition rate increased and the percentage of the deformed eggs declined in 851 medium as compared with 841 medium in 23 days' dynamic observation. Obviously, the normal reproductive function of the worms was maintained longer in 851 medium than that in 841 medium. After the peak of oviposition, the oviposition rate increased with each replenishment of media for a short time in both 841 and 851 media. The ultrastructure of the reproductive organs of both male and female worms was also observed. The mechanism by which some chemical compounds affect the oviposition and the possible application of our culture system are discussed.
CHARACTERIZATION OF 5'-METHYLTHIOADENOSINE METABOLISM IN FASCIOLA HEPATICA. C.T. AYER, M.K. RISCOE AND D.T. CLARK. PORTLAND STATE UNIVERSITY AND VETERANS ADMINISTRATION MEDICAL CENTER. PORTLAND, OR 97207.

Methylthioadenosine (MTA) is derived from S-adenosylmethionine (SAM) during the synthesis of the polyamines spermidine and spermine. Methionine is recycled from MTA by one of two mechanisms. In mammalian cells and some microorganisms, MTA is degraded in one step to adenine and methylthioribose-1-phosphate by MTA phosphorylase. However, in certain other microorganisms, MTA is catabolized in two steps: first to adenine and methylthioribose (MTR) via MTA nucleosidase followed by conversion of MTR to MTR-1-P via MTR kinase.

Phosphate-independent clearing activity (MTA nucleosidase) and an ATP dependent MPR kinase activity were demonstrated in cell-free extracts of Fasciola hepatica adults and in rediae containing cercariae. The optimum pH and temperature for the enzyme reactions was determined to be pH 9 and 57°C.

The unique presence of MTR kinase may provide a target for chemotherapeutic exploitation.


Anaplasma marginale is a tick-transmitted intraerythrocytic bacterium which causes significant morbidity and mortality (total losses of about 3 billion dollars) in cattle throughout the world, including the United States. Due to their small size, poor staining characteristics, and low parasitemias found in carriers, accurate diagnosis of anaplasmosis is problematic and rarely achieved. Our goal is to define oligonucleotides which can be used to diagnose anaplasmosis. Cytoplasmic ribosomal RNA (rRNA) is abundant and contains some sequences which are conserved, while others evolve rapidly and are unique to a given genus, species, or strain of organisms. As a preliminary to the construction of an Anaplasma-specific rDNA hybridization probe, we isolated intact DNA from a freeze-thawed infected blood sample using a cesium gradient to remove denatured DNA. A genomic library was constructed in M18R (USBiochemicals). Isolated plasmids were hybridized with kinase-labeled E. coli rRNA and a synthetic oligomer conserved among subbacterial rDNA. Ten positive clones hybridized to the rRNA probes, suggesting that plasmid clones containing rDNA from A. marginale have been isolated. Supported by a grant from the campus Research Board.
49.

DEVELOPMENT OF A WHOLE-WORM ELISA FOR HELIGMOSOMOIDES POLYGYRUS.
K.S. LARRICK, L.H. SEMPREVIVO AND J.P. TRITSCHLER II.
UNIVERSITY OF MASSACHUSETTS. AMHERST, MA. 01003.

Live Heligmosoides polygyrus fourth stage larvae (L4) and adults were used
to develop an enzyme-linked immunosorbent assay (ELISA) that measured anti-
body binding to the worm's surface. Six day postinfection L4 or adult
parasites were obtained from either the small intestine wall or lumen
(respectively) of BALB/c mice. Worms were placed in groups of 30 into 20 ml
glass scintillation vials. Fifty ul of primary sera obtained from orally-,
subcutaneously- or non-immunized mice were diluted 1:25 in 0.15 saline and
added to each vial. After 45 min of incubation at 37 C, the parasites were
rinsed three times with excess saline and 1ml/vial of the secondary antibody
(rabbit anti-mouse urease conjugate diluted 1:100 in saline) applied.
Incubation with the secondary antibody was conducted for 45 min at 37 C, the
parasites washed twice with excess saline, and either 4 L4 or 2 adults were
transferred to a 96 well millititer ELISA plate (Millipore Co., Bedford MA.)
To each well were added 200 ul of urease substrate and the parasites were
incubated overnight at 4 C. Forty ul of liquid were transferred from each well
to corresponding wells of a 96 well ELISA plate and read on a Titertek ELISA
reader (Cambridge Technology, Bedford MA.) at 590 nm. Using this procedure
it was demonstrated that sera from mice subcutaneously vaccinated with L4 had
significantly higher titers to the worm's surface than sera from naive mice
or mice immunized by the oral route. This system should more closely reflect
in vivo antigen-antibody interactions than using killed antigen preparations.

50.

INTESTINAL CELL GRANULES IDENTIFIED AS ZINC SULFIDE IN ANCYLOSTOMA CANINUM
DURING THE ADULT FORM OF THE NEMATODE. A.J. GIANOTTI AND D.T. CLARK.
PORTLAND STATE UNIVERSITY. PORTLAND, OR 97207.

The adults of the Superfamily Ancylostomatoides possess in their
intestinal cells an accumulation of birefringent crystals which are
surrounded with a thin layer of protein. The cytoplasmic inclusions
appear within the the anterior portion of the intestine during the first
months of infection, but their prevalence spreads to the more distal cells
during prolonged infection.

Two methods were utilized to identified the crystals as ZnS. The
crystalline structure of the granules was analyzed by x-ray powder
diffraction. Elemental analysis was preformed utilizing energy dispersive
spectrometric analysis (EDS) in the form of a microprobe associated with
a scanning electron microscop. These two methods provided results which
unequivocally support the ZnS identification when compared with known
samples of ZnS, and its naturally occuring beta form, sphalerite. These
results concur with those of Rogers (1940) and Clark (1956) who found zinc
sulfide in several species of Strongylus.

The granules seem to be the product of a detoxification function; binding
excess metallic ions, primarily zinc, with sulphydryl groups to
counteract the toxicity of the metallic ions.
A STUDY OF POSSIBLE INTERFERENCE BETWEEN ANTHELMINTIC TREATMENT SUPERIMPOSED ON LOW DOSE SUPPLEMENTATION WITH ANTIBIOTIC WHEN FED TO PIGS. A.R. DONOGHUE*, N. NONAKA, B. THACKER, T.W. SCHILLHORN VAN VEEEN. CLINICAL PARASITOLOGY LABORATORY, MICHIGAN STATE UNIVERSITY, E. LANSING, MI 48824

Drug interactions have been recognized in human medicine. In mammals, the effects of different drugs when given concurrently are not necessarily predictable on the basis of knowledge of the individual effects. Little research has been devoted to this topic in veterinary medicine. In many food animal production systems, antibiotics and anthelmintics are frequently given in the feed, and in order to facilitate feed management, it would be advantageous to give the two together. The objective of the present experiment was to determine the possible interaction between lincomycin (LI) or tylosin (TY) and fenbendazole (FBZ). One hundred twenty crossbred pigs were experimentally inoculated with Ascaris suum and Trichuris suis. Forty-two days later the pigs were divided into the following 12 groups and treated according to the following protocol: (1) control; (2) FBZ 3mg/kg-3 days; (3) FBZ 1.5mg/kg-6d; (4) FBZ 0.75mg/kg-12d; (5) LI+FBZ 3mg/kg-3d; (6) LI+FBZ 1.5 mg/kg-6d; (7) LI+FBZ 0.75mg/kg-12d; (8) LI 200g/T; (9) TY+FBZ 3mg/kg-3d; (10) TY+FBZ 1.5mg/kg-6d; (11) TY+FBZ 0.75mg/kg-12d; (12) TY 100g/T. Following treatment, pigs were necropsied and total parasite counts performed. Groups 1, 8, and 12 had higher A. suum and T. suis counts than all other groups (P<0.05). Also, group 4 had a higher T. suis count (P<0.05). It appears from these data that LI and TY do not interfere with the efficacy of fenbendazole. In an additional study, 36 pigs were divided into the following treatment groups: (A) control; (B) TY 100 g/T + FBZ 3 mg/kg-3d; (C) TY + FBZ 0.75 mg/kg-12d. Pigs were necropsied at 12, 24, 72 hours, and 6 days following completion of treatment. Liver samples were obtained and analyzed for residues of TY + FBZ. The residue patterns of both compounds did not seem to influence each other.

DETECTION OF IVERMECTIN IN PLASMA AND TISSUE SAMPLES OF TREATED ANIMALS BY RADIOIMMUNOASSAY. M. ELKASSABY, C. MARSCHKE*, T. GEARY*, T.W. SCHILLHORN VAN VEEEN AND J.F. WILLIAMS, DEPARTMENTS OF PATHOLOGY AND MICROBIOLOGY AND PUBLIC HEALTH, MICHIGAN STATE UNIVERSITY, EAST LANSING, MI, AND *THE UPJOHN COMPANY, KALAMAZOO, MI 49001

Conventional approaches to the detection of ivermectin in biological samples generally involve high performance liquid chromatography (HPLC) and fluorescence detection of the analyte after lengthy and labor intensive organic solvent extraction procedures have been applied. To facilitate studies on the uptake and distribution of ivermectin in treated subjects a radioimmunoassay for this drug was evaluated. Antibodies to ivermectin-carrier conjugates were prepared in rabbits and a competitive inhibition assay configured based on antibody binding to a H3 labelled ivermectin standard and a chemically purified unlabeled ivermectin preparation. Sensitivity to 2 ng/ml was achieved. Assay validation was conducted using data analysis on a modified Faden and Rodbard computer program. Recovery rates on spiked samples and correlation characteristics with HPLC analyses were determined using plasma, fat and muscle tissues from normal and dosed bovids. Plasma samples (50 ul) were processed for RIA without extraction; a variety of simple lipid solvent procedures were applied to optimize extraction of ivermectin from 100 mg tissue samples. Correlation coefficients on the order of +0.99 were obtained between RIA and HPLC in "blinded" comparisons of data from series of samples from treated animals. The RIA procedure appears to offer enhanced convenience and speed in the detection of blood and tissue residues of ivermectin, with an accuracy and sensitivity comparable to HPLC techniques. (This work was supported in part by NIH grant AI-16312)
PHARMACOKINETIC PROFILES OF ALBENDAZOLE METABOLITES AFTER INTRARUMINAL AND SUBCUTANEOUS ADMINISTRATION OF NETOBIMIN IN SHEEP. C. E. LANUSSE* AND R.K. PRICHARD. INSTITUTE OF PARASITOLOGY, McGILL UNIVERSITY, Qc, CANADA H9X 1C0

Netobimin (NTB) is a broad spectrum pro-benzimidazole anthelmintic compound. Its anthelmintic efficacy depends on its conversion to albendazole (ABZ) metabolites in the host. The pharmacokinetics and profile of urine excretion of NTB and its metabolites were investigated after intraruminal (IR) or subcutaneous (SC) administration in sheep at 20 mg/kg. Plasma and urine concentrations of NTB, ABZ, albendazole sulphoxide (ABZSO) and alberidazole sulphone (ABZS02) were measured serially over a 120-hour period by HPLC. NTB showed a similar pharmacokinetic profile after both treatments, with rapid absorption and detectable plasma levels up to 12 h post-treatment. ABZ was only detected in plasma after IR treatment. Low ABZSO and ABZS02 plasma concentrations were detected between 6-8 and 30 h after SC administration. High levels of these metabolites were detected from 0.5 up to 96 h after IR treatment, resulting in Cmax and AUC values significantly higher (P<0.05) than after SC administration. In both treatments, ABZSO Tmax (17.5 and 10 h for IR and SC, respectively) was achieved earlier than ABZS02 Tmax (25.5 and 11.5 h for IR and SC treatments, respectively). The percentage of total dose excreted by urine was 17.0 (IR) and 8.1 (SC). We conclude that there is a higher level of NTB conversion to ABZ metabolites after IR compared with parenteral administration. Research at the Institute of Parasitology is supported by NSERC and FCAR.

TARGET ANIMAL SAFETY STUDIES WITH ALBENDAZOLE. C. R. MILLER*, V. J. THEODORIDES, H. H. BIRKHEAD AND I. W. DALY1, SMITHKLINE BECKMAN ANIMAL HEALTH PRODUCTS, WEST CHESTER, PA 19380 AND BIO/DYNAMICS, INC.1, EAST MILLSTONE, NJ 08873

Albendazole is a broad spectrum anthelmintic activity in animals and man. Single doses of 75, 106, 150 or 300 mg/kg were administered orally to groups of four cattle. The NOEL was 75 mg/kg. Albendazole was administered to cattle for 5 days at doses of 7.5, 25 or 40 mg/kg. No overt signs of toxicity were observed in any of the animals. Groups of 27 cows received multiple oral doses of 25 mg/kg either on days 7 and 14 of gestation or on days 21, 31, 41, 51 and 61 of gestation. An additional group of 27 cows received 25 mg/kg ca 30 days prior to calving. The pregnancy rates after first mating were 85% for controls, 63 for cows dosed twice on days 7 and 14 and 85% for cows treated on days 21, 31, 41, 51 and 61 of gestation. All calves were normal. In a second study 90 heifers received a single dose of 25 mg/kg on either day 7 or 14 of gestation. The conception rates were 75% for controls, 61% for heifers treated on day 7 of gestation and 8% for heifers treated on day 14 of gestation. A group of 71 heifers received 15 mg/kg of albendazole 7 and 14 days after breeding. The conception rates were 72% for controls and 61% for the treated heifers. Bulls dosed with 22.5 mg/kg developed no adverse effects on spermatozoal production, lipido or testosterone levels.

In sheep albendazole was administered as a single oral dose of 18.8 to 500 mg/kg. Results indicated that the NOEL was 37.5 mg/kg. The LD50 was 100 mg/kg. Albendazole 7.5-30 mg/kg was administered to a large number of pregnant ewes, 7 to 43 days after breeding or under field conditions. Abnormal lambs were born to ewes treated on days 7, 17 and 21 of gestation; the NOEL was 11.4 mg/kg.
55.

HUMAN SAFETY STUDIES WITH ALBENDAZOLE. V. J. THEODORIDES*, I. W. DALY†, P. KRAEER, R. WANG AND C. J. DI CUOLLO. SMITHKLINE BECKMAN ANIMAL HEALTH PRODUCTS, WEST CHESTER, PA 19380 AND BIO/DYNAMICS INC.†, EAST MILLSTONE, NJ 08873

Albendazole has broad spectrum anthelmintic activity in animals and man. The oral LD_{50} in guinea pig, hamster, mouse, rabbit and rat was 900, 10,000, 3,000, 500 and 1320 mg/kg, respectively. In 13-week studies, no toxicity was observed at 30 mg/kg in dogs and rats and 20 mg/kg in mice; at higher doses in rats, transitory leukopenia and reduced testicular size were observed. In Beagle dogs after six months oral exposure at 5, 30 or 60 mg/kg, no effects were seen in the low dose group, only the white blood cell count reduced in the middle-dose group. Blood dyscrasias, depressed body weight and reduced bone marrow cellularity were seen in the high-dose group. In mice, rabbits and rats the no teratogenic effect dose levels were 30, 10 and 5 mg/kg. When rats were dosed for three generations at 30, 75 or 150 ppm in diet, the high dose group effects were decreased F_0 gestation length, and reduced F_1 pup survival. There was no evidence of carcinogenic activity for male and female mice dosed for 25 months with 25, 100 and 400 mg/kg/day, and male and female rats dosed for 28 months with 3.5, 7 and 20 mg/kg/day. In the Ames and CHO cells mutagenicity assays there was no genotoxicity. The residues in edible tissues at the suggested withdrawal time are very low and the bound residues are only slightly bioavailable.

56.

PHARMACOLOGY OF BENZIMIDAZOLE RESISTANCE IN HAEMONCHUS CONTORTUS.
G. W. LUBEKA* AND R. K. PRICHARD. INSTITUTE OF PARASITOLOGY, MCGILL UNIVERSITY, QC, CANADA H3X 1C0

The binding of tritiated mebendazole ([^3]H)[MBZ] and oxibendazole ([^3]H)[OBZ] to crude tubulin supernatants prepared from eggs, larvae or adults of thiabendazole susceptible (S) and thiabendazole resistant (R) strains of H. contortus has been examined. The R strain bound less drug than the S strain for all the three stages. Two receptors, a high affinity or specific binding (SB) and a low affinity binding (LAB) receptor, could be distinguished. The association constant (Ka) and maximum binding (Bmax) at infinite ligand concentration (i.e., the density of binding sites per mg protein) were calculated for SB by iterative curve fitting using the computer programme LIGAND. The Ka was similar for S and R but the Bmax of R was much less than that of S. Thus resistance was shown to be due to the loss of the SB receptors. Resistance did not affect LAB but caused a greater loss of SB for [^3]H)[OBZ than for [^3]H)[MBZ]. The Ka of SB was of the same order of magnitude (x 10^7 M^{-1}) for eggs, larvae and adults but eggs contained many more receptors per mg protein than the other stages. Research at the Institute of Parasitology is supported by NSERC and FCAR.
OVICIDAL EFFICACY AND ULTRASTRUCTURAL MODE OF ACTION OF FENOXYCARB ON FLEA EGGS. A.A. MARCHINTONDO* AND J.L. RINER, FERMENTA ANIMAL HEALTH COMPANY, KANSAS CITY, MO 64190; D.E. SONENSHINE AND K.F. ROWE, PROFESSIONAL LABORATORY, CAREPAKE, NC 27926; AND J.H. SLUSser, EASTERN VIRGINIA MEDICAL SCHOOL, NORFOLK, VA 23501.

Fenoxycarb, a MAAG Agrochemicals compound, is a non-neurotoxic insecticide which expresses its activity as an insect growth regulator. Household and pet products containing this highly photostable compound are currently under development by Fermenta Animal Health Company for the control of fleas. Like other insect developmental inhibitors, the activity of fenoxycarb is achieved through mimicry of the juvenile hormone of the insect. Typically, this results in the disruption of metamorphosis and prevents the final molt, pupation and emergence of new fleas. In addition to this mode of action, fenoxycarb also exhibits a marked residual activity due to an inhibitory effect on the hatching of flea eggs. Fenoxycarb applied to carpeting, which was seeded repeatedly every 28 days with 100 flea eggs in rearing medium, provided 100% control of adult flea emergence for greater than 5 months. Flea eggs recovered from fenoxycarb-treated carpeting appeared to be discolored and partially collapsed. Transmission electron microscopy of the embryonic and post-embryonic development of flea eggs exposed to fenoxycarb serves to document the inhibitory effects and ultrastructural changes responsible for the ovicidal activity of fenoxycarb.

ANTIGENIC DIVERSITY AMONG 13 GEOGRAPHIC ISOLATES OF EIMERIA MAXIMA
WILLIAM L. CURRENT, LILLY RESEARCH LABORATORIES, INDIANAPOLIS, IN 46285

Eimeria maxima is recognized as the most immunogenic of the coccidian parasites in chickens. Studies in our laboratory have shown that young (<one week old) chickens readily develop immunity to E. maxima. In order to survive, it seems reasonable that the parasite would have evolved mechanisms to readily alter antigens that are recognized by the host and that this survival strategy would result in the existence of distinct antigenic types within broiler houses. To determine the number of distinct antigenic types that exist in 15 isolates (cloned by single oocyst inoculation) of E. maxima obtained from broiler houses in different poultry producing regions of the US, two battery studies, involving 3060 broiler chickens, were conducted. In the first study, groups of 15 (5 birds/cage, 3 cages) one-day-old broiler chickens were immunized by oral inoculation of 2,000 sporulated oocysts of one of 8 different E. maxima isolates. On day 10, groups of immunized and coccidia-free, age-matched controls were challenged with 50,000 oocysts so that each isolate was tested for its ability to immunize against itself and the other 7 isolates. Total oocyst production on days 5-7 after challenge revealed 4 distinct antigenic types, 2 each containing 3 isolates and 2 each containing 1 isolate. Immunization followed by challenge with any isolate of the same antigenic type resulted in greater than 75% reduction in oocyst production (compared to non-immunized controls), whereas immunization followed by challenge with any isolate of a different antigenic type resulted in less than 25% reduction. Similar results were observed in lesion scores. Antigenic types were not readily distinguished on the basis of weight gain and feed-to-gain ratios. In the second study, using a similar design, one representative isolate from each of the 4 antigenic types was used to determine the antigenic type of 5 additional E. maxima isolates. Three of the 5 isolates were antigenically similar to a single isolate from the first study that comprised a distinct antigenic type, one was antigenically similar to another antigenic type already containing 3 isolates, and the fifth isolate was different from all of the others tested so that a single immunizing infection resulted in a low degree of resistance to homologous (same isolate) challenge (<25% reduction in oocyst production). These studies demonstrate that there are at least 4 distinct antigenic types of E. maxima in the 13 isolates tested and suggest that a multivalent rather than a monovalent vaccine may be more effective in controlling E. maxima.

Previous studies indicate that host genetics can influence the number of gastrointestinal nematode eggs in the feces (epg) of calves. To delineate the factors influencing epg values, calves were sampled at monthly intervals from April until weaning in September. The first significant (p<0.05) effect of the sire on calf epg values was in July. Worm recoveries from sentinel calves show that March to June transmission is restricted to Ostertagia ostertagi, Cooperia onchophora and Nematodirus helvetianus. Haemonchus placei and Oesophagostomum radiatum reach substantial levels by August begins and the effect of sire on epg values disappears at this time. As such, the observed regulation of epg values may be either species-specific or dose dependent. Based upon their epg profile, excess bull calves were treated to remove existing worms and challenged with 2 x 10^5 infective larvae of O. ostertagi. Calves with a low epg phenotype were found to harbor similar numbers of worms when compared to calves of a high epg phenotype, but, the number of eggs produced per worm by the high epg calves was approximately double that of low epg animals. Preliminary studies do not show a significant correlation between bovine leucocyte antigen (BoLA) class I haplotypes and epg values but infer that 2 haplotypes may be associated with high epg values.

SLA^a MINIATURE SWINE REACT AGAINST TRICHINELLA SPIRALIS ENCRYPTED MUSCLE LARVAE. K.B. MADDEN*, J K. LUNNEY, AND K.D. MURRELL. HELMINTHIC DISEASES LABORATORY, LPSI, ARS, USDA. BELTSVILLE, MD 20705.

Inbred miniature swine of the SLA^a/a (aa) phenotype reduce the number of encysted Trichinella spiralis muscle larvae (ML) established from a primary inoculum of 300 ML, upon secondary challenge with 10,000 ML (p<0.0003). To further elucidate the genetic basis and kinetics of the observed anti-encysted ML reactivity, responder aa and nonresponder dd, as well as the F_1 ac and ad. pigs received a primary and/or challenge inocula as above. At weeks 1, 2, 3.5, and 6 weeks post-challenge, the tongues and diaphragms of individual pigs were digested to determine ML burdens. By week 2 post-challenge, significant differences in the ML burdens of primary-infected dd versus aa and ac pigs were observed (p<0.01). After challenge, aa and ac pigs exhibited reduced encysted ML burdens when compared with unchallenged aa and ac pigs (p<0.002). In vitro analyses of antibody reactivity to, or lymphocyte blastogenesis induced by, T. spiralis antigens have revealed no differential responses unique to the aa pig. However, preliminary histologic data indicate that responder aa pigs exhibit a marked eosinophilia surrounding encysted ML at 2-3 weeks post-challenge. Studies are underway to determine which loci and which parasite antigens stimulate this novel reactivity against the encysted ML of T. spiralis, a stage previously considered to be resistant to host immune responses, and refractory to moderate anthelmintic therapy.
Little is known about the development of acquired immunity to parasitic nematodes in neonatal pigs, yet immunizations during this phase of growth have practical advantages in most management schemes where piglets are handled and infectious diseases are a problem. Experiments comparing the level of protective immunity to a challenge inoculation with A. suum eggs of piglets maintained for 3 weeks on a parasite contaminated dirt lot versus piglets maintained in an Ascaris-free environment in confinement showed reduced lung larval recoveries in the exposed pigs. An anamnestic antibody response was observed in exposed piglets following challenge. Immunity was long lived because parasite exposed piglets that were moved to an Ascaris-free environment had >60% protection against a challenge inoculation given 7 weeks after relocation. Additional experiments demonstrated that as little as 1 day of exposure of neonates on a contaminated dirt lot induced a protective response to a challenge inoculation given 3 weeks later. These experiments suggest that early immunization of neonatal pigs against A. suum induces significant protective immunity and could be a useful component of an integrated control scheme against Ascaris infection.

The murine nematode Heligmosomoides polygyrus is a common model for trichostrongyle infections of man and livestock, and has been used extensively in research aimed at developing vaccines for this family of parasites. Levels of immunity induced in such research often have been encouraging, but the methods used to generate immunity are frequently cumbersome or cause pronounced host mortality. Most vaccinating routines which protect against virulent challenge by H. polygyrus have employed the infective third stage larvae (L3) even though it is recognized that the tissue dwelling fourth stage larvae (L4) are the most immunogenic. We used the method of Ey et al. (Exp. Parasitol., 52:69-76) to isolate L4 larvae of different ages from the small intestinal wall of infected mice and implanted 5 or 10 of these larvae subcutaneously as vaccine in naïve BALB/c mice, 10 to 12 weeks old. Four weeks after immunization the mice were challenged orally with 30 H. polygyrus L3. The mice were killed 21 days later and their worm burdens quantified and compared to those of unvaccinated controls. The poorest protection was obtained using either 2 day old or 8 day old L4 (~60% in comparison to controls) whereas the best protection was obtained using 6 day old L4 (98.4% for 5 L4, and 100% for 10 L4). These results suggest that introduction of L4 may be an efficient means to immunize against many trichostrongyles, especially those with a significant tissue phase in the host gut wall.
A PRELIMINARY STUDY ON VACCINATION OF RHEUSUS MONKEYS AGAINST AMEBIASIS. HEMANT KUMAR* AND SOHAIL AHMAD. DEPARTMENT OF MICROBIOLOGY, J.N.MEDICAL COLLEGE, ALIGARH MUSLIM UNIVERSITY, ALIGARH - 202 002. INDIA.

Attempts were made to immunize rhesus monkeys (Macaca mulatta) with soluble extracts of whole Entamoeba histolytica prepared from an axenized strain, NIH:200. Animal immunizations through intraperitoneal routes were carried out with amebic extracts mixed with beta 1,3 glucan. The induction of protective immunity was demonstrable by a corresponding enhancement of humoral and cell mediated immune responses. A peak IHA titre was recorded in the fourth week following immunizations. The vaccinated monkeys were challenged with an amebic strain of proven pathogenicity. Immunizations with antigen-glucan combination conferred total protection to the monkeys, as they appeared completely normal until 120 days post infection. The expression of protective immunity was adjuvant dependent as control monkeys inoculated with antigen alone, or glucan only, showed typical disease symptoms soon after challenge. At necroscopy, gross and microscopic amebic lesions were clearly seen in the challenged tissues from unprotected animals.


The biological activities of eosinophils in tissues - where they are most common - remains little understood nor investigated. We present here a model (Angiostrongylus cantonensis in August rats) where it is possible to study the degranulation of these cells in tissues and the aftermath of this event. A. cantonensis in its natural host the rat only causes significant pathology when the nematode reaches the lung, having passed through the central nervous system. At the time of release of first stage larvae there is a major degranulation of the accumulated eosinophils and a breakdown of host tissues; only minor tissue changes are seen in the earlier migration through the CNS. The measurement of serum immunoglobulin and enzyme activity in the rats parallels the tissue events; serum mediated recognition of the cuticle by eosinophils only occurs at the time of the tissue degranulation events and aryl sulphotase levels in the blood also increase at this time. These events support the contention proposed for a number of nematode diseases, including onchocerciasis, that eosinophils acting in concert with antibodies affect parasite viability and damage host tissues. The role of this pathology in the continuation of this parasite's life cycle is discussed.
65.

Pathogenesis of Filarial Lymphadenopathy in Dogs. SHOHREH MILLER*, KAZUHIDE NAKAGAKI and BRUCE HAMMERBERG. COLLEGE OF VETERINARY MEDICINE, NORTH CAROLINA STATE UNIVERSITY, RALEIGH, NORTH CAROLINA 27606.

Brugia infected dogs were used to study the pathogenesis of verminous lymphatic disease. Nine beagle dogs were infected in the left rear paw with 10 L3 of *Brugia pahangi* six times at monthly intervals. Peripheral blood lymphocytes (PBL) and lymphocyte cell suspensions of popliteal node biopsy material from noninfected and parasite infected nodes were tested before and 3 days after perfusion with parasite excretory/secretory (ES) products in an in vitro blastogenesis assay. Strength of parasite-specific blastogenic response varied among dogs and between various lymphocyte population within the same dog, while all PBL and node samples showed adequate responses to mitogens. In most animals, the infected node revealed a higher lymphocyte blastogenic response to parasite antigens than the noninfected node. The perfusion of popliteal nodes with ES products significantly suppressed parasite-specific lymphocyte blastogenic response. (Supported by NIH grant # 860574).

66.


Cattle infested with *Hypoderma lineatum* develop specific humoral antibodies and a delayed hypersensitivity reaction to purified *H. lineatum* proteins. The peripheral blood lymphocytes (PBL) from cattle vaccinated with purified *H. lineatum* proteins and infested with *H. lineatum* respond strongly to Hypodermin A proteins when analyzed by blastogenic assays. To study the kinetic development of the T-cell response, 5 calves were vaccinated with native Hypodermin A (NHYA), 5 calves were vaccinated with denatured and reduced Hypodermin A (DRHYA), and 5 calves were unvaccinated. All calves were then infested with *H. lineatum*. The PBL were harvested biweekly during the vaccination period and biweekly for 2 months and then monthly during the infestation period and subjected to blastogenic analysis. Two of the calves vaccinated with NHYA and 2 of the calves vaccinated with DRHYA developed a positive response (SI > 2.0) to the NHYA antigen during the vaccination period. Positive responses to NHYA antigen were noted at various times during the infestation period in 4 of the NHYA vaccinated calves; in 5 of the DRHYA vaccinated calves and in 3 of the unvaccinated control calves. Positive responses to the DRHYA antigen were noted in all of the calves of all 3 groups at various times during the infestation period. The positive responses appeared stronger in the DRHYA vaccinated calves to both antigens tested during the infestation period. These results indicate that a T-cell response does develop in vaccinated and infested cattle to Hypodermin A.

Eight bovine hearts affected with eosinophilic myositis (EM), two unaffected hearts, and heartblood samples were collected at slaughter. Histologically, Sarcocystis cruzi was identified in all hearts except that of a newborn calf collected as a negative control.

Radioimmunoassay was used to determine Sarcocystis-specific IgG and IgE titers. Sarcocystis-specific IgG titers were 1:1280-1:2560 in EM affected cattle and 1:640-1:1280 in nonaffected cattle. Sarcocystis-specific IgE titers were all 1:640-1:1280.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis/Western blot analysis was used to compare antigen extracts and serum samples from EM affected vs nonaffected cattle. Eighteen bands, 22-215 kD, were consistently detected on blots using anti-bovine IgG. Seven bands, 37, 44, 53, 57, 94, 113, and 215 kD, were also consistently detected on blots using monoclonal anti-bovine IgE. One band, 61 kD, was consistently detected on IgE blots but only occasionally recognized on IgG blots. Sixteen protein bands were detected in silver-stained gels of S. cruzi-negative, newborn calf antigen, but none were recognized on Western blots. No consistent differences were found among antigen extracts or among serum samples from EM affected vs nonaffected cattle on silver-stained gels or Western blots.

MURINE NEUTROPHIL-MEDIATED KILLING OF SCHISTOSOMA JAPONICUM DURING CULTIVATION IN VITRO. J. CHENG, X. HUA*, Y. LI AND C. KONG. HUBEI MEDICAL COLLEGE, WUHAN, P.R. CHINA.

Killing effects of murine peritoneal neutrophils in the presence of immune sera and/or complement on mechanically transformed schistosomula were observed. The neutrophils alone caused no damages on the schistosomula, but killing ability of the neutrophils supplemented with immune sera and/or complement was significantly increased. The results suggest that neutrophils and serum immune factors were synergic in killing schistosomula. Additionally, killing effects of neutrophils from both normal and infected mice and susceptibility of schistosomula at different ages were compared respectively. There was no significant difference between killing ability of neutrophils from normal mice and those from infected ones. The susceptibility of 24 hour's old schistosomula to neutrophil-mediated killing was very significantly higher than those of 0 hour old ones. The mechanism of neutrophil-mediated killing of the schistosomula is discussed and the standardization of evaluation of cellular immunity to schistosomula is suggested.
69.
ULTRASTRUCTURAL OBSERVATION OF ANTIBODY- AND COMPLEMENT-DEPENDENT NEUTRAPHIL-MEDIATED DAMAGE TO SCHISTOSOMULA OF SCHISTOSOMA JAPONICUM IN VITRO. X. HUA*, J. CHENG, AND Y. LI. HUBEI MEDICAL COLLEGE, WUHAN, P.R. CHINA.

Schistosomula of Schistosoma japonicum were incubated with murine peritoneal neutrophils in media supplemented with or without immune sera and/or complement for 72 hours. The morphological change of the parasite was observed under scanning electron microscopy. It was noted that much more neutrophils adhered to the cercariae tails in the presence of the immune serum and complement than those to schistosomula. It was seen that neutrophils adhered to the surface of the schistosomula and then lysed the tegument to form a typical crater-like structure. However, neutrophils alone did not cause any damage to the schistosomula. The role of neutrophils in resistance against schistosomulum invasion is discussed.

70.
POTENTIAL VECTORS OF BLUETONGUE VIRUSES IN MIDDLE AMERICA. E.C. GREINER*, E.J. HOMAN, E.P.J. GIBBS, T.M. YUILL AND C. BARRETO. UNIVERSITIES OF FLORIDA AND WISCONSIN, GAINESVILLE, FL AND MADISON, WI AND OIRSA, SAN SALVADOR, EL SALVADOR

A combined study was begun in 1986 to examine the epidemiology of bluetongue viruses in the Caribbean region and Central America. Sentinel groups of young ruminants were established in Guatemala, Belize, Honduras, El Salvador, Nicaragua, Costa Rica, Panama, Jamaica, Barbados and Trinidad/Tobago. The regular bleeding of these sentinels has enabled us to follow seroconversion, make over 70 isolations of bluetongue virus from sentinels and examine fluctuations in the populations of the Culicoides fauna associated with these ruminants. The lack of the only proven nearctic vector, C. variipennis, throughout nearly the entire middle American region indicates that other species of Culicoides must be functioning as the vectors of these viruses. Evidence to date suggests C. insignis, C. pusillus and C. filariferus are the prime candidates. Pools of Culicoides from the region await attempted virus isolation presently and new procedures for detecting viral antigen are about to be tested in preserved Culicoides that were collected during the time of sentinel seroconversions. Data will be presented on the initial phases of this program from the arthropod vector perspective.
71.

A TRIBUTE TO THE DISCOVERERS OF TEXAS CATTLE FEVER. R.A. RONCALLI. MERCK SHARP & DOHME RESEARCH LABORATORIES, RAHWAY, NJ 07065

Between 1886 and 1893 four American scientists - Daniel Elmer Salmon, Theobald Smith, Fred Lucius Kilborne and Cooper Curtice - were involved, in one way or another, in a series of studies aimed at discovering the cause of Texas fever, a disease which for a long time had plagued the American cattle industry. Overall, each of the four scientists involved played a vital role in the discovery. Salmon, the first chief of the B.A.I., who in 1883-84 established the northern line of the cattle tick area, received credit for having assigned to Kilborne the responsibility for carrying out the investigations, and to Smith the task of studying the pathology of the disease. Kilborne, first alone and then in association with Smith, carried out the trials to test the hypothesis that ticks played a role in the transmission of Texas fever in northern cattle. In 1891, Smith reported that the organism producing the cattle fever was a protozoan. Curtice's major credit derived from his contribution towards the study of the life cycle of Boophilus. It was certainly most timely to have four scientists who, in spite of their different personalities and positions, and each working according to his own convictions, were able to generate a series of events, which culminated with the discovery of the nature, cause and prevention of Texas cattle fever.

72.

IDENTIFICATION AND CHARACTERIZATION OF CANDIDATE IMMUNOGENS OF BABESIA BOVIS AND B. BIGEMINA. STEPHEN A. HINES. UNIVERSITY OF FLORIDA. GAINESVILLE, FL.

The critical first step in the development of an antigenically defined vaccine against bovine babesiosis is the identification of potentially protective antigens. We have used monoclonal antibodies and immune sera to characterize surface exposed proteins of the merozoite, the blood stage that is infective for the erythrocyte and accessible to the host immune system.

Species-specific monoclonal antibodies that react with the surface of live merozoites by immunofluorescence immunoprecipitate seven major merozoite proteins of B. bovis and five major merozoite proteins of B. bigemina. Immune sera from protected cattle detect additional merozoite surface proteins of B. bovis and can be used to further characterize identified antigens. The four immunodominant merozoite surface proteins may be the best candidates for an improved vaccine against B. bovis. A 42 kDa integral membrane glycoprotein induces the highest antibody titer in protected animals and is immunoprecipitated by antibodies from two heterologous geographic isolates.
73.

CANINE EHRlichIOSIS (SYN. TROPICAL CANINE PANCYTOPENIA - TCP). Miodrag Ristic, University of Illinois, Urbana, IL 61801

Although Ehrlichia canis, the causative agent of canine ehrlichiosis has been known since 1935, it was not until 1968 that its full pathogenic potential for the dog was first recognized. It was in Vietnam during 1968-1970 that a severe outbreak of canine ehrlichiosis occurred among hundreds of U.S. military working dogs, with many of these cases resulting in death. This most severe and often fatal form of the disease was known as tropical canine pancytopenia (TCP). An organized and intensive research effort which followed at the military and university levels resulted in the development of methods for in vitro cultivation of E. canis and serologic identification of infected dogs. Over the past decade, the new means of disease detection made it possible to recognize ehrlichiosis as one of the most important canine infectious diseases in the United States, with outbreaks and isolated cases occurring in every state where the vector, the brown dog tick Rhipicephalus sanguineus, exists.

Recently, an E. canis-like agent has been incriminated as a human pathogen with disease that appears to be similar to Rocky Mountain spotted fever. Ehrlichia senetsu, on the other hand, is a strictly human pathogen. The identification of E. risticii, the causative agent of Potomac horse fever is further evidence of the importance of ehrlichiae as the cause of disease in man and animals.

74.

CLINICAL APPROACH TO FELINE DIROFILARIASIS. E.C. Hawkins. Dept of vet clin sciences, Purdue Univ, W Lafayette, IN 47907

Cats with dirofilariasis may be presented for peracute disease, chronic respiratory or GI signs, or nonspecific signs. Thoracic radiographs demonstrating enlarged pulmonary arteries and parenchymal changes often provide the first suggestion of disease. Microfilaria are absent or present in low numbers. Concentration techniques must be used for their detection. Occult tests for adult antigen are useful, but negative results can occur. Confirmation of the disease with nonselective angiography may be required.

Treatment of feline dirofilariasis is controversial. Adulticide therapy with thiacetarsamide appears to be effective but drug reactions and severe post-adulticide reactions can occur. In minimally symptomatic cats the treatment may carry more risk than the disease. Aspirin therapy is not advised in these cats. Corticosteroids may be useful in controlling signs of pneumonitis but may decrease the efficacy of adulticide therapy. Microfilaricide is only necessary in microfilaremic cats. Due to the natural resistance of the cat to infection, routine heartworm prevention is not recommended nor has it been investigated. Diethylcarbamazine and ivermectin have been used in highly endemic areas.
ANAPLASMOSIS, CURRENT RESEARCH TOWARDS VACCINATION. A.F. BARBET*, D. ALLRED, T. McELWAIN, T.C. McGUIRE+ AND G. PALMER+. UNIVERSITY OF FLORIDA, GAINESVILLE, FL 32610 AND +WASHINGTON STATE UNIVERSITY, PULLMAN, WA 99164.

We have employed a molecular approach towards development of an anaplasmosis vaccine. The goal is to avoid problems associated with current attenuated and killed vaccines and improve upon the protection obtained.

A surface antigen of Anaplasma marginale was identified by surface radiolabeling. This antigen consisted of a complex of two polypeptides, each of apparent molecular weight 105,000 in the Florida isolate. Two monoclonal antibodies which neutralized infectivity of the organism for cattle bound to an epitope on one of these polypeptides. The antigen complex was purified from infected erythrocytes and shown to be immunoprotective in cattle. We have cloned, expressed and sequenced the gene coding for each polypeptide. The gene coding for the polypeptide recognized by neutralizing monoclonal antibody contained a tandemly repeated sequence of 87 base pairs. A peptide specified by this sequence was synthesized and was recognized by the antibody in ELISA, immunoblot and radioimmunoassay. Cloning of the genes and identification of an immunoprotective epitope should allow several alternative approaches towards vaccine development and delivery.

LYMPE BORRELIOSIS. S.W. NIELSEN*, J.E. POST, S.D. WRIGHT AND E.E. SHAW. DEPT. OF PATHOBIOLOGY. THE UNIVERSITY OF CONNECTICUT. STORRS, CT 06268

This anthropozoonosis, recognized in Old Lyme, CT in 1975 is caused by a spirochete, Borrelia burgdorferi and vectored by the hard tick, Ixodes dammini and other biting anthropods. The human disease has three stages, the first, characterized by fever, malaise, fatigue, headache, and "erythema chronicum migrans." The second stage, a few weeks later, has either cardiac or neurologic signs, and the third stage, several months later, is a progressive oligoarthritis. Clinical signs resembling human Lyme disease have been recognized in dogs, cats, horses, and cattle (acute lameness, painful joints, and low grade fever). Most animals treated with tetracycline or penicillin recover within one week. Borrelia have been isolated from the urine, blood, milk, cerebrospinal fluid, myocardium, and kidney in one or more of these species. Borrelia associated lesions of synovitis, myocardiitis, and glomerulonephritis have been seen in dogs, whereas other species show less distinct lesions. The white-footed mouse (Peromyscus leucopus) is an ideal reservoir host for Borrelia by virtue of its abundance, the fact that it is frequently and heavily parasitized by both larvae and nymphs. It can become infected by contact with feces and urine containing B. burgdorferi and is able to sustain infection for many months without clinical signs or histopathological changes, and only a mild antibody response. Many large mammals including deer, fox, raccoon; dogs and cattle may act as hosts for the adult I. dammini.
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