PROCEEDINGS

AAVP

AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS

48th Annual Meeting
July 19-22, 2003
Denver, CO

and

MEMBERSHIP DIRECTORY

AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS AWARDS HISTORY

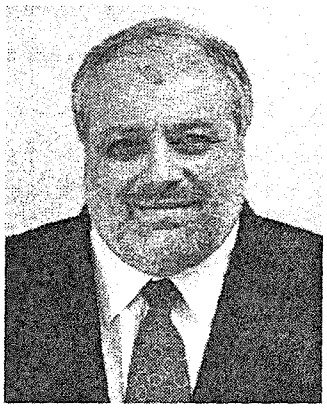
Distinguished Veterinary Parasitologists

1985	J. P. Dubey
1986	N. D. Levine
1987	E. J. L. Soulsby
1988	J. F. Williams
1989	K. D. Murrell
1990	W. C. Campbell
1991	J. H. Drudge and E. T. Lyons
1992	G. F. Otto
1993	T. R. Klei
1994	P. M. Schantz
1995	J. C. Williams
1996	T.B. Stewart
1997	J. O. D. Slocombe
1998	J. Ralph Lichtenfels
1999	R.K. Prichard
2000	E.L. Roberson
2001	B.L. Blagburn
2002	S.A. Ewing

2003 Distinguished Veterinary Parasitologist Award Winner

Louis C. Gasbarre Ph.D.

Biographical Sketch



LOUIS CHARLES GASBARRE

Louis C. Gasbarre the son of Gabriel and Eleanor Gasbarre was born in Ridgway, Pennsylvania, a very small town in northwestern Pennsylvania. He attended Indiana University of Pennsylvania where he majored in biology and received his first introduction to the field of Parasitology. During his sophomore year he enrolled in a course in Parasitology taught by Walter W. Gallati. This experience lead him to enroll in special research courses under the tutelage of Dr. Gallati for the remainder of his time at the University. The focus of these courses was a survey of the helminth parasites of small mammals in western Pennsylvania. Upon graduation, he enrolled in a MS program under the guidance of Dr. Leo Jachowski in the Department of Zoology of the University of Maryland in College Park Maryland. The focus of the MS thesis research was the induction of immunity in cestode infections in mice. These studies were interrupted by a stint as a hospital corpman in the United States Navy which afforded him the opportunity to work in a clinical Mycology laboratory for 2 years. Upon completion of the MS degree, he enrolled in a PhD program at the University of Maryland under the joint tutelage of A. James Haley of the Department of Zoology and Dr. John F. Finerty of the Laboratory of Microbial Immunity in the National Institute of Allergy and Infectious Disease of the National Institutes of Health in Bethesda, Maryland. The target of the dissertation research was the role of T lymphocytes in the elicitation of protective immune responses in mice infected with larval forms of the cestode *Hymenolepsis nana.* After completion of his graduate studies he received a Rockefeller Foundation Postdoctoral Fellowship to study at the World Health Organization Immunology Research and Training Center in Lausanne Switzerland. At WHO-IRTC he joined the laboratory of Dr. Jacques Louis and focused on the definition of T cell responses in mice inoculated with *Trypanosoma brucei* After a 2 year stay in Switzerland he returned to the USA where he was hired as a Research Associate through the University of Maryland to work with Dr. Joseph Urban at the Animal Parasitology Institute of the Beltsville Agricultural Research Center in Beltsville Maryland. While in this position he began studies of cellular immune responses in swine infected with the kidney worm, *Stephanurus dentatus* . Shortly after beginning this position a permanent position became available in the laboratory of Dr. Ronald Fayer to work on the immunobiology of *Sarcocystis* infections in cattle and horses. This led to his hire in 1981 as a Research Microbiologist in the Agricultural Research Service of the US Department of Agriculture, and he has remained in this organization ever since. After 2 years working in the *Sarcocytis* project an opportunity arose to move to studies of the immunobiology of gastrointestinal nematode infections in cattle, a topic that has remained the focus of his research efforts until the present. In the past few years this work has turned more to the genetic basis of parasite resistance in cattle and for the search for genes controlling this resistance. Recently Dr. Gasbarre was selected to act as the Research Leader for a new laboratory, the Bovine Functional Genomics Laboratory.

Dr. Gasbarre has been active in both the AAVP and the American Association of Veterinary Immunologists having served as president of both organizations. In addition, his career has extended him the opportunity to interact with a great number of outstanding scientists, practitioners, and producers both in North America and throughout the world. These scientific and social interactions have been a very important facet of the success of his career and have resulted in not only a level of productivity that could not have been obtained alone, but also in the generation of a thoroughly enjoyable research environment. He has been married for 33 years to his biggest supporter, Joyce, and has two children Christopher a dermatology resident at the Cleveland Clinic, and Julie a Program manager at a center for mentally disadvantaged adults. Without the unqualified support of these three important individuals, he would not have been able to attain the honor of being this year's AAVP Distinguished Veterinary Parasitologist.

AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS AWARDS HISTORY

Hoechst-Roussel Agri-Vet Company Graduate Student Research Award

1987	L. G. Rickard
1988	D. A. Cross
1989	S. C. Barr
1990	J. C. Parsons
1991	C. E. Lanusse
1992	D. G. Baker
1993	R. A. Cole
1994	R. M. Kaplan
1995	S. T. Storandt
1996	A. L. Willingham III
1997	C. C. Siefker
1998	R. M. O'Handley
1999	J.S. Matthew
2000*	S. Abner
2001	A. Cheadle
2002	No recipient
2003	M.G. Rossano

*As of 2000, this award was renamed the "Intervet Graduate Student Research Award".

Distinguished Service

1976	R. R. Bell
1983	T.J. Hayes
1987	N. F. Baker
1988	D. E. Cooperrider
1994	S. D. Folz
1997	H. R. Ciordia

AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS
Founded 1956

Officers 2002-2003

President	Joe Urban USDA, ARS ,BHNRC Beltsville, MD	Vice-President	Linda Mansfield Michigan State University East Lansing, MI
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1970-1972	H. J. Griffiths
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1979-1981	E. L. Roberson
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1996-1997	P. M. Schantz
1997-1998	J.C. Williams
1998-1999	L.C. Gasbarre
1999-2000	R. S. Rew
2000-2001	T.J. Kennedy
2001-2002	Anne M. Zajac
2002-2003	J.F. Urban, Jr.

AAVP 48TH ANNUAL MEETING
Westin Tabor Center Hotel

REGISTRATION
Tabor Auditorium Foyer
Saturday 19 July, 10:00-7:00 PM
Continental C Foyer
Sunday 20 July, 8:00 AM-6:00 PM
Monday 21 July, 8:00 AM-6:00 PM

SPEAKER READY ROOM
Teller Room, 7 AM-6 PM Daily
Westin Tabor Center Hotel

SOCIAL PROGRAM
Bayer Social
Saturday, July 19, 2003
Augusta Room
Westin Tabor Center Hotel
7:00-9:00 PM

Fort Dodge Animal Health Social
Sunday, July 20, 2003
Tabor Molly
Westin Tabor Center Hotel
7:00-9:00 PM

Novartis Animal Health Social
Monday, July 21, 2003
Augusta Room
Westin Tabor Center Hotel
7:00-9:00 PM

AAVP Graduate Student Luncheon/Mixer
Monday, July 21, 2003
Horace Tabor
Westin Tabor Center Hotel
Noon-1:30 PM

AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS

48TH ANNUAL MEETING SPONSORS

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*Provided Best Student Paper Presentation Awards
**Provided Honorarium for Distinguished Veterinary Parasitologist Award
***Provided in-kind meeting and annual Association support
****Provided Honorarium for Graduate Student Award
*****Provided support for the Merial Symposium

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The American Association of Veterinary Parasitologists (AAVP) gratefully acknowledges the above companies for their support and sponsorship of the 2003 annual meeting.

American Association of Veterinary Parasitologists
48th Annual Meeting
Westin Tabor Center Hotel, Denver, CO

SATURDAY JULY 19	SUNDAY JULY 20	MONDAY JULY 21	TUESDAY JULY 22
TELLER ROOM SPEAKER READY ROOM 1:00 PM - 6:00 PM	**TELLER ROOM** SPEAKER READY ROOM 7:00 AM - 6:00 PM	**TELLER ROOM** SPEAKER READY ROOM 7:00 AM - 6:00 PM	**CONVENTION CENTER** AVMA SPEAKER READY ROOM 7:00 AM - NOON
TABOR AUDITORIUM FOYER AAVP REGISTRATION TABLE 10:00 AM - 7:00 PM	**CONTINENTAL C FOYER** AAVP REGISTRATION TABLE 8:00 AM - 6:00 PM	**CONTINENTAL C FOYER** AAVP REGISTRATION TABLE 8:00 AM - 6:00 PM	
LAWRENCE A AAVP EXEC. CMTE. Board Meeting/Lunch 10:00 AM – 1:30 PM	**TABOR AUDITORIUM** Session IV: Equine Protozoal Encephalomyelitis (I) 8:00 – 10:00 AM **CONTINENTAL C** Session V: Companion Animal Parasitology (I) 8:00 – 10:00 AM	**CONTINENTAL B** Session: XI: Immunology and Immunopathology 8:00 – 10:00 AM **CONTINENTAL C** Session XII: Anthelmintics/Resistance 9:00 – 10:00 AM	**CONVENTION CENTER** AAVP/AVMA JOINT SYMPOSIUM Moderator: Joseph F. Urban 8:00 AM – Noon *" New technologies to address emerging infections in animals"* 8:00 – 8:45 AM. The use of Real-time PCR arrays in livestock Harry D. Dawson 8:45 – 9:30 AM. Parasitic and other infectious diseases of laboratory animals David G. Baker
TABOR AUDITORIUM OPENING REMARKS: President Joseph F. Urban VP/Program Chair: Linda S. Mansfield 2:00 PM - 2:15 PM			
TABOR AUDITORIUM Session I: MERIAL SYMPOSIUM *The Threat of Vectors and Vector borne Diseases* 2:15-2:45PM. The Problem with Flies Phillip Scholl 2:50-3:20PM. Mosquitos and foreign diseases. Edward Walker 3:25-3:55PM. Equine Babesiosis (piroplasmosis): Don Knowles 4:00-4:30PM. Canine leishmaniasis. Peter Schantz			
BREAK 4:30 – 5:15 PM	**BREAK 10:00 - 10:30 AM**	**BREAK 10:00 - 10:30 AM**	**BREAK 9:30 - 10:00 AM**
CONTINENTAL C Session II: ZOONOSES/EMERGING DISEASES 5:15 – 6:45 PM **TABOR AUDITORIUM** Session III: ECTOPARASITES 5:15 – 6:45 PM	**TABOR AUDITORIUM** Session VI: Apicomplexan Parasites (II) 10:30 AM – Noon **CONTINENTAL C** Session VII: Food Animal/Livestock Parasitology 10:30 AM – 12:15	**CONTINENTAL C** Session: XIII: AAVP SYMPOSIUM: What's the Future for Treatment of Parasitic Diseases? 10:30 – 12:00 PM	**CONVENTION CENTER** *"New technologies to address emerging infections in animals"* 10:00 – 10:45 AM. Molecular Tools to Evaluate Water-borne Zoonoses James Higgins 10:45 – 11:30 AM. Vaccine technology: DNA vaccines for livestock Guy H. Palmer
AUGUSTA ROOM **BAYER SOCIAL** 7:00 - 9:00 PM	**Lunch Break** Noon – 2:00 PM	**HORACE TABOR** AAVP Graduate Student Luncheon/Mixer Noon – 1:30 PM **Lunch Break** Noon – 1:30 PM	
	TABOR AUDITORIUM Session VIII: Round Table: EPM 2:00 – 3:30 **CONTINENTAL C** Session IX: Round Table: Parasitology 2:00 – 3:30 PM	**CONTINENTAL C** AAVP Business Meeting 1:30 – 3:00 PM	
	BREAK 3:30 – 4:00 PM	**BREAK 3:00 – 3:30 PM**	
	TABOR AUDITORIUM Session X: FORT DODGE ANIMAL HEALTH SYMPOSIUM 4:00 – 5:30 PM	**CONTINENTAL C** Presidential Address & Awards 3:30 - 4:30 PM **CONTINENTAL C** Session: XIV NOVARTIS ANIMAL HEALTH SYMPOSIUM *Parasitic Zoonoses – Old Diseases, New Threats* 4:40 – 5:40 PM	
	TABOR MOLLY **FORT DODGE ANIMAL HEALTH SOCIAL** 7:00 – 9:00 PM	**AUGUSTA ROOM** **NOVARTIS ANIMAL HEALTH SOCIAL** 7:00 – 9:00 PM	

AAVP PROGRAM, 2003

SATURDAY, JULY 19

I (2:15-4:30 PM) **MERIAL SYMPOSIUM - "The threat of vectors and vector borne diseases"**
Moderator: Dr. Doug Carithers

Session	Number	Speakers	Topic
I	01	P.J. Scholl	The problem with flies: myiasis, and the biology and control of stable flies
I	02	E.D. Walker	The threat of emerging mosquito-borne diseases
I	03	D. P. Knowles	Equine babesiosis (piroplasmosis): Is the regulatory impact on international movement of horses justified?
I	04	P.M. Schantz	Emergence of canine visceral leishmaniasis in North America, 2000-2003.

II (5:15 – 6:45 PM) **ZOONOSES/EMERGING INFECTIONS**
Moderators: A.A. Gajadhar and H. M. Elsheikha

Session	Number	Speakers	Topic
II	05	R. Alva	The Efficacy of FRONTLINE® Plus (fipronil/(s)-methoprene) for the Control of Aedes aegypti on Cats
II	06	L. Forbes	The food safety risk associated with non-commercial foods prepared from *Trichinella*-infected meat from marine mammals
II	07	H.M. Elsheikha (S)	A natural infection of miniature donkeys with the Sarcocystidaen Protozoa *Besnoitia bennetti.*
II	08	A.C. Rosypal (S)	Pathogenesis and immunological responses of beagles to 1-year experimental infection with an American isolate of *Leishmania infantum.*
II	09	G. Olah	Identification of an Anaplasmal protein associated with intraerythrocytic inclusion appendages.
II	10	A.S. Varela (S)	Detection of *Ehrlichia chaffeensis* in tissues of white tailed deer by PCR, in situ hybridization, and immunohistochemistry

III (5:15 – 6:45 PM) **ECTOPARASITES**
Moderators: B.L. Blagburn and C.M. McCoy

Session	Number	Speakers	Topic
III	11	P.A. Macedo (S)	Population profile of stable flies, *Stomoxys calcitrans*, captured on Alsynite traps in southeastern Nebraska– a 2-year study.
III	12	R.G. Arthur	Topically applied products to inhibit the feeding of mosquitoes on dogs.
III	13	B.L. Blagburn	Evaluation of a combination of imidacloprid and permethrin for prevention of B*orrelia burgdorferi* transmission from black-legged ticks (*Ixodes scapularis*) to *Borrelia burgdorferi* - free dogs
III	14	M.W. Dryden	IMIDACLOPRID (ADVANTAGE®) International Flea susceptibility monitoring: update 2003.
III	15	C.M. McCoy (S)	Blood feeding dynamics of cat fleas in flea feeding chambers attached to cats
III	16	A.A. Pérez de León	Evaluation technique for products against mosquitoes infesting horses.

SUNDAY, JULY 20

IV (8:00 – 10:00 AM) EQUINE PROTOZOAL ENCEPHALOMYELITIS - I
Moderator: D.S. Lindsay and J.S. Hoane

Session	Number	Speakers	Topic
IV	17	D.S. Lindsay	*Sarcocystis neurona* (Protozoa: Apicomplexa): Description of sporocysts, excystation and early in vitro development
IV	18	E.A. Kruttlin (S)	Activity of pyrantel tartrate on percoll-treated *Sarcocystis neurona* sporozoites
IV	19	M.G. Rossano (S)	Daily pyrantel tartrate does not prevent *S. neurona* infection in horses
IV	20	G.D. Gupta (S)	Development of recombinant *Sarcocystis neurona* SAG1 antigen in insect cells by using baculovirus expression system

IV	21	J.S. Hoane (S)	Modification of a recombinant antigen ELISA for detection of anti-*Neospora hughesi* antibodies in equine serum samples
IV	22	D.K. Howe	Examination of *Sarcocystis neurona* merozoite surface antigens, and their utilization for EPM diagnostics and vaccine development
IV	23	L.S. Mansfield	Evidence that horses are natural intermediate hosts for Sarcocystis neurona
IV	24	W. Saville	An equine protozoal myeloencephalitis (EPM) challenge model testing a second transport

V (8:00 – 10:00 AM) COMPANION ANIMAL PARASITOLOGY - I
Moderator: D.D. Bowman and S.E. Marley

Session	Number	Speakers	Topic
V	25	D.D. Bowman	Persistent Efficacy of Moxidectin Canine Sustained Release (SR) Injectable [ProHeart® 6] Against Experimental Infections of *Ancylostoma caninum* and *Uncinaria stenocephala* (Hookworm) Infections in Dogs
V	26	A.E. Marsh	*Cytauxzoon felis* in Missouri Ticks and Cats
V	27	R. Vasilopulos	Prevalence of and factors associated with shedding of *Giardia* spp. in domestic cats.
V	28	S.E. Little	Canine and feline clinical parasitology laboratory submissions, 1993-2002: a decade of diagnoses
V	29	C.R. Reinemeyer	A prevalence survey of antibodies to *Anoplocephala perfoliata* in horses from the Unites States
V	30	J.E. Holste	Dose selection of praziquantel and efficacy of an ivermectin (0.2 mg/kg) and praziquantel (1.0 mg/kg) combination paste against cestodes, nematodes, and bots when administered as a single oral dose to horses.
V	31	S.E. Marley	Efficacy of an ivermectin (0.2 mg/kg) and praziquantel (1.0 mg/kg) combination paste against cestodes, nematodes, and bots when administered as a single oral dose to horses.

V	32	J.E. Holste	Ivermectin/Praziquantel oral paste: Field efficacy against naturally acquired GI nematodes & cestodes of horses in North America.

VI (10:30AM - Noon) APICOMPLEXAN PROTOZOAN PARASITES – II
Moderator: E.C. Greiner and S.M. Mitchell

Session	Number	Speakers	Topic
VI	33	H.M. Elsheikha (S)	*Sarcocystis inghami* n.sp. (Sporozoa: Sarcocystidae) from the skeletal muscles of the Virginia opossum *Didelphis virginiana* in Michigan
VI	34	E.C. Greiner	Sarcocystis in free-ranging raccoons and striped skunks from Arkansas
VI	35	J.P. Dubey	Life cycle and transmission of a new species of *Besnoitia* from domestic rabbits
VI	36	C. MacKenzie	Clinical and epidemiological aspects of Besnoitiosis in donkeys
VI	37	A.A. Gajadhar	Experimental *Toxoplasma gondii* infection in grey seals, and food safety implications
VI	38	S.M. Mitchell (S)	Efficacy of Ponazuril in the prevention and treatment of toxoplasmosis in mice

VII (10:30AM – Noon) FOOD ANIMAL/ LIVESTOCK PARASITOLOGY
Moderator: R. Fetterer and Dellaney Rudolph

Session	Number	Speakers	Topic
VII	39	T.M. Craig	The effect of *Fasciola hepatica* on reproductive efficacy in prepubertal beef heifers
VII	40	P.L. Kellerhouse	In Vitro Culture of *Eimeria Tenella* in Primary Chicken Kidney Cells (PCKC) and Quantification of Parasite Growth by 3H-Uracil Uptake.
VII	41	R. Fetterer	Characterization of a 19 kDa protein conserved in *Eimeria* species
VII	42	O. Slocombe	Efficacy of 2% diatomaceous earth (DE) against gastro-intestinal parasites in calves in a clinical trial.

VII	43	R.M. O'Handley	Prevalence of *Giardia* in Adult Cattle from Prince Edward Island
VII	44	J.M. Trout	Genotypes of *Giardia* in Dairy Calves and Pastureland Wildlife

VIII Round Table – EPM Society: New advances in understanding and treatment of EPM
Moderator: Dr. Dorsey Kordick

IX Round Table: What will the field of parasitology look like in the 21st century
Moderator: Dr. Craig Reinemeyer

X (4:00 – 5:30PM) FORT DODGE SYMPOSIUM
Moderator: Dr. David Rock, Fort Dodge Animal Health

Session	Number	Speakers	Topic
X	45	C. Reinemeyer	Dose confirmation and field efficacy studies of a 2% moxidectin / 12.5% praziquantel oral gel formulation against *Anoplocephala* spp. and other equine parasites
X	46	R. D. Rulli	Tolerance and Toxicity Evaluation of Moxidectin/ Praziquantel Equine Oral Gel in Foals and Yearlings
X	47	S. Grubbs	Field evaluation of moxidectin/praziqantel equine oral gel in horses
X	48	D. Rock	CYDECTIN (moxidectin) 1% Injectable Solution for Beef and Non-lactating Dairy Cattle: Safety and Efficacy Data Summary

MONDAY, JULY 21

XI (8:00 – 10:00AM) IMMUNOLOGY AND PATHOLOGY OF PARASITIC INFECTIONS
Moderators: Joan Lunney and Helene Kringel

Session	Number	Speaker	Topic
XI	49	C. Almazan Garcia(S)	Identification of protective antigens for the control of *Ixodes scapularis* infestations using cDNA expression library immunization
XI	50	S. Almeria	Cytokine gene expression at the materno-fetal interface in pregnant cows infected with *Neospora caninum*

XI	51	R. Cook (S)	Evaluation of *Schistosoma mansoni* antioxidant enzyme DNA vaccines in mice
XI	52	J. Lunney	Immune responses controlling *Toxoplasma gondii* infection in pigs.
XI	53	C. Panuska	Lymphocyte subsets in the intestines of calves with a primary infection of *Oesophagostomum radiatum*
XI	54	D.D. Rudolph (S)	Characterization of humoral IgG response in llamas infected with *Fasciola hepatica*
XI	55	H. Kringel (S)	Localized increases in IL-4 and IgE mRNA correlate inversely with whipworm burden in pigs
XI	56	J.F. Urban	The effects of dietary probiotics on immunity to *Ascaris suum* in pigs

XII (8:30 – 10:00AM) ANTHELMINTICS/ RESISTANCE
Moderators: R. Kaplan and R. Tandon

Session	Number	Speaker	Topic
XII	57	R. Kaplan	Comparison of fecal egg count reduction test and DrenchRite© larval development assay for detection of anthelmintic resistance on goat farms.
XII	58	R. Tandon (S)	Cloning, Characterization and Phylogenetic analysis of the glutamate gated chloride channel (GluCl) gene in *Cylicocyclus nassatus*.
XII	59	R.M. Zaya	Preliminary investigations into the establishment of equine small strongyle (Cyathostominae) infections in the jird (*Meriones unguiculatus*) as a potential laboratory model
XII	60	J. Tritschler	Survey of anthelmintic usage and efficacy, preliminary Results
XII	61	C.M. Tate (S)	Susceptibility of White-Tailed Deer to Experimental Infection with Anaplasma (Ehrlichia) phagocytophilum, the Etiologic Agent of Human Granulocytic Ehrlichiosis.

XIII (10:30AM – Noon) **AAVP SYMPOSIUM: "What's the future for treatment of parasitic diseases?**

Moderator: L.S. Mansfield

Session	Number	Speaker	Topic
XIII	62	T.G. Geary	Frontiers in drug discovery: novel drug targets in Parasites
XIII	63	R.M. Kaplan	The parasites fight back: resistance mechanisms in Parasites
XIII	64	C. Mackenzie	Control Programs for helminth diseases: Experiences with Oncocerciasis and other infections

(1:30 – 3:00PM) **AAVP BUSINESS MEETING**

(3:30 – 4:30PM) **PRESIDENTIAL ADDRESS & AWARDS**

XIV (4:40 – 5:40PM) **NOVARTIS ANIMAL HEALTH SYMPOSIUM**
Moderator: Dr. David G Stansfield, Novartis Animal Health

Session	Number	Speaker	Topic
XIV	65	S.C. Parks	Nitenpyram in review: A summary of the efficacy studies conducted with Capstar® (nitenpyram) from sources around the world, with emphasis on its use in conjunction with lufenuron.
XIV	66	M. Dryden	Evaluation of the efficacy of lufenuron-nitenpyram and imidacloprid to control Flea Allergy Dermatitis and Flea Populations in a simulated home environment Study

TUESDAY, JULY 22
AVMA: ADVANCES IN VETERINARY MEDICINE
AAVP PRESIDENT'S SYMPOSIUM: New technologies to address emerging infections in animals
Moderator: President Joseph F. Urban

Session	Number	Speaker	Topic
AVMA/AAVP	67	Harry D. Dawson	The use of Real-time PCR arrays to delineate immunological and metabolic gene expression pathways in pigs and cattle.

AVMA/AAVP	68	David G. Baker	Laboratory Animal Applications: Selected natural parasitic and other infectious diseases of laboratory animals and their potential effects on
AVMA/AAVP	69	James Higgins	Molecular Tools to Evaluate the Epidemiology and Control of Water-borne Zoonotic Infections
AVMA/AAVP	70	Guy H. Palmer	Vaccine technology: Improving DNA vaccine efficacy in livestock

ABSTRACTS

01

The problem with flies: Myiasis, and the biology and control of stable flies.

PHILIP J. SCHOLL. USDA/ARS, MIDWEST LIVESTOCK INSECTS RESEARCH UNIT, UNL-EAST CAMPUS, LINCOLN, NE 68583-0938.

Flies are classified in the Family Diptera and are so named because of their common feature of having two wings as adults (di = two; ptera = wing). Myiasis is defined as the infestation of live human and vertebrate animals with dipterous larvae, which at least for a certain period of time feed on the host's living or dead tissue, liquid body-substances, or ingested food. Historically, the most important myiasis fly was the New World Screwworm, *Cochliomyia hominivorax*, which has been successfully eradicated from North America using the Sterile Insect Technique. Other important myiasis-producing flies from the Americas include the agriculturally important oestrid flies (e.g. cattle grubs, horse bots, sheep nose bots, and *Dermatobia* from South America), all of which are easily controlled by administration of macrocyclic lactones, especially the avermectins. Stable flies, *Stomoxys calcitrans*, have recently become, by most estimates, the arthropod responsible for the greatest production losses to cattle in the U.S. Stable flies were once considered to be pests of confined beef only with no long-term, effective chemical solution. Recently, probably due to increased round bale feeding, they have also been implicated as being responsible for serious losses to pastured cattle. The total annual loss attributed to this pest when considering both confined and pasture cattle has been estimated to be nearly one billion dollars.

02

The Threat of Emerging Mosquito-Borne Diseases.

EDWARD D. WALKER, DEPT OF MICROBIOLOGY AND MOLECULAR GENETICS, MICHIGAN STATE UNIVERSITY, EAST LANSING, MI 48824 WALKER@MSU.EDU

Emerging mosquito-borne diseases are caused by particular pathogens, associated with mosquitoes as biological vectors, which have recently shown increases in prevalence of infection, often increase in natural range distribution, and often an expanded host and vector species range. An extraordinary example is introduction of avian malaria, avian poxvirus, and the mosquito Culex quinquefasciatus into Hawai's, resulting in the decimation of native birds owing to vector transmission. Another real-time example is the invasion of West Nile virus into North America, leading to high rates of infection and death in corvids and other birds, and an epidemic of severe disease in humans and horses. The unfolding scenario of the West Nile virus invasion into North America will be used in the majority of this presentation as an example of an emerging, epidemic mosquito-borne disease. Spatial and temporal facets of the epidemic will be discussed, in particular the association of the virus with urban environments; the occurrence of epizootics in corvids prior to the human epidemic curve as a surveillance and monitoring tool; and the application of geographic information systems to uncover landscape associations.

03

Equine Babesiosis (piroplasmosis): Is the Regulatory Impact on International Movement of Horses Justified?

DONALD P. KNOWLES, DEPARTMENT OF VETERINARY MICROBIOLOGY AND PATHOLOGY, COLLEGE OF VETERINARY MEDICINE, WASHINGTON STATE UNIVERSITY, PULLMAN, WA.

Equine babesiosis (piroplasmosis), a disease of equids in many regions of the world, is caused by the tick-borne hemoprotozoans *Babesia equi* and *Babesia caballi*. There is concern about the risk and consequences of entry of these parasites through international movement of horses into the United States were piroplasmosis is limited to Florida and apparently not eliminated. The horse population with the United States is presumed to be entirely susceptible to piroplasmosis, therefore management safeguards against the entry and/or dissemination of piroplasmosis is mandatory. It has been known for many years that the current serological test [complement fixation test (CFT)] used internationally for regulating equine movement lacks sensitivity. Comparison of the CFT to recently developed cELISAs for serologic detection of *B. equi* and *B. caballi* supported the previous reported performance problems of the CFT. Considering the past and current policy restricting movement of infected horses into the United States recent research efforts have been directed at discovering antigens expressed by the sexual stages of equine *Babesia* sp. within its tick vector. The hypothesis being tested is that such antigens will stimulate transmission-blocking immunity.

04

Emergence of Canine Visceral Leishmaniasis in North America, 2000-2003.

P.M.SCHANTZ, CENTERS FOR DISEASE CONTROL & PREVENTION, ATLANTA, GA

Visceral leishmaniasis (VL) is a vectorborne infection of global importance caused by protozoa of the genus *Leishmania* that infect humans, dogs, and other mammals. VL is endemic in many countries of the world and, until recently, was thought to be an imported disease in North America. Beginning in the late summer of 1999, this infection was identified as the agent causing high morbidity and mortality among Foxhounds in a kennel in New York State. An investigation was initiated to determine the geographic extent, prevalence, incidence and modes of transmission of leishmaniasis in Foxhounds and other canids in North America. Serologic testing of over 12,000 foxhounds, other breeds of dogs, and wild canids in 35 states and 4 Canadian provinces was performed; several rounds of testing were offered during three years. *Leishmania* infections detected by serodiagnosis were confirmed whenever possible by culture of the organism. Foxhounds infected with *L. infantum* were identified in 21 states and 2 Canadian Provinces. Possible zoonotic infection of humans associated with infected dogs was assessed by voluntary serologic testing of persons associated with these animals. No evidence of infection was found in humans associated with infected Foxhounds or in other breeds of dogs and wild canids. Transmission appears to occur directly from dog to dog, including from pregnant dam to pups, and via blood transfusion. *Trypanosoma cruzi*, another potentially zoonotic agent of infection, was also detected in Foxhounds in many states and, because of serologic cross-reactivity, it was necessary to use specific serologic assays to distinguish infections by these two agents. Protozoa of the *L donovani* complex, agents of visceral leishmaniasis in humans, are established and widely

distributed in Foxhounds but not in other breeds of dogs or humans in North America. Transmission of the protozoal agent in North America appears limited to direct dog-to-dog mechanisms, however, if the organism becomes adapted for vector transmission by indigenous phlebotomines, the possibility of exposure of humans will be greatly increased.

05

The Efficacy of FRONTLINE® Plus (fipronil/(s)-methoprene) for the Control of *Aedes aegypti* on Cats.

R. ALVA[1]*, J.W. MCCALL[2], J. IRWIN[1], A. BOECKH[3], AND M. SOLL[3]. [1]MERIAL LIMITED, ATHENS GA, 30601, [2]UNIVERSITY OF GEORGIA COLLEGE OF VETERINARY MEDICINE ATHENS GA 30602, [3]MERIAL LIMITED, DULUTH GA

The objective of this study was to evaluate the ability of FRONTLINE Plus to control mosquitoes, *Aedes aegypti*, on cats. Fourteen Domestic Short Hair cats were included in this controlled trial. Cats were randomly assigned to two treatment groups based on descending body weight. Cats in Group 1 were not treated. Cats in Group 2 received 0.50 mL FRONTLINE Plus, administered topically, once on Day 0. On Days 1, 2, 7, 14, 21, 28 and 35, cats were sedated and exposed to approximately 100 (avg. 92-106), laboratory reared, four-day-old female mosquitoes. After 60 minutes, mosquitoes were aspirated into cartons, and the cartons were placed in an insectary. Dead mosquitoes were counted and removed approximately 24 and 48 hours after each exposure. Any remaining live mosquitoes were frozen and counted approximately 48 hours after each exposure. The geometric means of live mosquito counts were significantly lower ($p \leq 0.01$) for the FRONTLINE Plus treated group than for the untreated control group for five weeks after treatment. Treatment of cats with FRONTLINE Plus provided control of *Aedes aegypti* mosquitoes for five weeks in this study. ®FRONTLINE is a registered trademark of Merial Limited, Duluth, GA USA.

06

The food safety risk associated with non-commercial foods prepared from *Trichinella*-infected meat from marine mammals.

LORRY FORBES *, CANADIAN FOOD INSPECTION AGENCY, CENTRE FOR ANIMAL PARASITOLOGY, SASKATOON, CANADA; LENA MEASURES, FISHERIES AND OCEANS CANADA, MONT-JOLI, CANADA; DANIEL LECLAIR, MICROBIOLOGY RESEARCH DIVISION, HEALTH CANADA, OTTAWA, CANADA; ALVIN GAJADHAR, CANADIAN FOOD INSPECTION AGENCY, CENTRE FOR ANIMAL PARASITOLOGY, SASKATOON, CANADA; CHRISTIAN KAPEL, ROYAL VETERINARY AND AGRICULTURAL UNIVERSITY, DANISH CENTRE FOR EXPERIMENTAL PARASITOLOGY, COPENHAGEN, DENMARK.

Outbreaks of human trichinellosis in the arctic are frequently associated with the consumption of meat from marine mammals. The causative agent, *Trichinella nativa*, is commonly found in polar bears and walruses, with rare reports in seals and a single beluga whale. Most of the recently described human cases have been associated with the consumption of walrus meat in a traditionally prepared fermented meat dish called igunaq. Post-outbreak analysis of two such preparations using digestion techniques revealed the presence of *T. nativa* larvae, but these were non-infective after bioassay in mice and guinea pigs. Although it appeared that infectivity

decreased with time, no data existed to confirm the presence or duration of infective larvae in similar food products produced under northern conditions. In order to better evaluate the food safety risk, grey seals (*Halichoerus grypus*) were successfully infected with *T. nativa* and resultant muscle tissue was used to produce igunaq, nikku (air dried raw meat) and sausage. These foods were bioassayed over time using mice and cats. Infective *T. nativa* larvae survived for at least 5 months in igunaq, nikku and raw frozen sausage, and in poorly cooked sausage. Although the risk of infection for experimental animals decreased with time in this experiment, this may not be the case for animals and humans under field conditions. Variation in the number and age of larvae in infected meat, variable production techniques, and non-controlled storage conditions are some of the factors that could unpredictably extend the survival of larvae. These data indicate a significant food safety risk for foods prepared non-commercially using *T. nativa*-infected meat, and originate from the first experimental trichinellosis infection in seals.

07

A natural infection of miniature donkeys with the Sarcocystidaen Protozoa *Besnoitia bennetti*.

*HANY M. ELSHEIKHA, CHARLES D. MACKENZIE, JUDITH V. MARTENIUK, BARBARA STEFICEK, SHARON WINDSOR, A. MAHDI SAEED, AND LINDA S. MANSFIELD. MOLECULAR EPIDEMIOLOGY LABORATORY, DEPARTMENT OF PATHOBIOLOGY AND DIAGNOSTIC INVESTIGATION, LARGE ANIMAL CLINICAL SCIENCES, DIAGNOSTIC CENTER OF POPULATION AND ANIMAL HEALTH, CROSSROADS DONKEY RESCUE, AND DEPARTMENT OF MICROBIOLOGY AND MOLECULAR GENETICS, COLLEGE OF VETERINARY MEDICINE, MICHIGAN STATE UNIVERSITY, MI 48824, USA

Besnoitiosis is an uncommonly detected protozoal infection of equids. Fourteen miniature donkeys (*Equus asinus*) in a herd of 38 animals resident in a mid-Michigan farm presented with clinical signs of besnoitiosis. These animals showed different intensities of a similar clinically picture. Small (3-5 mm), raised, hard, nodular lesions ("pearls") were observed or palpated in the ocular sclera, the sub-mucosa of the inner lip, and the eyelids and external nares of some animals. Focal areas of rough lichenified skin with raised, small dermal nodules were present on the head, the base of the ears, the shoulders and in the inner aspects of the hind limbs as well as the perineal and perivulvar regions. These lesions did not appear to be pruritic. Examination by light and ultrastructural microscopy of isolated scleral nodules and skin biopsies showed cysts with protozoal organisms consistent in morphology with that of *Besnoitia bennetti* bradyzoites. The common histopathological change seen was the presence of many 100-200 um diameter, thick walled, *Besnoitia* cysts together with a chronic cellular response. Minor peri-vascular cellular accumulations of plasma cells, lymphocytes and scattered eosinophils were present around some vessels. Degenerating cysts, when present, were involved in granulomatous cellular responses and were most common in the animals undergoing treatment with trimethoprim and sulphamethoxazole (1.0mg/Kg and 20mg/Kg daily for 42 day); this treatment resulted in some clinical improvement. Although this infection has been reported infrequently in equids in Africa and the USA, this is the first report of a significant outbreak in a herd of donkeys. Therefore, Besnoitiosis should be considered more often in the differential diagnosis of cutaneous nodular diseases in the donkey. The presence of the distinct ocular signs (scleral pearls) together with the buccal sub-mucosal cysts and the nodular dermal lesions appear to be strong and consistent clinical indicators of the presence of this infection.

08

Pathogenesis and Immunological responses of beagles to 1-year experimental infection with an American isolate of *Leishmania infantum*.

A.C. ROSYPAL*, G.C. TROY, R. GOGAL, A.M. ZAJAC, AND D.S. LINDSAY, VIRGINIA TECH, BLACKSBURG, VIRGINIA

Previously considered an exotic disease in the United States, canine leishmaniasis caused by *Leishmania infantum* has emerged within the foxhound population in the United States. *Leishmania infantum* is the etiologic agent of visceral leishmaniasis in many areas of the world and dogs are considered a major reservoir host for human *Leishmania* infections. We isolated *L. infantum* from 3 naturally infected foxhounds from Virginia and examined the pathogenic and immunological responses of American *L. infantum* for dogs. We inoculated (IV) 8 five-month-old female purpose bred beagles with promastigotes (high dose = 2 x 10^8; low dose = 1 x 10^7) of our LIVT-1 strain of *L. infantum*. Dogs that received the high dose of promastigotes developed clinical signs associated with leishmaniasis including weight loss, skin abnormalities, ocular discharge, and polyarthritis. Promastigotes were cultured from bone marrow or lymph node aspirates by 13 weeks post inoculation (PI) from all high dose dogs. Promastigotes were cultured from lymph node aspirates from 2 dogs in the low dose at 51 weeks PI. Lymphocyte subsets (CD4+ and CD8+) were quantified by flow cytometric analysis. Seroconversion was evaluated by an indirect fluorescent IgG antibody test. These data may help to characterize the pathogenicity of American *L. infantum* and to improve diagnosis of canine leishmaniasis in the United States. Supported in part by grant DO1CA-16 from the Morris Animal Foundation to DSL and AMZ. ACR is a Morris Animal Foundation Fellow.

09

Identification of an Anaplasmal Protein Associated with Intraerythrocytic Inclusion Appendages.

GLENN OLAH*[1], GUY PALMER[2], KATHERINE KOCAN[3], MARCUS FECHHEIMER[4], TRAVIS MCGUIRE[2] AND ROGER W. STICH[1], [1]DEPARTMENT OF VETERINARY PREVENTIVE MEDICINE, THE OHIO STATE UNIVERSITY, COLUMBUS, OH 43210, [2]DEPARTMENT OF VETERINARY MICROBIOLOGY AND PATHOLOGY, WASHINGTON STATE UNIVERSITY, PULLMAN, WA 99164, [3]DEPARTMENT OF VETERINARY PATHOBIOLOGY, OKLAHOMA STATE UNIVERSITY, STILLWATER, OK 74078, AND [4]DEPARTMENT OF CELLULAR BIOLOGY, UNIVERSITY OF GEORGIA, ATHENS, GA 30602

Anaplasmal inclusion appendages are actin-laden structures that are associated with many isolates of *Anaplasma marginale*. It has been hypothesized that these structures represent actin-based motility of the pathogen in the blood meal, facilitating contact with the tick midgut epithelium prior to infection of the vector. Previous work has indicated that at least one anaplasmal protein is associated with the inclusion appendage, as has been indicated for other intracellular prokaryotic parasites such as vaccinia virus, *Listeria monocytogenes*, *Shigella flexneri*, and *Rickettsia* spp. Identification and characterization of the anaplasmal and host cytoskeletal protein components of anaplasmal inclusion appendages is required before the molecular mechanism of their formation and their biological function can be fully understood.

Two *A. marginale*-specific monoclonal antibodies that recognize inclusion appendages were used to identify a posited anaplasmal actin associated protein (AAAP). Both monoclonal antibodies were confirmed to co-localize with *A. marginale* DNA and host cell actin in infected erythrocytes, and found to recognize the same protein by 2-D gel electrophoresis of infected erythrocytes followed by Western analysis. MALDI and tandem mass spectrometry of the putative AAAP spot provided peptide sequence information sufficient for identification of an open reading frame within the *A. marginale* St. Maries isolate genome database. Work is underway to overexpress, confirm the identity and characterize the recombinant form of this protein.

10

Detection of *Ehrlichia chaffeensis* in tissues of white tailed deer by PCR, *in situ* hybridization, and immunohistochemistry.

A.S. VARELA*[1], E.W. HOWERTH[2], M. D. MURPHY[2], AND S.E. LITTLE[1]. [1]DEPARTMENT OF MEDICAL MICROBIOLOGY AND PARASITOLOGY, COLLEGE OF VETERINARY MEDICINE, UGA, ATHENS, GA 30602; [2] DEPARTMENT OF PATHOLOGY, COLLEGE OF VETERINARY MEDICINE, UGA, ATHENS, GA 30602.

Ehrlichia chaffeensis, causative agent of human monocytic ehrlichiosis (HME), is maintained in nature through a cycle involving white-tailed deer (*Odocoileus virginianus*) as a natural reservoir host and lone star ticks (*Amblyomma americanum*) as the principal vector. Following primary exposure, deer develop persistent infection and demonstrate recrudescent rickettsemia for as long as nine months. In order to determine cell and tissue tropism of *E. chaffeensis* in the deer reservoir host, 7 deer were inoculated with *E. chaffeensis*, tissues were harvested at 31 (n=1) or 85 (n=6) DPI, and then spleen, mandibular lymph node, prescapular lymph node, inguinal lymph node, and bone marrow were assayed by PCR and *in situ* hybridization; spleen and mandibular lymph nodes were also examined by immunohistochemistry. At the time of collection, at least one and as many as 5 different tissue samples from each inoculated deer had evidence of *E. chaffeensis* by PCR assay of a variable-length PCR target. However, *in situ* hybridization using an *Ehrlichia*-wide riboprobe failed to definitively identify morulae in any of the tissues evaluated from the 7 deer. Immunohistochemistry using primary polyclonal anti-*E. chaffeensis* goat antibody also failed to detect morulae in any tissue sections examined. These data suggest that the level of organism in tissues of chronic, persistently infected deer is very low, and below the detection level of microscopic examination of tissue sections. Adaptation of a real-time PCR assay used to quantify *E. chaffeensis* in cell culture for use in tissue samples may allow identification of the preferred site for persistent infection of the deer reservoir host with this pathogen.

11

Population profile of stable flies, *Stomoxys calcitrans*, captured on Alsynite traps in southeastern Nebraska – a 2-year study.

*PAULA A. MACEDO[1], PHILIP J. SCHOLL[2], JOHN B. CAMPBELL[1] AND DAVID TAYLOR[2], [1]UNIVERSITY OF NEBRASKA, LINCOLN, NE 68583, AND [2]USDA/ARS, MIDWEST LIVESTOCK INSECTS RESEARCH UNIT, UNL-EAST CAMPUS, LINCOLN, NE 68583-0938.

Stable flies, *Stomoxys calcitrans*, have long been recognized as important pests of confined cattle, inflicting a painful bite, to which cattle respond by bunching together, thereby increasing heat stress and reducing feed intake. Ranchers and veterinarians, as well as livestock entomologists, university scientists, and animal health companies concur that stable flies have, in recent years, also emerged as a serious problem for grazing cattle in pastures and rangelands of North America. The extent of movement on regional scales and corresponding breeding structure of populations of these flies in North America are unclear. In a 2-year study (2001-2002), seasonal abundance of stable flies was studied in southeastern Nebraska. Stable flies were active from April to November in both years, and were captured using Alsynite sticky traps. In 2001, total adult populations followed the typical bi-modal pattern, whereas in 2002, a tri-model pattern was observed due to an unusually high rainfall event in early August. Total numbers of captured flies per week, female/male sex ratio, recency of blood meal, and physiological age-grades of females were recorded and analyzed. Males consistently outnumbered females in a 2 to 1 ratio, and there was a negative relationship between physiological age of females and total adult population profile.

12

Topically applied products to inhibit the feeding of mosquitoes on dogs.

*R.G. ARTHER[1], S. CARROLL[2], L.R. CRUTHERS[3], R.L. SLONE[3], AND D.R YOUNG[4]. [1]BAYER HEALTHCARE LLC, ANIMAL HEALTH, SHAWNEE MISSION, KS, [2]CARROLL-LOYE BIOLOGICAL RESEARCH, DAVIS,CA, [3]PROFESSIONAL LABORATORY & RESEARCH SERVICES, CORAPEAKE, N.C., [4]YOUNG VETERINARY RESEARCH SERVICES, TURLOCK, CA

Dogs may become infected with West Nile Virus following exposure to infected mosquitoes. While the clinical significance of West Nile in dogs needs further investigation treatment options are needed for dogs exposed to mosquitoes. Two studies were conducted to evaluate the four topically applied insecticides/acaricides to inhibit the feeding of *Culex pipiens* and *Aedes aegypti*. Dogs were pre-tested to assure they were suitable for hosts for mosquitoes and allocated to treatment groups (6 dogs /group): Advantage®(Adv) 9.1% imidacloprid, Kiltix ®(Kt) 45 % permethrin, K9 Advantix™ (K9 Atx) 8.8 % imidacloprid + 44 % permethrin, Frontline® Plus (Fp +) 9.8 % fipronil + 8.8% (S)-methoprene, control (untreated). Treatments were applied per label instructions on test day 0. For mosquito challenges, dogs were sedated and placed in Plexiglass or aluminum screen chambers (~ 24" x 24"x 24"). Fifty unfed female mosquitoes were released into the chamber with a dog for one hour. Live and dead mosquitoes were then counted and examined for the presence of blood. Mosquito evaluations were conducted on test days 1, 3, 7, 14, 21 and 28/30. K9 Atx provided 96.5–100 % blood feeding inhibition by *C.*

pipiens through test day 30. FP + provided 83.3 % inhibition of *C. pipiens* on test day 1 and 0-63.5 % inhibition between days 3 and 30. K9 Atx provided greater blood feeding inhibition against *A. aegypti* than either Ad or Kt.

13

Evaluation of a combination of imidacloprid and permethrin for prevention of *Borrelia burgdorferi* transmission from black-legged ticks (*Ixodes scapularis*) to *Borrelia burgdorferi* – free dogs.

JENNIFER A. SPENCER[1], JAMIE M. BUTLER[1], CHRISTINE C. DYKSTRA[1], KIRBY C. STAFFORD[2], MARGARET B. POUGH[3], STEVEN A. LEVY[4], DAVID L. BLEDSOE[5], AND BYRON L BLAGBURN*[1]. [1]COLLEGE OF VETERINARY MEDICINE, AUBURN UNIVERSITY, AL 36849, [2]CONNECTICUT AGRICULTURAL EXPERIMENT STATION, NEW HAVEN, CT 06504, [3]CORNELL UNIVERSITY, ITHACA, NY 14853, [4]DURHAM VETERINARY HOSPITAL, DURHAM, CT, [5]BAYER ANIMAL HEALTH, SHAWNEE, KS.

Adult Beagle dogs confirmed to be free from *B. burgdorferi* exposure via IFAT testing, were randomly assigned to two groups (treated and nontreated control) of 8 animals each and housed separately in a BL-2 facility. K-9 Advantix™ was applied according to label instructions to one or several sites on the dorsal midline of all 8 treated dogs. Field caught adult *Ixodes scapularis,* with an average infectivity rate of 57.6%, were used as a source of *B. burgdorferi* organisms. One week post treatment 100 ticks per dog were placed on the backs of the dogs and encouraged into the hair coat. Serum antibody titers to *B. burgdorferi* (determined by ELISA or IFAT) were obtained for weeks 2 through 13. By week 6 all nontreated control dogs had seroconverted and all treated dogs continued to remain antibody negative. Skin punch biopsies obtained from control dogs for PCR testing were positive for *B. burgdorferi* DNA. K-9 Advantix™ is effective 7 days after treatment in the prevention of transmission of Lyme borreliosis from infected *I. scapularis* ticks to dogs.

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IMIDACLOPRID (ADVANTAGE®) International Flea susceptibility monitoring: update 2003.

M.W. DRYDEN[4*], B.L.BLAGBURN[1], D.L. BLEDSOE[2], R. BOND[6], I. DENHOLM[3], D. E. JACOBS[6], H. MEHLHORN[7], N. MENCKE [2], P. PAYNE [4], M.K. RUST[5], I. SCHROEDER[2], AND M.B.VAUGHN[2]; [1]AUBURN UNIVERSITY (AU), [2]BAYER ANIMAL HEALTH (BAH), [3]IACR-ROTHAMSTED, UK, [4]KANSAS STATE UNIVERSITY (KSU), [5]UNIVERSITY OF CALIFORNIA, RIVERSIDE (UCR), [6]ROYAL VET COLLEGE, LONDON, UK (RVC), [7]HEINRICH-HEINE UNIVERSITY, DÜSSELDORF, GERMANY.

An Imidacloprid flea susceptibility monitoring team was established in 1999 with the aim to develop and validate a bioassay to effectively monitor and document susceptibility of cat flea (*Ctenocephalides felis*) isolates collected from the field to imidacloprid. Thompson (2001) referred to this initiative as an example to illustrate the importance of basic research to safeguard the continued efficacious use of parasiticides. Thompson concluded that strategic partnerships between scientists and pharmaceutical companies are important in achieving this goal. A larval bioassay was developed, standardized, validated and agreed upon by the team as the reference

diagnostic test kit. A discriminating dose of 3 ppm imidacloprid was determined from evaluating year 2000 field isolates, which was approximately 2 times the highest LC_{95} of the control laboratory flea strains. During 2001 and 2002 this standardized bioassay was used to test field isolates collected from veterinary clinics within the USA, the UK and Germany. Over 130 field isolates were collected from the USA, UK and Germany in 2002. Fleas collected from one multi-cat household in California with limited history of flea control products indicated larval survivorship at 3 ppm and was tested further with a full dose-titration assay (0.005 ppm to 3 ppm). It was concluded that this flea population had an LD_{95} which was not higher than a baseline isolate previously tested. All 2002 field collected flea isolates were determined to be as susceptible to imidacloprid as the previously collected field isolates used as a baseline. The results obtained from this project clearly demonstrated the importance of close cooperation between the pharmaceutical industry and academia in assuring the continued efficacy of marketed drugs and insecticides. Ref: RCA Thompson (2001) The future of veterinary parasitology: a time for change? Vet. Parasitology 98: 43.

15

Blood feeding dynamics of cat fleas in flea feeding chambers attached to cats.

C.M. MCCOY[1]*, M.W. DRYDEN[2], AND A.B. BROCE[1]. [1]DEPARTMENT OF ENTOMOLOGY, KANSAS STATE UNIVERSITY, MANHATTAN, KS, [2]DEPARTMENT OF DIAGNOSTIC MEDICINE/PATHOBIOLOGY, KANSAS STATE UNIVERSITY, MANHATTAN, KS.

A modified flea feeding chamber technique was used that confined fleas but also allowed unhindered feeding directly on cats. A series of studies were conducted to find the bionomic parameters that allow for optimal feeding at 5, 60 and 240 minutes. The Drabkins reagent method was used to determine the amount of hemoglobin consumed and the hemoglobin (g/dL) count from each cat was then used to determine actual volume of blood consumed. Parameters evaluated included sex ratio, density and flea age post-emergence. As each parameter was evaluated the dynamic (i.e. sex ratio 1:2, 1:1, 2:1) that produced the steepest feeding slope was then used in each subsequent experiment. Baseline levels of hemoglobin were also tested on unfed fleas. The optical density ranged from 0.000 in a single unfed flea to 0.061 in 100 unfed fleas. Cat blood had hemoglobin optical density values ranging from 0.008 in 1 uL of blood to 0.413 in 80 uL of blood. Evaluations of male and female fleas showed that ten male and ten female fleas feeding for five minutes produced optical density values of 0.012 and 0.018 respectively. While 10 male and 10 female fleas feeding for 240 minutes produced optical density values of 0.021 and 0.068 respectively.

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Evaluation technique for products against mosquitoes infesting horses.

A.A. PÉREZ DE LEÓN*. STILLMEADOW, INC. SUGAR LAND, TX 77478

Techniques to evaluate products against mosquitoes infesting horses were developed to obtain consistent and reliable results. Each horse was tied to a stanchion located inside a chamber with vinyl net walls supported by a plastic frame. This was followed by the release of ~200 adult female *Aedes albopictus*. The infestation period lasted 60 minutes. Two infestation chambers were set up inside a vinyl tent providing a semi-controlled environment. Mosquitoes were collected in catch bottles using an insect vacuum for at least 30 minutes following completion of the exposure period. Mosquito collection resumed for at least 10 more minutes after the horse was

removed. Catch bottles were transported in a cooler with ice packs and stored frozen at the laboratory. Defrosted mosquitoes were sorted, examined under a dissecting microscope, and counted after crushing to ascertain blood-feeding. Behavioral features of the host (stomping, defecation, urination) and ectoparasite (resting on floor and surreptitious places) also decreased the number of mosquitoes recovered. A horse with 25% of the live mosquitoes recovered showing evidence of blood-feeding was considered to be an acceptable host. This system allows testing of products on horses under GLP to obtain a direct label claim for vectors of West Nile virus.

17

***Sarcocystis neurona* (Protozoa: Apicomplexa): Description of sporocysts, excystation and early in vitro development.**

DAVID S. LINDSAY*, AND J.P. DUBEY. DEPARTMENT OF BIOMEDICAL SCIENCES AND PATHOBIOLOGY, VIRGINIA TECH, BLACKSBURG, VIRGINIA AND USDA, PARASITE BIOLOGY, EPIDEMIOLOGY AND SYSTEMATICS LABORATORY, BELTSVILLE, MARYLAND

Equine protozoal myeloencephalitis is a major cause of neurological disease in horses from the Americas. Horses are accidental intermediate hosts. The structure of sporocysts of the causative agent, *Sarcocystis neurona*, has never been described. Sporocysts of *S. neurona* were obtained from the intestines of a laboratory-razed opossum fed skeletal muscles from a raccoon that had been fed sporocysts. Sporocysts were 11.3 by 8.2 μm and contained 4 sporozoites. The appearance of the sporocyst residuum was variable. The residuum of some sporocysts was composed of many dispersed granules, while some had granules mixed with larger globules. Excystation was by collapse of the sporocyst along plates. Sporozoites were weakly motile and contained a centrally or posteriorly located nucleus. No retractile bodies were present but globules about 1 μm in diameter were occasionally present in the anterior portion of sporozoites. Sporozoites contained 2 rhoptres and other organelles typical of apicomplexan zoites. Sporozoites entered host cells in culture and underwent schizogony with in 3 days. Supported by USDA/CSREES. Animal Health and Disease grant VA-137180 to DSL.

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Activity of pyrantel tartrate on percoll-treated *Sarcocystis neurona* sporozoites.

E.A. KRUTTLIN*, M.G. ROSSANO, A.J. MURPHY, R.A. VRABLE, J.B. KANEENE, H.C. SCHOTT II, AND L.S. MANSFIELD. MICHIGAN STATE UNIVERSITY, COLLEGE OF VETERINARY MEDICINE, EAST LANSING, MI 48824.

The protozoan parasite *Sarcocystis neurona* is the etiologic agent of the neurological disease equine protozoal myeloencephalitis (EPM). *S. neurona* sporocysts contain sporozoites that are released in the digestive system of the horse after accidental ingestion. Previously, we found that the asexual merozoite stage exposed to concentrations of pyrantel tartrate higher than 0.0025M ($8.91X10^{-4}$ g/ml) had no growth in equine dermal cells while those exposed to similar concentrations of the tartrate salt or media alone had significant growth. We hypothesized that the drug also has activity against sporozoites. However, methods for *in vitro* excystation do not separate sporocysts from sporozoites, which is necessary to evaluate the effects of chemical compounds on the infective stage. This study describes a new technique for separating

sporocysts from sporozoites *in vitro* and the effect of the drug against the sporozoite stage. Sporocysts were collected from opossums fed *S. neurona* infected cat tissue and excysted (Murphy and Mansfield, 1999). This material was placed onto a 60% Percoll, 40% Hank's balanced salt solution gradient and centrifuged for 1 hour at 12,000 RPM. The sporozoite solution below the colored marker bead line representing a density of 1.05 g/ml was removed, and examined microscopically. This method resulted in pure sporozoites free of intact sporocysts. Percoll purified sporozoites were treated in replicate with either pyrantel tartrate at a concentration of 0.005M or DMEM medium for 24 hours and viability assessed in an *in vitro* plaque assay (Kruttlin et al., 2001). At 3 weeks post-inoculation, many plaques were seen in all 6 wells inoculated with DMEM-treated sporozoites, while all 6 wells inoculated with pyrantel-treated sporozoites showed zero plaque growth. This preliminary *in vitro* study shows pyrantel tartrate is effective against the sporozoite stage of the parasite at the dose of 0.005M. The new separation technique is valuable for drug screening studies against the sporozoite stage of *S. neurona* to find a pharmaceutical preventative for EPM. Work supported by Pfizer Inc 71-2954.

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Daily pyrantel tartrate does not prevent *Sarcocystis neurona* infection in horses.

M.G. ROSSANO[1]*, H.C. SCHOTT I[1], J.B. KANEENE[1], A.J. MURPHY[1], E.A. KRUTTLIN[1], M.T. HINES[2], D.C. SELLON[2], J.P. DUBEY[3] & L.S. MANSFIELD[1]. [1]COLLEGE OF VETERINARY MEDICINE, MICHIGAN STATE UNIVERSITY, EAST LANSING, MI; [2]DEPT. OF VETERINARY CLINICAL SCIENCES, WASHINGTON STATE UNIVERSITY, PULLMAN, WA; [3]USDA, ARS, BARC, BELTSVILLE, MD.

Equine protozoal myeloencephalitis (EPM) is caused by the protozoan parasite *Sarcocystis neurona*. *In vitro* studies have shown that pyrantel tartrate is lethal to *S. neurona* merozoites. The purpose of this study was to determine whether daily administration of pyrantel tartrate prevents *S. neurona* infection in horses. 24 mixed breed weanling horses, confirmed negative for antibodies to *S. neurona,* were group housed at the Washington State University (WSU) Animal Resource Unit in outdoor pens and fed locally produced feeds. Horses were randomized into two groups: group A received pyrantel tartrate (Strongid C 2X) at the label dose and group B received a placebo. 10 adult horses from WSU tested negative for antibodies to *S. neurona* and were monitored as untreated, uninfected controls. Experimental horses were orally inoculated with 100 sporocysts per day for 28 days, 500 sporocysts per day for 28 days and 1,000 sporocysts per day for 56 days. Pyrantel tartrate was administered to the horses immediately following dosing with sporocysts. Blood was collected weekly and spinal fluid was collected monthly. All samples were tested for antibodies to *S. neurona*. At 134 days post-infection 10/12 horses in each treatment group were seropositive for antibodies to *S. neurona*. 4/12 horses in group A and 3/12 horses in group B had antibodies to *S. neurona* in their CSF. No significant difference was found between groups A and B for serum or spinal fluid tests (Fisher's exact test). Control horses remained seronegative. We conclude that daily administration of pyrantel tartrate does not prevent infection with *S. neurona* in horses when given according to label directions. Supported by Pfizer grant #71-2954.

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Development of recombinant *Sarcocystis neurona* SAG1 antigen in insect cells by using baculovirus expression system.

G.D. GUPTA*, A.E. MARSH. COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF MISSOURI, COLUMBIA, MO, 65211.

Equine Protozoal Myeloencephalitis (EPM) is a well-recognized neurological disease of horses in North and South America caused primarily by *Sarcocystis neurona*. Many EPM testing methods utilize culture-derived parasites as antigen for detecting cerebrospinal fluid (CSF) and serum antibodies against *Sarcocystis neurona*. The sera from exposed animals react strongly to ~29 kilodalton *S. neurona* Surface Antigen 1 (SnSAG1) by immunoblot analysis suggesting that this is an immunodominant surface antigen. We hypothesize that insect baculovirus expressed recombinant SnSAG1 antigen will be recognized by these same the antibodies. The *SnSAG1* gene was expressed as recombinant antigen in insect cells using the baculovirus system. Sera from naturally infected and experimentally immunized horses with *S. neurona* recognized the recombinant antigen and not the insect cell proteins by immunoblot analysis. Similar results were observed with CSF from confirmed *S. neurona* infected horses. In addition, the *S. neurona* monoclonal antibody 2A7-18 and sera from other *S. neurona* inoculated species (skunk, rabbit, cat and opossum) recognized this recombinant antigen. The recombinant antigen has also been tested against *Sarcocystis falcatula, Toxoplasma gondii, Neospora* and *Hammondia* antibodies to evaluate cross-reactivity. Based on these results, the developed reagents and samples are undergoing optimization for a non-species specific competitive enzyme-linked immunosorbent assay (ELISA) for detection and quantification of antibodies to *S. neurona*.

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Modification of a recombinant antigen ELISA for detection of anti-*Neospora hughesi* antibodies in equine serum samples.

JESSICA S. HOANE*[,1], MICHELLE R. YEARGAN[1], SHELBY STAMPER[1], DAVID S. LINDSAY[2], WILLIAM J.A. SAVILLE[3], JENNIFER K. MORROW[4], DANIEL K. HOWE[1]. [1]DEPARTMENT OF VETERINARY SCIENCE, UNIVERSITY OF KENTUCKY, LEXINGTON, KY 40546, [2]VIRGINIA-MARYLAND REGIOINAL COLLEGE OF VETERINARY MEDICINE, VIRGINIA TECH, BLACKSBURG, VA 24061, [3]DEPARTMENT OF VETERINARY CLINICAL SCIENCES, THE OHIO STATE UNIVERSITY, COLUMBUS, OH 43210, AND [4]EQUINE BIODIAGNOSTICS, INC, LEXINGTON, KY, 40511.

Neospora spp. are protozoan pathogens that have been associated with equine abortion, neonatal encephalomyelitis, visceral neosporosis and encephalomyelitis. To aid in the investigation of *Neospora* infection in horses, we have modified an existing enzyme-linked immunosorbent assay (ELISA) that was originally based on the *Neospora caninum* major surface antigen NcSAG1. The homologous antigen from *Neospora hughesi*, NhSAG1, was cloned and expressed as a recombinant protein in *E. coli*. The purified NhSAG1 was implemented into an antibody-capture ELISA, and the assay was tested with a set of characterized serum samples for optimization and to establish a reliable positive-negative cutoff. We are in the process of screening sample sets with the ELISA to assess the prevalence of *Neospora* exposure in horses from a variety of geographic regions (e.g., Kentucky, Ohio and Saskatchewan). Additionally, previous work has demonstrated

that antigenic differences exist between the NcSAG1 and NhSAG1 homologues. Accordingly, we are investigating whether the two complementary assays can be used to discriminate infections by the two parasite species. These recombinant ELISAs offer ease of testing large numbers of samples, and their design makes it possible to screen for anti-*Neospora* antibodies in a variety of potential host animals. It is hoped that use of these assays for examining exposure to *Neospora* will facilitate our understanding of this pathogen's importance for equine health.

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Examination of *Sarcocystis neurona* merozoite surface antigens, and their utilization for EPM diagnostics and vaccine development.

DANIEL K. HOWE*, MEAGHAN MROZ BARRETT, RAJSHEKHAR Y. GAJI, SHELBY STAMPER, JESSICA HOANE, AND MICHELLE YEARGAN, DEPARTMENT OF VETERINARY SCIENCE, UNIVERSITY OF KENTUCKY, LEXINGTON, KY 40546-0099

A search of the *Sarcocystis neurona* expressed sequence tag database revealed a family of at least four paralogous genes that exhibit clear homology to the extensive family of SAG and SRS surface antigens in *Toxoplasma gondii*. The four *S. neurona* genes were expressed in *E. coli* as recombinant proteins, and these were used to immunize rabbits and rats for the production of monospecific polyclonal antisera. Western blot analysis of Triton X-114 partitioned parasites indicated that all four proteins are membrane-associated, and surface biotinylation assays confirmed that the proteins are displayed on the merozoite surface. Multiple sequence alignments revealed that the *S. neurona* proteins possess multiple conserved cysteine residues, consistent with what has been described previously for *T. gondii* surface antigens and which are likely important for the tertiary structure of the proteins. Due to their surface localization and obvious homology to *T. gondii* surface antigens, these *S. neurona* proteins have been designated SnSAG1, SnSAG2, SnSAG3, and SnSAG4. Western blot analysis of *S. neurona* merozoites demonstrated that SnSAG1 and SnSAG4 co-migrate with one another and jointly represent the 30 kDa immunodominant parasite antigen that has been referred to previously as Sn30. The western blot also suggested that SnSAG2 is the immunodominant 16 kDa antigen referred to as Sn16. The four recombinant SnSAGs have been utilized to develop enzyme-linked immunosorbent assays, and these ELISAs are currently being used to examine antibody responses in *S. neurona*-infected horses. Additionally, we have initiated studies to investigate the role of the SnSAGs in attachment and invasion of host cells and their potential for use in the development of a protective vaccine.

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Evidence that horses are natural intermediate hosts for *Sarcocystis neurona*.

T.P. MULLANEY, M. KIUPAL, A.J. MURPHY, J.BELL & *L.S. MANSFIELD. COLLEGE OF VETERINARY MEDICINE, MICHIGAN STATE UNIVERSITY, EAST LANSING, MI 48824

Equine Protozoal Myeloencephalitis (EPM) is a neurological disorder in horses mainly caused by the protozoan *Sarcocystis neurona*. Opossums (*Didelphis spp.*) are the definitive host for *S. neurona* and shed sporocysts in feces. True intermediate hosts (cats, raccoons, skunks and armadillos) ingesting these sporocysts develop asexual stages (schizonts and subsequently sarcocysts) and transmit the parasite to opossums during predation. Horses also acquire the parasite by ingestion of

feed or water contaminated by opossum feces. However, horses are classified as aberrant intermediate hosts because the terminal asexual sarcocyst stage required for transmission to the definitive host has not been found in their tissues despite extensive efforts. Here, in a young colt with neurological disease consistent with EPM, we demonstrate both mature schizonts in the brain and spinal cord and mature sarcocysts in the tongue with genetic and morphological characteristics of *S. neurona*. Histological and Immunohistochemical findings on schizonts and sarcocysts, and EM findings on sarcocysts are identical to published features of *S. neurona* (Stanek et al., 2002). DNA from schizonts and sarcocysts of this horse produced *Sarcocystis* specific 334 bp PCR products and RFLP analysis of these PCR products showed banding patterns characteristic for *S. neurona* (Tanhauser, et al., 1999). Sequencing and alignment of this nucleic acid sequence with that of a previous *S. neurona* isolate from Michigan (Mansfield, 2001) showed 100% similarity. When compared with the sequence from a California isolate (GeneBank AF093158), the two Michigan isolates were 100% similar except for a 2 base insert. The finding of mature, intact *S. neurona* schizonts and sarcocysts in the tissues of this single horse is highly suggestive that horses have the potential to act as intermediate hosts. Further studies are needed to demonstrate Koch's postulates with repeated transfer of *S. neurona* between opossums and horses. Supported by the Diagnostic Center for Population and Animal Health, MSU.

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An equine protozoal myeloencephalitis (EPM) challenge model testing a second transport.

WILLIAM SAVILLE*, CHERYL SOFALY, STEPHEN REED, JITENDER DUBEY, MICHAEL OGLESBEE, VERONIQUE LACOMBE, ROBERT KEENE, KIM GUGISBERG, STEVE SWENSEN, RONALD SHIPLEY, YU-WEI CHIANG, HSIEN CHU, & TERRY NG. DEPARTMENT OF VETERINARY PREVENTIVE MEDICINE, COLLEGE OF VETERINARY MEDICINE, THE OHIO STATE UNIVERSITY, COLUMBUS, OH 43210

A transport-stress model where the study animals were dosed with *Sarcocystis neurona* sporocysts on the day of arrival has been developed at Ohio State University. Recent studies suggested a second transport following inoculation with *S. neurona* sporocysts would result in more severe signs of EPM. To test this hypothesis, horses were randomly assigned to 1 of 4 groups. Group 1 horses were transported 4 days after inoculation (DAI); Group 2 at 11 DAI; Group 3 at 18 DAI; and Group 4 horses were not transported a second time (controls). An overall neurologic score was determined based on a standard numbering system used by the masked examiner. All scores range from 0 to 5/5, 5/5 being the most severely affected animal. The mean neurologic scores for the Groups ranged from 2.4/5 to 3.3/5. The Group 4 horses did not have a second transport, therefore, they were compared to all other groups. Using nonparametric statistics, statistically different scores were present between Group 4 and Groups 1 and 2. There were no differences in the time of seroconversion between groups, however, there was a difference between the time of onset of first clinical signs between Groups 1 and 4, likely due to the different examination days. Differences in housing and handling at the 2 study sites may have accounted for the differences in severity of clinical signs. This study demonstrated consistent, significant clinical signs in all horses at approximately the same time period post inoculation, most severe in horses that did not experience a second transport.

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Persistent Efficacy of Moxidectin Canine Sustained Release (SR) Injectable [ProHeart® 6] Against Experimental Infections of *Ancylostoma caninum* and *Uncinaria stenocephala* (Hookworm) Infections in Dogs.

D.D. BOWMAN[1,2]*, T. ROCK[3], K. HEANEY[3], N. NEUMANN[1], M. ULRICH[1], D. AMODIE[3]. [1]CHK-R&D, STANWOOD, MI; [2]DEPARTMENT OF MICROBIOLOGY & IMMUNOLOGY, COLLEGE OF VETERINARY MEDICINE, CORNELL UNIVERSITY, ITHACA, NY; [3]FORT DODGE ANIMAL HEALTH, FORT DODGE, IA.

The efficacy of ProHeart® 6 on hookworm infections induced 3, 4, 5, and 6 months after dogs received their injections was examined. On Day 0, forty dogs were orally inoculated with 300 *Ancylostoma caninum* larvae and on Day 1 with 400 *Uncinaria stenocephala* larvae. Necropsies were on Day 21. Groups were treated on Days -173, -145, -117, and -89 or not treated. Percent efficacies for egg reductions were (geometric means): Day -89 (99.2%); Day -117 (97.0%); Day -145 (84.8%), and Day -173 (81.2%). The mean reductions in *A. caninum* worm counts using geometric means at 3, 4, 5, and 6 months were: 94.7%, 90.3%, 82.0%, and 60.2%, respectively. The mean reductions in *U. stenocephala* worm counts using geometric means at 3, 4, 5, and 6 months were: 94.6%, 85.3%, 71.6%, and 48.2%, respectively. Reductions in hookworm egg shedding remained excellent to very good throughout the full six months (99.2% reduction at 3 months, 97.0% at 4 months, 84.8% at 5 months, and 81.2% at 6 months).

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***Cytauxzoon felis* in Missouri Ticks and Cats**

PETER J. BONDY, JR., *ANTOINETTE E. MARSH, and LEAH A. COHN. COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF MISSOURI-COLUMBIA, MO.

Cytauxzoon felis, a protozoal organism, causes a mortality approaching 100% in domestic cats. The clinical disease and associated pathology are well described in previous work, yet several questions still remain concerning the life cycle of the organism. Although the adult brown dog tick, *Dermacentor variabilis*, is capable of experimental transmission, there is no information on other tick species that could harbor the organism or act as competent vectors for transmission. Additionally, sequence variation in organismal DNA is unreported. We hypothesize that in addition to *D. variabilis*, other species of hard-bodied ticks will be identified as natural carriers. Over 3,000 ticks have been collected from Missouri counties with previously confirmed cases of *C. felis*. These ticks have been speciated and will be evaluated for *C. felis* DNA using a polymerase chain reaction (PCR) designed in our laboratory. Initial results indicate that our designed primers are specific and sensitive for the DNA encoding for the small subunit ribosomal RNA (ssu-rRNA) of *C. felis*. Furthermore, amplification of *C. felis* DNA from ticks, notably *Amblyomma americanum,* from various counties has been achieved. Determined sequences of amplified products from both ticks and a confirmed infected cat have been compared to a published sequence and are 100% identical. This further supports our primers' ability to amplify *C. felis* DNA. Future work will focus on evaluating the ticks collected for the presence of *C. felis* using PCR. Amplified *C. felis* products will be sequenced and compared to a published gene sequence for potential sequence variation. Although identifying carriers is not

equivalent to identifying vector competence, it is the first step in gaining information about the natural life cycle of this organism. (Funded, in part, by Merial Companion Animal)

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Prevalence of and factors associated with shedding of *Giardia* spp. in domestic cats.

R. VASILOPULOS*, L.G. RICKARD, A. MACKIN, C. HUSTON, AND G.T. PHARR. COLLEGE OF VETERINARY MEDICINE, MISSISSIPPI STATE UNIVERSITY, MISSISSIPPI STATE, MS.

Fecal samples from 154 domestic cats of various breeds, gender, ages, and with clinical signs from northeastern Mississippi and northwestern Alabama were examined for the presence of both *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts using a direct immunofluorescent assay. The overall prevalence for *Giardia* was 20% (30/154; 95% CI = 14-27%) while the prevalence of *Cryptosporidium* was slightly lower at 16% (25/154; 95% CI = 7-22%). Twenty-three cats were concurrently shedding cysts and oocysts of both parasites, 8 cats were shedding *Giardia* cysts only, and 2 cats were shedding *Cryptosporidium* oocysts only. The prevalence of *Giardia* in apparently healthy cats was 13% (12/92;, 95% CI = 7-22%) while the prevalence in cats with any gastrointestinal signs was 29% (18/62; 95% CI = 18-42%). Factors associated with having a sample positive for *Giardia* cysts included concurrent infections with *Cryptosporidium* sp. ($p < 0.001$), the presence of diarrhea ($p < 0.001$), and age, with kittens < 6 months more likely to be shedding *Giardia* cysts than older cats ($p<0.001$). There was, however, no association between type of housing or gender and the presence of *Giardia* cysts. Our study reports a prevalence of *Giardia* in cats from the northeastern Mississippi region that is higher than that typically reported. Our study also found a strong association between *Giardia* and *Cryptosporidium* infections in cats, supporting the possibility that infection with one protozoal parasite may predispose cats to concurrent infection with the other protozoal parasite or, alternatively, that the two protozoal infections share common risk factors.

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Canine and feline clinical parasitology laboratory submissions, 1993-2002: a decade of diagnoses.

S. E. LITTLE* AND D. L. AMBROSE. DEPARTMENT OF MEDICAL MICROBIOLOGY AND PARASITOLOGY, COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF GEORGIA, ATHENS, GEORGIA, 30602-7387.

Case accession records available from all canine (n=3,045) and feline (n=1,133) fecal samples submitted to the Clinical Parasitology Laboratory of the Athens Diagnostic Laboratory, College of Veterinary Medicine, University of Georgia from January 1993 through December 2002 were reviewed. At least one parasite was detected in 23.2% (706/3,045) of fecal samples from dogs and 21.1% (239/1,133) of fecal samples from cats; 5.7% of canine fecal samples and 3.4% of feline fecal samples contained evidence of 2 or more parasites. *Anyclostoma caninum* (268/706; 38.0%), *Trichuris vulpis* (165/706; 23.4%), *Toxocara canis* (122/706; 17.3%), and *Isospora* spp. (116/706; 16.4%) were the most frequently diagnosed parasites in canine fecal samples. However, other less common parasites, including *Uncinaria stenocephala* (21/706; 3.0%), also were detected. In feline fecal samples, *Toxocara cati* (90/239; 37.7%), *Isospora* spp. (56/239;

23.4%), *Ancylostoma tubaeforme* (29/239; 12.1%), and *Giardia* (25/239; 10.5%) were the most common parasites detected. Cestodes, including *Dipylidium caninum*, *Taenia* spp., and *Spirometra,* were found in 2.7% (81/3,045) of canine samples and 4.3% (49/1,133) of feline samples examined. Intestinal parasites remain a common finding in dogs and cats despite the availability of highly efficacious treatment and control products.

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A prevalence survey of antibodies to *Anoplocephala perfoliata* in horses from the United States. C.R. REINEMEYER[1*], A.W. FARLEY[1], S.A. KANIA[2], B.W. ROHRBACH[2], AND R.H. DRESSLER[3]. [1]EAST TENNESSEE CLINICAL RESEARCH, KNOXVILLE, TN, [2]UNIVERSITY OF TENNESSEE COLLEGE OF VETERINARY MEDICINE, KNOXVILLE, TN, [3]PFIZER ANIMAL HEALTH, BARKSDALE, TX.

The 48 contiguous states were divided into 10 regions, each comprising approximately 10% (range 9.1% to 11.3%) of the total U.S. equine population. In one (TX) or two states within each region, laboratories were asked to provide serum from samples that had been submitted for Equine Infectious Anemia testing. Between August, 2002 and April, 2003, 19 laboratories each provided sera from ~160 horses (320 from TX and CA; 108 from FL), along with information about the donor's age, sex, breed, and state of residence. Sera were analyzed by an ELISA that measured IgG(T) antibodies against *Anoplocephala perfoliata* (sensitivity 81.0%; specificity 95.1%). Antibody concentrations were expressed as a percentage of the positive control titer. The regional prevalence of antibodies to *A. perfoliata* ranged from 12.7% along the Pacific coast to 95.8% in the upper Midwest, and was greater than 30% in 8/10 regions. Based on 3308 samples, overall national prevalence was 54.2%. The prevalence of antibodies was significantly less ($P<0.01$) in intact males, and in Arabians, Mustangs, and Quarter Horses compared with mixed breed horses. Seroprevalence was significantly greater ($P<0.01$) in horses older than 15 years. Differences by sex and age were attributed to variations in management, specifically to the extent of pasture exposure.

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Dose selection of praziquantel and efficacy of an ivermectin (0.2 mg/kg) and praziquantel (1.0 mg/kg) combination paste against cestodes, nematodes, and bots when administered as a single oral dose to horses.
S.E. MARLEY[1], J.E. HOLSTE[1*], D.E. HUTCHENS[2], A.J. PAUL[2]. [1]MERIAL LIMITED, DULUTH, GA, USA, [2]UNIVERSITY OF ILLINOIS, VET. MED., URBANA, IL, USA

The efficacy in horses of praziquantel (PZQ - 1.0 mg/kg bwt) combined as a paste with ivermectin (IVM – 0.2 mg/kg bwt) against cestodes, nematodes, and bots was tested when the paste was administered as a single oral dose. The effective cestocidal dose of PZQ in the IVM + PZQ combination was determined to be 1.0 mg/kg bwt. A dose confirmation study was conducted to confirm the efficacy of the IVM + PZQ combination. Animals were not treated (Control) or received IVM + PZQ paste, orally, once on Day 0. Compared to Controls, horses treated with IVM + PZQ had significantly ($p<0.05$) fewer (>99% reductions) adult *Anoplocephala perfoliata, Coronocyclus coronatus, C. labiatus, C. labratus; Cyathostomum catinatum; Cylicocyclus leptostomum, C. nassatus, C. insigne; Cylicostephanus calicatus, C.*

longibursatus, & C. minutus; *Strongylus vulgaris; Triodontophorus nipponicus;* and L2 & L3 *Gasterophilus intestinalis*, L3 *G. nasalis*; and L4 Cyathostominae genera *Cyathostomum, Cylicocyclus, Coronocyclus,* and *Cylicostephanus* at necropsy ~2 weeks post-treatment.

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Efficacy of an ivermectin (0.2 mg/kg) & praziquantel (1.0 mg/kg) combination paste against cestodes, nematodes, and bots when administered as a single oral dose to horses.

S.E. MARLEY[1*], C.R. REINEMEYER[2], J.S. HUNTER, III[1], J. IRWIN[1]. [1]MERIAL LIMITED, DULUTH, GA, USA, [2]EAST TN CLIN RES, INC., KNOXVILLE, TN, USA.

The efficacy of praziquantel (PZQ - 1.0 mg/kg bwt) combined as a paste with ivermectin (IVM – 0.2 mg/kg bwt) was tested as a single oral dose against cestodes, nematodes, and bots in horses. A component dose confirmation study was conducted to confirm the efficacy of the IVM + PZQ combination and each component individually. Animals were not treated (Control), or received IVM alone, PZQ alone, or IVM + PZQ combination paste, orally, once on Day 0. At necropsy ~ 2 weeks post-treatment, compared to Controls, horses treated with IVM + PZQ had significantly ($p<0.05$) fewer (>99% reductions) adult *Anoplocephala perfoliata, Coronocyclus coronatus, C. labiatus, C. labratus; Cyathostomum catinatum; Cylicocyclus leptostomum, C. radiatus, C. nassatus; Cylicostephanus goldi, C. calicatus, C. longibursatus, C. minutus*; *Oxyuris equi*; *Poteriostomum imparidentatum;* L2 & L3 *Gasterophilus intestinalis & G. nasalis*. IVM alone was equally efficacious against nematodes & PZQ alone was similarly effective against tapeworms. Activity of each single entity, compared to the combination, establishes non-interference in this formulation.

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Ivermectin/Praziquantel oral paste: Field efficacy against naturally acquired GI nematodes and cestodes of horses in North America.

S.E. MARLEY[1], S. REHBEIN[1], J.E. HOLSTE[1*], M.Y. DOUCET[2], A. PAUL[3], C.R. REINEMEYER[4], C. FENGER[5], S. YOON[1]. [1]MERIAL LIMITED, DULUTH, GA; [2]FAC. MÉD. VÉT., UNIV. MONTRÉAL, SAINT-HYACINTHE, CAN; [3]UNIV. OF IL, URBANA, IL; [4]EAST TN CLIN RES. INC., KNOXVILLE, TN; [5]EQUINE INTERNAL MED. CONSULTING, GEORGETOWN, KY.

Efficacy & acceptability of an ivermectin (IVM) + praziquantel (PZQ) oral paste formulation was assessed under field conditions in North America. Six study sites were included, involving 149 horses aged ~3 months to 25 years across a wide variety of breeds. Horses were infected with strongyles (100%) & tapeworms (56%) as confirmed by positive fecal egg counts. Per replicate on Day 0, 3 horses received IVM (0.2 mg/kg BW) + PZQ (1 mg/kg BW) paste, orally, and one horse served as a control (non-treated). Fecal samples were examined at various times pre-treatment & 7, 8, 9, 14, 15, and 16 days post-treatment. Pre-treatment strongylid and cestode egg counts were similar for horses allocated to treated and control groups ($p>0.10$). Across study sites, horses treated with IVM + PZQ had significantly lower post- treatment strongylid & cestode egg counts (reductions ≥97% at all but one time point) than the controls. These results demonstrated IVM + PZQ paste to be highly effective against GI nematode & cestode infections; there were no adverse or treatment-related effects observed.

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***Sarcocystis inghami* n.sp. (Sporozoa: Sarcocystidae) from the skeletal muscles of the Virginia opossum *Didelphis virginiana* in Michigan.**

*HANY M. ELSHEIKHA, SCOTT D. FITZGERALD, LINDA S. MANSFIELD & A. MAHDI SAEED. MOLECULAR EPIDEMIOLOGY LABORATORY, DIAGNOSTIC CENTER OF POPULATION AND ANIMAL HEALTH, AND DEPARTMENT OF MICROBIOLOGY AND MOLECULAR GENETICS, COLLEGE OF VETERINARY MEDICINE, MICHIGAN STATE UNIVERSITY, MI 48824, USA

This report describes the newly identified *Sarcocystis inghami* n. sp. from the skeletal muscles of opossums (Mammalia: Didelphidae) that were collected from south central Michigan (42°43′-42°79′N, 84°18′-84°86′W), USA. The new species is distinguished from all species described from North and South American opossums by the distinctive morphology of the villar protrusions on the cyst wall. Sarcocysts of *S. inghami* are microscopic, up to 700 μm long and 110 μm wide. The sarcocyst wall is up to 7 μm thick, with long, stalked protrusions, which average 5.5 × 1.2 μm. These are constricted at the base, expanded laterally, rounded off distally and occasionally bifid. The villar protrusions have numerous microtubules without electron-dense bodies that extended from the tips into the granular layer. Bradyzoites are 10.7 × 4.3 (8-12 × 4-5) μm. This is the second species of *Sarcocystis* sarcocyst described from the Virginia opossum in North America.

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Sarcocystis in free-ranging raccoons and striped skunks from Arkansas.

E.C. GREINER[1]*, T. YAZWINSKI[2], C. SULSONA[1], K. GILLIS[1], C. TUCKER[2], J. ROBINS[2], AND J.DAME[1]. [1]COLLEGE VETERINARY MEDICINE, UNIVERSITY OF FLORIDA, GAINESVILLE, FL; [2]UNIVERSITY OF ARKANSAS, FAYETTEVILLE, ARKANSAS.

With the determination that skunks and raccoons can serve as intermediate hosts for *Sarcocystis neurona*, we decided to determine the prevalence of this parasite in a free-ranging population of these hosts. The skunks and raccoons were collected in Arkansas from the autumn of 2002 through the spring of 2003. These were collected as road kills and necropsied at the University of Arkansas. Muscle samples (legs, heart, diaphragm, and tongue) were harvested and sent to the University of Florida. Muscles were examined under a stereomicroscope for sarcocysts and tissues were fixed and sectioned for hematoxylin and eosin stained slides. Two types of sarcocysts were detected with both hosts. A short sarcocysts of up to1 cm in length and a long form up to 25 mm long. These were submitted for PCR amplification and DNA sequencing of the ITS region, as well as, for TEM. The short forms were consistent with *S. neurona* and the long forms for *S. felis*. The *Sarcocystis spp.* prevalence in striped skunks was 39/56 = 64.6% and 23/41 = 56.9%. The prevalence of individual species are still under investigation.

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Life cycle and transmission of a new species of *Besnoitia* from domestic rabbits

J. P. DUBEY *. USDA, ARS, ANRI, PBESL, BUILDING 1001, BELTSVILLE, MD 20705

Besnoitia ssp. are coccidian parasites affecting livestock and wild animals. A species of *Besnoitia* from naturally infected rabbits from Argentina was propagated experimentally in mice, gerbils, rabbits, cats, and cell cultures. Cats fed tissue cysts from rabbits shed oocysts with a prepatent period of 9 to13 days. Sporulated oocysts were infective to gerbils, rabbits, outbred Swiss Webster and interferon gamma gene knockout mice. Bradyzoites were infective orally to gerbils and cats. Tachyzoites were successfully cultivated and maintained *in vitro* in bovine monocytes and African green monkey kidney cells. Schizonts were seen in the lamina propria of the small intestine of cats fed tissue cysts; the largest ones measured 52 x 45 µm. Schizonts were also present in mesenteric lymph nodes, livers, and other extra-intestinal organs of cats fed tissue cysts. Oocysts were 10–14 x 10-13 µm in size. This rabbit-derived species of *Besnoitia* resembled *B. darlingi* of the North American opossum, *Didelphis virginiana* with an opossum-cat cycle, but it was not transmissible to *D. virginiana,* and *B. darlingi* of opossums was not transmissible to rabbits. Based on biologic, serologic, antigenic, and molecular differences between the rabbit and the opossum *Besnoitia*, the parasite from domestic rabbits from Argentina, is distinct from *B. darlingi.*

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Clinical and epidemiological aspects of Besnoitiosis in donkeys.

CHARLES MACKENZIE*, HANY ELSHEIKA, JUDY MARTENUIK, LINDA MANSFIELD. COLLEGE OF VETERINARY MEDICINE, MICHIGAN STATE UNIVERSITY, EAST LANSING, MI 48824

Besnoitia bennetti infection is a rarely detected infection of equids in Africa and America. It was first described in Sudan in the early 20th century and has only been recorded in a few cases. Its treatment remains ill defined. Here, we describe the clinical presentation of an outbreak of 14 cases in a small donkey herd in Mid-Michigan in 2002. The infection was confirmed by skin biopsy with the detection of cysts containing morphologically distinct *B. bennetti* parasites. The major clinical presentations were: a) loss of general condition in heavily infected animals, b) nodular, minimally pruritic, focal dermatitis, c) presence of conjunctivitis and fine scleral nodules, and d) fine submucosal nodules. The extent and intensity of the nodules (parasitic cysts) varied with the location on the body. Specific areas were more commonly affected. The patterns of affected areas of skin can be used as an aid to diagnosis and differentiation of the disease. The location of nodules ("pearls") in the ocular tissues also provide for simple diagnostic detection. Thus, the clinical presentation and its pattern can be used for preliminary screening for the presence of infection. Analysis of the relationship between infected and uninfected animals in the herd indicates that there was likely a period of intense transmission 1-2 years ago, which took place on this farm. The possibilities for the mode of transmission include a range of feral animals and may be related to the physical conditions and housing layout of the farm. There is a need for better diagnostic tests including immunologically based approaches. Such tests will facilitate the needed investigation into the significance and prevalence of this infection in the USA and elsewhere in the world. The present observations suggest that this infection is more common

than thought. Equine clinicians and parasitologists should consider *Besnoitia* more frequently in differential diagnosis of dermatitis in donkeys and possibly other equids.

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Experimental *Toxoplasma gondii* infection in grey seals, and food safety implications.

A.A. GAJADHAR*[1], L.B. FORBES[1], L. MEASURES[2], C. KAPEL[3], J.P. DUBEY[4]
[1]CENTRE FOR ANIMAL PARASITOLOGY, CFIA SASKATOON LABORATORY, SK, CANADA; [2]FISHERIES AND OCEANS CANADA, LMI, MONT-JOLI, QC,CANADA; [3]DANISH CENTRE FOR EXPERIMANTAL PARASITOLOGY, RVAU, DENMARK; [4]ANIMAL PARASITIC DISEASES LABORATORY, USDA, BELTSVILLE, MD.

Despite several published reports of marine mammals naturally infected with *Toxoplasma gondii*, the source of infection and the impact on the health of these animals are unknown. The objectives of this study were to determine experimentally the susceptibility of seals *(Halichoerus grypus)* to *T. gondii* oocysts and the viability of stages which develop in seals, and to assess the infectivity of the parasite in country foods prepared with infected seal meat. Eight weaned seals approximately 4 weeks old were captured and raised in confinement tanks. Five weeks later, each of 4 seals were orally inoculated with 100 or 10,000 oocysts of *T. gondii* (VEG strain), and another 4 seals served as negative controls. Occasionally, mild behavioural changes were observed in infected seals, but not in control animals. Blood samples were collected weekly from all animals, and at 5 or 10 weeks post-inoculation seals and mice were killed. A modified agglutination test revealed the presence of antibodies to *T. gondii* in sera collected from inoculated seals and mice. No stage of the parasite was found on an extensive histological examination of sections collected from seal tissues, and immunohistochemical staining of numerous sections revealed a single cyst in one of the low-dose inoculated seals. Control mice inoculated with 10 oocysts became serologically and histologically positive to *T. gondii*. Cats fed brain or muscle tissues from inoculated seals had *T. gondii* oocysts in their feces within two weeks of feeding. However, cats fed infected seal meat products prepared by drying (nikku or jerky), fermenting (igunaq), or salting (sausage) and bioassayed in cats did not shed oocysts. This study provides experimental evidence that *T. gondii* oocysts can establish viable infection in seals, and supports the hypothesis that toxoplasmosis in marine mammals can be acquired from oocysts in surface water runoff and sewer discharge. Results also suggest that *T. gondii* might not survive processing conditions used in some country foods.

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Efficacy of Ponazuril in the prevention and treatment of toxoplasmosis in mice.

*SHEILA M. MITCHELL, ANNE M. ZAJAC, WENDELL L. DAVIS, AND DAVID S. LINDSAY, VIRGINIA TECH, BLACKSBURG, VIRGINIA, AND BAYER HEALTHCARE, ANIMAL HEALTH DIVISION, SHAWNEE, KANSAS, USA.

Toxoplasmosis is an important zoonotic disease. Abortions are a common clinical sign in small ruminants and swine. Acute systemic disease is also common in other susceptible mammals. We have previously shown that ponazuril, a major metabolite of toltazuril, is active against the RH strain of *Toxoplasma gondii* in cell cultures. The current study was done to determine the efficacy of ponazuril in preventing and treating acute toxoplasmosis in outbred CD-1 mice. We

used 1,000 tachyzoites of the RH strain per mouse in the present study. In our laboratory system all non-treated mice develop severe toxoplasmosis and die within 8 to 11 days when inoculated subcutaneously with 1,000 tachyzoites. Groups of 5 female mice were used. Mice were weighed daily and ponazuril administered orally in a suspension. Mice given 10 or 20 mg/kg BWT ponazuril 1 day prior to infection and then daily for 10 days were completely protected against acute toxoplasmosis. Relapse did not occur after treatments were stopped. Eighty percent of mice treated with 10 mg/kg ponazuril at 3 days after infection and 100% of mice treated with 20 mg/kg ponazuril 3 days after infection and then daily for 10 days were protected from fatal toxoplasmosis. Sixty percent of mice treated with 10 mg/kg ponazuril at 6 days after infection and 100% of mice treated with 20 mg/kg ponazuril 6 days after infection and then daily for 10 days were protected from fatal toxoplasmosis. Relapse did not occur after treatments were stopped. The results demonstrate that ponazuril is effective in preventing and treating toxoplasmosis in mice. It should be further investigated as a safe and effective treatment for this disease in animals. Supported by a grant from Bayer HealthCare, Animal Health.

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The effect of *Fasciola hepatica* on reproductive efficacy in prepubertal beef heifers.

M. PACZKOWSKI, T.M. CRAIG*, D. MAGEE, J. A. THOMPSON, D. W. FORREST TEXAS A&M UNIVERSITY, COLLEGE STATION, TEXAS

The common liver fluke, *Fasciola hepatica,* may have effects on the reproductive potential of cattle. In females the effect is seen by the delay of puberty that is not just associated with lower rates of growth. A trial was conducted on prepubertal (ca. 4 month old) mixed breed, Angus sired, beef heifers (n+21) that were kept in a facility where natural fluke transmission could not occur. One-half of the heifers were administered 600 metacercariae each. Serum chemistry, reproductive steroids and fluke eggs were evaluated during the trial. Indications of infection were first detected by the elevation of serum gamma glutamltranspeptidase (ggt) and aminotransferase (ast) levels in infected calves. Fecal egg counts were variable in individuals and observed only in infected calves beginning 92 days post infection. Body weights, estradiol and progesterone levels were not significantly different between groups. Only 91% of the infected heifers exhibited estrus where 100% of the controls exhibited estrus by 13 months of age. By this time 50% of the control and 36% of the infected heifers were confirmed pregnant. There did not appear to be an association between numbers of eggs observed and the onset of puberty or conception.

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In Vitro Culture of *Eimeria tenella* in Primary Chicken Kidney Cells (PCKC) and Quantification of Parasite Growth by 3H-Uracil Uptake.

P.L. KELLERHOUSE*, J.S. MATHEW, D. KERSTEN, C.M. BROWN, T. BIFTU, AND D.R. THOMPSON, MERCK, SOMERVILLE, NEW JERSEY.

Attempts were made to grow *Eimeria tenella* (ET) in primary chicken kidney cells (PCKC) and to quantitate the intracellular growth of the parasite in vitro by parasite-specific incorporation of 3H-uracil. PCKC's isolated from 1-2-week old birds were seeded in 96-well plates and were allowed to grow to a monolayer for 48h. Freshly excysted ET sporozoites were added to the wells 48h after

seeding cells. 3H-uracil was added 24 or 48 h after infection in order to quantify parasite growth. The contents of 96 well plates were harvested on to a glass fiber Filtermat using Tomtec harvester 5 days following infection. Radioactivity of the contents of each well was measured using a liquid scintillation counter. Results of this study show that PCKC readily supported development of ET and oocysts were recovered from the culture plates 8-9 days after infection. Incorporation 3H-uracil by the parasites appears to be useful in quantifying parasite growth.

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Characterization of a 19 kDa Protein Conserved in *Eimeria* species.

R. FETTERER*, M. JENKINS, K. MISKA
ANIMAL PARASITIC DISEASES LABORATORY, ARS/USDA, BELTSVILLE MD 20705.

A wide range of recombinant proteins from *Eimeria* species have been reported to offer some degree of protection against infection and diseases, but biological function of these proteins is largely unknown. Previous studies have demonstrated the presence of a 19 kDa protein in sporozoites (SZ1) and merezoites (3-1E) of *Eimeria acervulina* that can be used to confer partial protection against coccidiosis. Sequence analysis indicates these proteins are identical. Rabbit antisera to recombinant SZ1 recognized an approximately 19 kDa protein from extracts of *Eimeria tenella* sporozoites, merezoites, sporulated oocysts and oocysts in various stages of sporulation. Although staining was weaker than with *Eimeria* species, anti-SZ1 antibody also recognized a 19 kDa protein from tachyzoite extracts prepared from *Toxoplasma gondii* and *Neospora caninum*. Immuno-fluorescence antibody staining indicated specific staining of *E.tenella*, *E. maxima* and *E. acervulina* sporozoites, *E. tenella* and *E.~~imeria~~ maxima* merozoites. Staining was most intense in the cytoplasm of the anterior ends of the parasites. No specific staining of *N. caninum* tachyzoites was noted. 2D-gel analysis indicated the antibody recognized a single protein from *E. tenella* sporulated oocysts with a Mr of 19 kDa and pI of about 4.2. The predicted pI of the SZ1 is 4.37. Comparison of the sequence of SZ1 with sequences in the standard data bases indicates a strong conserved domain consistent with an actin-binding protein, profilin In addition, a moderate amount of identity was noted with several profilins of plant origin. The sequence of SZ1 has nearly complete identity with a sequence from the *E. tenella* genome. The current results suggest a hypothesis that SZ1 is a highly conserved protein in all asexual stages of *Eimeria* and possibly other coccidians and may be related to the actin-binding protein profilin.

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Efficacy of 2% diatomaceous earth (DE) against gastro-intestinal parasites in calves in a clinical trial.

OWEN SLOCOMBE*, DEPARTMENT OF PATHOBIOLOGY, ONTARIO VETERINARY COLLEGE, UNIVERSITY OF GUELPH, GUELPH, ONTARIO, CANADA. N1G 2W1.

In Ontario, Canada during March-April 1997 10 Hereford cross one year old calves naturally infected with gastro-intestinal parasites were in two treatment groups in a loose housing barn receiving grain and hay *ad libitum*. On Days -2, 7, 14 and 21 of the study a 5 gram fecal sample was taken from each calf to determine parasite eggs/gram (epg) with the Cornell-Wisconsin Centrifugal Flotation technique. The epg on Day -2 was used to randomize calves into the

groups. On Day -2 and 21, a 20 gram fecal sample from each calf in a group was pooled for coproculture. On Day 0 through 20, one group was fed daily grain mixed with a ready to use insecticide dust (Protect-It®) containing 90% DE. A 2% DE-grain ration was prepared daily and just before feeding. The primary egg type seen was the GIN (Gastro-Intestinal Nematode=strongyle=trichostrongyle). Mean epgs on Day -2 and Day 21 for the DE calves were 11.8 and 13.3 respectively and for the control group 10.4 and 8.3. *Ostertagia* and *Cooperia* spp., were the primary producers of those eggs. A few calves had a few *Nematodirus* spp., eggs and all calves shed low numbers of *Eimeria* spp. DE caused no reduction in the number of eggs or coccidia oocysts in the feces of calves.

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Prevalence of *Giardia* in Adult Cattle from Prince Edward Island

R.M. O'HANDLEY*[1], H.W. BARKEMA[1], N. BUCHANAN[1], AND J.M. TROUT[2]
[1]ATLANTIC VETERINARY COLLEGE, UNIVERSITY OF PRINCE EDWARD ISLAND, CHARLOTTETOWN, PEI, CANADA, [2]USDA, ARS, ANRI, ANIMAL WASTE PATHOGEN LABORATORY, BELTSVILLE, MD

The prevalence of *Giardia* in dairy and beef calves has been well documented. However, few studies have examined the prevalence of *Giardia* in adult cattle. In this study fecal samples were collected from cows >1 year of age (n=195) on 6 farms on Prince Edward Island (Canada). *Giardia* cysts were isolated from 20 g of feces using sucrose flotation and centrifugation, then stained with a *Giardia* specific FITC-labelled mAb and enumerated under a fluorescent microscope. Additional fecal samples were collected from one herd and examined for *Giardia* every two weeks over a three-month period. The overall prevalence of *Giardia* in the cows was 38%, with the prevalence on individual farms ranging from 21% to 52%. Mean number of *Giardia* cysts excreted by positive cows was 21 cysts per gram of feces. The percentage of cows excreting *Giardia* cysts in the herd that was sampled every two weeks fluctuated over time, ranging from 60% to 20% with a cumulative prevalence in the herd of 65%. The mean number of *Giardia* cysts excreted by this herd also fluctuated over time, ranging from 15 to 47 cysts per gram of feces. The results of this study demonstrate *Giardia* was highly prevalent in these adult cattle. Despite a low number of cysts excreted per gram of feces, adult cattle may still act as a significant reservoir for *Giardia* due to the large amount of feces they produce. Currently, *Giardia* isolates from these cattle are undergoing DNA sequence analysis to determine their zoonotic potential.

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Genotypes of *Giardia* in Dairy Calves and Pastureland Wildlife.

J.M. TROUT[1]*, M. SANTIN[1], AND R. FAYER. [1]USDA/ARS, BUILDING 173, BARC-EAST, BELTSVILLE, MD.

Fecal samples were collected from 2-6 week-old dairy calves from two farms in each of the following states: VT, NY, PA, MD, VA, NC, and FL, as well as from white-tailed deer in MD and coyote in PA. For analysis by immunofluorescent microscopy and PCR, fifteen gram portions of each sample were mixed with water, passed through a 45 micron screen, mixed with CsCl, and centrifuged. The top 4 ml of suspension from the tubes was collected, pelleted by centirfugation, washed with distilled water, and resuspended in 100 µl of distilled water. Fifty µl

was stained with a commercial *Giardia* specific mAb. The remianing 50 µl was subjected to DNA extraction using a commercial tissue DNA extraction kit. For coyotes, duodenal scrapings were also obtained. A portion was subjected to DNA extraction as described above, while the remainder was fixed and examined by light microscopy for the the presence of trophozoites. PCR amplification was done for the TPI, 16S, and β-giardin genes, using a nested PCR for TPI and 16S. PCR products were sequenced and the *Giardia* gene sequences obtained from animals in this survey were compared to each other and to previously sequenced *Giardia* isolates. PCR and gene sequence analysis revealed that calves were infected primarily with the hoofed-livestock genotype, however, a small percentage of Assemblage A genotype was also detected. Assemblage A *Giardia* was also detected in one white-tailed deer. Preliminary sequencing of the 16s gene suggests the coyote *Giardia* may also fall into Assemblage A.

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Dose confirmation and field efficacy studies of a 2% moxidectin / 12.5% praziquantel oral gel formulation against *Anoplocephala* spp. and other equine parasites.

R. CLEALE[1], K. HEANEY[1], D. HUTCHENS[2], M. HUTCHINSON[3], T. KLEI[4], G. LEVOT[5], C. REINEMEYER[6]*, D. RULLI[1], AND R. SWAIN[7]. [1]FORT DODGE ANIMAL HEALTH, PRINCETON, NJ; [2]UNIV. OF ILLINOIS, URBANA, IL; [3]ROYAL VET. COLL., HERTFORDSHIRE, U.K.; [4]LOUISIANA STATE UNIV., BATON ROUGE, LA; [5]NSW AGRICULTURE, MENANGLE, NSW, AU, [6]EAST TN CLINICAL RESEARCH, KNOXVILLE, TN; [7]MOREDUN SCIENTIFIC, LTD., EDINBURGH, UK.

Dose confirmation studies with an oral gel formulation of 2% moxidectin (MOX) plus 12.5% praziquantel (PRZ) were conducted at 4 sites (IL, LA, TN, UK). Horses naturally infected with *Anoplocephala* spp. and *Gasterophilus intestinalis* were ranked by endoscopically-estimated bot numbers (IL, LA, TN) or by body weight (UK) and allocated randomly to be untreated controls or to receive gel product at dosages of 0.4 mg/kg MOX and 2.5 mg/kg PRZ. Horses were necropsied for total parasite counts 12 to 14 days post-treatment. The efficacy of MOX/PRZ was ≥99.8% against *Anoplocephala* spp. at all sites, ≥94.9% against *G. intestinalis* at 3 of 4 sites (IL, 84%), and ≥96.5% against Cyathostominae adults and larvae (IL, LA, TN), *G. nasalis* (UK), *H. muscae* (LA), *P. equorum* (UK), and *P. mamillana* (UK). In one non-interference trial (TN), the efficacy of MOX/PRZ against bots or tapeworms, respectively, was no different than MOX or PRZ separately. In 3 field trials in the UK or AU, MOX/PRZ gel was 100% effective against *Anoplocephala* spp. and reduced strongylid egg counts >95% for 140 days (UK) and 12 weeks (AU). No adverse reactions accompanied MOX/PRZ treatments.

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Tolerance and Toxicity Evaluation of Moxidectin/Praziquantel Equine Oral Gel in Foals and Yearlings.

R. DINO RULLI[1]*, STEVEN GRUBBS[1], KATHLEEN HEANEY[1], CHARLES E. HEIRD[2], ERIN IVEY-WEICH[2], AND CRAIG REINEMEYER[3]. [1]FORT DODGE ANIMAL HEALTH, PRINCETON, NJ; [2]SOUTHWEST BIO-LABS, LAS CRUCES, NM; [3]EAST TENNESSEE CLINICAL RESEARCH, KNOXVILLE, TENN.

Three studies were conducted to evaluate the safety of an equine anthelmintic containing 0.4 mg moxidectin and 2.5 mg praziquantel per kg bodyweight. In the first study no adverse reactions

were noted in four month old foals (3 controls, 3 given 2X and 3 given 3X) treated once a week for three weeks. In the second study four foals (3 to 5 ½ months) and two "yearlings" (6 ½ - 8 ½ months) were treated with a 5X dose, and two "yearlings" were treated with a 6X dose. Two 5X foals and one 5X yearling did not show any adverse reactions. The other animals showed signs of mild lethargy, ataxia and depression starting no earlier than 14 hours post treatment and completely resolved by 48 hours. There were no treatment-related findings in blood or urine parameters. A third study had 4 groups of 8 foals (4 males/4 females) 3 to 5 months of age treated once a week for three weeks with either 1X, 3X, 4X dose, or a 4X sham-treatment. There were no chronic adverse reactions to the test material. Some animals in the 3X and 4X groups had transient mild ataxia, lethargy and depression. Two foals in the 1X group had mild reactions following their first dose, but no adverse reactions following their third dose. This is in contrast to the 3X and 4X group animals that had increased reactions after multiple doses. There were no test article-related effects on blood and urine parameters, body weight, food consumption, or gross and microscopic examinations following necropsy.

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Field evaluation of moxidectin/praziquantel equine oral gel in horses.

S. GRUBBS*, D. AMODIE, M. WULSTER-RADCLIFF, D. RULLI, FORT DODGE ANIMAL HEALTH, PRINCETON, NJ; C. REINEMEYER, EAST TENNESSEE CLINICAL RESEARCH, KNOXVILLE, TN; T. YAZWINSKI, C. TUCKER, UNIVERSITY OF ARKANSAS, FAYETTEVILLE, AR; D. HUTCHENS, UNIVERSITY OF ILLINOIS, URBANA, IL; L. SMITH, LODI, WI; D. PATTERSON, NEWTOWN, PA.

The safety and efficacy of a 2% moxidectin/12.5% praziquantel oral equine gel formulation in horses at multiple sites was studied under field use conditions. Horses were fecal sampled and eggs counted prior to treatment and on Day 14. Three hundred horses were administered moxidectin/praziquantel at a dose of 0.4 mg moxidectin/kg b.w. and 2.5 mg praziquantel/kg b.w, and one hundred horses were treated with vehicle (control). All study personnel, as well as the horse owners, were masked to treatment assignment. The safety and efficacy of the product under field use conditions was evaluated by multiple observations for adverse reactions of each horse and by the pretreatment to Day 14 reduction in fecal nematode and cestode eggs, respectively. Moxidectin/praziquantel was well tolerated, based on the fact that no adverse reactions to treatment were observed. Based on fecal egg count geometric means, moxidectin/praziquantel reduced Anoplocephala spp by >99% and provided a statistically significant reduction (>98% efficacy) in the strongyle egg count ($P < 0.05$). Based on fecal egg counts on Day 14, the reduction in *Parascaris* egg counts was variable across sites.

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CYDECTIN® (Moxidectin) 1% Injectable Solution for Beef and Nonlactating Dairy Cattle: Safety and Efficacy Data Summary.

D. ROCK*, R. DELAY AND M. GLIDDON, FORT DODGE ANIMAL HEALTH, PRINCETON, NEW JERSEY, USA.

Moxidectin, a macrocylic lactone of the milbemycin class of compounds, is available globally in a number of parenteral, topical and oral formulations for the treatment and control of internal and

external parasites of a variety of food producing and companion animals. CYDECTIN (moxidectin) 1% injectable is a non-aqueous formulation that is available in the US and several other countries in North, Central and South America. Results of pre-clinical studies comparing the non-aqueous formulation with an earlier aqueous-based injectable formulation were used to determine the experimental parameters required to establish the effective dose of the nonaqueous injectable. A dose of 1 ml/50 kg body weight to provide 0.2 mg moxidectin/kg body weight by subcutaneous injection was established as optimum for control of economically important endo- and expternal parasites. This dose also protects cattle against reinfection with *Dictyocaulus viviparus* and *Oesophagostomum radiatum* for 42 days, *Haemonchus placei* for 35, and *Ostertagia ostertagi* and *Trichostrongylus axei* for 14 days after treatment. This injectable formulation was shown to be safe for use in a variety of classes of cattle, including animals of breeding age, and to the environment. A withdrawal period of 21 days has been established for this product.

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Identification of Protective Antigens for the Control of *Ixodes scapularis* Infestations using cDNA Expression Library Immunization

CONSUELO ALMAZÁN*, KATHERINE M. KOCAN, DOUGLAS K. BERGMAN, JOSE C. GARCIA-GARCIA, EDMOUR F. BLOUIN AND JOSÉ DE LA FUENTE
DEPARTMENT OF VETERINARY PATHOBIOLOGY, COLLEGE OF VETERINARY MEDICINE, OKLAHOMA STATE UNIVERSITY, STILLWATER, OK, 74078.

Identification of antigens that induce an immune response against tick infestations is required for the development of vaccines against these economically important ectoparasites. In order to identify protective antigens against *Ixodes scapularis*, a cDNA expression library from a continuous cell line (IDE8) that was initially derived from *I. scapularis* embryos was constructed. cDNA clones were subjected to several rounds of screening in which mice were immunized with individual pools and then challenge-exposed by allowing *I. scapularis* larvae to feed on the immunized and control mice. Immunity against tick infestation was determined by the reduction in the ability of the larvae to attach, repletion and molt to the nymphal stage. Individual clones in pools that induced immunity to larval infestations were partially sequenced and grouped according to their putative protein function by comparison with sequence databases. The screening identified several individual antigens that induced a protective immune response against *I. scapularis* infestations. Our studies demonstrated for the first time that cDNA expression library immunization (ELI) combined with sequence analysis is a powerful and efficient tool for identification of candidate antigens for use in vaccines against ticks.

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Cytokine gene expression at the materno-fetal interface in pregnant cows infected with *Neospora caninum*.

S. ALMERIA[1]*, H DAWSON[2], J.P. DUBEY[3], L. GASBARRE[4]. [1]AUTONOMOUS UNIVERSITY EDIFICI V, CAMPUS UAB, BELLATERRA, BARCELONA; [2]USDA, ARS, BHNRC, BELTSVILLE, MD; [3]USDA, ARS, PBESL, BELTSVILLE, MD; [4]USDA, ARS, IDRL, BELTSVILLE, MD.

Neospora caninum is a protozoan parasite considered a major cause of abortion in cattle. The present study was performed to define the immune responses at the materno-fetal interface of pregnant cows infected with *N. caninum*. Four cows were infected early in the second trimester of pregnancy (110 days of gestation) intravenously with 10^7 cattle strain culture derived tachyzoites and three pregnant cows were kept as uninfected controls. Animals were euthanized at 3 weeks after infection. Caruncular endometrium, intercaruncular endometrium and cotyledonary tissues were collected from the materno-fetal interface of control and infected animals. Cytokine gene expression of bovine cytokines was performed by real time PCR using bovine-specific TaqMan® probes for IL-10, IL-4, IFN-γ, TNF-α, IL-12 (p40 and p35), IL-1, IL-2, IL-6, IL-8 and TGF-β. No abortion was observed in any infected dam at the time of the killing, however, transplacental infection had already taken place in all infected fetuses. Main changes of cytokine gene expression were observed in the caruncular tissues with increased expression of IFN-γ and IL-12, but also IL-6 and IL-10 in infected animals compared to control uninfected animals. In the cotyledon IFN-γ and IL-12 expression increased in the infected animals, while TGF-β expression was decreased in infected animals compared to the controls ($p \leq 0.1$). In the intercaruncular tissues while IFN-γ expression was higher in infected animals ($p \leq 0.1$), IL-8 and TGF-β significantly decreased in infected animals ($p \leq 0.05$). No IL-4 expression was detected in any of the analyzed tissues. The observed changes in cytokine gene expression indicated that the main inflammatory related responses were observed at the maternal side of the placenta. Cytokine gene expression analyzed by real time RT-PCR showed increased expression of both Th1 and Th2 cytokines in *N. caninum* infected animals compared to uninfected ones. This cytokine expression could have a role in the transplacental transmission of the parasite and/or mediate tissue damage.

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Evaluation of *Schistosoma mansoni* antioxidant enzyme DNA vaccines in mice.

ROSEMARY COOK*, CLAUDIA CARVALHO-QUEIROZ AND PHILIP T. LOVERDE. DEPARTMENT OF MICROBIOLOGY, STATE UNIVERSITY OF NEW YORK, BUFFALO, NEW YORK 14214

Schistosoma mansoni, an intravascular parasite, lives in a hostile environment in close contact with host humoral and cellular cytotoxic factors. To establish itself in the host, the parasite has evolved a number of immune evasion mechanisms. Production of antioxidant enzymes, for example, allow the parasite to evade oxidative damage caused by radicals released from immune cells such as eosinophils. *In vitro* studies using reactive species such as hydrogen peroxide and superoxide anion have shown that while the larval stages are highly susceptible to oxidant

damage, the adult is not. Our laboratory demonstrated that transcription and translation of certain antioxidant enzymes are developmentally regulated, with the highest levels present in the adult worm, and preliminary studies on mice vaccinated with naked DNA constructs containing Cu/Zn cytosolic superoxide dismutase (CT-SOD), or glutathione peroxidase (GPX) showed significant levels of protection compared to a control group (XPG). Based on the above research, we hypothesized that the adult stage of the parasite can be targeted for immune elimination using these antioxidant enzyme vaccines. Groups of BALB/c mice were primed and boosted with 100μg of CT-SOD, GPX, or XPG (control) DNA. To answer the question whether or not the adult stage of *S. mansoni* could be targeted for immune elimination, the vaccinated mice were challenged by surgical transfer. This technique bypasses the larval stages and the accompanying immune responses engendered by them. The average decrease in worm recovery in the experimental groups compared to the control groups (XPG, and not vaccinated) was determined to be the percent protection. In three independent experiments, mice vaccinated with CT-SOD DNA had a significant percent protection (42.83%, 35.92%, and 38.01%, respectively). Mice vaccinated with the GPX construct in two independent experiments experienced a decrease in worm burden, however not as significant as the CT-SOD group (12.57%, and 25.96%). Taken together, these data indicate that the adult stage of *S. mansoni* can be targeted for immune elimination using antioxidant enzymes as vaccine candidates in this mouse model. This research offers a major advance over existing paradigms in schistosome vaccinology. By targeting adult worms, the model offers the potential of a therapeutic as well as a prophylactic vaccine. Studies are now being directed at determining the immune mechanism(s) stimulated in this vaccine model by antibody analysis, analysis of cytokine production levels, and the use of knockout mice.

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Immune responses controlling *Toxoplasma gondii* infection in pigs.

S. NISHI[1], H. DAWSON[2], J.P. DUBEY[3], J.F. URBAN[2] AND J. LUNNEY[1]*. [1]IDRL, ANRI, BARC, ARS, USDA; [2]NRFL, BHNRC, BARC, ARS, USDA; [3]APDL, ANRI, BARC, ARS, USDA

Toxoplasma gondii (Tg) infection induces a strong interferon-gamma (IFNg) dominated, T helper-1 (Th1) response in pigs with immune pathology that quickly resolves. Pigs were infected with 4.5 x 10^6 Tg oocysts and various tissues were collected at 2, 4, 7 and 14 days after infection (DAI). Tg organisms were detected in mesenteric lymph nodes (MLN) at 2DAI and, at 4 DAI, in liver, spleen, lung and ileum, by IHC and Real-Time PCR. At the same infection times changes in inflammatory and Th1 immune mRNA expression were observed by Real-Time RT-PCR. Increased IFNg mRNA and protein expression were observed in MLN and hepato-splenic (HS) LN cells. Changes were observed in IL1b, IL-6, NRAMP1, IFNg, INDO and STAT-1 expression at 2DAI. Responses were more intense at 4DAI and also included TNFa, Arginase, IRF-1, IL-10, SOCS-1, SOCS-3, and IFNa activation. Liver showed the most intense changes in gene expression in response to Tg infection; PMBC had the lowest changes. High serum haptoglobin and NO levels at 4DAI ($p<0.01$) confirms inflammatory and macrophage activation. Up-regulation of inflammatory and Th1-dominated markers indicates mechanisms involved in the host defense against acute toxoplasmosis. Supported by ARS funds, and National Pork Board and BRDC grants.

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Lymphocyte subsets in the intestines of calves with a primary infection of *Oesophagostomum radiatum.*

C. PANUSKA*, COLLEGE OF VETERINARY MEDICINE, MISSISSIPPI STATE UNIVERSITY, MISSISSIPPI STATE, MS.

Oesophagostomum radiatum is notable because an initial infection, in which nematodes are successfully established, results in a strong immunity to re-infection. Understanding the changes in the immune system of the bovine host that result in such acquired immunity is foundational to the development of nematode control methods utilizing immune modulation. In this study, changes in subpopulations of T lymphocytes were assessed using indirect immunohistochemical staining. T cells were counted in intestinal tissue from uninfected calves, calves with immature infections (day 10 post-infection) and calves with mature infections (day 45 post-infection). Significantly fewer CD4+ T cells were present in the villi of the small intestine of infected animals than in the villi of uninfected animals. This was the result of a significant reduction in the CD4+ population in the lamina propria of infected animals. No significant reduction occurred in CD4+ cells associated with the villus epithelium of infected animals. Significant differences were not detected in numbers of CD8+ or TCR+ T cells in the villi of the small intestine of infected and uninfected animals. T cell populations within the large intestine are being evaluated. Supported by NRI-CGP/USDA Grant No. 2001-35208-10811.

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Characterization of humoral IgG response in llamas infected with *Fasciola hepatica.*

DELLANEY D. RUDOLPH*, LORA G. RICKARD, AND CARLA PANUSKA, COLLEGE OF VETERINARY MEDICINE, MISSISSIPPI STATE UNIVERSITY.

Fasciola hepatica is a parasitic trematode that is the causative agent of liver fluke disease in llamas. Although infections can be fatal, the immunobiology of this host-parasite relationship is unknown. Within the Camelidae, the IgG isotypes have unique characteristics. In addition to conventional IgG, two functional IgG subclasses devoid of light chains and the first constant domain, CH1, are present. Liver flukes produce the protease cathepsin during migration. These proteinases are known in other animals to cleave IgG in the hinge region preventing antibody-mediated attachment of immune effector cells to the parasite. Currently the role cathepsins play in liver fluke infections in llama is unknown. Using monoclonal and polyclonal antibodies in an ELISA, preliminary data indicates that the magnitude and duration of the immune response differed between isotypes; however, due to cross-reactivity of the monoclonals produced, serum was fractioned by protein column separation and tested by ELISA to further assess isotype differences. In Western blotting, all IgG isotypes recognized bands of *F. hepatica* somatic antigen at the same molecular weights as cathepsin L1 and L2. Further investigation is required to determine if these bands are cathepsins.

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Localized increases in IL-4 and IgE mRNA correlate inversely with whipworm burden in pigs

HELENE KRINGEL[1*], ETHIOPIA BESHAH[2], JOSEPH F. URBAN, JR.[2], HARRY D. DAWSON[2] [1]DANISH CENTER FOR EXPERIMENTAL PARASITOLOGY, THE ROYAL VETERINARY AND AGRICULTURAL UNIVERSITY, DYRLAEGEVEJ 100, DK-1870 FREDERIKSBERG C, DENMARK; [2]USDA, ARS, BELTSVILLE HUMAN NUTRITION RESEARCH CENTER, BELTSVILLE, MD, USA

Humans infected with *Trichuris trichiura* express local and peripheral blood eosinophilia, enhanced Th2-derived cytokines from ex vivo-activated immune cells, and parasite antigen specific IgE antibody levels that negatively correlate with whipworm egg output indicating that type 2-immunity is protective. We have previously shown that *T. suis* induces a localized parasite antigen specific IgG and IgA antibody secreting cell response in the proximal colon mucosa (PCM) and colonic lymph nodes (CLN) of pigs. A recently developed gene expression array for swine immune markers has been used in the current study to expand this evaluation and test the hypothesis that a type 2-immune response is protective against whipworm in pigs. RNA was extracted from 12 different tissues collected from *T. suis*-infected and un-infected pigs at 3 and 8 weeks post-inoculation (pi), and quantitative gene expression for Th1-, Th2-, or humoral immune responses was determined by real-time PCR. The fold difference in gene-specific mRNA between non-infected and infected pigs was related to worm burden at time of sampling. *Trichuris suis* significantly ($P<0.05$) up-regulated IL-4 and IgE gene expression in the PCM and CLN at 3 weeks pi that was inversely related to worm burden. Thus, a localized type 2-immune response correlates with protective immunity against swine whipworm.

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The effects of dietary probiotics on immunity to *Ascaris suum* in pigs.

G. SOLANO-AGUILAR[1], H. DAWSON[1], T. LEDBETTER[1], T. SHEA-DONOHUE[1], N. SCHOENE[1], J. CALL[2], E. BESHAH[3], W. HARE[4], J. URBAN, JR*[1]. USDA/ARS [1]BELTSVILLE HUMAN NUTRITION RESEARCH CENTER, NUTRIENT REQUIREMENTS & FUNCTIONS LABORATORY; [2]EASTERN REGIONAL RESEARCH CENTER, MICROBIAL FOOD SAFETY RESEARCH UNIT, WYNDMOOR, PA; ANIMAL & NATURAL RESOURCES INSTITUTE, [3]IMMUNOLOGY & DISEASE RESISTANCE LABORATORY; [4]VETERINARY SCERVICES, BELTVILLE, MD 20705

A probiotic is a product containing viable, defined microorganism in sufficient numbers to alter the micro flora of the host by implantation or colonization, and exert beneficial effects on host health. The objective of the study was to validate the pig as a model for the efficacy of human-derived dietary probiotic strains such as *Lactobacillus rhamnosus* GG (LGG) (ATCC 53103) and *Bifidobacterium lactis* (Bb12) on immunity to nematode parasites. The strains were evaluated for 1) effective delivery to the gastrointestinal tract, 2) enumeration at various locations in the host intestine, and 3) for effects on immune and intestinal function. A clear association between LGG and the intestinal mucosa was observed along with enhanced production of pro-inflammatory cytokines after in vitro mitogen stimulation of blood mononuclear cells and the intracellular signaling molecule NF-κB in ex vivo blood and bronchoalveolar lavage cells. There was no

effect on differential blood cell counts or serum liver function enzymes, but a positive effect on weight gain suggesting improved neonatal growth and health. Probiotic treatment with Bb12 did not alter absorption of glucose in the small intestine however; it did attenuate *A. suum*-induced inhibition of glucose absorption. This suggests that probiotics can modulate immune function and ameliorate the effect of parasitic disease on intestinal function in pigs. This work provides a basis for the use of probiotics in promoting swine health as well as a model for effects on allergic disease in swine and humans.

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Comparison of fecal egg count reduction test and DrenchRite© larval development assay for detection of anthelmintic resistance on goat farms. R. M. KAPLAN[1*], S.B. HOWELL[1], L. L. MORTENSEN[2], L. H. WILLIAMSON[1], T.H. TERRILL[3], AND M. LARSEN[2]. [1]COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF GEORGIA, ATHENS, GA; [2]DANISH CENTER FOR EXPERIMENTAL PARASITOLOGY, ROYAL VETERINARY & AGRICULTURAL UNIVERSITY, DENMARK; [3]AGRICULTURAL RESEARCH STATION, FORT VALLEY STATE UNIVERSITY, FORT VALLEY, GA.

The most common method for determining resistance status on a farm is the fecal egg count reduction test (FECRT), but this test is very labor intensive and is not practical on farms with small numbers of goats. An alternative to the FECRT is the larval development assay (LDA). We compared results of FECRT and LDA performed as part of an anthelmintic resistance prevalence study conducted on 18 goat farms in Georgia. Methods: Nematode eggs were recovered from pooled feces collected from 8 to 10 untreated goats on each farm and were placed into wells of a Drench Rite plate. Plates were incubated for 7 days at 25°C with nutritive media being added after 24 hr. After 7 days Lugols iodine was added to all wells, contents of wells were transferred to a clean 96-well plate, and plates were examined using an inverted head microscope. Resistance status was determined based on LD_{50}, which were calculated for each drug by logistic regression. Results: Reliable LDA data was obtained on 13 of 18 farms. There was strong agreement between FECRT and LDA for benzimidazole drugs and ivermectin. Ivermectin monosaccharide appeared to be slightly more sensitive than ivermectin aglycone in distinguishing susceptible from resistant farms. Results with levamisole were less consistent. There was tendency for LDA to be less sensitive in finding resistance. The cause of this discrepancy is not known but may be due to a number of factors. However, LDA clearly detected the 1 farm with high-level levamisole resistance (FECR=58%) and demonstrated the existence of a subpopulation of high-level levamisole-resistant *Haemonchus* on all but 1 farm. Conclusions: The Drench Rite LDA is a very useful test for detecting anthelmintic resistance in GIN of goats. However, with levamisole there were minor discrepancies between the results of the 2 methods. Further analysis is needed to sort out the reasons for these differences.

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Cloning, Characterization and Phylogenetic analysis of the glutamate gated chloride channel (GluCl) gene in *Cylicocyclus nassatus*.

*RITESH TANDON AND R.M.KAPLAN. DEPARTMENT OF MEDICAL MICROBIOLOGY AND PARASITOLOGY. COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF GEORGIA, ATHENS, GA 30602. USA.

Small strongyles or cyathostomins including *C. nassatus* are now considered as the most important intestinal parasites and principle parasitic pathogen of horses. Drug resistance in cyathostomins has been reported for phenothiazine, benzimidazoles, febantel, piperazine, and pyrantel pamoate but not for Avermectin/Milbemycins (AMs). The invertebrate glutamate gated chloride channels (GluCl) are the putative receptor molecules and target for the antiparasitic drug ivermectin and are most probably involved in mechanisms of drug-resistance. Selection at the GluCl gene in response to drug treatment can be ascertained by comparing frequencies of GluCl gene alleles in drug-selected and susceptible isolates of worms. Our hypothesis is that particular alleles are associated with selection for resistance to drug. Full length Glucl alpha subunit gene of *C. nassatus* and a fragment of beta subunit were cloned and sequenced in this study using degenerate primers based on amino acid homology in related organisms. Phylogenetic analysis using UPGMA, Neighbor-joining or Maximum Parsimony yielded similar results with beta subunits from different organisms forming one clade and alpha subunits forming another clade. Intron sequences in alpha and beta subunits of the GluCl gene in *C. nassatus* were identified by amplifying genomic DNA and cDNA using same primer pairs. Further work to characterize the allelic variation of GluCl gene in *C. nassatus* is in progress.

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Preliminary investigations into the establishment of equine small strongyle (Cyathostominae) infections in the jird (*Meriones unguiculatus*) as a potential laboratory model.

R.M. ZAYA*[1] AND C.R.REINEMEYER[2]. [1]PFIZER ANIMAL HEALTH, KALAMAZOO, MI, AND [2]EAST TENNESSEE CLINICAL RESEARCH, KNOXVILLE, TN.

The lack of a model system for the study of equine small strongyles (cyathostominae) has prompted attempts at infecting jirds (*Meriones unguiculatus*) with these parasites. Initial efforts to infect jirds were successful in that some larvae were found in cecal and/or colon tissue of infected jirds up to 15 days post infection. Although these infections were considered successful, the rate of these first infections was prohibitively low for many practical purposes. This limited initial success stimulated continued investigations to optimize model conditions to achieve the highest possible worm burden. Experimental variables studied to enhance larval establishment included the number of larvae in the inoculum, the use of ensheathed and exsheathed larvae, trickle infections versus bolus infections, immunosuppression of jirds, the age of the larval culture, the source of the culture, and infecting fasted versus fed. This report describes how these variables affected the rate of cyathostome infection in the jird.

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Survey of anthelmintic usage and efficacy, preliminary results.

JOSEPH TRITSCHLER*, MICHAELA DISMANN, AND ANNE ZAJAC. VIRGINIA STATE UNIVERSITY, PETERSBURG 23806 AND VA-MD REGIONAL COLLEGE OF VETERINARY MEDICINE, BLACKSBURG 24061.

On-farm efficacy testing and phone surveys are being conducted with small ruminant producers in the Mid-Atlantic region. Although a purpose of the phone survey is to investigate differences in demographics or management practices that might be associated with perceived anthelmintic failure, preliminary analysis did not reveal any significant or suggestive differences. This may be attributed to difference response patterns between goat and sheep producers, particularly those who had beef cattle. On-farm anthelmintic efficacy was evaluated for the five compounds most used by surveyed producers: albendazole, fenbendazole, levamisole, ivermectin and moxidectin. Fecal Egg Count Reduction (FECR%) tests were conducted by comparing fecal egg counts (EPG) prior to and 7-14 days following treatment, adjusted to an initial level of > 50 EPG and a minimum of zero percent FECR. Anthelmintics were administered orally to goats at 1.5-3.0 times the oral dosage indicated for sheep (or cattle). Efficacies of fenbendazole ranged from 22-78% (6 farms) and 72-73% (3 farms) at 10 mg/kg and 15 mg/kg doses, respectively. Albendazole (20 mg/kg) showed 40% and 78% FECR on 2 farms. Efficacies of ivermectin ranged from 26-65% (5 farms) and 72-86% (2 farms) at 400 mcg/kg and 600mg/kg, respectively. In contrast, efficacies of levamisole ranged from 85-100% (4 farms) and 65-100% (5 farms) at 12 mg/kg and 16 mg/kg, respectively. Efficacies of moxidectin ranged from 83-100% (6 farms) at 500 mcg/kg. Data suggest anthelmintic resistance may be a serious problem in the Mid-Atlantic region.

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Susceptibility of White-Tailed Deer to Experimental Infection with *Anaplasma* (*Ehrlichia*) *phagocytophilum*, the Etiologic Agent of Human Granulocytic Ehrlichiosis.

*CYNTHIA M. TATE(1,2), M. PAGE LUTTRELL(1), ELIZABETH W. HOWERTH(1,3), ULRIKE G. MUNDERLOH(4), WILLIAM R. DAVIDSON(1,2). SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY(1), DEPARTMENT OF MEDICAL MICROBIOLOGY AND PARASITOLOGY(2) AND DEPARTMENT OF PATHOLOGY(3), COLLEGE OF VETERINARY MEDICINE, THE UNIVERSITY OF GEORGIA, ATHENS, GA 30602 AND DEPARTMENT OF ENTOMOLOGY(4), UNIVERSITY OF MINNESOTA, ST. PAUL, MN 55108.

Although approximately 1220 cases of human granulocytic ehrlichiosis (HGE) have been diagnosed in the United States since 1994, knowledge of the natural history of Anaplasma phagocytophilum (formerly *Ehrlichia phagocytophila*) remains incomplete. Because antibodies reactive to and PCR products identical with A. phagocytophilum have been found in wild white-tailed deer and because white-tailed deer are the main host for adult stages of Ixodes scapularis, the vector of HGE, we experimentally inoculated four white-tailed deer with a human isolate of HGE. Two deer served as negative controls. Infection dynamics were monitored by culture, PCR, serology and examination of granulocytes in Giemsa-stained whole blood smears. Cell culture and/or reverse transcriptase nested polymerase chain reaction (RT-nPCR) results from

peripheral blood indicated that A. phagocytophilum circulated for at least 17 days in all deer. Moreover, the organism was consistently re-isolated from two deer at each of 4 sampling dates during a 23-day period between 16 and 39 DPI. Morulae were not observed in granulocytes, and there was no indication of clinical disease. These results confirm that white-tailed deer can support an A. phagocytophilum infection, with a resulting rickettsemia of sufficient duration to infect tick vectors. The relative role of white-tailed deer as competent reservoirs in the enzootic maintenance of A. phagocytophilum merits investigation.

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Frontiers in drug discovery: anthelmintic pharmacology

*TIMOTHY G. GEARY. PFIZER ANIMAL HEALTH, KALAMAZOO, MI 49001

Whether new anthelmintic drugs for veterinary use will be discovered and developed during the next decade will depends upon the resolution of many business and scientific questions. Relevant issues include:

(a) Developing a more comprehensive understanding of the basic pharmacology of drug resistance in parasitic nematodes;

(b) Determining if work done on model organisms (such as *C. elegans*) can improve the economics of endectocide discovery; and

(c) Identifying key pharmaceutical attributes that can be used to predict which anthelmintic analogs in a given series most warrant development.

Examples of specific experimental questions pertinent to each category will be used to illustrate opportunities for investigation.

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The parasites fight back: resistance mechanisms in parasitic nematodes.

R.M. KAPLAN*, DEPARTMENT OF MEDICAL MICROBIOLOGY & PARASITOLOGY, COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF GEORGIA, ATHENS, GA.

Most species of gastrointestinal nematode parasites have enormously large population sizes and genetic diversity that provide a huge pool of alleles upon which selection can act. Drug resistance is defined as a change in allele frequency within a population of organisms caused by drug selection, whereby more drug is required to exert its effect than was necessary prior to selection. So-called 'resistance genes' are alleles of relevant genes that confer resistance, and evidence suggests that in most cases these alleles preexist in populations of parasitic nematodes prior to the introduction of the drug. How quickly resistance will appear after a drug is first introduced depends on many genetic, biological, biochemical and pharmacokinetic parameters. Therefore each parasite species will develop resistance to different drugs at different rates. However, it is considered that in all cases resistance is an inevitable consequence of drug treatment. Currently there are 3 major classes of anthelmintics in use: benzimidazoles (BZ), imidazothiazoles/ tetrahydropyrimidines, and avermectin/milbemycins (AM). Anthelmintic resistance has been documented in multiple nematode species to all 3 classes, and is considered a major threat to current and future nematode parasite control in small ruminants and horses. Resistance has been slower to develop in nematodes of cattle, but the problem may be more severe than is commonly recognized. Diagnosis of resistance in its early stages, when resistance-gene frequencies are still

low and intervention to prevent full-fledged resistance can still be made, requires molecular-based tests. Development of such tests requires knowledge of the molecular basis for resistance. Unfortunately, except for BZ, our knowledge in this area remains profoundly insufficient. Current knowledge of molecular mechanisms believed to be associated with anthelmintic resistance to the major drug classes will be reviewed.

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Control Programs for helminth diseases: Experiences with Oncocerciasis and other infections.

CHARLES MACKENZIE*, FILARIAL DISEASES UNIT, MICHIGAN STATE UNIVERSITY, EAST LANSING MI 48824.

Can we eliminate helminth diseases? There have been a number of attempts in the past to rid the medical and veterinary world of specific helminths but none have succeeded to date. There are currently three major control and/or elimination programs against human helminths in action: the onchocerciasis control, the lymphatic filariasis elimination and the guinea worm eradication programs. Are there insights into the outcomes of these programs in the veterinary world, and are their lessons from the human public health field for veterinary medicine? This presentation will describe the background, the successes and challenges facing the three human helminth control programs. The onchocerciasis program has been in place for 15 years and uses a single annual dose of ivermectin (Mectizan®) to control clinical disease. The program has now shown success in its aim to reduce eye and skin disease. The lymphatic filariasis mass drug administration has been in place for four years in some countries and takes an "elimination" approach using either Mectizan® or diethylcarbamazine with albendazole. There are unexpected responses emerging from the experiences to date. The guinea worm program uses an environmental/human behavioral approach by filtering the infected water; this is a successful program, which has significantly reduced or eradicated the infection form a number of countries. These differing approaches to reducing the parasite raise a number of important questions and challenges, as well as important lessons; such factors also are applicable to many veterinary helminths and their control. Comparison of the different of the approaches that have been used in veterinary medicine and those in human medicine suggests that certain factors must be considered both in the short and long terms.

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Nitenpyram in review: A summary of the efficacy studies conducted with Capstar® (nitenpyram) from sources around the world, with emphasis on its use in conjunction with lufenuron

*S. CRAIG PARKS, NOVARTIS ANIMAL HEALTH, US, INC.,GREENSBORO, NC.

Flea control options for pet owners continue to grow and diversify. The concurrent use of nitenpyram and lufenuron offers another option for both pet owners and veterinarians in their attempt to control fleas. Nitenpyram is a neo-nicotinoid that provides safe, simple, fast acting and efficient adulticidal flea control without residue or residual, making it unique among the flea adulticides. Nitenpyram has been in the marketplace since 2000 in various parts of the world. Used in conjunction with lufenuron, another popular flea control product with unique

characteristics, an interesting synergy for flea control is available to pet owners. Efficacy studies of various types have been conducted in the US, Europe, and Australia. Study designs range from simple (laboratory) dose titration/confirmation formats, to controlled clinical trial evaluations. An overview of these data generated from around the world, emphasizing those where nitenpyram and lufenuron have been used concurrently, will be presented.

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Evaluation of the efficacy of lufenuron-nitenpyram and imidacloprid to control Flea Allergy Dermatitis and Flea Populations in a simulated home environment study. MICHAEL DRYDEN[1]*, MELINDA J. WILKERSON[1], PATRICIA PAYNE[1], MARY BAGLADI-SWANSON[2]; [1]DEPART. DIAGNOSTIC MEDICINE PATHOBIOLOGY AND [2]DEPART. CLINICAL SCIENCES, COLLEGE OF VETERINARY MEDICINE, KANSAS STATE UNIVERSITY

A study was conducted to evaluate the efficacy of lufenuron-nitenpyram and imidacloprid to control FAD in a simulated home-environment. Twenty dogs with previously documented FAD were skin-tested by intradermal injections and exposed to ≈25 confined fleas. Fleas were analyzed for blood and dogs examined for skin reactions. Dogs were distributed into 10 indoor-outdoor runs (2 dogs/run) with two-carpet swatches/run by ranked dermatology scores. Dogs were then infested with 50 cat fleas, on day –29 and –26, then 200 fleas eggs were placed into carpet swatches in each run for 3 days. Dogs were then treated with nitenpyram. Blinded dermatology and pruritic behavior assessments were conducted once weekly. Dogs were monitored for flea infestation by combing. Ten dogs in group 1 were administered nitenpyram orally every two days for 6 weeks and lufenuron with a meal on days 0 and 30. Dogs in treatment group 2 were treated topically with imidacloprid on days 0 and 30. All dogs reacted to the provocative challenge with wheal-flare reactions. Fleas fed rapidly with 89.88% testing positive for blood after the 5 min challenge. Mean flea comb counts in groups 1 and 2, respectively were 25.8 and 24.2, (day 0) 29 days after first infestation. Flea numbers rapidly decreased following treatment. Mean dermatology scores on day 0 were 18.1 and 19.5 for dogs in Group 1 and 2, respectively. By day 7 dermatology scores were reduced by 45.3% and 40% for dogs in groups 1 and 2, respectively. Pruritic behavior scores on Day –1 were 75.5 and 50.2 for dogs in groups 1 and 2, respectively. By day 8, scores were reduced by 69.1% and 73.7% for dogs in groups 1 and 2, respectively. Dermatology lesions and pruritus dramatically decreased within 14 to 21 days after treatment. Mean dermatology scores on day 21 were similar to scores on day –7.

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The Use of Real-time PCR Arrays to Delineate Immunological and Metabolic Genes in swine and cattle. HARRY DAWSON, PH.D.*, U. S. DEPARTMENT OF AGRICULTURE, AGRICULTURAL RESEARCH SERVICE, BELTSVILLE HUMAN NUTRITION RESEARCH CENTER, NUTRIENT REQUIREMENTS AND FUNCTIONS LABORATORY BELTVILLE, MD 20705

Real-time PCR is commonly used to verify microarray expression data. We have designed over 200 real-time PCR assays for large scale primary analysis of genes involved in immunological or nutritional function. Compared with microarray, these assays have a greater linearity, specificity and sensitivity. The immunological foci of these assays are on pathways involved in apoptosis, toll

receptor signaling and Th1/Th2 differentiation. The nutritional foci are on genes involved in iron, vitamin A and vitamin D metabolism. In conjunction with these assays, we have developed an annotated database for data mining using microarray analysis software packages. Using subsets of these assays, we profiled pig responses to *Toxoplasma gondii, Ascaris suum, Trichurus suis, Oesophagostomum dentatum, Salmonella typhimurium* and *Campylobacter jejuni* and cattle responses to *Neospora caninum. T. gondii* elicited a generalized Th1 response, typified by high level induction of IFN-γ, TNF-α, and iNOS. *A. suum* infection elicited a generalized Th2-associated response including increased IL-4, IL-5, IL-13 and mast cell tryptase expression. *T. suis* infection elicited a limited Th2-associated response. This response included localized increased IL-4 and IgE expression that was inversely correlated with worm burden inferring protection by these two molecules. The impact of this tool lies in the expanding access to genetic information between mammalian species allowing hypothesis testing of infection resistance mechanisms using quantitative expression analysis of large numbers of genes at multiple tissue sites. There is also potential application to the measurement of therapeutic efficacy for a variety of immunological and nutritional interventions against disease problems of veterinary importance.

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Selected Natural Parasitic and other Infectious Diseases of Laboratory Animals and their Potential Effects on Research Results.

DAVID G. BAKER*, DVM, MS, PHD, DACLAM. LOUISIANA STATE UNIVERSITY

Improvements in animal husbandry, disease surveillance, and veterinary care, have resulted in a tremendous decline in the number of pathogens found in laboratory animal facilities. In spite of these, natural pathogens continue to be found infecting or infesting laboratory animals in even the best facilities. When this happens, a first natural response from the research scientist is, "How will that pathogen affect my research?". The answer to that question is the subject of this presentation. The presentation begins with an overview of the historical struggle against pathogens of laboratory animals. Thereafter, examples are presented of viral, bacterial, and parasitic infections known to have altered host physiology, and therefore compromised research findings. The presentation concludes with a discussion of how specific treatments may also alter host physiology and introduce unwanted variability.

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Molecular Tools to Evaluate the Epidemiology and Control of Water-borne Zoonotic Infections.

JAMES A. HIGGINS*, PHD. USDA-ARS, BELTSVILLE, MD.

At the USDA-ARS Animal Waste Pathogen Laboratory in Beltsville, MD, we are evaluating molecular detection methods for bacterial (*E. coli*) and protozoal (*Cryptosporidium*) pathogens that can be transmitted by water. Since March 2002, we have been testing water samples-- collected from streams associated with a variety of ecosystems--for the presence of pathogenic *E. coli* (EPEC). Our protocol involves culturing total coliforms from the water samples, extracting DNA from the cultures, and detecting the pathogenicity-related *tir* gene using PCR. As of February 2003 coliform cultures from over 450 samples have been assayed and over 63% found to contain EPEC by *tir* gene PCR. DNA sequencing of the ~450 bp amplicon from 30 of these samples yields two

clades: a major one, consisting of sequences with a high degree of similarity to that of *E. coli* strain O157:H7; and a minor clade with similarity to *E. coli* strains O26, B10, and 95ZG1. Among the myriad PCR assays published for use in detection of *Cryptosporidium*, we have focused on the use of an RT-PCR targeting the RNA of a viral symbiont present in the nucleus of this protozoan. When fresh oocysts are used as template, the assay is capable of detecting as few as one oocyst. It is feasible to distinguish between strains of *C. parvum* by exploiting differences in the sequences of their viral symbionts. The viral symbiont RT-PCR assays is being evaluated for its ability to distinguish between aged (and nonviable) oocysts and those that are younger and viable.

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Vaccine Technology: Improving DNA vaccine efficacy in livestock.

GUY H. PALMER, PHD.*, COLLEGE OF VETERINARY MEDICINE, WASHINGTON STATE UNIVERSITY, PULLMAN, WA.

DNA-based immunization is a contemporary strategy for inducing cellular and humoral immunity against infectious organisms in animals and humans. DNA vaccine encoded antigens are synthesized *de novo* within the transfected cell and are preferentially processed by proteosomes and presented by MHC class I molecules. However, some endogenous antigens are directly targeted or chaperoned to the endocytic pathway where they are processed and presented to $CD4^+$ T cells by MHC class II molecules. MHC class II-restricted $CD4^+$ T cell responses are critical for generation of high affinity neutralizing antibody, immunoglobulin class switching, stimulation of macrophage killing of intracellular pathogens, and optimal expansion of $CD8^+$ cytotoxic T lymphocytes. Consequently, enhancing $CD4^+$ T cell responses is fundamental in optimizing vaccine-induced immunity against a broad spectrum of microbial pathogens.
DNA vaccines are very efficacious in murine models, but deployment of DNA vaccines in outbred animals and humans, which are the actual populations to be protected, is mainly hindered by the requirement for multiple immunizations using high vaccine doses. This requirement is partly due to poor efficacy of DNA vaccines in recruiting dendritic cells to the immunization site, which results in sub-optimal dendritic cell antigen presentation to $CD4^+$ T lymphocytes. We have focused on enhancing early events in antigen processing and presentation. Our data generated in calves representing diverse MHC class II haplotypes show that intradermal injection of DNA-encoding Flt3L and GM-CSF significantly increases dendritic cell recruitment to the immunization site with peak recruitment occurring as early as 10 days post-inoculation. Importantly, administration of DNA-encoding Flt3L and GM-CSF followed by DNA vaccination significantly increases ($p<0.05$) the population of antigen-specific effector/memory cells in PBMC as measured by ELISPOT. Furthermore, this treatment significantly enhances antigen-specific $CD4^+$ T cell proliferation and IFN-γ secretion as compared with MHC class II *DR*-matched calves vaccinated identically but without Flt3L and GM-CSF. In these studies, we identified specific $CD4^+$ T cell epitopes presented by defined MHC class II allelic gene products. Consequently, we are able to evaluate the immunogenicity of the modified DNA vaccine independent of the MHC influence and hence overcome a critical impediment to DNA vaccine optimization in cattle. More broadly, this *in vivo* read-out system provides an opportunity for a systematic evaluation of further modifications that will enhance DNA vaccine immunogenicity in cattle. Current progress in enhancing immune responses using these modified vectors will be presented.

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