

Wildlife Disease Association

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PO Box 886

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Proceedings are distributed to all meeting attendees. The Wildlife Disease Association does not regard the program abstracts as a publication and they should not be quoted in scientific literature.

1997 Wildlife Disease Association

Conference Agenda

(All sessions will be held at the Florida Marine Research Institute, if not stated)

Sunday, August 10

- 8:30 - 12:00 Journal of Wildlife Diseases Editorial Board Meeting (Harbor View Room)
12:00 - 1:00 Lunch for Members of Editorial Board and Council
1:00 - 5:00 Council Meeting (Harbor View Room)
12:30 - 5:00 Registration (Hilton Main Lobby)
5:30 - 7:30 Reception Hilton Poolside; Dinner on Your Own

Monday, August 11

- 6:30 - 8:00 Continental Breakfast at Hilton
8:30 - 8:45 Welcome and Opening Comments
8:45 - 12:00 Special Papers and Zoonoses
12:00 - 1:00 Lunch (On Campus Near Pool Area)
1:00 - 4:40 Biotoxin Symposium and General Papers
4:15 - 5:15 Posters (Fourth Floor, FMRI)
6:00 Bus Pick up at Hilton
6:30 - 11:00 Beach Party and Video Night at Ft. Desoto Park

Tuesday, August 12 Poster Session During Morning and Afternoon Breaks

- 6:30 - 8:00 Continental Breakfast at Hilton
6:30 - 8:00 Prayer Breakfast at Hilton
8:00 - 11:30 Student Presentations; General Papers
11:30 - 1:00 Lunch (On Campus Near Pool Area)
1:00 - 4:45 Surveillance Symposium

5:00 - 6:00 AAWV Meeting; Dinner on Your Own.

8:00 - 12:00 WDA Auction at Hilton

Wednesday, August 13

6:30 - 8:00 Continental Breakfast at Hilton

8:00 - 11:45 Birds

11:45 - 1:00 Lunch (On Campus Near Pool Area)

1:00 - 5:00 Carnivores, Pinnipeds and Whales

5:00 - 6:00 Business Meeting

7:00 - 8:00 Cocktails, Cash Bar, Hilton

8:00 - 9:00 Banquet and Awards (Dali Room)

9:00 - Live Band

Thursday, August 14

6:30 - 8:00 Continental Breakfast at Hilton

8:00 - 12:00 Big Game Species

12:00 - 1:00 Lunch (On Campus Near Pool Area)

1:00 - 5:00 Forensics Workshop

5:00 Adjourn

Presentation Schedule

Monday, August 11

Special Talks and Zoonoses Symposium

Moderator: Scott Wright

- 8:30 - 8:45 Welcome & Opening Remarks
- 8:45 - 9:15 Carlton M. Herman Memorial Lecturer
- (1) Environmental Contaminants and Alligator Embryos, Louis J. Guillette, Jr.
- 9:15 - 9:30 (2) A Tribute Carlton M. Herman, Founder of the Wildlife Disease Association, Louis N. Locke
- 9:30 - 10:00 American Association of Wildlife Veterinarians: Cutting Edge Speaker
- (3) Enterohemorrhagic Escherichia coli O157:H7: A Growing Public Health Problem, Michael P. Doyle
- 10:00 - 10:15 Break

Moderator: Buffy Howerth

- 10:15 - 10:30 (4) Experimental Escherichia coli 0157:H7 in White-Tailed Deer, J.R. Fischer, M.P. Doyle, T. Zhao, C.A. Brown, and C.T. Sewell
- 10:30 - 10:45 (5) Zoonoses and Students, Richard G. Botzler
- 10:45 - 11:00 (6) Wildlife Rabies in the USA: Ecological Opportunity and Viral Constraint, C.E. Rupprecht, J.S. Smith, J. Krebs, M. Niezgoda, C. A. Hanlon, J. Hensley, J. Childs
- 11:00 - 11:15 (7) Oral Wildlife Rabies Vaccination: Potential Application Strategies and Unresolved Issues, Cathleen A. Hanlon and Charles E. Rupprecht
- 11:15 - 11:30 (8) Rabies Surveillance and Control in the Northwest Territories, Canada: Establishment of a "Lay Vaccinator Program", Brett T. Elkin
- 11:30 - 11:45 (9) Historical Presence and Epizootiology of Hantavirus in Rodent Populations of Rocky Mountain National Park, Colorado, USA, Robert G. McLean, Sonya R. Ubico, Thomas G. Ksiazek
- 11:45 - 12:00 (10) Sylvatic Plague in White-Tailed Prairie Dog Colonies in Wyoming, Elizabeth S. Williams, Bob Luce, Sandy Pistono
- 12:00 - 1:00 Lunch

Biotoxin Symposium and General Papers

Moderator: Karen Steidinger

- 1:00 - 1:20 (11) Biotoxins in the Marine Environment: A Question of Recognition, Karen A. Steidinger
- 1:20 - 1:40 (12) Exposure of Marine Animals to Biotoxins: Is There a Chronic Disease Problem? Jan H. Landsberg
- 1:40 - 2:00 (13) Quantitative Methodologies for Assessing Biotoxin Accumulation, Daniel G. Baden
- 2:00 - 2:20 (14) Brevetoxicosis in Manatees (*Trichechus manatus latirostris*) from the 1996 Epizootic: Gross, Histologic and Immunohistochemical Features, Gregory D. Bossart, Daniel G. Baden, Ruth Ewing, Brenda Roberts, Scott D. Wright
- 2:20 - 2:35 (15) Avian Pox Virus in Forest Birds from Mauna Loa on the Island of Hawaii, Charles Van Riper III, Sandra G. Van Riper, Wallace R. Hansen
- 2:35 - 2:50 (16) Eastern Box Turtle (*Terrapene carolina carolina*) Mycoplasma Serosurvey, Paul P. Calle, Jim McDougal, John L. Behler, Isabella Schumacher, and Daniel Brown
- 2:50 - 3:05 Break

Moderator: Bill Samuel

- 3:05 - 3:25 (17) Demonstration of a Heartwater Carrier State in Wild African Ruminants, Michael Burrige, Trevor Peter, Suman Mahan, and Euan Anderson
- 3:25 - 3:45 (18) Health Evaluation of Free-Ranging Guanaco, William B. Karesh, Marcela M. Uhart, Ellen S. Dierenfeld, W. Emmett Braselton, Alfonso Torres, Carol House, Helena Puche, and Robert A. Cook
- 3:45 - 4:00 (19) Diagnosis of Tuberculosis and Brucellosis by Antigen-Specific IFN-Gamma Assays in Bovidae in Belgian Zoos, Jacques Godfroid, Michel Desmecht, Karl Walravens, Frank Boelaert, Emile Nduwamahoro, Hamidou Traore, Francoise Portaels, Vincent Weynants and Jean-Jacques Letesson
- 4:00 - 4:15 (20) The Pathology of Natural Bovine Tuberculosis in European Badgers (*Meles meles*): A Review, D. Gavier-Widen, John Gallagher, Steve Gillgan

Poster Presentations (Fourth Floor, FMRI)

4:15 - 5:00

- (21) Cutaneous Viral Papillomatosis in a West Indian Manatee (*Trichechus manatus latirostris*), Ruth Ewing, Gregory D. Bossart, Mark Lowe
- (22) Novel Ehrlichia-Like Organism in Wild White-Tailed Deer Associated with Lone-Star Tick Parasitism, A.R. Brandsma, S.E. Little, J.M. Lockhart, W.R. Davidson, D.E. Stallknecht, and J.E. Dawson

- (23) Ostium Secundum atrial Septal Defects in the Florida Panther (*Felis concolor coryi*), Mark W. Cunningham, Mike R. Dunbar, Claus D. Buergelt, Bruce Homer, Sharon K. Taylor, Melody Roelke-Parker, Robert King, Scott B. Citino, and Carolyn Glass
- (24) Evidence of Exposure in Alaskan Moose (*Alces alces*) to *Brucella suis* biovar IV, Philip H. Elzer, Felicia M. Ward, Todd M. O'Hara
- (25) Characterization of the Virulence and Colonization Profile of *Brucella abortus* Strain RB51 in Bison, Philip H. Elzer, Matthew D. Edmonds, Gerhardt G. Schurig, and Donald S. Davis
- (26) Concentrations of Lead, Selenium, and Mercury in Blood of Emperor Geese J.Christian Franson, Lynn H. Creekmore, Joel A. Schmutz, and Ada C. Fowler
- (27) A New Equine Herpesvirus Isolated from the Outbreak of Epizootic Encephalitis of Thomson's Gazelles, Hideto Fukushi, Akiko Taniguchi, Seiji Namihira, Takako Tomita, Tokuma Yanai, Toshiaki Masegi, Tsuyoshi Yamaguchi and Katsuya Hirai
- (28) Hydronephrosis, Hydroureter and Pyelonephritis in a Virginia Opossum Due to Metastatic Neoplastic Infiltration of the Urinary Bladder by an Intestinal Carcinoma with Paneth Cell Differentiation, Joseph Gaydos, Robert Duncan, Reneé Prater, and John Mugass
- (29) Seroconversion of Snow Geese Following Two Avian Cholera Epizootics on Arctic Breeding Grounds, Diana R. Goldberg, Daniel J. Shaddock, Michael D. Samuel, Louis Sileo, and John Y. Takekawa
- (30) Occurrence of Haematozoa in Finnish Grouse, Tuula Hollmén, Pekka Helle, and EllisGreiner
- (31) Management of Urban Exotics; On the Edge of a Potential -P.R. Nightmare, Little Liedblad, Dean Bollinger, Chris Carey
- (32) Oral Rabies Vaccine in a Field Trial: Contact by Target and Nontarget Species in West-Central Florida, Cathy Olson, Patricia A. Wemer
- (33) Effects of Translocation Treatments on Survival and Health of Native and Non-Native Passerines, Thierry M. Work, N. Gregory Massey, Luanne Johnson and Paul C. Banko
- (34) Geographic Information Systems (GIS) in Wildlife Health Research in Hawaii, Thierry M. Work, and Robert A. Rameyer
- (35) Serum Chemistry and Hematology of Summer-Acclimatized Desert-Dwelling Ringtails (*Bassariscus astutus*) (Carnivora: Procyonidae) from the McDowell Mountains in Central Arizona, USA, Cary D. Chevalier, Terry L. Morris

Tuesday, August 12

Student Papers and General Papers

Moderator: Todd O'Hara

Winner of WDA Travel Award (Paper will not be given as author was unable to attend)

- (36) Lake Whitefish, *Coregonus clupeaformis*, from the St. Lawrence River, Quebec, Canada; A Bioindicator of Environmental Contamination, Igor Mikaelian and Daniel Martineau, Yves De Lafontaine and Chantal Ménard, John C. Harshbarger
- 8:00 - 8:15 (37) Altered Behavior and Predation Susceptibility of Fishes Infected with Larvae of *Eustrongylides ignotus*, Donald F. Coyner, Sarah R. Schaack, Marilyn G. Spalding, and Donald J. Forrester
- 8:15 - 8:30 (38) Causes of Mortality in Juvenile Northern Elephant Seals (*Mirounga angustirostris*) at a Rehabilitation Center From 1992-1997, Deborah Fauquier, Frances Gulland, Martin Haulena, and Linda J. Lowenstine.
- 8:30 - 8:45 (39) The Chorioallantoic Membrane as a Target Organ of Petroleum Crude Oil Toxicity to Mallard Duck Embryos, Wanjala S.Lusimbo, Frederick A. Leighton and Gary A. Wobeser
- 8:45 - 9:00 (40) Evaluation of the Extent and Duration of Alterations in Hematological and Serum Biochemical Parameters Resulting from Oil Exposure and Rehabilitation of American Coots, Scott H. Newman, Dan W. Anderson, Michael H. Ziccardi, John G. Trupkiewicz, Paul R. Kelly, J.M. LaPoint, Keith Lewis, Sebastian Herzog and Liz Brusati
- 9:00 - 9:15 (41) In situ Characterization of Street Strains of Rabies Virus in Formalin-fixed Tissue, Charles W. Wright
- 9:15 - 9:30 (42) The Epidemiology of Newcastle Disease in a Breeding Colony of Double-Crested Cormorants on Doré Lake, Saskatchewan, 1994-1996, Thijs Kuiken, Frederick A. Leighton, Gary Wobeser, José Riva and Robert A. Heckert
- 9:30 - 9:45 (43) In situ characterization of a Lyssavirus from Australia using formalin-fixed tissue, Heidi A. Shoemaker
- 9:45 - 10:00 (44) Bluetongue Virus and Epizootic Hemorrhagic Disease Virus Infection of White-Tailed Deer Lymphocytes and Endothelial Cells with Emphasis on the Interferon Response, Mark J. Abdy, Elizabeth W. Howerth, and David E. Stallknecht
- 10:00 - 10:15 Break (Visit Posters on Fourth Floor, FMRI)

Moderator: Sarah Shapiro-Hurley

- 10:15 - 10:30 (45) Viremia and Distribution of Virus in Tissues Following Experimental Infection of Deer Mice (*Peromyscus maniculatus*) with Vesicular Stomatitis Virus New Jersey Serotype, Todd E. Cornish, David E. Stallknecht and Elizabeth W. Howerth
- 10:30 - 10:45 (46) Seroprevalence of Antibodies to Six Viruses in Wild Caspian Seals (*Phoca caspica*), C. Rosa, F.J. Dein, P. Have, J.L. Bengtson

----- General Paper -----

- 10:45 - 11:00 (47) Use of Recombinant Cell Bioassay for the Detection of Aromatic Hydrocarbons in the Serum of Free-Ranging Wildlife, Michael Ziccardi, W. J. Rogers, I.A. Gardner, J.P.Giesy, J.A. Davis and Michael S. Denison
- 11:00 - 11:15 (48) Immunotoxicology of dietary bleached kraft pulp mill effluent on mink (*Mustela vison*), Judit E. Smits, B. Blakley, H.B. Schiefer, and G.A. Wobeser
- 11:15 - 11:30 (49) Hyperplastic Foveolar Gastropathy and Hyperplastic Foveolar Gastritis in Baboons, Gene B. Hubbard, C.A. Rubio
- 11:30 - 1:00 Lunch

Wildlife Disease Surveillance Symposium

Moderator: Ted Leighton

- 1:00 - 1:15 (50) Symposium on Wildlife Disease Surveillance: Introduction, Frederick A. Leighton
- 1:15 - 1:30 (51) Surveillance of Wildlife Diseases in France: The Sagir Network, Francois Lamarque
- 1:30 - 1:45 (52) The Sagir Network - Part II: Observations and Information from the Surveillance Program, Marc Artois
- 1:45 - 2:00 (53) Multifaceted Wildlife Disease Surveillance in the Real World, Victor F. Nettles
- 2:00 - 2:15 (54) Utility and Deficiencies of National Wildlife Health Center Wildlife Mortality Databases, Milton Friend
- 2:15 - 2:30 (55) Surveillance for Chronic Wasting Disease in Colorado, M.W. Miller, E.S. Williams, M.A. Chechowitz
- 2:30 - 2:45 (56) A Proposed Pilot Program of Surveillance in Sub-Saharan Africa: Diseases Hared by Farmed, Wild and Captive/Bred Animals, Dorothy B. Preslar
- 2:45 - 3:00 (57) Establishment of a National System for Surveillance of Wild Animal Diseases in Mexico Luis Miguel del Villar

- 3:00 - 3:15 Break (Visit Posters on Fourth Floor, FMRI)
- 3:15 - 3:30 (58) The Disease Surveillance Program of the Canadian Cooperative Wildlife Health Centre, Frederick A. Leighton, Gary Wobeser, Ian K. Barker, Pierre-Yves Daoust, Daniel Martineau
- 3:30 - 3:50 (59) Surveillance Strategies for Co-Infecting Vector-Borne Zoonoses Andrew Spielman
- 3:50 - 4:05 (60) Wildlife Disease Surveillance in National Parks, A. Alonso Aguirre
- 4:05 - 4:20 (61) The Overall Role of OIE in Wildlife Disease Surveillance, Michael Woodford
- 4:20 - 4:35 (62) Promed-ahead and Global Awareness of Wildlife Diseases, Martin Hugh-Jones

Wednesday, August 13

Birds

Moderator: Marilyn Spalding

- 8:00 - 8:15 (63) AMDUCA and Extra-label Drug Use: Rights and Responsibilities for the Zoo and Wildlife Veterinarian Linda Wilmot
- 8:15 - 8:30 (64) Update on the Animal Medicinal Drug Use Clarification Act of 1994 Regulations and Meat Withdrawal Times for Wildlife Mark L. Drew
- 8:30 - 8:45 Question and Answer Session on AMDUCA Wilmot/Drew
- 8:45 - 9:00 (65) Pathology of Recurrent Bald Eagle Mortality at Degray Lake, Arkansas Nancy J. Thomas, Carol U. Meteyer, Lou Sileo and Kimberly J. Miller
- 9:00 - 9:15 (66) Pathogenicity, Host Immunity, and Diagnosis of Plasmodium relictum Infections in Hawaiian Forest Birds: Lessons for Field Studies of Avian Malaria, Carter T. Atkinson, Julie K. Lease, Robert J. Dusek, Nicholas P. Shema, Susan I. Jarvi
- 9:15 - 9:30 (67) The Effect of Environmental Temperature on the Elevational Distribution of Avian Malaria in Hawaii, Dennis A. LaPointe, M. Lee Goff, and Carter T. Atkinson
- 9:30 - 9:45 (68) Malaria at the Seney National Wildlife Refuge in the Upper Peninsula of Michigan Revisited, John N. Stuht
- 9:45 - 10:00 (69) Manipulation of Reproductive Success Modifies Antibody Responses of Kestrels, Victor Apanius
- 10:00 - 10:15 Break

Moderator: Carter Atkinson

- 10:15 - 10:30 (70) Health Evaluation of Free-ranging and Hand-reared Macaws in Peru, William B. Karesh, Alvaro del Campo, W. Emmett Braselton, Helena Puche, and Robert A. Cook
- 10:30 - 10:45 (71) Transmission of *Mycoplasma gallisepticum* Between House Finches and Chickens, D.E. Stallknecht, M.P. Luttrell, J.R. Fischer, and S.H. Kleven
- 10:45 - 11:00 (72) Effects of Avian Cholera on Survival of Lesser Snow Geese, Michael D. Samuel, John Y. Takekawa, Vasily V. Baranyuk, Lou Sileo
- 11:00 - 11:15 (73) Type C Botulism in Fish-Eating Birds at the Salton Sea, California, Tonie E. Rocke
- 11:15 - 11:30 (74) The Effect of Dietary Aflatoxin on Wild Turkey Poults, Charlotte F. Quist, Jeremy V. Kilburn, Denise I. Bounous, Victor F. Nettles, Roger D. Wyatt
- 11:30 - 11:45 (75) Does Mercury Contamination Limit Breeding in Everglades Wading Birds? Marilyn G. Spalding, Peter C. Frederick
- 11:45 - 1:00 Lunch

Carnivores and Pinnipeds

Moderator: Torsten Morner

- 1:00 - 1:15 (76) Mortality, Morphometrics, Physical Condition, and Comparison of Aging Methods of Mountain Lions Necropsied in California from 1976 - 1996, Kristin G. Charlton, David W. Hird, and E. Lee Fitzhugh.
- 1:15 - 1:30 (77) A Review of Causes of Mortality of the Florida Panther (*Felis concolor coryi*), 1972-1997, Sharon K. Taylor, Claus Buegelt, E. Darrell Land, David S. Maehr, Melody Roelke-Parker, Bruce Homer, Mike Dunbar, Mark W. Cunningham, and Carolyn Glass
- 1:30 - 1:45 (78) The Occurrence and Possible Effects of Mercury and Other Contaminants in the Florida Panther (*Felis concolor coryi*), Michael R. Dunbar, and Colin M. Gillin
- 1:45 - 2:00 (79) Dermatophyte (*Trichophyton mentagrophytes*) Infection and Treatment with Itraconazole in a Florida Panther (*Puma concolor coryi*), David S. Rotstein, Mike R. Dunbar, and Kelly Helmick
- 2:00 - 2:15 (80) Efficacy of Modified-Live Versus Killed FVR C-P Vaccines in Captive Bobcats (*Felis rufus*), Debra L. Miller, Benny J. Woody, Bruce D. Leopold, and Carolyn Cray
- 2:15 - 2:30 (81) *Toxoplasma* and *Trichinella* in Hunter-Killed Black Bears (*Ursus americanus*) from Eastern North Carolina, Felicia. Nutter, Jay Levine, Jitender Dubey, and Michael Stoskopf.
- 2:30 - 2:45 (82) Oral Mycoplasmal Infections in Canadian Pinnipeds, Lena Measures

2:45 - 3:00 (83) Epidemiology of an Ocular Disease of Unknown Etiology in Hawaiian Monk Seals (*Monachus Schauinslandi*), A. Alonso Aguirre, Bob Braun, Mark Hanson Amy Sloan, Cindy P. Driscoll

3:00 - 3:15 Break

Moderator: Don Forrester

3:15 - 3:30 (84) Beluga Whales from the St. Lawrence: an Overview of Postmortem Findings (1983-1997), Igor Mikaelian, Marie -Pier Tremblay, Daniel Martineau, Sylvain De Guise, Stéphane Lair, Lena Measures

3:30 - 3:45 (85) National Marine Fisheries Service - Marine Mammal Health Programs, Cindy P. Driscoll, Teri Rowles

3:45 - 4:00 (86) Determination of Cause(s) of Death in Large Whales in the U.S., Cindy P. Driscoll

4:00 - 4:15 (87) Viral Serologic Survey of Alaskan Eskimo Harvested Bowhead Whales (*Balaena mysticetus*), Todd M. O'Hara, Robert S. Suydam, John C. George

4:15 - 4:30 (88) Isolation and Characterization of a New *Brucella* spp. In a Minke Whale (*Balaenoptera acutorostrata*), Jacques Godfroid, Chantal Clavareau, Vincent Wellemans, Frank Boclaert, and Karl Walravens

4:30 - 4:45 (89) High Prevalence of Gastrointestinal Adenocarcinomas in Stranded Beluga Whales (*Delphinapterus leucas*) from the St. Lawrence Estuary, Stéphane Lair, S. De Guise, I. Mikaelian, L. Chouinard, D. Martineau

4:45 - 5:00 (90) Frequency of Killer Whale (*Orcinus orca*) Bites, Ship Collisions, and Entanglements Based on Scarring on Bowhead Whales (*Balaena mysticetus*), John Craighead George and Todd M. O'Hara

Thursday, August 14

Neartic Big Game

Moderator: Tom Thorne

8:00 - 8:15 (91) Long-Term Experimental *Ehrlichia chaffeensis* Infection in White-Tailed Deer, W.R. Davidson, J.M. Lockhart, D.E. Stallknecht, S.E. Little, E.W. Howerth

8:15 - 8:30 (92) Detection of *Ehrlichia chaffeensis*, the Agent of Human Monocytotropic Ehrlichiosis, in Archived Tissue of White-Tailed Deer (*Odocoileus virginianus*) by PCR, S.E.Little and E.W. Howerth

- 8:30 - 8:45 (93) Lack of Seroreactivity to Ehrlichia chaffeensis in Southeastern Rodent Populations, J. Michael Lockhart, William R. Davidson, David E. Stallknecht, and Jacqueline E. Dawson
- 8:45 - 9:00 (94) Serologic Survey for Antibodies to Borrelia burgdorferi in White-Tailed Deer in Southern Ontario, Canada, G. James Gallivan, Ian K. Barker, Harvey Artsob, Louis A. Magnarelli, Jeffrey T. Robinson, and Dennis R. Voigt
- 9:00 - 9:15 (95) An Unusual Syndrome in Black-Tail Deer, Jim E. Everman, Mike Garner, P. Briggs Hall
- 9:15 - 9:30 (96) Adenovirus Hemorrhagic Disease in Deer: Natural and Experimental Infection, Leslie W. Woods and Pamela K. Swift
- 9:30 - 9:45 (97) Heart Rate as an Indicator of Stress in Bighorn Sheep, Margaret A. Wild, Dan L. Baker, and David C. Bowden
- 9:45 - 10:00 (98) The Potential for False-Positive Diagnosis of Meningeal Worm Infection by Extraction of Larvae from Feces, Michael S. Duffy, Nathan J. Keppie and Michael D. B. Burt

10:00 - 10:15 Break

Moderator: Randy Davidson

- 10:15 - 10:30 (99) Chronic Wasting Disease in White-Tailed Deer (Odocoileus virginianus) from Wyoming, Elizabeth S. Williams, Roger Bredehoft, Glenn Stout, Sandy Pistono, and E. Tom Thorne
- 10:30 - 10:45 (100) An Update on Bovine Tuberculosis in Free-Ranging White-Tailed Deer in Michigan, Stephen M. Schmitt, Scott D. Fitzgerald, Thomas M. Cooley, Thomas F. Carlson, Colleen S. Bruning-Fann, Debbi A. Donch, Dale E. Berry, and James G. Sikarskie
- 10:45 - 11:00 (101) Chemical Immobilization of Large Numbers of Pronghorn in an Urban Environment Using Carfentanil and Xylazine, Terry J. Kreeger, Robert Lanka, E. Tom Thorne, Tom Smith, F. E. Warren, and Tom G. Smeltzer.
- 11:00 - 11:15 (102) Evaluation of the Vaccine Efficacy of Brucella abortus Strain Rb51 in Elk, Philip H. Elzer, Gerhardt G. Schurig, Fred M. Enright, and Donald S. Davis
- 11:15 - 11:30 (103) Pathogenicity of Intramuscularly Injected Brucella abortus Strain RB51 in Male Elk Calves, Walter E. Cook, Elizabeth S. Williams, E. Tom Thorne, Terry J. Kreeger, Glenn Stout, Sandy Pistono, Fred Enright and Phil Elzer, and Gerhardt Schurig
- 11:30 - 11:45 (104) RB51 Vaccine Update: Safety study of RB51 Vaccine in Pregnant Bison from Yellowstone National Park, Jerry Zaugg, Tom Roffe, Mike Gilsdorf, David Hunter
- 11:45 - 12:00 (105) Biosafety of Parenteral Brucella abortus Strain RB51 Vaccine in Bison (Bison bison) Calves, Thomas J. Roffe, Steven C. Olsen, Allen E. Jensen, Mitchell V. Palmer, Thomas Gidlewski, Royce Huber

12:00 - 1:00 Lunch

1:00 - 3:00 (106) Wildlife Forensics Seminar, Richard K. Stroud

3:00 - 3:15 Break

3:00 - 4:00 Wildlife Forensics Seminar (Continued)

4:00 - 5:00 Question and Answer Session on Wildlife Forensics

Carlton M. Herman Memorial Lecturer

(1) Environmental Contaminants and Alligator Embryos

Louis J. Guillette, Jr, Department of Zoology, University of Florida Gainesville, FL 32611 USA

Since the onset of the industrial age, environmental contaminants have posed a major threat to wildlife health. The focus of our concern on the health consequences of environmental pollution have, in the last three decades, been on lethal, carcinogenic and/or extreme teratogenic manifestations. Evidence from a number of sources suggests that another mechanism, endocrine-disruption must also be examined. There is excellent laboratory and field evidence that man-made chemicals - xenochemicals - released into the environment act as hormones or antihormones - endocrine disrupting contaminants (EDCs). The release of EDCs occurred in the past and continues today. We have used reptiles - primarily the alligator - as an ecosystem monitor for it exhibits limited mobility and feeds at the top of the food chain. Recent studies show that reptiles living in contaminated environments exhibit (1) population declines due to lethal and reproductive effects of the contaminants on embryos, juveniles or adults, (2) developmental abnormalities of embryos, including subtle effects in the reproductive system of alligators, and (3) abnormalities of the endocrine system. I will examine the data available on abnormalities of the reproductive system in reptiles induced by endocrine-disrupting xenobiotics. I will discuss the role of these xenobiotics in light of experimental evidence showing that estrogenic steroids are capable of stimulating sex reversal -- male to female -- in developing reptilian embryos exhibiting environmental sex determination. A hypothesis will be presented suggesting that any compound that disrupts the normal steroid milieu of the developing embryo will have significant, life long, consequences on sex determination and the organization and function of the reproductive system. The relative relationship between these abnormalities of reproduction and those currently reported in other wildlife populations will be discussed.

(2) A Tribute Carlton M. Herman, Founder of the Wildlife

Disease Association

Louis N. Locke, National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin
53711

Carlton M. Herman, founder and long-time supporter of the Wildlife Disease Association, died on April 5, 1997 after a period of declining health. He was born in 1909 in New York City. In 1935 he received his M.S. degree from Syracuse University, studying blood parasites of birds under the guidance of R.D. Manwell and in 1938 he completed his Sc.D. in parasitology working with Robert Hegner at the Johns Hopkins School of Hygiene and Public Health. Carlton then became a research associate of the New York Zoological Society and traveled to Kenya where he studied blood parasites in birds. In 1940, he joined the hospital staff of the San Diego Zoo and then for 9 years, beginning in 1942, he was employed by the California Division of Fish and Game in charge of disease investigations. Much of his research was with deer and quail. Carlton joined the U.S. Fish and Wildlife Service at the Patuxent Wildlife Research Center in 1950 and worked there until his retirement in 1971. Much of his research, and that of the people he supervised, was related to diseases of waterfowl. It was at the 1949 meeting of the Wildlife Society in San Francisco, California that Carlton first discussed with colleagues the feasibility of a society in the discipline of wildlife diseases. The Wildlife Disease Association (WDA) was founded under Carlton's direction in 1951. He became its first chairman and established a monthly mimeographed newsletter. By 1959, Carlton and David E. Davis, with financial support from the American Institute of Biological Sciences (AIBS), coordinated the transition from a mimeographed newsletter to microcards and subsequently to microfiche. This was an experimental program to establish the effectiveness of microfiche as a medium for scientific communication. The arrangements with AIBS included an agreement that dues in the WDA would remain at \$1.00/year during the experimental use of

microfiche. Membership boomed! To continue qualifying for grants to publish the microfiche, called Wildlife Disease, a more formal organizational structure was required. A committee drew up the constitution and by-laws and Carlton served as the first president from 1959 to 1961. During his years with the U.S. Fish and Wildlife Service, Carlton lectured and attended conferences extensively in other countries. This led to his encouraging increased international cooperation among wildlife disease specialists and increased international involvement through the WDA. Carlton was instrumental in establishing the first WDA International Conference held in 1961 at Schwanga Lodge in the Catskill Mountains of New York State. The career of Carlton Herman and the history of the Wildlife Disease Association are inextricably entwined. As acknowledgment and thanks for his numerous and significant contributions, the Association presented him with the Distinguished Service Award in 1969 and the Emeritus Award in 1971.

American Association of Wildlife Veterinarians

Cutting Edge Speaker

(3) Enterohemorrhagic Escherichia coli O157:H7: A Growing

Public Health Problem

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The public health significance of enterohemorrhagic E. coli O157:H7 (O157) continues to grow, with major foodborne disease outbreaks occurring throughout the world. Unusual properties such as acid tolerance are contributing to the bacteria's threat as a foodborne pathogen. Furthermore, strains of O157 are acquiring antibiotic resistance which increases their potential seriousness as human pathogens. In addition to ground beef, other vehicles of transmission are being reported. Examples include venison, water, fermented sausage, lettuce, radish sprouts, calves, dogs, and people. Surveys indicate that cattle are important sources of O157, with about 3% of dairy calves and 2% of feedlot beef cattle carrying E. coli O157:H7 or O157:NM in their intestinal tract. New evidence indicates that deer may be carriers of O157 and that water may be an important vehicle of transmission among cattle. Recent outbreaks of O157 infection associated with swimming in recreational lakes suggest that the pathogen may survive for extended periods of time in water. This pathogen's apparent hardiness, low infectious dose, and increased occurrence in the environment and in other sources make O157 a serious threat to public health.

(4) Experimental *Escherichia coli* O157:H7 in White-Tailed

Deer

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Cattle are considered the primary carrier of *E. coli* O157:H7; however, recent events have raised questions regarding a possible role for free-ranging deer in the epidemiology of *E. coli* O157:H7. The objectives of this study were to characterize experimental *E. coli* O157:H7 carriage by white-tailed deer (*Odocoileus virginianus*) by evaluating clinical response, duration and magnitude of fecal shedding, sites of bacterial localization, and associated lesions. Six 3-month old deer were orally inoculated with 10⁸ CFU of *E. coli* O157:H7, two received a similar inoculum of non-toxigenic *E. coli*, and one received no inoculum. Inoculated deer remained clinically normal during the trial and were shedding 10³ to 10⁵ CFU *E. coli* O157:H7/g feces by 1 day post inoculation (PI). Magnitude of fecal shedding decreased during the first 2 weeks PI, and lower numbers of *E. coli* O157:H7 were detected for the remainder of the 4-week trial. To assess contact transmission, an uninoculated deer was placed with an inoculated deer shedding approximately 10² CFU *E. coli* O157:H7/g feces at 12 days PI. Fecal shedding of *E. coli* O157:H7 by the introduced deer was detected within 2 days. Deer were necropsied at intervals throughout the trial. Moderate numbers of *E. coli* O157:H7 were recovered from the forestomachs, small intestines, and large intestines of all inoculated deer necropsied from 4 - 12 days PI and from 1 deer at 26 days PI. Low numbers of *E. coli* O157:H7 were detected using enrichment techniques in the large intestines of 2 deer necropsied at 25 days PI. Significant gross or microscopic lesions were not apparent in any deer. Results of this study indicate that deer can carry and shed *E. coli* O157:H7 similarly to other ruminants such as cattle and sheep.

(5) Zoonoses and Students

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University students conducting field and laboratory studies often are at risk for exposure to a variety of zoonotic diseases; examples can include rabies, bubonic plague, Lyme disease, and hantavirus infection. Commonly, little effort is made to address these diseases with students in the classroom prior to their conducting their work. Yet, these issues provide educators a valuable opportunity for preparing students with essential tools to address a set of field and laboratory-associated problems they can encounter throughout their professional lives. Proposed general guidelines for the classroom include providing relatively detailed discussions of any specific health risks that students might encounter in the field and laboratory for a class, before students start the work; avoiding host species and sites known to harbor serious health risk agents, when possible; and training students in specific field and laboratory protocols to minimize their risk of contacting zoonotic agents during their work. While the specific protocols used would vary with each particular disease, some required protocols might include the wearing of sterile moisture-proof gloves and other protective clothing (e.g. lab aprons) with all handling of potentially contaminated animals or materials; disinfection of traps; disinfection of work areas before and after the field or lab work; not bringing rodents indoors; avoiding downwind exposure to rodents and their excreta; and having students sign a release form indicating they are aware of the health risks, are not immunocompromised, and are aware of the safety protocols involved.

(6) Wildlife Rabies in the USA: Ecological Opportunity and
Viral Constraint

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Rabies in the United States and its territories persists as an acute encephalomyelitis due to infection with specific viral variants among insectivorous bats and carnivores (raccoons, skunks, foxes, coyotes, mongoose and dogs). Besides compartmentalization to these few taxa, numerous examples of spillover infection to other, non-reservoir species exist. No mammals are known to be completely resistant to infection, and with the exception of the marine representatives (e.g., manatee, cetaceans and pinnipeds), all indigenous mammalian Orders and most major mammalian Families are documented in historical reports since 1960, including such examples as armadillo, badger, bison, black bear, bobcat, caribou, coati, deer, fisher, insectivores (e.g., shrews), javelina, lagomorphs (e.g., rabbits and hares), mink, moose, mountain lion, ocelot, opossum, otter, pronghorn antelope, ringtail, rodents (e.g., beaver, chipmunk, gopher, mice, muskrat, nutria, porcupine, prairie dog, rats, squirrels, woodchuck, etc.), weasel and wolf. A variety of poorly understood mechanisms at the viral, host and population level are thought to be responsible for the maintenance of the dynamics between reservoir, vector or victim status. However, as exemplified by the emergence of coyote rabies in south Texas during the late 1980's, the ability to reliably predict and appropriately respond to additional outbreaks in new hosts is less than ideal. While at least one biologic has been licensed in 1997 for the oral vaccination of raccoons against rabies (whose use may be expanded to several other carnivore species), no such product has shown efficacy under field conditions for skunks. Similarly, it is doubtful if such technologies will be applied in the near future for bat rabies control. Coupled with the threat of international introduction and interstate translocation of other related lyssaviruses, the enhanced surveillance, diagnostic vigilance and novel management of wildlife rabies will continue to tax the capabilities of a modern cadre of biomedical professionals. A more basic appreciation of the pathogenesis and epizootiology of these agents in

relevant hosts, as to how and why new viral variants arise, would afford significant progress towards this end well into the next century.

(7) Oral Wildlife Rabies Vaccination: Potential Application

Strategies and Unresolved Issues

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The raccoon rabies epizootic currently affects approximately 1 million sq. km from Florida to Maine, including recent westward expansion into Ohio, with a potentially affected human population of more than 90 million, or 35% of the entire USA population. Traditional public health measures, consisting of human rabies postexposure prophylaxis (PEP), domestic animal vaccination, educational efforts, etc., have averted human mortality due to this rabies virus variant but with substantial increases in PEP rates and associated costs. As an adjunct to traditional control, oral wildlife vaccination has been under field evaluation in the USA since 1990. To date, more than 1.5 million baits containing a recombinant rabies vaccine have been distributed. The growth rate in annual number of baits targeting raccoons has been nearly exponential since the first experimental release in 1990 of 3,000 vaccine-laden baits on Parramore Island, off the eastern shore of Virginia. For example, more than 750,000 baits were distributed in the one year period from August 1996 through 1997. However, consistent data in support of reliable rabies control remain limited and strategies vary. Bait densities have ranged from 160/sq km to 50/sq km. Baiting frequency is typically annual or semi-annual. Seasonal timing of bait distribution has included all seasons, but generally occurs in spring or autumn. The length of the bait distribution period has been acute (e.g., several days to weeks), with one exception extending over several months. Bait distribution patterns can be characterized as either homogeneous or habitat-targeted. Distribution methods have ranged from hand-distribution on foot, from ground vehicles and helicopters, to mechanized distribution from fixed-wing aircraft, applied solely or in various combinations. Assessment of outcome has ranged in effort from several full-time dedicated project personnel to no apparent additional effort beyond routine passive public health rabies surveillance. Although this is an emerging novel control strategy, pre-existing recommendations provide a framework for the evaluation of such field programs, including, for example, increased rabies surveillance, particularly as the disease apparently decreases. While oral vaccination

may initially be welcomed as a panacea, simplistic conveyance of unrealistic benefits coupled with field applications designed within financial, jurisdictional, or political constraints may lead to overt "failure" of projects and premature, inappropriate dismissal of a potentially powerful adjunct rabies control measure.

(8) Rabies Surveillance and Control in the Northwest

Territories, Canada: Establishment of a “Lay Vaccinator Program”

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Rabies is endemic in arctic fox populations across northern Canada. There is a relatively high rate of positive rabies cases among domestic dogs in the Northwest Territories, where working sled dogs remain an important component of traditional subsistence lifestyles and the renewable resource economy. The combination of remote communities in close contact with wildlife, a large number of working sled dogs, and the tendency to tie sled dogs outside year-round provides a good opportunity for the spread of rabies from foxes to dogs. Rabies control efforts in the NWT are directed at protecting people against exposure to the disease through public education, control of stray dogs, identification and testing of potential rabies cases, and a dog vaccination program. Rabies is a federally reportable disease under Canada’s Health of Animals Act, which makes provisions for special vaccination programs in “remote areas where veterinary services are not readily available”. A rabies vaccination program was first established in northern Canada in the 1950's to protect working sled dogs used by the Royal Canadian Mounted Police, and was expanded to all remote communities across the north that had no access to private veterinary service. This federal program was discontinued in 1995, and a new NWT Lay Vaccinator Program was developed cooperatively by the GNWT Departments of Renewable Resources and Health & Social Services in consultation with Agriculture & Agri-Food Canada. Under this program, each community identifies an individual to be trained to serve as the local “lay vaccinator”. This volunteer is responsible for implementing the rabies control program and vaccinating dogs in their community. The GNWT provides all vaccine, supplies, training and technical support required for the program. A series of audiovisual training and reference material has been developed in both English and Inuktitut. The success of the program depends on the universal availability and widespread use of vaccine, its’ safe and effective

administration, and the diligent efforts of the local program coordinators.

(9) Historical Presence and Epizootiology of Hantavirus in
Rodent Populations of Rocky Mountain National Park,
Colorado, USA

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Sin Nombre Virus (SNV, Hantavirus, Bunyaviridae), the etiologic agent of hantavirus pulmonary syndrome in humans, is associated primarily with the deer mouse (*Peromyscus maniculatus*) throughout its range in North America. Serum samples from rodents captured in Rocky Mountain National Park (RMNP), Colorado, in 1975 and in 1994-95 were tested by enzyme-linked immunosorbent assay for antibody reactive to SNV to determine the historical presence and epizootology of hantavirus.

In 1994, we captured 48 deer mice, 41 golden-mantled ground squirrels (*Spermophilus lateralis*), 45 Richardson's ground squirrels (*S. richardsonii*), 32 least chipmunks (*Tamias minimus*), 6 Unita chipmunks (*T. umbrinus*), and 1 yellow-bellied marmot (*Marmota flaviventris*) at a variety of sites in RMNP. Antibody was detected only in deer mice (19%, 9/48) from two natural sites, two buildings, and a campground. In 1995, 7 of 53 (13%) deer mice from one natural site, one building, and a different campground in RMNP had SNV antibody. Whereas, 15 golden-mantled ground squirrels, 7 Richardson's ground squirrels, 27 least chipmunks, and 1 pine squirrel (*Tamiasciurus hudsonicus*) were all negative for antibody. A retrospective examination of serum samples from deer mice captured in 1975 during a previous study in RMNP revealed that 32% (92/285) were SNV antibody positive. These mice were captured from the same two natural sites and campground that were sampled in 1994. Our results are in agreement with previous studies in which the deer mouse was identified as the principal reservoir host species for SNV and sciurid rodents were not infected. Infected deer mice were found at numerous sites within the lower montane forest habitat of RMNP and deer mice regularly invaded dwellings and other buildings. The detection of SNV antibody in deer mice in the 1975 samples confirms the historical presence of hantavirus

in RMNP and identifies established foci of infection.

(10) Sylvatic Plague in White-Tailed Prairie Dog Colonies in
Wyoming

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Sylvatic plague, caused by *Yersinia pestis*, is common throughout the range of white-tailed prairie dogs (*Cynomys leucurus*). This disease was recognized in 1986 in Shirley Basin, the first reintroduction site for black-footed ferrets (*Mustela nigripes*). Activity and distribution of plague has been monitored since 1991 because of the potential impact of this disease on prairie dogs and reintroduced black-footed ferrets. Monitoring methods included examination of prairie dogs and ground squirrels (*Spermophilus elegans*) found dead in Shirley Basin, serologic surveys of coyotes (*Canis latrans*) and badgers (*Taxidea taxus*), and evaluating prairie dog abundance. In 1995 and 1996 surveys of deer mice (*Peromyscus maniculatus*) and grasshopper mice (*Onychomys leucogaster*) for antibodies against *Y. pestis* were incorporated into our surveillance program to provide more discrete information on plague distribution. Hemagglutinating antibodies were detected in 259 (86%) of 300 coyotes and 173 (84%) of 207 badgers. Seroprevalence was similar in all years. Thirty-two (10%) of 325 mice were seropositive in 1995 and two (1%) of 341 mice were seropositive in 1996. Sylvatic plague was probably responsible for a decline in the white-tailed prairie dog population in Shirley Basin in 1995. Monitoring exposure of mice to *Y. pestis* appears to be valuable for detecting local activity of plague in white-tailed prairie dog colonies.

(11) Biotoxins in the Marine Environment: A Question of
Recognition

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Fish belong in schools and birds belong in flocks

But here lie their carcasses

Waiting for necropsy as scientists apply their smocks.

What was the culprit?

Mercury? Aero monas? A virus? Something unknown?

What were the tell-tale signs that were shown?

Were there external signs that were easy to see?

Or were the clues microscopic, in cells and such - Could that be?

And what about the environment - Any tell-tale signs there?

A chemical spill? Oil spill? Was the pollution too much to bear?

Were the fish and birds hungry? Was there no prey to eat?

Or was the prey that they ate toxic - or nutritionally incomplete?

Was it microalgae - the kind that blooms in large patches,

The kind that might be toxic and decrease the fishermans daily catches.

Who can really say? Who can ask, recognize, test, and identify?

These potential culprits - and answer the question "why".

Are we prepared?

Are we armed with the right tests and know-how?

If not, then listen up, because Im going to explain it in more detail right now...

In the past few years, marine mammal, bird, fish, and other mortality events have been directly associated
with microalgal biotoxins. The studies done in response to these events have left many questions. We

know there are lytic compounds, agglutinating compounds, neurotoxins and other bioactive compounds, but many acute and/or chronic effects of biotoxins on marine wildlife remain unknown. Some toxins are still uncharacterized. How prevalent are biotoxins in chronic and acute disease events? How do we verify the pathways and pathologies?

(12) Exposure of Marine Animals to Biotoxins: Is There a
Chronic Disease Problem?

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The effects of harmful algal blooms on marine animals usually considered are direct toxicity during or immediately following the acute planktonic phase. Such direct impacts on specific groups of marine animals include water-borne exposure to the toxin through algal cell lysis (fish, shellfish); bioaccumulation by ingestion of toxic animals (fish, birds, turtles, mammals); inhalation of aerosolized toxin (mammals, turtles, birds); creation of toxic sediment sinks (benthic animals); and consumption of toxic benthic stages (shellfish). Acute exposure to lethal doses of toxins can result in massive animal mortalities over a relatively short time. However, there is little information concerning chronic lethal or sublethal effects on marine organisms caused by bioaccumulated or biomagnified algal toxins nor whether such effects render organisms susceptible to disease. The potential for some biotoxins to act as immunomodulators has not been well explored. Numerous unexplained marine animal mortalities and disease processes may be attributable to chronic exposure to biotoxins. Chronic exposure can lead to impaired feeding, avoidance behavior, physiological dysfunction, increased susceptibility to disease, reduced growth and reproduction, and, ultimately, pathological effects and death. Examination of selected fish, shellfish, and turtle mortalities, disease, or incidences of neoplasia with unexplained or incomplete etiologies suggests strong circumstantial evidence for exposure to biotoxins. The linkages between chemical contaminants and neoplasia or disease susceptibility in marine animals have been relatively well described. However, studies of the epizootiology of disease and neoplasia in marine animals should also consider environmental factors. In addition to routine collections of tissues for such studies, scientists should also consider developing specific protocols for sampling water, sediments, and other biota. It is critical to relate incidences of disease and neoplasia to the distribution of harmful algal blooms and the potential for accumulation of biotoxins.

(13) Quantitative Methodologies for Assessing Biotoxin

Accumulation

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Marine biotoxins present an ever increasing threat to the health and well-being of wild species. Epizootics ranging from loons, to gannets, to bottlenose dolphins, to monk seals, to manatees, have all been reported in the last decade. There has been considerable debate surrounding identification of the causative agent(s) and resolution of differences has been hampered by (1) lack of detailed baseline data, to differentiate acute intoxication from chronic exposure, (2) low levels of sophistication in some of the tests used to assess exposure, (3) lack of correlation of analytical analyses (chemical or physical) with receptor-ligand based assays (biochemical or immunocytochemical). The purpose of this presentation is to present an overview of the specific methodologies that have been developed to detect natural marine biotoxins in tissues obtained following epizootics. Examples of patch clamp electrophysiology, radio- and enzyme-linked-immunoassays, molecular pharmacological receptor binding assays, and immunocytochemical tests will be provided for comparison with high performance liquid chromatography, supercritical fluid extraction, and liquid chromatography/mass spectrometry

(14) Brevetoxicosis in Manatees (*Trichechus manatus*
latirostris) from the 1996 Epizootic: Gross, Histologic and
Immunohistochemical Features

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In 1996, at least 149 manatees (*Trichechus manatus latirostris*) died in an unprecedented epizootic along the southwest coast of Florida. At about the same time, a bloom of the brevetoxin-producing dinoflagellates, *Gymnodinium breve*, were present in the same area. Grossly, severe nasopharyngeal, pulmonary, hepatic, renal and cerebral congestion was present in all cases. Nasopharyngeal and pulmonary edema and hemorrhage were also seen. Consistent microscopic lesions consisted of catarrhal rhinitis, pulmonary hemorrhage and edema, multiorgan hemosiderosis and nonsuppurative leptomeningitis. Immunohistochemical staining using a polyclonal primary antibody to brevetoxin (GAB), showed intense positive staining of lymphocytes and macrophages in the lung, liver and secondary lymphoid tissues. Additionally, lymphocytes and macrophages associated with the inflammatory lesions of the nasal mucosa and meninges were also positive for brevetoxin. These findings implicate brevetoxicosis as a component of and the likely primary etiology for the epizootic. The data suggests that mortality resulting from brevetoxicosis may not necessarily be acute but may occur after chronic inhalation

and/or ingestion. Immunohistochemical staining with IL-1 beta interleukin converting enzyme showed positive staining with a cellular trophism similar to GAB. This suggests that brevetoxicosis may initiate apoptosis and/or the release of inflammatory mediators which culminate in fatal toxic shock.

(15) Avian Pox Virus in Forest Birds from Mauna Loa on the
Island of Hawaii

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Forest birds were captured from 1977-1980, from sea level to tree line in mesic and xeric habitats, to determine host incidence and altitudinal distributions of avian pox virus infections. Potential mosquito vector distributions and activity cycles were determined. Pox virus infections were concentrated in the mid-elevational ranges in the ecotonal areas where vectors and native birds had the greatest overlap. Native forest birds were: 1) more likely to be infected than were introduced species; 2) most likely to be infected in the wet vs. dry forests; 3) most likely to have active lesions during the wetter winter months. Laboratory challenges were made to determine host susceptibilities and revealed that infections with avian pox virus will kill native birds. Temporal and elevational differences in wild bird avian pox prevalence were apparent throughout the annual cycle, a result of differing host and parasite responses to biotic and abiotic factors.

(16) Eastern Box Turtle (*Terrapene carolina carolina*)

Mycoplasma Serosurvey

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Infectious diseases are significant in chelonian conservation programs, especially for release and translocation projects. *Mycoplasma agassizii* upper respiratory tract infection has caused widespread morbidity in free-ranging desert (*Gopherus agassizii*) and gopher (*Gopherus polyphemus*) tortoises. It is speculated that release of infected tortoises may have introduced the organism. Eastern box turtles (*Terrapene carolina carolina*) (n=49) from a free-ranging population were evaluated between 13 Jun and 1 Sep 1996 as part of a mark recapture study. All were in excellent physical condition except that three had aural abscesses. Blood samples were collected by jugular venipuncture and evaluated for *Mycoplasma* sp exposure by a plasma enzyme-linked immunosorbent assay (ELISA) validated for the detection of *Mycoplasma agassizii* antibodies in desert tortoises. Twenty one turtles (43%) were seropositive, 12 (24%) were serologically suspect, and 16 (33 %) were seronegative. Nasal wash samples from seropositive turtles were evaluated by mycoplasma culture in SP4 mycoplasma broth and polymerase chain reaction (PCR) testing utilizing *Mycoplasma* genus specific reaction conditions. No *Mycoplasma* sp were isolated in culture or identified by PCR testing. These serologic results suggest that there is a mycoplasma species infecting this population of eastern box turtles but until an organism is isolated it's impossible to determine if it is the same *Mycoplasma* sp as previously identified in the desert and gopher tortoise. *Mycoplasma agassizii* has, however, recently been isolated from a box turtle with upper respiratory tract disease in another population. Because novel pathogens may have devastating consequences when introduced into naive populations, care should be taken in the release or translocation of seropositive box turtles. They should not be introduced to populations which are seronegative or of unknown serologic status and contact between them and other chelonians should be avoided. This project was funded by a grant from the Wildlife Conservation Society's Freed Foundation

Species Survival Fund.

(17) Demonstration of a Heartwater Carrier State in Wild

African Ruminants

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Four species of wild African ruminants, eland (*Taurotragus oryx*), giraffe (*Giraffa camelopardalis*), kudu (*Tragelaphus strepsiceros*) and wildebeest (*Connochaetes taurinus*), were experimentally infected with *Cowdria ruminantium*, the tickborne rickettsia causing heartwater. Once infections were established, all of which were asymptomatic, nymphal bont ticks (*Amblyomma hebraeum*) were allowed to feed on each species and, after molting, the resultant adults were fed on sheep. Fatal heartwater was transmitted to sheep by ticks fed on all four wildlife species, demonstrating that eland, giraffe, kudu and wildebeest are all capable of becoming carriers of *C. ruminantium* infection. These results clearly illustrate the risks associated with movement of wild African ruminants from heartwater-endemic areas to heartwater-free areas such as the United States which have large populations of susceptible domestic, exotic and wild ruminant hosts and have indigenous tick species which are proven experimental vectors of heartwater. Research is in progress to develop realistic options to protect domestic and exotic livestock and wild ruminants in the United States from heartwater.

(18) Health Evaluation of Free-Ranging Guanaco

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Twenty free-ranging guanaco (*Lama guanicoe*) in Chubut Province, Argentina were immobilized with either carfentanil or tilletamine/zolazepam for health evaluations. All but two animals appeared to be in good condition. Hematology, serum chemistry, vitamin, and mineral levels were measured, and fecal ova and parasites evaluated. Serology tests included bluetongue, brucellosis, bovine respiratory syncytial virus, bovine viral diarrhea/mucosal disease, equine herpesvirus 1, infectious bovine rhinotracheitis, Johne's disease (paratuberculosis), foot and mouth disease, leptospirosis (17 serovars), parainfluenza-3, and vesicular stomatitis. No guanaco had positive test results. There was no apparent difference in external appearance or condition, or statistical differences in blood test values between the ova positive and ova negative animals. Blood samples from 20 domestic sheep maintained in the same reserve with the guanaco were also collected at the same time for serology tests. Sheep were found to have antibody titers to Bovine respiratory syncytial virus, Johne's disease, leptospirosis, and parainfluenza 3.

(19) Diagnosis of Tuberculosis and Brucellosis by Antigen-Specific IFN-Gamma Assays in Bovidae in Belgian Zoos

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Reintroduction of bovine brucellosis (*Brucella abortus*) and bovine tuberculosis (*Mycobacterium bovis*), in domesticated cattle herds in the European Union, from non-conventional sources, i.e. ruminants in zoological parks, mainly bovidae, make testing compulsory for this latter species. A practical cost-effective *in vitro* assessment of the specific cellular mediated immunity (CMI) should overcome, at least partially, the repeated immobilization problems of wild animals and other inherent limitations linked to the *in vitro* nature of the skin tests for brucellosis and tuberculosis. We adapted a commercial IFN-g test (*Mycobacterium tuberculosis* IFN-g test kit) developed in Australia (Rothel et al., 1990) in the recent context of tuberculosis for the diagnosis of brucellosis (Weynants et al., 1995) in cattle. The reactivity of IFN-g has been shown to be species specific in the bovidae family. A group of Scottish Highlanders has been classified brucellosis positive by the IFN-g test (confirmed by *B. abortus* isolation). We detected tuberculosis (confirmed by *M. bovis* isolation) in a group of American bison (*Bison bison*), a group of European bison (*Bison bonasus*) and in a group of water buffalos (*Bubalus bubalus*) by the IFN-g test. Restriction Fragment Length Polymorphism (RFLP) studies based on the Insertion Sequence IS6110 have been carried out on the *M. bovis* isolates. All zoo isolates showed a one band profile. During our study, a major tuberculosis outbreak occurred in domestic cattle. All *M. bovis* isolates originating from domestic cattle showed a unique nine bands profile. These results suggest that there was no possible epidemiological link between tuberculosis in the zoo and the 1996 tuberculosis outbreak in domestic cattle.

(20) The Pathology of Natural Bovine Tuberculosis in European

Badgers (*Meles meles*): A Review

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European badgers (*Meles meles*) are naturally infected with *Mycobacterium bovis* in local areas in Great Britain. They can excrete mycobacteria for prolonged periods and act as maintenance hosts for the infection. Contamination of pastures with tuberculosis sputum, feces, urine and exudates of badgers may result in indirect infection of cattle and has serious consequences for the control of tuberculosis in this latter species. The most frequent route of infection in the badger is via the respiratory system, followed by infective bite wounds. Pulmonary forms of the disease are the most common, with single or multiple gross lung lesions, from 1-2 mm to several centimeters in diameter up to confluent areas of chronic pneumonia. Necrosis of bronchial and mediastinal lymph nodes with no detectable pulmonary lesions also occur. Chronic generalized disease involving most lymph nodes and organs are occasionally observed. Hematogenous spread and military tuberculosis are more common following bite wound infections. The kidneys appear to be predilection sites for metastatic dissemination and in advanced kidney infections large numbers of mycobacterium are excreted in urine. In some areas the most frequent presentation of tuberculosis in the badger is the silent form "NVL" (no visible lesion). In these badgers *M. bovis* is cultured from the lymph nodes but no gross lesions are detected at the post mortem examination. NVL badgers may account for 80% or more of the tuberculosis badgers in some statutory badger removal operations. Histologically, the tuberculosis lesion is a cellular, solid, epithelioid cell granuloma with granulocytes, macrophages and lymphocytes, little necrosis in early lesions and more extended in larger granulomas, mild or absent fibrotic encapsulation, and minimal mineralization. Langhans giant cells are not observed. Serial thin slicing of the whole formalin fixed lungs of NVL badgers has revealed 0.5 to 1mm white foci. Histologically, these foci are fibrocacific nodules of the type

observed in humans with arrested forms of lung tuberculosis. Acid fast bacilli were detected in these foci and reactivation of these lesions has been observed, suggesting that contained latent infections may recrudescence and develop productive forms. Bovine tuberculosis does not appear to be prevalent in badger populations in other European countries, even though cattle tuberculosis is still a problem in many areas in Europe. The peculiarities of the morphology of bovine tuberculosis in the badger, particularly the NVL forms, make diagnosis difficult. It is recommended that mycobacterial culture accompany the post-mortem examination, even in apparently healthy badgers, in areas endemic for bovine tuberculosis.

(21) Cutaneous Viral Papillomatosis in a West Indian Manatee

(*Trichechus manatus latirostris*)

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Papillomaviruses are a group of DNA viruses, many of which are generally species specific, which can induce solitary or multiple cutaneous, oral, genital or gastric tumors. A captive, adult, seven year old, female West Indian manatee (*Trichechus manatus latirostris*) presented with multiple raised, firm, verrucoid, sessile and pedunculated, cutaneous growths on the anterior portion of her body, near the left eye lid margin and the left pectoral flipper. Histologically, the skin had marked papillary epidermal hyperplasia involving all zones along moderately dense fibrovascular stalks. Transmission electron microscopy revealed occasional intranuclear spherical to hexagonal virions which measured approximately 45-50 nm. in diameter, and were arranged in dense arrays to multiple scattered virion aggregates. The keratinocytes also had diffuse cytoplasmic proteolysis, cellular swelling, karyoplasm rarefaction, nucleolar disaggregation, and nucleolar disintegration. The viral particles in conjunction with the histological and ultrastructural changes are characteristic of viral papillomatosis. This is the first report of a virally induced disease in a West Indian manatee.

(22) Novel Ehrlichia -Like Organism in Wild White-Tailed Deer

Associated with Lone-Star Tick Parasitism

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White-tailed deer (*Odocoileus virginianus*) and the lone star tick (LST), *Amblyomma americanum*, are involved in maintenance and transmission of *Ehrlichia chaffeensis*, the causative agent of human monocytotropic ehrlichiosis. Recently, a novel Ehrlichia-like organism not known to cause human disease has been found in wild deer from the southeastern United States. To evaluate whether LST infestation was associated with the presence of this novel Ehrlichia-like organism in deer, two retrospective studies were conducted using specific nested PCR to test archived deer serum samples. In the first study, serum samples collected over 14 years from the same population were tested. The Ehrlichia-like organism was not detected in serum samples from deer collected prior to 1986, when LSTs were rare or absent on deer, but was present in samples collected from 1986 and every year thereafter, when LSTs were present and abundant. Both prevalence of infection of the Ehrlichia-like organism in deer and prevalence of infestation of deer with LSTs increased over time from 1986-1996. In the second study, serum samples from 120 deer from 24 sites in 14 southeastern states were tested. Serum samples from deer populations without evidence of LST infestation consistently tested negative for the Ehrlichia-like organism, whereas those from populations infested by LSTs had PCR-evidence of infection ranging from 0 to 80%. These data indicate that lone star ticks and white-tailed deer are involved in the natural history of at least two Ehrlichia spp., and may have implications for future studies into the epidemiology of the agents of the human ehrlichioses.

(23) Ostium Secundum atrial Septal Defects in the Florida

Panther (*Felis concolor coryi*)

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Ostium secundum atrial septal defects (ASD) were observed in 7 of 28 (25%) necropsied Florida panthers and have been implicated in the deaths of 3 (11%). Defects ranged from 3 to 15 mm in diameter with a mean of 11.5 and 4.0 mm in severe and mild cases of ASD respectively (overall mean = 8.28 mm). Gross pathological changes attributed to ASD in severely affected panthers (n = 4) included mild to severe right atrial and ventricular hypertrophy and dilatation (n = 3), acute pulmonary hemorrhage and edema (n = 3), and pulmonary vascular hypertrophy (n = 1). One panther with severe ASD also had congenital tricuspid valve dysplasia contributing to cardiac and pulmonary pathology. Panthers with mild ASD had no associated pathological changes. Electrocardiography in 1 panther with severe ASD showed a right ventricular hypertrophy pattern; contrast angiography in another revealed significant left to right shunting. Heart murmurs ranging in grade from I-V/VI were detected in 9 of 16 (56%) Florida panthers examined before necropsy. Four of 10 (40%) panthers without ASD or other cardiac disease had systolic, primarily right-sided, grade I-II murmurs. Two of 3 (67%) panthers with mild ASD had systolic right sided grade I-II murmurs. All panthers examined with severe ASD (n = 3) had systolic right-sided grade II-V

murmurs. Nine panthers in this study were not auscultated while living or results were not recorded. The detection of heart murmurs varied with the examining veterinarian, field conditions, and presence of other cardiac anomalies. Degree of secondary cardiac changes affecting shunt dynamics may also have been responsible for differences in murmur grades for panthers with severe ASD. Hematocrits tended to be higher in panthers with severe ASD (40.35%) versus panthers without ASD (36.5%). Panthers with ASD also had a higher rate of cryptorchidism (100%) than panthers without (64.3%). Morphological abnormalities commonly seen in all panthers in this study include a kink in the last vertebrae of the tail and dorsal midline cowlick. Heredity and a low genetic diversity are suspected predisposing factors for ASD in Florida panthers. One panther with mild ASD is the suspected sire of at least 3 of the remaining 6 panthers with ASD. Field detection and evaluation of ASD in Florida panthers may include portable electrocardiography and ultrasonography. Genealogy may identify those panthers most at risk.

(24) Evidence of Exposure in Alaskan Moose (*Alces alces*) to

Brucella suis Biovar IV

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Five populations of moose from regions of northern Alaska were sampled and serologically tested for the presence of antibodies to *Brucella* spp. using standard diagnostic tests. It was found that there were high incidences of serologically positive animals in these groups, ranging from 2.8% to 17.0%. Blood samples taken from a majority of these animals were not suitable for the recovery of brucellae, and all other samples were culture negative. Therefore, the purpose of this study was to use Western blot analysis with cell lysates from various *Brucella* spp. to determine the antibody profile of each animal to gain insight as to which species of *Brucella* had infected the animals. Due to the distribution of the A and M antigens in the LPS of *Brucella*, the following strains were selected for use: *B. abortus* (A antigen), *B. melitensis* (M antigen), and *B. suis* IV (M&A antigens) to differentiate the antibody responses. All of the positive animals reacted with all three of the antigens tested, demonstrating that the animals were exposed to *Brucella*. The intensity of the reactions was timed and measured; it was found that the animals reacted initially and strongest with *B. suis* IV, then *B. abortus*, and finally *B. melitensis*. Absorptions were performed on the sera with the above antigens, and the blots repeated. This again demonstrated that the animals had responses primarily to *B. suis* IV. Serological evidence indicates that these particular moose were exposed to *B. suis* IV.

(25) Characterization of the Virulence and Colonization Profile
of *Brucella abortus* Strain RB51 in Bison

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For this study, 31 American bison (*Bison bison*) were obtained from a brucellosis reactor herd in Kansas. Of the 31 animals, only 3 had positive reactions on the standard brucellosis serology tests. It has been previously demonstrated that bison vaccinated with *Brucella abortus* strain RB51 had increased colonization, pathology, and possible fetal pathogenesis when compared to vaccinated cattle. Therefore the purpose of this study was to evaluate the colonization and virulence of RB51 in sexually immature calves, sexually mature males, and pregnant females. The adult males (10) and calves (7) received 30×10^9 colony forming units of RB51 subcutaneously, and all of the adult females (14) received 1×10^9 colony forming units of RB51 subcutaneously. The adult males and calves plus 5 non-pregnant cows were divided into 2 groups; group one was slaughtered at 13 weeks post vaccination and group 2, at 16 weeks post vaccination. At slaughter, various tissues, including liver, spleen, lymph nodes, and the entire reproductive tract, were collected and cultured for *Brucella*. RB51 was not cultured from any of the vaccinated animals, and one adult bull was culture positive for a field strain of *B. abortus*. To date, of the 9 pregnant vaccinated cows, 4 have delivered healthy calves; and one animal delivered a dead, full-term calf. Serology results are still pending regarding RB51 antibodies. These results suggest that RB51 administered to a high-risk bison herd (*Brucella* positive) probably does not cause prolonged colonization (disease) and fetal pathogenesis.

(26) Concentrations of Lead, Selenium, and Mercury in Blood
of Emperor Geese

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The emperor goose (*Chen canagica*) is an arctic species that inhabits the Bering Sea coasts of western Alaska and northeastern Russia. Alaska's emperor goose population has declined by about 50% since the mid-1960s and concern regarding contaminants exposure, one of several factors identified as potential contributors to the decline, led the Pacific Flyway Council to recommend that emperor geese be monitored for exposure to toxic substances. The primary nesting area for emperor geese is the Yukon-Kuskokwim Delta (YKD) of Alaska, where lead poisoning from lead shot ingestion has been identified in eiders. During late July of 1996, we collected blood samples from emperor geese on the YKD for lead, selenium, and mercury analysis. Lead was detected (>0.02 ppm wet weight) in 42 of 235 (18%) samples, but only one bird had a blood lead concentration (0.67 ppm) above that which is generally accepted as the threshold of normal background exposure in waterfowl (0.20 ppm). Selenium concentrations in 36 blood samples from adult emperor geese ranged from 0.25 to 8.65 ppm (mean + SD = 3.28 ppm + 2.05). Little interpretive data are available for wild birds but, in experimental studies, blood selenium concentrations of 12 ppm were associated with death of mallards (*Anas platyrhynchos*) and selenium concentrations in the blood of control birds were <0.40 ppm. Mercury was detected in the blood of 31 of 36 adults, but was <0.10 ppm in each sample.

(27) A New Equine Herpesvirus Isolated from the Outbreak of
Epizootic Encephalitis of Thomson's Gazelles

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We encountered an acute disease in a herd of Thomson's gazelles (*Gazella thomsoni*) kept at a zoological garden in Japan. Two gazelles died with no sign of disease. Remaining seven gazelles showed neurological symptoms including rotation movements and spasm. Five of the affected gazelles died within 4 days after the onset of symptoms. Histological changes were found in the central nervous system. The major findings were ischemic changes of the neurons accompanying acidophilic or amphophilic intranuclear inclusion bodies, spongiosis consisted of diffuse or nodular hyperplasia of microglia and perivascular infiltration of mononuclear cells in the cerebral cortex. A herpesvirus was isolated from brain of the affected gazelles. The virus, gazelle herpesvirus 1 (GHV-1), was serologically related to equine herpesvirus 1 (EHV-1). However, DNA fingerprints of GHV-1 were different from those of EHV-1 and other equine herpesviruses. Southern hybridization revealed differences in the DNA restriction profiles throughout the entire genome. Nucleotide sequencing of glycoproteins B, D, E, G, and I showed 93 to 97% homology to EHV-1, indicating that GHV-1 is closer to EHV-1 than any other herpesvirus. GHV-1 was virulent to BALB/c mice, syrian hamsters, guinea pigs, goats and dogs by intranasal inoculation, causing neurological symptoms and death, and less virulent to horses, causing nonsuppurative encephalitis with respiratory symptoms and no sign of neurological disease. These results suggest that GHV-1 is a new type of equine herpesvirus with strong neurotropism and can cause emerging infection to domestic and companion animals as well as wild animals. The range of the natural host(s) of GHV-1 should be investigated.

(28) Hydronephrosis, Hydroureter and Pyelonephritis in a
Virginia Opossum Due to Metastatic Neoplastic Infiltration
of the Urinary Bladder by an Intestinal Carcinoma with
Paneth Cell Differentiation

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A 23- month- old wild captured Virginia opossum (*Didelphis virginiana*) was presented for weight loss, anorexia, diarrhea, and odiferous urine. The opossum was part of a group studied for evidence of an endogenous circannual fat cycle. On physical exam the opossum had significant muscle loss over vertebrae and pelvis, tachypnea with increased bronchovesicular noises over both lung fields and a gassy abdomen per palpation. Complete serum biochemical profile and electrocardiogram were within normal limits. Survey radiographs revealed a diffuse pulmonary interstitial pattern bilaterally and a diffusely gas filled intestine. A urine sample could not be obtained. Due to poor prognosis, the opossum was euthanized. Gross necropsy and histopathology revealed infiltration of the stomach and intestinal serosa, muscularis, and submucosa by nests of carcinoma. Paneth cell differentiation was present. The urinary bladder showed infiltration of the serosa and the muscularis tunics with nests of pleomorphic carcinoma cells similar to those in the stomach and intestine. The kidneys showed marked dilatation of the renal pelvis with subacute to chronic active pyelonephritis. Hydroureter was also present. Paneth cell differentiation suggests the carcinoma arose in the intestines. Distant metastasis to the urinary bladder with scirrhous response to the tumor rendered the bladder nondistensible and partially obstructed the ureters leading to hydroureter, hydronephrosis, and pyelonephritis. Intestinal neoplasia with Paneth cell differentiation is very rare. This is the first case of intestinal carcinoma with Paneth cell differentiation that we have encountered in a Virginia opossum.

(29) Seroconversion of Snow Geese Following Two Avian
Cholera Epizootics on Arctic Breeding Grounds

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Avian cholera is an infectious bacterial disease of wild waterfowl that commonly causes explosive epizootics on the wintering and staging grounds in the U.S. and Canada. Less frequently, it has been reported to occur during the breeding season for some colony nesting birds. In the summer of 1995 and 1996 there were large avian cholera dieoffs in a nesting colony of 400,000 snow geese on Banks Island, Canada. Sera collected from adult geese one month following the epizootics showed an increased prevalence of exposure, as measured by enzyme-linked immunosorbent assay (ELISA), compared to samples taken in 1994 when no dieoff occurred. The prevalence of exposure of Banks Island snow geese following the epizootics was also greater than that found in snow goose sera collected from Wrangel Island, Russia, a nesting colony where avian cholera has never been reported. Differences between years and nesting colonies will be examined.

(30) Occurrence of Haematozoa in Finnish Grouse

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Finnish grouse belong to the family Tetraonidae, and form a group of relatively closely related species. Haemoparasites of four species of grouse were identified from 306 blood samples collected at various locations throughout Finland in 1995-1997. Prevalences of infection were compared among host species and geographic locations, and the year-to-year variation of infection prevalence was further characterized in a wild population of black grouse (*Tetrao tetrix*). The seasonal rhythm of infections was studied in captive populations of black grouse and capercaillie (*Tetrao urogallus*). Three species of blood-inhabiting protozoa (*Leucocytozoon lovati*, *Haemoproteus masoni*, *Trypanosoma avium*) and a filariid nematode (microfilariae) were found in grouse. All four parasites were geographically widely distributed among Tetraonidae in Finland. *Leucocytozoon lovati* was the most common haemoparasite encountered regardless of host species, geographic area, or host population density (infection rates ranged from 64% to 100%), whereas *Haemoproteus masoni* had the lowest prevalence with infection rates of up to 20%. Preliminary findings suggest that the prevalence of microfilarial infection increases when host population density decreases. Seasonal changes in blood parasitemia occurred in captive birds: during the winter months (November-April), prevalences of *Leucocytozoon* and microfilariae decreased from 100% to 80% and from 50% to 40%, respectively. *Haemoproteus* and *Trypanosoma* were not observed in blood samples collected during the winter. Future studies aim to explain the observed differences in prevalence. Currently, work is in progress to investigate differences in vector distribution and prevalence.

(31) Management of Urban Exotics; On the Edge of a Potential

-P.R. Nightmare

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Mute swans (*Cygnus olor*) are an exotic species introduced to the United States from Europe in the late 1800's. The history of mute swans within Bend parks has dated back to the 1930's. The population was stable with fewer than 6 birds until 1988 when the numbers steadily increased to 40 swans by 1995. The 1995 Audubon bird count registered 6 nesting pairs along a 2 mile stretch of river with 28 juvenile birds being confined to a quarter mile section of river in a downtown park. In 1995 a Waterfowl Advisory Committee was created by the Bend Metro Parks and Recreation District to address the overpopulation of waterfowl. In 1996 the advisory committee began implementing a swan management plan based on the humane society's protocol of neuter and adoption. Seventeen cobs and six pens have been surgically altered, with all birds being pinioned. A xylazine/ketamine/isoflurane combination was used to anesthetize the birds. An intraabdominal incision was made and the gonads were clamped and extracted from the body cavity. The ribs were approximated with #1 maxon and the muscle layers and skin were closed with absorbable suture. Six pens were ovariectomized, one female died from intraoperative hemorrhage and two died from peritonitis probably as a result of trying to reduce hemorrhage. Fifteen cobs were castrated, with one dying from peritonitis, two were euthanized and one died of unknown causes. In addition, two cobs were vasectomized to allow the normal breeding behavior, with their mated pens being pinioned only. The plan is continuing with the vasectomy of the cobs in the four remaining mated pairs and surgical alteration of the juvenile unmated birds.

(32) Oral Rabies Vaccine in a Field Trial: Contact by Target and
Nontarget Species in West-Central Florida

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Oral rabies vaccination, targeting raccoons (*Procyon lotor*), has been conducted in Pinellas County, Florida since 1995. During the spring of 1997, a field trial was conducted attempting to determine the fate of vaccine-laden baits. Inked tracking plates were used to estimate the frequency of animal contact with fishmeal polymer baits containing an oral rabies vaccine. A total of 413 baited tracking plates were placed in 4 land use associations including single residential, multiple residential, industrial/commercial and natural areas. A bait was considered to have been contacted if it was missing from the tracking plate or at least partially consumed. A total of 254 (61%) of the baits were contacted by target or nontarget animals, or a combination of the two. Bait contact by raccoons was highest in natural areas (58%; 87/151) and lowest in industrial/commercial areas (6%; 2/31). Bait contact by nontarget species, including opossums (*Didelphis virginiana*), cats (*Felis catus*), dogs (*Canis familiaris*), and rodents was highest in multiple residential areas (69%; 18/26) and lowest in natural areas (26% 40/151). In order to increase the percentage of baits contacted by raccoons in urban areas, the baiting strategy may have to be altered from that used in natural areas.

(33) Effects of Translocation Treatments on Survival and
Health of Native and Non-Native Passerines

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Translocation is useful to re-introduce endangered and threatened birds into new habitats. In Hawaii, translocations of small passerines have met with variable success. This is due in part to lack of understanding of the effects of translocation procedures on such birds. We used two surrogate passerines, the non-native Japanese white eye and the native common amakihi to assess their response to two generic translocation regimens involving 4 hour transport by vehicle and holding in captivity for 48 hours. During each translocation scenario, we monitored for each bird food intake, weight, fecal output, hematology and serum chemistry. We found that transport of birds for 4 hours offered minimal risk. In contrast, the first 24 hours of holding in an aviary and low aviary temperatures provided the highest risk to birds. During translocations, small passerines should be held for a minimum of 48 hours or be transported immediately to the release site.

(34) Geographic Information Systems (GIS) in Wildlife Health

Research in Hawaii

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We used a global positioning system (GPS) in tandem with a geographic information system (GIS) during a lead exposure study in Laysan albatross on Midway Atoll. The GPS-GIS systems allowed us to plot albatross nests and study plots on Sand Island to within sub-meter accuracy. Using GPS and previously surveyed benchmarks, we were also able to geotransform computer graphic maps of Sand and Eastern Islands on Midway so as to make them geographically accurate and usable in GIS systems. Known latitude and longitude of nest sites offers us a variety of options to explore soil lead contamination and avian lead exposure within plots on a spatial scale. We can also navigate back to particular plots or nests in the future. If the limitations of the system are recognized, GPS/GIS could provide powerful tools for investigating and quantifying spatial events in wildlife health studies.

(35) Serum Chemistry and Hematology of Summer-Acclimatized Desert-Dwelling Ringtails (*Bassariscus astutus*) (Carnivora: Procyonidae) from the McDowell Mountains in Central Arizona, USA

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Standard serum chemistry and hematological values were measured on 17 field-fresh ringtails (7 females, 10 males) from the McDowell mountains in the Sonoran Desert approximately 15 km north of Phoenix, Arizona. The purpose of this study was to establish reference standards of serum chemistry and hematology of this population of ringtails under manual restraint. The following serum components were measured (mean and standard deviation, SD, in parentheses: glucose (mg/dL; 146.11, SD = 8.09), sodium (Na; mmol/L; 152.47, SD = 1.04), potassium (K; mmol/L; 4.57, SD = 0.09), Na/K ratio (33.6; SD = 0.81), chloride (mmol/L; 118.17, SD = 1.29), CO₂ (mmol/L; 13.53, SD = 0.9), blood urea nitrogen (BUN; mg/dL; 19.11, SD = 0.75), creatinine (mg/dL; 0.93, SD = 0.05), BUN/creatinine ratio (21.7, SD = 1.54), uric acid (mg/dL; 2.41, SD = 0.15), calcium (Ca; mg/dL; 9.81, SD = 0.12), phosphorus (P; mg/dL; 5.05, SD = 0.31), Ca/P ratio (2.06; SD = 0.11), alkaline phosphatase (IU/L; 155.42, SD = 36.52), direct bilirubin (mg/dL; 0.05, SD = 0.01), indirect bilirubin (mg/dL; 0.22, SD = 0.04), total bilirubin (mg/dL; 0.27, SD = 0.04), aspartate transferase (AST; formerly SGOT; IU/L; 262.95, SD = 134.6), alanine transferase (ALT; formerly SGPT; IU/L; 193.32, SD = 76.5), lactate dehydrogenase (LDH; mg/dL; 1513.58, SD = 245.28), cholesterol (mg/dL; 129.32, SD = 7.26), triglyceride (mg/dL; 20.05, SD = 3.77), total protein (g/dL; 8.01, SD = 0.16), albumin (A; g/dL; 3.89, SD = 0.07), globulin (G; g/dL; 4.11, SD = 0.13), and A/G ratio (0.96, SD = 0.03). Hematological values measured included: leukocytes (10³/mm³; 2.88, SD = 0.49), erythrocytes (10⁶/mm³; 8.15, SD = 0.16), hemoglobin (g/dL; 13.26, SD = 0.25), hematocrit (%; 39.8, SD = 0.66), mean cellular volume (MCV; fL; 48.8, SD = 0.61), mean corpuscular hemoglobin (MCH; pg; 16.24, SD = 0.13), mean corpuscular hemoglobin concentration (MCHC; g/dL; 33.34, SD = 0.31),

erythrocyte distribution width (EDW; %; 9.67 , SD = 0.2), platelets (103/mm³; 520.94, SD = 23.97), % neutrophils (0), % lymphocytes (44.39, SD = 4.0), % monocytes (2.8, SD = 0.46), % eosinophils (4.58 , SD = 1.1), % basophils (0) y % banded neutrophils (%; 1.73, SD = 0.45). Females had significantly higher glucose values (P = 0.034) than males, while males had significantly higher values than females for MCV (P = 0.042) and % lymphocytes (P = 0.002). These data are also compared to information from other populations of ringtails from Isla Espiritu Santo off the coast of La Paz, Baja California Sur, Mexico. Blood chemistry and hematology profiles are important for establishing normal health profiles which, in turn, are essential for monitoring population health and for understanding the dynamics of disease, energy, and nutrition in wildlife populations.

(36) Lake Whitefish, *Coregonus clupeaformis*, from the St.

Lawrence River, Quebec, Canada; A Bioindicator of

Environmental Contamination

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As part of a survey of fish diseases in the St.-Lawrence River, lake whitefish (*Coregonus clupeaformis*) were collected in the fall of 1995 and 1996 to assess the prevalence of liver lesions. A total of 355 fish were necropsied. The prevalences of foci of vacuolated hepatocytes, foci of eosinophilic hepatocytes, hepatocellular carcinoma, cholangioma and cholangiocarcinoma were 15.8 %, 0.3 %, 1.9 %, 0.6 % and 2.8 %, respectively. These results represent the first report of a series of hepatic tumors in a wild salmonoid species. Other significant changes included four cases of true hermaphroditism, a mucinous pyloric adenocarcinoma, a Sertoli cell tumor, a meningioma and an intestinal leiomyoma. Lake whitefish and other fish species from the St.-Lawrence River were collected to measure the level of tissular chemical contaminants. Lake whitefish had 3-10 fold more Aroclor 1254, pp'-DDE, mirex, photomirex, copper and endrine tissue concentrations than other fish species. These results suggest an etiologic role for environmental carcinogens and reproductive disruptors in the lesions found in lake whitefish.

(37) Altered Behavior and Predation Susceptibility of Fishes

Infected with Larvae of *Eustrongylides Ignotus*

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Eustrongylides ignotus is a parasitic nematode whose definitive host is the piscivorous bird. Several species of small fishes serve as intermediate hosts, while larger predatory fish may be paratenic hosts. Birds acquire the parasite by consuming infected fish. In some areas of Florida (USA), 80% mortality of nestling wading birds (Ciconiiformes), due to infection with *E. ignotus* has been reported, although the prevalence of infected fishes in foraging areas is often = 1%. As part of a 3-year study of the life cycle of *E. ignotus*, we examined predation susceptibility of infected mosquitofish (*Gambusia holbrooki*) to three species of predatory fishes, including juvenile largemouth bass (*Micropterus salmoides*) (n = 6), warmouth (*Lepomis gulosus*) (n = 4), and bluegill (*Lepomis macrochirus*) (n = 2). A 55-gallon aquarium with removable plexiglass divider and remote observation windows was constructed. Predatory fish were allowed to acclimate to one half of the tank, while one infected and one uninfected mosquitofish were placed in the other. The divider was removed and an observer recorded the number of capture attempts and time required for capture. Predators were observed for behavioral alterations for 4-days post ingestion of infected mosquitofish, then necropsied. Over a 3-month period, 38 trials were performed. Infected prey were preferentially selected in 31 (82%) of trials. The number of capture attempts ($\bar{x} \pm SE$) were 2.7 ± 0.2 for infected fish and 3.9 ± 0.4 for uninfected fish. Mean time of capture was 12.4 ± 1.6 minutes for infected fish and 21.7 ± 2.9 for uninfected fish. In addition, aberrant behavior including lethargy, convulsions, and buoyancy abnormalities were observed in 8 (67%) of the predators. At necropsy, larvae of *E. ignotus* were recovered from the coelomic cavity, viscera, and air bladders of predators. Infected mosquitofish were more susceptible to predation ($P = 0.01$) than uninfected fish. Post infection, predatory fishes exhibited signs and lesions which may predispose them to predation by wading

birds. Parasite induced behavior modification of intermediate hosts may facilitate the transmission of this nematode in free-ranging animal populations.

(38) Causes of Mortality in Juvenile Northern Elephant Seals

(*Mirounga angustirostris*) at a Rehabilitation Center From

1992 - 1997

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From January 1, 1992 to May 31, 1997, of 784 northern elephant seals (*Mirounga angustirostris*) that stranded alive along the California coast and were transported to a rehabilitation center, 241 died. Of the dead animals, there were 4 pups (1 - 30 days old), 195 weaners (between 1- 12 months old), 39 yearlings (12 - 24 months old), 2 sub-adults (between 2-4 years old) and one 14 year old adult. There were 134 males and 107 females. Among the pups, two animals died of meningoencephalitis, one died from pneumonia and one animal died with a congenital heart defect. The most common cause of death of the weaners was *Otostrongylus circumlitis* infection associated with arteritis and pneumonia with or without disseminated intravascular coagulation (DIC) (43.6%). The second and third most common causes of death of weaners were septicemia (14.4%) and bacterial pneumonia (11.8%). Among the yearlings, "Northern elephant seal skin disease", a generalized ulcerative and hyperkeratotic skin condition of unknown etiology resulting in septicemia was responsible for 38.5% of the deaths. Another 33.3% of the yearling deaths were caused by *Otostrongylus circumlitis* infection associated with obstructive bronchitis and pneumonia with or without DIC. Bacteria isolated from lesions in dead juvenile seals included *Klebsiella pneumoniae*, *Salmonella newport*, *Plesiomonas shigelloides*, *Escherichia coli*, *Edwardsiella tarda*, *Enterobacter cloacae*, *Streptococcus viridans*, *Micrococcus* sp., *Corynebacterium* sp., *Pseudomonas aeruginosa*, *Moraxella* sp., *Morganella* sp. and *Listeria ivanovii*.

(39) The Chorioallantoic Membrane as a Target Organ of

Petroleum Crude Oil Toxicity to Mallard Duck Embryos

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Chorioallantoic membranes from mallard duck embryos that had been externally oiled with 10 μ l of a Prudhoe Bay crude oil on day 12 of incubation were examined by light and transmission electron microscopy on days 12, 13, 14, 16, 18, 20 and 24 of incubation. There were diffuse morphological changes in the chorioallantoic membranes on both the side of external contamination and on the uncontaminated side. A high proportion of chorionic epithelial cells were necrotic. There was locally extensive irregular chorionic epithelial cell hyperplasia and loss. Mesodermal fibroblasts had severe hydropic change. Necrosis of vascular endothelium was observed in 17% and 33% of embryos on day 20 and 24 of incubation. The mesodermal layer was expanded and had swollen fibroblasts, fibrosis, multifocal hemorrhage and mild inflammation. Blood vessels in the mesoderm were significantly reduced in density per millimeter of chorioallantoic membrane. The allantoic layer was hyperplastic only on day 24 of incubation. On transmission electron microscopy, there were abnormal cytoplasmic vesicles, degenerate mitochondria, and pyknotic nuclei of the three cell types in the chorionic epithelium, and loss of villi on allantoic cells. These observations support the hypothesis that chorioallantoic membrane is a target organ of crude oil toxicity to avian embryos.

(40) Evaluation of the Extent and Duration of Alterations in
Hematological and Serum Biochemical Parameters
Resulting from Oil Exposure and Rehabilitation of
American Coots

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In order to determine long term physiological effects of oil exposure and rehabilitation on avian species, 22 hematological parameters and 23 serum biochemical parameters for both rehabilitated (RHB) birds and comparison (CON) birds were examined at monthly intervals (April, May, June and July) after the Unocal-MetroLink oil spill (February 21, 1995). The majority of significant differences ($p < 0.05$) between RHB and CON birds occurred in April (less than 2 months after the spill) including: white blood cell count (WBC), mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, creatine kinase (CK), alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, calcium, total protein, globulin, and albumin:globulin (A:G) ratio. Values from RHB birds were lower than CON birds with the exception of WBC, A:G ratio and calcium concentration. Creatine kinase concentrations significantly differed ($p < 0.05$) in both April (CON > RHB) and July (RHB > CON). Abnormalities associated with liver enzymes, muscle enzymes, immunocompetence and red blood cells consist of the main alterations detected in this study. The biological significance of these results and their predictive value as it relates to long term post-release survival will be presented.

(41) In situ Characterization of Street Strains of Rabies Virus in
Formalin-Fixed Tissue

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Recently improved techniques for identifying rabies virus RNAs (both mRNA and genomic RNA) and antigen in situ in formalin-fixed (FF) tissue have become available. To date these procedures have been used primarily to study the pathology of laboratory strains of rabies. I investigated the utility of these techniques for the examination of street strains of rabies in FF tissues. Laboratory mice were infected with rabies viruses isolated from fox, raccoon, skunk, and three species of North American bats. On the first day of clinical signs of rabies in the mice, the mice were euthanized and their brains were fixed for 24 hours in formalin. These FF tissues were examined using digoxigenin-labeled RNA probes homologous to each of the five genes in the CVS laboratory strain of rabies virus. The distribution of rabies mRNA and genomic RNA for each of the street strains of rabies was different from that for the laboratory strains examined previously. These preliminary results suggest that these procedures may be useful in understanding the pathogenesis of rabies in various wildlife reservoirs.

(42) The Epidemiology of Newcastle Disease in a Breeding

Colony of Double-crested Cormorants on Doré Lake,

Saskatchewan, 1994-1996

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We studied causes of mortality, including Newcastle disease (ND), in a colony of about 4500 pairs of double-crested cormorants (*Phalacrocorax auritus*) and 3000 pairs of American white pelicans (*Pelecanus erythrorhynchos*) on Doré Lake, Saskatchewan, from 1994 to 1996. We visited the site every third day during the breeding season using an above-ground tunnel with multiple blinds, and collected moribund and dead birds for necropsy and virological examination by inoculation of individual tissues (brain, trachea, lung, liver, spleen, kidney, jejunum, bone marrow) into embryonated chicken eggs. Antibody titres to Newcastle disease virus (NDV) were determined in egg yolks and juvenile cormorant sera. In 1995 there was an epidemic of velogenic ND in juvenile cormorants with an estimated mortality rate of 65%. No clinical signs of ND were seen nor was NDV isolated from other bird species on or near the site. The most obvious clinical sign in juvenile cormorants was paresis or paralysis of one or more limbs. The main histological lesions were non-suppurative encephalomyelitis and generalized atrophy of lymphoid tissues. Velogenic NDV was isolated more frequently and at higher concentrations from the kidney than from the brain and jejunum, and was not isolated from any other tissue tested. Captive 4-month-old cormorants infected with this virus had no clinical signs of ND, but NDV was detected in cloacal swabs up to 28 days after infection. Juvenile cormorants were seronegative in 1994 and 1996; however, 30% of those sampled after the beginning of the ND outbreak in 1995 were seropositive. The proportion of seropositive eggs was significantly higher in 1996 than in 1995.

(43) In situ characterization of a Lyssavirus from Australia
using formalin-fixed tissue

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An isolate of a previously unrecognized subtype of Lyssavirus was received at CDC in Atlanta for characterization in August 1996. The virus, named pteropid bat virus (PBV), had been isolated from a fruit bat (*Pteropus alecto*) in eastern Australia in May 1996. At CDC the virus was used to infect mice, and the formalin-fixed (FF) brain tissues from the mice were examined using in situ methods for the detection of rabies virus. PBV was compared with a laboratory strain (CVS) of rabies virus using recently developed methods for the detection of rabies viral antigen and RNAs (both genomic and mRNA) in FF tissue. Digoxigenin-labeled RNA probes homologous to the N, P, and G genes of CVS hybridized to PBV in situ. Probes for the M and L genes of CVS did not hybridize in situ. Evaluation of formalin-fixed PBV-infected mouse brain using both monoclonal and polyclonal antibodies against rabies virus is currently in progress. Results from these in situ procedures, coupled with results from other established diagnostic procedures, show that PBV is closely related to, yet genetically distinct from, the subtype of Lyssavirus known as rabies virus. These techniques should be useful in future investigations of PBV and related pathogens in their natural hosts.

(44) Bluetongue Virus and Epizootic Hemorrhagic Disease

Virus Infection of White-Tailed Deer Lymphocytes and

Endothelial Cells with Emphasis on the Interferon

Response

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There are considerable differences in the clinical disease presentation of bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) infecting white-tailed deer (*Odocoileus virginianus*), cattle and sheep. This research examined two possible factors responsible for this variability. These included the production of Type I interferon (IFN) and the ability of these viruses to replicate in endothelial cells (EC). Type I IFN was produced *in vitro* in all species when lymph node mononuclear cells were infected with either BTV or EHDV. Deer cells appeared to have an intermediate response when compared to cattle and sheep cells, with cattle mononuclear cells producing the lowest IFN titers and sheep the highest. There was replication of both BTV and EHDV within EC of all species, with deer producing intermediate viral titers and IFN titers, whereas cattle had the highest viral titers but the lowest IFN titers, and sheep had moderate viral titers in spite of a high IFN response. In sheep, there appeared to be a difference in the ability of BT and EHD viruses to replicate EC, with BT viruses having higher titers; a similar difference was not seen with virus replication in deer and cattle EC. Pretreatment of EC with IFN did not appear to affect the ability of the viruses to replicate in the cells of any of the species.

(45) Viremia and Distribution of Virus in Tissues Following
Experimental Infection of Deer Mice (*Peromyscus*
maniculatus) with Vesicular Stomatitis Virus New Jersey
Serotype

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Vesicular stomatitis (VS) is an important disease of cattle, horses, and swine that occurs frequently in
Central and South America and occasionally in outbreaks in the western United States. Despite the
significant economic and regulatory impacts associated with this disease, the epizootiology of the disease
remains largely unidentified. Rodents have been proposed to play a potential role in the epizootiology of
VS outbreaks based on a few serological surveys and some experimental work, largely confined to
laboratory rodent species. In this study, we began investigation of the potential for native New World
rodents to play a role in the epizootiology of VS. Ninety deer mice (*Peromyscus maniculatus*) were
inoculated with Vesicular Stomatitis Virus (VSV) New Jersey (NJ) serotype (low passage Ossabaw strain)
via intranasal instillation. The mice were sampled sequentially over a 7 day period and VSV-NJ was
isolated from various tissues from all but four mice from post-inoculation days 1 through 7. Viremia was
detected via virus isolation during post-inoculation days 1 through 3. Virus was isolated from the lungs
early in the experiment, followed by lymph nodes and hearts in a few mice, with eventual localization in
the central nervous system in all positive mice. Virus isolation results were largely matched by
immunohistochemical detection of VSV-NJ in similar, sequentially sampled, fixed tissue sections. This
research indicates that *P. maniculatus* does develop a detectable viremia following infection, and may
serve to be a useful model for further experiments investigating the pathogenesis of VSV-NJ infection in
wild New World rodents. Furthermore, this work suggests that this species may serve as a useful model
for study of the potential role of wild rodents in the epizootiology of VS outbreaks.

(46) Seroprevalence of Antibodies to Six Viruses in Wild

Caspian Seals (*Phoca caspica*)

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Morbillivirus, seal herpesvirus and influenza A have been causative agents involved in the mortality of thousands of seals. The 1988 epizootic of phocine distemper virus killed approximately 18,000 common seals (*Phoca vitulina*) and 1000 gray seals (*Halichoerus grypus*) in the waters of northwestern Europe. Many elements were involved in this outbreak, including the previously mentioned viral diseases, environmental contaminants and other anthropogenic factors. The Caspian Sea is the solitary range of the Caspian seal (*Phoca caspica*). This isolated population's numbers were estimated to be at a level of more than one million animals in the early 20th century, but have fallen to below four hundred thousand in the most recent published estimate. With reported percentages of barren females estimated to be between 64 and 70%, the present day population density is likely to be significantly lower. The population decline has been attributed to a combination of disease outbreak, environmental pollution and commercial exploitation. As a part of preliminary disease investigation in this species, blood samples were collected from forty-four wild Caspian seals and analyzed for antibodies to six viruses. No neutralizing antibodies against phocine distemper virus or canine distemper virus were found. An additional immunoperoxidase test was employed to rule out the presence of morbillivirus antibodies. Seven (16%) of the samples reacted with phocine distemper virus under this testing regime. All sera were tested against influenza A virus by immunoperoxidase test, with eighteen (41%) of the seals displaying a positive result. The sera were also tested for antibodies against feline herpesvirus, a virus closely related to and commonly cross-reacting with seal herpesvirus. Seven reactors (16%) were discovered in this group. None of the sera tested were found to have detectable antibodies against canine parvovirus or canine coronavirus. These

results demonstrate exposure to several disease entities in the Caspian seal population. The absence of neutralizing antibodies against phocine or canine distemper virus, in addition to the presence of reaction upon immunoperoxidase testing indicate the possibility of another distinct morbillivirus in marine mammals.

(47) Use of Recombinant Cell Bioassay for the Detection of
Aromatic Hydrocarbons in the Serum of Free-Ranging
Wildlife

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Halogenated and polycyclic aromatic hydrocarbons (HAHs and PAHs) are a diverse group of widespread and persistent environmental contaminants. These chemicals have been shown to cause a variety of detrimental effects in both people and wildlife, including tumorigenesis, endocrine abnormalities, organ dysfunction and lethality; many of which have been directly linked to activation of the Ah receptor (AhR). Currently, although several methods are available to detect exposure in vertebrates, they are expensive, labor intensive and require large amounts of tissue for analysis. A recombinant cell line, which responds to HAHs/PAHs with the induction of firefly luciferase, has recently been developed as a bioassay for the direct detection of such AhR ligands in natural and manufactured products. This assay, through optimization and validation, has also been found to be effective as a “bioassay of exposure” in both people and animals through the analysis of small amounts of serum, both directly and through simple liquid-phase extractions (thereby significantly increasing assay “sensitivity”). Several additional methods, such as acid denaturation of samples (to remove PAH components, thereby differentiating exposure classes) and the incorporation of a potent Ah receptor antagonist (to correct for non-ligand dependent induction of cells) continue to be examined to improve the diagnostic value of the assay for use with people and wildlife. Here we report data on several analyses of sera from wildlife populations experiencing differing exposures to HAHs/PAHs.

(48) Immunotoxicology of Dietary Bleached Kraft Pulp Mill

Effluent on Mink (*Mustela vison*)

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Semi-aquatic predators are exposed to anthropogenic contaminants directly, or by bioaccumulation of compounds through the food chain. In this study, dietary bleached kraft pulp mill effluent (BKME) exposure in mink (*Mustela vison*) was examined. In two 8-month studies, mink (15 in year 1; 30 in year 2) were fed diets containing fish caught downstream of a BKME discharge point. Drinking water contained 25% BKME. Control mink were fed nutritionally matched 'clean' fish diets. Clinical, biochemical, hematological, pathological, and reproductive factors were examined. Hepatic enzyme induction was measured by ethoxyresorufin-O-diethylase (EROD) activity. The immunotoxicity of BKME was studied through mitogen stimulated proliferative response of peripheral blood mononuclear cells (PBMC), delayed type hypersensitivity (DTH) response, and antibody response to mycobacterial antigen, assessed by enzyme-linked immunosorbent assay (ELISA). Except for MFO induction and immune function, there were few differences between exposed and control groups for the other variables. EROD activity was greater in exposed females and males, compared with controls. Immunotoxicological assessment showed no difference in PBMC proliferation between control and BKME-exposed mink. The DTH response was impaired in the BKME-exposed mink, while the antibody response was enhanced. This observed immune deviation, expressed as suppression of cell mediated immunity and enhanced antibody production, could interfere with competitive fitness of mink in their natural environment.

(49) Hyperplastic Foveolar Gastropathy and Hyperplastic

Foveolar Gastritis in Baboons

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Five cases of gastric hyperplasia out of 215 baboon stomachs examined were found incidentally at necropsy. Two stomachs had diffuse giant mucosal folds and three had multiple giant mucosal nodules. The increased mucosal thickness in those areas was due to giant hyperplasia of the foveolar epithelium. The former two specimens were classified as Menetrier's disease (MD) and the latter three as Varioliform Lymphocytic Gastritis (VLG), since they were histologically identical to those gastropathies in humans. Whereas MD had diffuse giant hypertrophic faveoli without lymphocytic infiltration ($>16/100$ epithelial cells) and prominent glandular cysts, VLG had nodules due to giant hyperplastic faveoli with intraepithelial lymphocytosis ($>20/100$ epithelial cells). Since the causes leading to gastric MD and VLG in humans remain unknown, the spontaneous occurrence of these two conditions in the baboon may open new avenues for investigation of possible etiologic factors in the laboratory. It also shows how a previously new unknown conditions can be found incidentally in animals even after years of study.

(50) Symposium on Wildlife Disease Surveillance: Introduction

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This symposium was organized to focus attention on wildlife disease surveillance. Disease surveillance can be viewed as consisting of four different activities: detection of disease and disease-causing agents, diagnosis of disease, assembly and analysis of relevant data and use of the analysed information for the original purpose of the surveillance program. Surveillance can be limited to one or a few diseases, or it can be general and consider all diseases. Sampling of populations can be passive, based on the routine activities of field personnel and diagnostic laboratories, or active, based on statistical sampling of populations. Wildlife disease surveillance has the potential to contribute useful information in the areas of wildlife management and conservation, domestic animal health and public health. International trade in animals and animal products and heightened interest in emerging diseases have placed a new emphasis on disease surveillance. This symposium will explore the practice, strengths, weaknesses and usefulness of surveillance of diseases in wild animals.

(51) Surveillance of Wildlife Diseases in France: The Sagir

Network

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Created in 1986 by the “Office National de la Chasse” (ONC), a government agency in charge of wildlife, the SAGIR network is a national surveillance system of wildlife diseases unique in Europe. It is organized as a cooperative venture among ONC, the National Center for Veterinary and Alimentary Studies (CNEVA) in Nancy, the toxicology laboratory of the National Veterinary School in Lyon (ENVL), the “Departmental” Veterinary Laboratories (LVD) and the “Departmental” Federations of Hunters (FDC). As a warning system, SAGIR worked perfectly for the last ten years. The network can be credited with some significant achievements as far as wildlife health is concerned. However, a few limiting factors are still preventing the SAGIR network from becoming a real epidemiological-surveillance system. A plan is currently being implemented to improve the network.

(52) The Sagir Network - Part II: Observations and Information

from the Surveillance Program

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Given the strengths and weaknesses of SAGIR, described previously, the network is more effective in some activities than in others. It has succeeded as a watchdog; outbreaks of new diseases have been quickly recorded, as was the case with European Brown Hare Syndrome (EBHS) in 1986 and classical swine fever (hog cholera) in wild boar in 1992. Recent epizootics of botulism and pesticide poisoning also have been detected. For species that are regularly examined in large numbers, it has been possible to describe temporal trends in major mortality factors. For the Brown Hare, a limited number of specific diseases are of importance: pseudotuberculosis (yersiniosis), pasteurellosis, EBHS and tularemia. In contrast, mortality in Roe Deer has been dominated by opportunistic internal parasitism and miscellaneous bacterial infections. While successful in identifying such important mortality factors, the network has not secured the data necessary to assess their impact on host populations. The distribution of certain infectious agents present in wildlife has been more precisely defined. Diseases such as Aujeszky's disease (pseudorabies), trichinosis and brucellosis are persist among wild boar without evident impact on host populations, but with potentially important impacts on domestic animals. SAGIR thus serves an important role as the wildlife health surveillance organization of the French government.

(53) Multifaceted Wildlife Disease Surveillance in the Real
World

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There is a distorted perception among the general public that wildlife health professionals have large data bases for every disease condition in wildlife and these data bases are coupled to active surveillance programs. In truth, the inherent difficulties in monitoring wildlife health and the relatively few dollars that are applied to this activity place extreme limitations on what is possible and force wildlife health scientists to conduct wildlife disease surveillance in numerous small but resourceful ways. Major tools used to monitor diseases in the "real world" associated with uncooperative wild animals and restricted resources are diagnostic investigations of mortality events, surveys for baseline information that may be either general or focused but usually are short-termed, and "networked" projects. Data from diagnostic cases can be valuable for wildlife surveillance, but there are sampling biases and reporting systems for this information are poorly developed. Although there are countless disease and parasite surveys done in wild animals, most are "snapshot" research projects that provide useful data, but no trends for wildlife population managers. Furthermore, the information is scattered throughout the literature and is difficult to track. Networked projects involve the assimilation of wildlife disease data from a variety individuals or laboratories via correspondence, e.g., questionnaires or requests for donation of data. Two examples are the Nationwide Hemorrhagic Disease Questionnaire and the Wild Swine Brucellosis and Pseudorabies Data Bases. Practical solutions to the current shortfalls in wildlife surveillance include greater efforts toward centralized reporting of diagnostic findings, greater extension of information on wildlife health issues to field personnel and the public to enhance recognition, continued research in wildlife health to determine what are the important elements and to improve technology for recognition, and of course, increased funding to do the work.

(54) Utility and Deficiencies of National Wildlife Health Center

Wildlife Mortality Databases

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Wildlife specimens from across the nation have been submitted for cause of death evaluations to the National Wildlife Health Center since program initiation in 1975. The cumulative total of these specimens numbers in the tens of thousands. This presentation explores how these data have been utilized in addressing wildlife management issues; deficiencies in these data for assessing status and trends in causes, geographic distributions, and population assessments; and ethical considerations associated with accountability and use of these data by others. The latter is of growing concern relative to electronic access and dissemination of information.

(55) Surveillance for Chronic Wasting Disease in Colorado

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Chronic wasting disease (CWD) is a spongiform encephalopathy of mule deer, white-tailed deer, and Rocky Mountain elk. The only documented foci of CWD are in northcentral Colorado and southeastern Wyoming. Passive surveillance of free-ranging cervids in enzootic areas has been ongoing for over 16 yr, but active surveillance began in Colorado in 1990. Our current surveillance program is primarily designed to provide data on distribution and prevalence of CWD, although several secondary benefits are recognized. Two strategies are currently employed: 1) A statewide targeted survey relies on recognition, collection, and submission of suspect CWD cases to veterinary diagnostic laboratories by wildlife managers. The CWD "suspect" profile includes deer or elk >18 mo of age that are emaciated and show abnormal behavior, \pm increased salivation, tremor, ataxia, incoordination, difficulty in swallowing, or polydipsia/polyuria; suspects are evaluated via brain histopathology and, in more recent years, by immunocytochemistry, SAF, or Western blot. Using this approach, about 70 CWD-positive cervids have been detected among 5 management units in northcentral Colorado since 1981; at least 175 additional "suspects" submitted from throughout Colorado have been examined and found to be affected by maladies other than CWD. 2) Population surveys rely on collections of heads from deer and elk harvested in enzootic areas; brains are examined by histopathology and, since 1995, by immunocytochemistry. Estimated prevalences from 1992-1996 harvest surveys were 0-1%/elk management unit/yr and 0-7.3%/deer management unit/yr in enzootic areas. Surveillance data have been used in public information campaigns, regulation and policy development, and research and management planning. Based on results obtained to date, this combination of targeted and population surveys appears effective in detecting, studying, and monitoring CWD in free-ranging cervid populations in Colorado.

(56) A Proposed Pilot Program of Surveillance in Sub-Saharan

Africa: Diseases Hared by Farmed, Wild and Captive/Bred

Animals

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By current assessments, Sub-Saharan Africa is the most likely part of the world to spawn infectious animal diseases. And despite the fact that substantial portions of its economies are food production and eco-tourism, it does not have a cohesive animal disease surveillance program. Moreover, the region operates in a continuing 'crisis' mode, depending in large part on outside resources. The AHEAD project is exploring the feasibility of establishing a pilot program that would begin with four or five contiguous countries and perhaps one other which would establish a base for expansion in the region. While ProMED and AHEAD are more visible for their electronic-mail networks than for policy planning and implementation, both are actively engaged in proposing true surveillance programs. The SSA program envisioned by AHEAD would link syndrome-based surveillance of the shared diseases at the clinical level to one or more diagnostic reference laboratories in the region, and incorporate electronic communications for reporting through official country channels to response resources. The outcome of a survey of various facilities in the region and a tentative plan of "first steps" will be presented, focusing on what is possible out of what is needed, particularly in the livestock-wildlife intersects.

(57) Establishment of a National System for Surveillance of
Wild Animal Diseases in Mexico

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No Abstract

(58) The Disease Surveillance Program of the Canadian
Cooperative Wildlife Health Centre

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The Canadian Cooperative Wildlife Health Centre (CCWHC) is an organization among Canada's four veterinary colleges to provide wildlife health-related services to wildlife agencies and to increase knowledge about wildlife health and disease in Canada. Disease surveillance is a major activity of the CCWHC. The purpose of this surveillance program is to develop and monitor the inventory of diseases and affected species and to make this information available to decision makers for programs and policies in wildlife management and conservation, domestic animal health and public health. Detection of disease is done largely by Canada's cadre of professional and avocational field personnel and is supported by a toll-free information telephone service, a variety of publications and short courses on health-related topics. Diagnosis is provided by CCWHC personnel and by provincial and federal veterinary laboratories. A national database of disease occurrences serves as the central repository of surveillance data. Information is provided to decision makers through special advisories, reports and annual meetings with federal, provincial and territorial wildlife directors. The services of the CCWHC are funded by annual allocations from sponsors; there are no direct charges to field personnel or to the public. The surveillance program has succeeded in expanding knowledge about wild animal diseases in Canada and in providing useful information that is increasingly taken into account in relevant public policies and programs. Many aspects of the program require improvement or enhancement to meet the current demands for CCWHC services. (Reference: Leighton, F.A., et al., 1997. The Canadian Cooperative Wildlife Health Centre and

surveillance of wild animal diseases in Canada. Canadian Veterinary Journal 38: 279-284.)

(59) Surveillance Strategies for Co-Infecting Vector-Borne

Zoonoses

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Because people tend to be exposed to diverse potentially pathogenic microbes when they are in contact with wildlife, attempts to monitor risk of zoonotic disease in a site should consider pathogen associations that may be synergistic. The deer-associated pathogens of New England provide an ideal example of such synergy. Human babesiosis, transmitted by deer ticks (*Ixodes dammini*), exacerbates pathogenesis due to the agent of Lyme disease, and the agent of human granulocytic ehrlichiosis may exacerbate babesiosis. A co-infecting Powassan-like virus and a *Babesia* of deer (*odocoilei*) share the same rodent reservoir and tick vector hosts. Where these infections are enzootic, mosquito-borne arboviral pathogens of deer (Cache Valley virus and Jamestown Canyon virus) may simultaneously infect human beings. An array of monitoring strategies will contribute to efforts designed to protect human populations from this array of zoonotic pathogens.

(60) Wildlife Disease Surveillance in National Parks

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The National Park Service (NPS) is in charge of a system of 337 parks covering 23 million hectares (79 million acres) in the United States. Wildlife diseases represent significant management concerns in a number of these parks. Diseases may be a threat to human health (i.e. Lyme disease, sylvatic plague, hantavirus infection, rabies); may be of primary concern to domestic livestock on adjacent park lands (i.e. bovine brucellosis, paratuberculosis); and may threaten wildlife populations (i.e. hemorrhagic disease, lungworm-pneumonia complex, distemper) within these protected areas. Historically, national parks have not systematically surveyed or monitored animal diseases. The need for a surveillance program in parks nationwide is evident. The systematic collection of specimens is required to determine the nature of infectious agents, including their geographic distribution, sources, reservoirs and vectors. Examples of disease surveillance in park ecosystems will be discussed including the monitoring of sylvatic plague in rodent populations; bovine respiratory viruses in ungulates; and rabies and Lyme disease in other mammals. Issues relating to domestic animal, human and wildlife health will be addressed within national park policy. Seroepidemiologic surveys are the first step and a useful tool to investigate disease and infection in park populations. Disease management should be integrated within ecosystem-level management. Disease monitoring and control are justifiable since the overall goal of the NPS is to conserve and protect natural ecosystems. The NPS must play a proactive role in management of disease issues by participating in state and regional disease surveillance programs. This surveillance system could provide background information on animal disease interactions which could then be integrated in policy-making decisions.

(61) The Overall Role of OIE in Wildlife Disease Surveillance

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It is surprising that epizootic disease in wildlife, particularly in endangered species, is so often overlooked by conservation authorities. Rinderpest entered Africa from Asia at the end of the last century and decimated wild and domestic ungulates alike. Such a pandemic occurring again today would surely cause the immediate extinction of several currently threatened species which now exist only in vulnerable isolated pockets. Similar consequences with other diseases in other places can be foreseen. Disease detection, control and eradication in domestic animals on an international scale, which are OIE's main objectives, prevents the loss of both domestic and wild animal genetic resources.

Wildlife involvement in the epidemiology of many of the major livestock and poultry diseases has long been known. When wildlife was plentiful, the disease threat from wild to domestic animals was the primary preoccupation of the world's national Veterinary Services in their attempts to control and eradicate the serious epizootic diseases which are shared by wildlife and domestic livestock. Hundreds of thousands of wild ungulates were thus slaughtered in southern Africa in an attempt to control trypanosomiasis. Luckily veterinary science and ethics no longer support these draconian measures and nowadays alternative environmentally acceptable methods are employed.

The OIE International Animal Health Code specifies controls for the most important diseases of domestic and wild animals and their products in international commerce. Future updates of the companion OIE Manual of Standards for Diagnostic Tests and Vaccines will increasingly include technical information on those diseases which commonly affect both wild animals and their domestic relatives. A separate Code and Manual for Aquatic Animals is now under preparation.

Because of the increasing role of veterinarians in the conservation of wildlife, the OIE has dedicated

several editions of its quarterly Scientific and Technical Review to the diseases of free-ranging wild animals. The organization's role includes disease surveillance and control and is thus a contribution to the conservation of wild fauna as a valuable resource for biological equilibrium.

The establishment in 1992 of an OIE Working Group on Wildlife Disease, comprised of internationally known specialists from all over the world, has increased the organization's ability to detect, report and where necessary, control outbreaks of wildlife disease. The members of the Working Group, representing many disease specialities and geographic regions, attempt to sensitize the officers of national veterinary services to the importance of wildlife as sentinels for disease surveillance in their domestic herds.

The Working Group reports to the 143 Member Country Veterinary Services information on disease of greatest risk to wildlife populations and suggests techniques for their surveillance, prevention and control. The Group also consults with CITES on matters of mutual interest.

In conclusion, almost every genetic resource is afflicted by disease agents. The world's officially organized national Veterinary Services, through their international organization, contributes to the maintenance of biodiversity by surveillance, prevention and control of epizootic diseases of both wild and domestic animals.

(62) Promed-Ahead and Global Awareness of Wildlife

Diseases

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ProMED-mail was started in 1995 with a handful of members. It now has over 13,000 members in 120 countries. As it became more popular and engendered more support, the communication rate increased, along with the general awareness that animal diseases and zoonoses are a major component of the 'emerging' diseases. Thus a special part crystallized out and was organized as Animal Health/Emerging Animal Diseases (AHEAD), with its own funding within ProMED. Like the parent structure it functions on various levels, and specifically in policy recommendation and electronic communications. The speaker is a moderator for AHEAD. In 1966 there were 1,145 AHEAD postings with 67% being specific reports or discussions on disease outbreaks and directly related events: the other third covered conferences, new books, websites, and miscellaneous reports. In all just over three postings a day. Of the disease outbreaks reported, 15% were in Africa, 45% in the Americas, 15% Asia, 18% Europe, and 7% in Oceania. Obviously, ProMED is informed of a minor fraction of what is known but it has become, in its way, the "CNN" of disease outbreaks with increasing recognition by governments, international health agencies (who were in fact participants from the start), and news and television industries world wide. But it is only as useful as its members make it through their participation and support. These and other aspects of AHEAD will be discussed.

(63) AMDUCA and Extra-Label Drug Use: Rights and Responsibilities for the Zoo and Wildlife Veterinarian

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Prior to October 1994, section 512 of the Federal Food, Drug, and Cosmetic Act provided that a new animal drug (NAD) was deemed unsafe unless it was subject to an approved application and the drug, its labeling and its use conformed to such approved application. Therefore, use of a new animal drug without approved application or in a manner different from that set forth in an approved application resulted in the drug being unsafe under this act. The Animal Medicinal Drug Use Clarification Act or AMDUCA, for which regulations were published in the Federal Register in November 1996 and for which implementing regulations took effect in December 1996, allows veterinarians to prescribe extra-label (ELU) uses of FDA-approved animal drugs and approved human drugs for animals.

Zoo species and free range wildlife species which are not harvested for human food fall into the non-food animal arena; therefore, the AMDUCA allows veterinarians to use whatever drug (approved human or animal) they need to effectively practice. Extra-label drug approved human or animal drug is permitted when there is no animal drug approved for the intended use, when there is an animal drug approved for the intended use, but the approved drug is not in the required dosage form or concentration or when an approved drug has been found to be clinically ineffective when used as labeled. Furthermore, when the intended use involves administration to a non-food animal, an approved human drug can be used.

Free-roaming wildlife species which may be hunted are viewed differently. The agency understands that some of these animals may be harvested for human food, and, therefore, they are considered to be food animals. When considering ELU in these animals, veterinarians must be in conformity with the provisions of the regulations applicable to food animals.

In food animals, “first resort” for ELU is an approved animal drug rather than an approved human drug. ELU that doesn’t require compounding is “first resort” over ELU that does. Where compounding is appropriate for use in food animals, “first resort” is to an approved animal drug rather than an approved human drug. All other requirements of the regulations must also be met, i.e., adequate recordkeeping, appropriate withdrawal time, etc. For example, certain drugs are prohibited from use in food animals. These include chloramphenicol, clenbuterol, diethylstilbestrol, dimrtridazole, ipronidazole, other nitroimidazoles, furazolidone (except for approved topical use), nitrofurazone (except for approved topical use); and sulfonamide drugs in lactating dairy cattle (except approved use of sulfadimethoxine, sulfabromamethazine, and sulfaethoxy pyridazine). Fluoroquinolones, and glycopeptides are in process of being added to the list of prohibited drugs.

In addition, the timing of ELU should take into consideration periods of harvest (e.g., hunting seasons). Determinations of the appropriate withdrawal time is the responsibility of the veterinarian, taking into account the species, their range, published withdrawal times in approved species, and local hunting season.

The agency believes that Congress intended that veterinarians be responsible for overseeing the extra-label use of drugs. The agency recognizes the unique applicability of the veterinary-client-patient (VCP) relationship to free-ranging wildlife. With respect to use of drugs by non-veterinarians, such as wildlife biologists who are typically state or Federal employees, it is noted that they are usually under the general supervision of a veterinarian, who also may be a government employee. Such relationships fall within the scope of a valid VCP relationship.

The preceding comments are general. There are limitations provided in the AMDUCA prohibiting extra-label use of certain drugs under specific conditions and other special circumstances. Interested parties are encouraged to contact the Center for Veterinary Medicine for further information.

(64) Update on the Animal Medicinal Drug Use Clarification

Act of 1994 Regulations and Meat Withdrawal Times for

Wildlife

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With the passage and advent into law of the Animal Medicinal Drug Use Clarification Act of 1994 in 1996, several questions remain unanswered with regard to the implications of this act for the practice of veterinary medicine in the field of wildlife management. The practical application of the provisions of this act for wildlife species that are defined as food producing animals are largely left to the discretion of the veterinarian. Therefore prior to the extra-label use of a drug, consideration must be given to the following questions and/or situations: 1) Does a valid veterinarian-client-patient relationship exist? 2) Does the veterinarian have supervision over lay personnel who are administering drugs in an extra-label manner? 3) Is there sufficient information to justify the extra-label use of a drug (reasonable basis, lack of approved drugs, drug metabolism data)? 4) Is the veterinarian prepared to be responsible for consequences of extra-label use of a drug including adverse reactions and residue problems? 5) Are adequate records being kept by both the veterinarian and lay personnel the administer drugs for extra-label use? If the veterinarian can fulfill the requirements of AMDUCA the use of drugs in an extra-label manner in wildlife should present no major difficulties for wildlife health workers.

(65) Pathology of Recurrent Bald Eagle Mortality at Degray

Lake, Arkansas

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Unprecedented mortality occurred in bald eagles (*Haliaeetus leucocephalus*) at DeGray Lake, Arkansas, during the winters of 1994-95 and 1996-97. The first eagles were found dead during November, soon after arrival from Fall migration and mortalities continued at a slow rate into January in both episodes. In total, 29 eagles died at or near DeGray Lake in winter 1994-95 and 26 in winter 1996-97, while no eagle deaths were noted in the intervening winter or in the past history of the lake. During the mortality events sick eagles were observed overflying perches or colliding with rock walls. No consistent abnormalities were seen on gross necropsy; but by light microscopy, each eagle examined had striking, bilaterally symmetrical, spongy degeneration of the white matter in the central nervous system. Cellular inflammatory response to the lesion was distinctly lacking. Transmission electron microscopy revealed that status spongiosus in the white matter was comprised of intramyelinic vacuoles, formed by splitting of the myelin lamellae at the intraperiodic line. This lesion is characteristic of toxicity from hexachlorophene, triethyl tin, isonicotinic acid hydrazide, and certain exotic plant toxins; however, despite exhaustive testing, no etiology has been determined for the Degray Lake mortalities.

(66) Pathogenicity, Host Immunity, and Diagnosis of
Plasmodium relictum Infections in Hawaiian Forest
Birds: Lessons for Field Studies of Avian Malaria

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We tested the susceptibility of 5 species of native Hawaiian forest birds to isolates of *Plasmodium relictum*. Mortality in Hawaii Amakihi (*Hemignathus virens*), Iiwi (*Vestiaria coccinea*), Apapane (*Himatione sanguinea*), and Maui Creeper (*Paroreomyza montana*) ranged from 50-90% after exposure to a single infective mosquito bite. Infections in Omao (*Myadestes obscurus*) were transient and non-fatal. Surviving birds were immune when rechallenged and developed antibodies to a common suite of malarial antigens that have persisted as long as 2.5 years after infection. We evaluated the sensitivity and specificity of PCR, immunoblots, ELISA, and microscopy for diagnosing malarial infections. No test was completely reliable; however, a combination of microscopy, PCR and immunoblot techniques appears to be the most powerful approach for detecting and quantifying both acute and chronic infections. Use of multiple diagnostic techniques is essential for accurately determining prevalence of *Plasmodium* in forest bird populations.

(67) The Effect of Environmental Temperature on the
Elevational Distribution of Avian Malaria in Hawaii

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Avian malaria (*Plasmodium relictum capistranoea*) is considered a major factor contributing to the population decline and extinction of endemic Hawaiian forest birds. Distributional anomalies of remaining bird populations have been correlated to vector mosquito distributions. Between 1992 and 1994 we trapped adult mosquitoes over an elevational transect and microscopically examined them for plasmodial infection. Vector relative abundance and infection rate varied over elevation. However, vector abundance is only one of many disease-limiting factors that vary with elevation. We examined the effect of environmental temperature on vector survivorship, gonotrophic cycle and parasite development. In field and laboratory studies we found that vector survivorship increases, while the rate of parasite development decreases with decreasing environmental temperature. Parasite cycle ceases at 13 °C. The duration of the gonotrophic cycle also increases with lower temperature. These results suggest that mid-elevation temperatures may be optimal for transmission and that high elevation refugia may be more a result of parasite thermal constraints than vector distribution. Current concerns of mosquito range expansion are overemphasized and vector control measures should be directed at mid-elevational forests.

(68) Malaria at the Seney National Wildlife Refuge in the
Upper Peninsula of Michigan Revisited

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Epizootics of a malaria-like disease attributed to *Leucocytozoon simondi* occurred regularly in giant Canada geese (*Branta canadensis maxima*) on the Seney National Wildlife Refuge in the 1960s and early 1970s. Mortality of goslings exceeded 90% in some years. There have been no apparent epizootics of leucocytozoonosis, or any other disease, on the refuge since 1972. Annual gosling mortality on the refuge has remained relatively low despite the continued existence of leucocytozoon. The ecological character of the refuge was changed in 1991 when trumpeter swans (*Cygnus buccinator*) were released to try and establish a resident population. It is not known if the swans can be infected with leucocytozoon or what role they play in the ecology of *L. simondi* on the refuge. Production and survival of cygnets has varied, but the flock seems to be doing well. It appears that they have been spared thus far from the serious disease problem encountered by giant Canada geese in the past. A series of investigations in the mid 1970s in the Upper Peninsula of Michigan pointed to the existence of a pathogenic form of *L. simondi* on the Seney refuge that did not exist elsewhere on the peninsula. These same studies also showed that different strains of *L. simondi* behaved differently in the invertebrate hosts as well. The life cycle of *L. simondi* as presently described would suggest that pathogenicity would not favor survival of this parasite. Why then does an apparently highly pathogenic form exist at the Seney National Wildlife Refuge? Why have regular epizootics of leucocytozoonosis on the refuge ceased? A new investigation into the ecology of *L. simondi* in the Upper Peninsula of Michigan is being designed to answer these questions and others. The purpose of the study is to develop a conceptual model that shows how and why a normally benign parasite becomes highly pathogenic.

(69) Manipulation of Reproductive Success Modifies

Antibody Responses of Kestrels

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Previous research suggested that naturally or artificially increased reproductive effort influenced host control of hematozoan infections. It was hypothesized that the increased energy turnover associated with increased parental effort had a negative effect on host immunocompetence. To directly test this hypothesis, brood size was manipulated in wild Eurasian kestrels and then daily energy expenditure and anti-SRBC antibody responses were measured in the field. Daily energy expenditure of the parents was positively related to the experimental brood size, with the highest levels in parents with enlarged broods. Antibody responses were negatively related to experimental brood size, with lower responses in parents with enlarged broods. Finally, antibody responses were negatively related to daily energy expenditure of the parents. These results suggest that artificially increasing avian reproductive effort has negative consequences in terms of humoral immunity and possibly parasite resistance. In terms of endangered species management, reproductive manipulations may provide short term benefits due to increased recruitment but may be associated with decreased survival of parents due to the cost of reproduction phenomenon.

(70) Health Evaluation of Free-ranging and Hand-Reared

Macaws in Peru

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As part of ongoing ecological studies and reproduction enhancement efforts for macaws in southwest Peru, a health survey of parent- and hand-reared Scarlet Macaws (*Ara macao*) and Blue and Gold Macaws (*Ara ararauna*) was conducted in 1994. Thirty-three birds were examined during handling procedures, and blood samples collected from 27 (9 parent-reared, 18 hand-reared) for laboratory analysis. All but one bird appeared to be in good condition with no abnormalities noted during physical examination. Hematology, plasma chemistries, and plasma vitamin and mineral levels were measured and correlated with the results of bacterial and viral serology. Positive antibody titers for *Salmonella* and psittacine herpesvirus were found. These diseases have the potential to affect wildlife population dynamics, and *Salmonella* may have public health significance. Serological tests for avian influenza, infectious laryngotracheitis, paramyxovirus-1, -2, -3, polyoma virus, chlamydiosis, and aspergillosis were negative. Significant differences in disease prevalence were not found between the two species or rearing situations, but none of the parent-reared chicks had positive titers for *Salmonella* while seven out of ten hand-reared, year-old free-flying birds were positive.

(71) Transmission of *Mycoplasma gallisepticum* Between
House Finches and Chickens

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Until recently, *Mycoplasma gallisepticum* (MG) infections were associated only with domestic poultry flocks, and free-living birds were not considered an important component in the epizootiology of this disease. All of this changed in 1994, when chronic conjunctivitis associated with MG infections was diagnosed in house finches (*Carpodacus mexicanus*). Specific objectives of this study were: 1) To determine if chickens can be infected with MG via direct, across wire, and proximity (across room) contact with naturally infected house finches; 2) To determine if house finches can be infected through direct contact with chickens experimentally infected with the finch MG strain; and 3) To determine if other poultry-derived MG strains will infect house finches. Results indicated that chickens can be infected with the finch strain of MG through direct contact with naturally infected house finches. Transmission of MG from naturally infected house finches to chickens was detected by seroconversion, polymerase chain reaction (PCR), and culture of MG. Transmission of MG from infected chickens to house finches also occurred but was only detected by positive serum plate agglutination (SPA) results after 8 weeks of direct contact with MG-infected chickens. Finches were susceptible to experimental infection with other vaccine and poultry strains of MG, and in these cases infection was confirmed by both PCR and culture.

(72) Effects of Avian Cholera on Survival of Lesser Snow

Geese

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Avian cholera, caused by the bacterium *Pasteurella multocida*, is one of the most important diseases affecting waterfowl in North America, but little is known about the impacts of this disease on annual survival rates. We studied the epidemiology and mortality from avian cholera on lesser snow geese (*Chen caerulescens*) wintering in the Pacific Flyway. We banded birds on their breeding areas at Wrangel Island, Russia and Banks Island, Canada. Birds were banded with aluminum legbands and individually coded plastic neckbands or radio transmitters. We experimentally vaccinated half of the neckbanded geese to provide protection from avian cholera for up to 1 year following banding.

Observations of neckbanded geese and telemetry tracking of radioed geese were primarily conducted on the wintering areas in California and Oregon to determine causes of mortality, seasonal movement patterns, and survival rates. We compared the causes of mortality and survival rates of vaccinated and unvaccinated geese to estimate the impact of avian cholera on survival of both snow goose populations and to determine the effectiveness of our vaccine in wild waterfowl. Avian cholera significantly reduced survival of snow geese, but impacts varied in relation to the weather and habitat conditions in the Central Valley of California.

(73) Type C Botulism in Fish-Eating Birds at the Salton Sea,
California

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In August through October of 1996, over 13,000 pelicans, herons, and other fish-eating birds became sick or died from type C botulism at the Salton Sea in the Imperial Valley of southern California.

Because fish-eating birds are not typically associated with type C botulism outbreaks, an extensive investigation was conducted to determine the potential sources of botulism toxin for these birds. Dead and dying fish (tilapia) were collected from various locations on the Salton Sea and live, presumably healthy fish were captured using gill nets or minnow traps, and immediately refrigerated or frozen until processing. The viscera were removed, ground in saline, and tested for type C botulism toxin by ELISA or mouse bioassay. Samples from fish caught live were also cultured for botulism spores.

Type C botulism toxin was detected in 35% of all the dead fish sampled and in 26% of sick fish that were captured just prior to death, however only about 6% of the healthy fish sampled had botulism spores in their guts. These findings suggest that toxin in the dead and dying fish was ingested prior to death and not the result of post-mortem formation. Laboratory studies indicated that tilapia were more sensitive to type C botulism toxin than previously thought. This is the first documented epizootic of type C avian botulism associated with fish and the largest die-off of pelicans ever reported in the U.S.

(74) The Effect of Dietary Aflatoxin on Wild Turkey Poults

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Aflatoxins, which are toxic metabolites of either common fungus *Aspergillus flavus* or *Aspergillus parasiticus*, have long been known to cause poor feed utilization, decreased weight gains, depressed immune function, liver dysfunction, coagulation abnormalities, cancer, and death in a wide variety of species including humans. In the past, aflatoxicosis in wildlife has not been a major concern as exposure to the toxins is rare under natural conditions. However, increased management, feeding, and baiting increases the potential of exposure of wildlife to aflatoxins through contaminated grain such as corn. Under these artificial conditions, wild turkeys are commonly fed corn, yet the susceptibility of these birds to ingested aflatoxin is unknown. The objective of this study was to evaluate the effect of dietary aflatoxin on feed intake and weight gains, liver function, blood coagulation and carotinoid levels, and immune function of wild turkey poults.

Four groups of 4-month-old wild turkeys were fed 0, 100, 200, or 400 ppb aflatoxin incorporated into the feed over a two-week period. Initial body weights were measured, and baseline blood parameters obtained. Feed consumption of each group was monitored. After two weeks, the birds were weighed; blood samples were collected for coagulation assays, complete blood counts, and blood chemistry profiles; and the birds were euthanized and necropsied. Liver, kidney, pancreas, and bursa weights were measured. The results indicate that aflatoxin-fed birds had decreased weight gains and feed consumption as compared to control birds. Blood chemistry alterations attributable to liver dysfunction varied depending on the dosage of aflatoxin. Decreased liver weights, liver enzyme alterations, slightly altered blood coagulation patterns, and mild histologic changes indicated low-level liver damage. Immune function, particularly cell-mediated immune function, was compromised. The effects were apparent in all treatment groups, but statistically significant effects were most often found

at 400 ppb aflatoxin. This study shows that short-term aflatoxin ingestion can have an effect on wild turkeys, and indicates that feeds containing even 100 ppb aflatoxin should be avoided for use as feed for wild turkeys.

(75) Does Mercury Contamination Limit Breeding in
Everglades Wading Birds?

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Mercury contamination has been put forth as a possible explanation for the reduction of breeding of Everglades wading birds. We have attempted to examine this hypothesis with a number of different approaches. The pervasive contamination of the animals in the ecosystem, and migratory nature of the species, does not allow for any comparisons with “contaminant free” birds. Non-breeding birds are difficult to identify and even more difficult to collect samples from. We compared mercury concentrations in feathers of breeding great blue herons, with those of nonbreeding adults collected during the breeding season. Feathers collected at nests from breeding birds had higher mercury concentrations than those collected from birds with no evidence of breeding condition. In addition, feather mercury concentrations in all birds showed a distinct peak during the breeding season, suggesting increased exposure during this part of the year. We discuss several explanations for these results.

(76) Mortality, Morphometrics, Physical Condition, and
Comparison of Aging Methods of Mountain Lions
Necropsied in California from 1976 - 1996

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Records of 564 mountain lions (*Puma concolor*) necropsied in California from 1976 to 1996 were analyzed for sex- and age-related differences in mortality, morphometrics, and physical condition. Regional and temporal differences were also examined. In the prime adult age class, male lions were more likely to be killed for domestic animal depredation than females and prime adult female lions were more likely to be killed by motor vehicles than males. No sex-related differences in mortality sources were detected in the 2-year-old and old adult age classes. Mortality due to depredation increased with mountain lion age while mortality due to motor vehicles was inversely related to mountain lion age. Female mountain lions appear to continue growing until just before their third year while male mountain lions appear to grow until just after their fourth year. Body weight, tail length, body length, chest girth, head length and width, neck circumference, front foot pad length and width, rear foot pad length and width, and front and rear foot width measurements were larger in adult male lions than in adult female lions. Chest girth of mountain lions was linearly related to body weight and a regression equation was derived to predict body weight based on chest girth. Weight/body length ratios of mountain lions differed according to physical condition. Mountain lions less than two years old were more likely to be in poor physical condition than prime adult mountain lions. Female mountain lions in the old adult age class were more likely to be in poor physical condition than prime adult females, whereas this relationship was not detected in male lions. Except for lions determined to have died of disease or debilitating injury, physical condition did not differ by cause of death. Body

weights and conditions of prime adult mountain lions did not differ by season, year (1990-1996), decade, or geographical region of California. Cementum annuli analysis and subjective assignment of age by field biologists were comparable for estimating the ages of mountain lions.

(77) A Review of Causes of Mortality of the Florida Panther

(*Felis concolor coryi*), 1972-1997

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6Deceased.

The Florida panther (*Felis concolor coryi*) is one of the most endangered mammals in the world with the free-ranging population estimated to consist of only 30-50 adult animals. These animals inhabit south Florida and a subset of the population has been studied using radio telemetry since 1981.

Between 1972 and 1997, there were 68 Florida panther carcasses recovered for postmortem evaluation. The cause of mortality was undetermined in 16 (24 %) panthers due to autolysis or insufficient carcass recovered. Of the 52 panther in which carcasses were suitable for evaluation the cause of mortality or predominant contributing factor to mortality were: Vehicular collision 25 (48 %), Aggression (intraspecific or self inflicted) 12 (23 %), Illegal shooting 5 (9 %), Congenital defects - atrial septal defect 3 (6 %), Research activities - capture 2 (4 %), and Infectious diseases - rabies 1 (2 %) and pseudorabies 1 (2 %). Toxicosis was suspected but not yet proven in 3 (6 %) panthers.

(78) The Occurrence and Possible Effects of Mercury and
Other Contaminants in the Florida Panther (*Felis
concolor coryi*)

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The Florida panther (*Felis concolor coryi*), an endangered subspecies, once inhabited the entire southeastern United States and was contiguous with other cougar populations. However, the current population consists of 30 to 50 adults and associated young inhabiting the Big Cypress Swamp and Everglades ecosystems of south Florida. This isolated and apparently inbred population is characterized by a low genetic diversity which has been implicated as the primary cause of several congenital anomalies including cardiac atrial septal defects, defects in sperm morphology, low sperm count, cryptorchidism, and low reproductive success. However, many of these clinical signs and physical anomalies found in the Florida panther have been reported in other species that were exposed to a variety of chemical compounds, including mercury and those that have been identified as environmental endocrine disruptors. Specific sources of mercury contamination (air-borne, agricultural point source, or naturally occurring) in wildlife in southwest Florida are unknown. Research has shown that mercury accumulates in aquatic habitats and animal species and bioconcentrates in carnivores at the top of the food web. No known acute deaths have been identified in the Florida panther due to mercury, however, mercury levels have been monitored in captured Florida panthers to determine if mercury is chronically affecting this endangered population. From 1985-95, blood mercury levels were elevated in Florida panthers in their southern most range (≈ 0.96 ppm) compared to animals north (≈ 0.22 ppm) of Everglades National Park (ENP). In 1989, mercury toxicosis was suspected as a possible cause of death in one adult female Florida panther with a liver concentration of 110 ppm in ENP. Since 1992, blood mercury levels have appeared to decrease in Florida panthers in ENP but increases have occurred in some panthers north of ENP. One possible

explanation may be the selection of aquatic carnivore prey species, such as raccoon (*Procyon lotor*), by panthers when more preferred prey, such as wild hogs (*Sus scrofa*) or white-tailed deer (*Odocoileus virginianus*), are less abundant. Raccoons, which are a major prey item for Florida panthers, bioaccumulate mercury and may also concentrate lipophilic, endocrine disrupting compounds like methoxy chlor, or P, P1-DDE in their body fat. It is possible that the reproductive abnormalities reported in the Florida panther may be from the exposure of developing kittens to xenobiotic endocrine-disrupting contaminants or mercury bioaccumulated by the dam via an aquatic food chain. Evidence of exposure of Florida panthers to environmental contaminants such as organochlorines and PCB's has been lacking until recently. Data from archived fat samples from Florida panthers that died from 1982 to 1992 indicated an exposure to a variety of potentially harmful chemicals including P,P1-DDE and Aroclor 1260. Both of these chemicals, which have been reported to be estrogenic in other species, were found in some panthers, especially those inhabiting ENP. Regardless of the success of the on-going genetic restoration program, the environmental contamination issue must be addressed to assure the recovery of the Florida panther.

(79) Dermatophyte (*Trichophyton mentagrophytes*) Infection
and Treatment with Itraconazole in a Florida Panther
(*Puma concolor coryi*)

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A 6-year-old male free-ranging Florida panther (*Puma concolor coryi*) from south Florida USA was diagnosed with a severe case of dermatophyte (*Trichophyton mentagrophytes*) infection. Clinical signs were progressive over a 2-year period from 1994 to 1996 and originally included moderate alopecia of the head, ears, and rear limbs. Clinical signs in 1996 involved 85% hair loss with excoriations, ulcerations, patchy pyoderma and lichenification of the skin, and two digits had nail loss. Otherwise, the panther was in good body condition. The animal was captured and kept in captivity for a period of 6 weeks and received treatment with itraconazole at a dose of 9.5mg/kg once daily placed in food. Clinical signs resolved except for the patchy areas of hair loss and the animal was released. Two other Florida panthers from the same area were diagnosed with dermatophyte infections (*T. mentagrophytes* and *Microsporum gypseum*) involving moderate alopecia, but clinical signs resolved spontaneously with no treatment. The significance of infection with *T. mentagrophytes* in the Florida panther population is not known and requires further investigation.

(80) Efficacy of Modified-Live Versus Killed FVR C-P

Vaccines in Captive Bobcats (*Felis rufus*)

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Historically, captive wildlife facilities engaging in vaccination programs have used predominantly killed virus strains of the vaccines because of the potential for morbidity when using modified-live strains. However, concern is also relevant about whether killed vaccines elicit an immune response in the recipient. We collected serum samples from 13 (8 males and 5 females) unchallenged bobcat (*Felis rufus*) kittens at 3 serial FVR C-P vaccination periods given at 4 week intervals beginning at 1 month of age. Kittens were divided by gender and then randomly assigned to either the modified-live or killed vaccination group. Immunofluorescent antibody testing was performed on all serum samples. Seven of 7 kittens from the modified-live group demonstrated positive responses at a 1:250 dilution. None of the kittens receiving the killed vaccine demonstrated antibody titers of 1:250. Six, 2 and 3 of 6 kittens had no protection for calicivirus, feline rhinotracheitis and panleukopenia, respectively at the 1:50 dilution. The remaining kittens had weakly positive responses at the 1:50 dilution after 2 serial injections. These results suggest that modified-live FVR C-P vaccine offers greater immunity than killed FVR C-P vaccine in bobcats and the killed vaccine may not provide enough immunity to be effective against disease. Further study is needed to test the safety of modified-live FVR C-P vaccines in non-domestic felines to allow for their use in captive wild felids.

(81) *Toxoplasma* and *Trichinella* in Hunter-Killed Black Bears

(*Ursus americanus*) from Eastern North Carolina

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Infection with the parasites *Toxoplasma* and *Trichinella* can cause serious illness in human beings. Both can be acquired by handling or consuming raw or undercooked meat containing the infective stages. The role of wildlife in the transmission of these diseases is not fully understood, but several species have been implicated as sources of human infection. Since black bears are hunted and the meat is often used as human food, our objective was to determine the prevalence of *Toxoplasma* and *Trichinella* in North Carolina black bears and to evaluate carcass handling and meat preparation practices. During the 1996 bear season in eastern North Carolina, 79 serum samples were collected from hunter-killed animals. *Toxoplasma* antibodies detected by the modified agglutination method were found in 83.7% of samples. Sera were tested at dilutions of 1:25, 1:50 and 1:500, and a significantly greater ($p=0.0006$) proportion of females than males had titers of 1:25 or more. *Trichinella* antibodies were not detected in any of the samples by modified ELISA. Surveys of hunters showed that 96% butcher the carcasses themselves, 100% consume the meat or share with someone who does, and 20% prepare the meat in a manner that may be inadequate to inactivate infective stages. Black bears have the highest reported seroprevalence of any mammal studied to date and serve as a potential source of human infection.

(82) Oral Mycoplasmal Infections in Canadian Pinnipeds

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Bacterial swabs of the tooth-gum interface in the oral cavity of four species of pinnipeds from eastern Canadian waters were collected and cultured for *Mycoplasma* spp. Three species of *Mycoplasma* and unidentified species of *Mycoplasma* and *Ureaplasma* were detected. Eight of 12 (67%) grey seals (*Halichoerus grypus*), 20 of 35 (57%) harbour seals (*Phoca vitulina*), 31 of 36 (86%) hooded seals (*Cystophora cristata*) and 8 of 27 (30%) harp seals (*Phoca groenlandica*) were infected with *Mycoplasma phocacerebrale*. *Mycoplasma phocidae* and *M. phocarhinis* were also detected in some seals. Young harbour (N = 3), grey (N = 2), harp (N = 10) and hooded seals (N = 10) from a few days to six months old were negative. Seals held captive in salt water or fresh water aquaria were also infected. *Mycoplasma phocacerebrale* has been implicated in human infections known as “seal-finger”. Human infections can be acquired by being bitten by infected seals or by handling live or dead infected seals. Some recent “seal-finger” infections in biologists and successful treatment are described.

(83) Epidemiology of an Ocular Disease of Unknown Etiology
in Hawaiian Monk Seals (*Monachus schauinslandi*)

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The Hawaiian monk seal (*Monachus schauinslandi*) is considered the most endangered pinniped in North America. As a result of a severe population decline, the species has been protected since 1972. In 1984 a rehabilitation program was initiated consisting of taking undersized, weaned female pups and holding them in captivity on Oahu. Rehabilitated seal pups were then released into the Northwestern Hawaiian Islands after 8-10 months. An eye disease of unknown etiology has been documented in 11 of 12 female seal pups brought into captivity for rehabilitation during 1995. The seals have not been released because of the risk of spreading the disease to the wild population. This study summarizes the investigations into the potential cause(s), origin, and nature of the ocular condition characterized by three stages including a period of blepharoconjunctivitis, blepharism and photophobia (initial clinical signs) that was most notable 1-4 weeks after arrival on Oahu. Corneal opacities and "blue eye" developed about three months later; and bilateral cataracts and blindness approximately 12 months following onset of corneal opacities. The prevalence, incidence, time course, association patterns and treatments are described based on daily observations for the past 23 months.

(84) Beluga Whales from the St. Lawrence: An Overview of
Postmortem Findings (1983-1997)

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A population of approximately 500 beluga whales (*Delphinaterus leucas*) inhabits the St. Lawrence Estuary and the Saguenay Fjord. This population has been chronically exposed to a complex mixture of industrial pollutants. In 1983, a research program based on the necropsy of stranded beluga whales was initiated. From 1983 to 1997, 17 cancers have been diagnosed among 93 necropsied beluga whales. One animal was affected by two cancers. This represents an overall prevalence of 18 % animals examined and represents 45% (17/38) of all cancers reported in cetaceans throughout the world. Six of these cancers affected the proximal intestinal mucosa. Other cancers included two granulosa cell tumors, two metastatic carcinomas of unknown origin, two gastric adenocarcinomas, one carcinoma of the urinary bladder, one hepatocellular carcinoma, one adenocarcinoma of the salivary glands, one adenocarcinoma of the mammary gland, and one ovarian dysgerminoma. Sudden and fatal dissecting aneurysms of the pulmonary trunk affected three adult males. Two congenital anomalies of the reproductive system were observed: one true hermaphrodite and one male pseudohermaphrodite. Because high tissue concentrations of organochlorine compounds, a well known group of reproductive disruptors, have been demonstrated in tissues of beluga whales from the St. Lawrence River, the presence of congenital anomalies of the reproductive tract may be related to pollution. Sixteen animals were affected by marked verminous bronchopneumonia due to *Halocercus monoceris* infection. These animals were generally young individuals and appeared emaciated. In 1995 and 1996, two mature females died from wounds caused by a propeller from a small motor-boat. As no stranded beluga examined prior to 1995 had evidence of propeller wounds, these two recent

deaths raise concerns that small motorized vessels may pose a new hazard for this endangered population of beluga. Large fibrous masses with osseous metaplasia were found in the epiploon and/or in the spleen in 4 cases and may represent chronic inflammatory reactions. Degenerative, hyperplastic and benign neoplastic changes were commonly encountered within the adrenal cortex, thyroid glands and the pituitary gland and could be age-related and/or represent a response to exposure to chemical contaminants. Other causes of death included large abscesses in diverse locations (5), severe peritonitis of unknown etiology (4), dystocia (3), "orphaned" calves (3), aborted fetuses (3), severe suppurative pneumonia (3), severe hepatitis (2), fatal perforating gastric ulcer (2), ulcerative esophagitis and gastritis (1), intestinal diphyllbothriosis (1), omphalophlebitis (1), and presumptive congenital defect of the larynx (1).

(85) National Marine Fisheries Service - Marine Mammal

Health Programs

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The Marine Mammal Health and Stranding Response Program at NMFS incorporates directed live animal sampling as well as examining beach cast carcasses when collecting data from marine mammals. Through live capture/release a complete suite of health assessment parameters is collected, analyzed, and assimilated into databases for government/management use. Efforts in recovering data and useful tissues from dead stranded animals can be challenging. Often carcasses are in remote areas, in various states of decomposition, or are vandalized before complete examinations are conducted. As stewards of our nations marine resources, proactive education/outreach is a growing element of the NMFS Marine Mammal Health and Stranding Response Program. Recent efforts to educate the public on appropriate approach behavior with marine mammals in the wild have been well received in the Southeastern US.

(86) Determination of Cause(s) of Death in Large Whales in
the U.S.

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While the magnitude of a post mortem examination on a large whale can seem daunting, it can be a useful method of assessing anthropogenic impacts to the endangered species that the National Marine Fisheries Service is mandated to monitor and protect. Within the last 10 years deaths attributed to human-related causes have been on the rise in humpback and right whale populations. Fisheries entanglements, ship strikes, and degraded habitats have contributed to the decline of large whales. Through consistent examination, sampling, database development, as well as appropriate management action, the NMFS is beginning to address these growing threats while mitigating efforts to reduce impacts to critical population/habitats. The North Atlantic Humpback population has increased slowly with protective efforts since the halt of commercial whaling. However, the North Atlantic Right Whale remains the most endangered large whale in the world. With only 300-350 individuals remaining, this dwindling population may be on the verge of extinction. The National Marine Fisheries Service is working cooperatively with fishing, and shipping industries as well as with other government agencies to identify causes of death in all large whales.

(87) Viral Serologic Survey of Alaskan Eskimo Harvested

Bowhead Whales (*Balaena mysticetus*)

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Serum samples from 21 of 36 Eskimo harvested bowhead whale (*Balaena mysticetus*) were positive (by virus neutralization; 50% endpoint titer $>1:28$ and 100% endpoint titer $>1:20$) for antibodies to at least one virus serotype from the calicivirus family, vesicular exanthema of swine virus (VESV) and San Miguel sea lion virus (SMSV). Many animals were positive to more than one serotype when using the Spearman-Kärber (S-K) method for calculating antibody titers. Of the serotypes detected, VESV F55 was the most common (antibody titers of 1:20-1:80) with 6 of 36 (16.7%) by the Monto and Bryan (MB) method, and 17 of 36 (47.2%) by the S-K method. VESV 1934B antibody was detected in 3 of 36 (8.3%) and 5 of 36 (13.9%) whales using the MB and S-K methods, respectively. VESV J56 antibody was detected in 3 of 36 (8.3%) by the S-K method only. All whales $<8.5\text{m}$ (estimated yearlings, $n=6$) were seronegative for J56 and 1934B while 10.0% and 16.7% of the whales $>8.5\text{m}$ were positive, respectively. Whales assumed to be sexually mature whales ($>13\text{m}$) had a higher incidence of antibody to 1934B and SMSV8 than those $<13\text{m}$. Gender had an effect on seroprevalence of antibody to VESV 1934B as titers $>1:28$ occurred in 18.2% of the females and 7.1% of the males, although the interaction of season, sex, and length was significant for VESV 1934B. Antibody to other serotypes (SMSV 8 and 12) occurred less frequently ($<5.6\%$) at an antibody titer $>1:28$ by the S-K method. All 36 whale sera were negative for antibody to VESV-A48, B51, C52, D53, E54, G55, H54, I55, and K54; Tillamook calicivirus and dolphin morbillivirus; and SMSV-1, 2, 4, 5, 6, 7, 9, 10, 11, and 13 at an antibody titer $>1:28$ by the S-K method.

(88) Isolation and Characterization of a New *Brucella* spp. In

a Minke Whale (*Balaenoptera acutorostrata*)

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We succeeded in isolating a novel unknown *Brucella* species from a minke whale (*Balaenoptera acutorostrata*) that has been caught during a commercial whaling offshore the Norwegian coast of Finnmark in May 1995. *Brucella* spp. isolation in seals, dolphins, and porpoises along the UK coast have been reported these last years. In a preliminary study, we have seen serological evidences of *Brucella* spp. infections in seals but also in whales caught in the Northern Atlantic. We have cultured spleen and liver samples from a minke whale classified positive by brucellosis serological tests. So far, cultural characteristics, agglutination, and biochemical tests are consistent with the diagnosis of smooth *Brucella* spp. Preliminary DNA work has shown positive PCR results for the specific *Brucella* 16S-23S Spacer Sequence and Insertion Sequence IS 6500. RFLP studies based on the IS6501 have shown a unique profile in comparison to the profiles described for *B. melitensis*, *B. abortus*, *B. suis*, *B. neotomac*, *B. canis* and *B. ovis*. This report is to our knowledge, the first description of the isolation of *Brucella* spp. in rorquals. The isolation of a novel agent responsible for reproductive disorders (in domestic animals) in endangered or threatened species, i.e. baleen whales, as well as its potential zoonotic importance is of concern. Finally, the isolation of *Brucella* spp. In whale, seals, dolphins and porpoises puts question marks on the lineage between marine *Brucella* species and on the source(s) of contamination.

(89) High Prevalence of Gastrointestinal Adenocarcinomas
in Stranded Beluga Whales (*Delphinapterus leucas*)
from the St. Lawrence Estuary

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Between 1983 and 1996, we examined 93 stranded beluga whales in order to establish the health status of the endangered population found in the St. Lawrence Estuary. Seventeen cancers were diagnosed in 16 animals, eight animals being affected by adenocarcinomas of the gastrointestinal tract. Overall, 8.6% (8/93) of the necropsied beluga whales were affected by malignant glandular epithelial gastro-intestinal tumors. Six adenocarcinomas were located in the intestine and two were found in the stomach. The prevalence of gastrointestinal adenocarcinomas in the stranded St. Lawrence Estuary beluga whale is high compared to the prevalence of these tumors in other animal species. Approximately 90% of the gastrointestinal adenocarcinomas reported in cetaceans have been found in this population of 500 animals. Different hypotheses could explain this high prevalence: 1) Since St. Lawrence beluga whales are exposed to environmental carcinogens, different xenobiotics, such as benzo(a)pyrenes, may have contributed to the etiology of these cancers. 2) Since the genetic variability of this population is reduced when compared to the Mackenzie Delta beluga whale population, genetic susceptibility to cancer might play a role in carcinogenesis. 3) An infectious etiology cannot be ruled out.

(90) Frequency of Killer Whale (*Orcinus orca*) Bites, Ship
Collisions, and Entanglements Based on Scarring on
Bowhead Whales (*Balaena mysticetus*)

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Bowhead whales of the Bering-Chukchi-Beaufort Seas stock harvested by Alaskan Eskimos were examined for scars from spring 1980 to 1997. Scarring patterns and critical measurements were assessed to determine the most likely cause of the trauma. We estimated the frequency of scars for killer whale bites, ship collisions, and entanglements to be 6.2% (n=194), 1.7% (n=235), and 8.5% (n=235), respectively. For orca attacks, the spaces between rake marks were within the range of interdigital measurements of known killer whale specimens. The frequency of killer whale trauma is significantly greater on mature animals (>13m). The most likely explanation is that the younger whales succumb to the actual and/or the longer whales simply have more exposure time to potential predation. In some cases, evidence of the actual material entangling the whale was retrieved and suspected to be from fisheries operations (likely crab pot lines). Other less frequent causes of significant trauma and eventual scarring are subsistence hunting (2), possibly satellite tag deployment (1), and walrus (tusk) attack (2). The documentation of scarring with respect to anthropogenic sources is critical to monitor as increased fisheries and shipping activities may result in a higher prevalence of scarring and possibly more severe lesions and impact on this large endangered arctic baleen whale. Many marine mammal species are threatened by increased trauma and it is most relevant for this to be presented in Florida, a site of major concern.

(91) Long-Term Experimental *Ehrlichia chaffeensis* Infection
in White-Tailed Deer

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Field and experimental studies have confirmed that white-tailed deer (*Odocoileus virginianus*) are vertebrate reservoir hosts of *Ehrlichia chaffeensis*, the cause of human monocytotropic ehrlichiosis; however, many aspects of this host-pathogen relationship are unknown and experimental data are available only from 3 short-term (<60 d) studies. Using a strain of *E. chaffeensis* (15B-WTD-GA) originally isolated from wild white-tailed deer, we conducted a long-term experimental infection in young captive deer. Four, 4-month-old seronegative deer each were inoculated intravenously with *E. chaffeensis*-infected DH82 cells and monitored for 270 days post-inoculation (DPI). These deer became seropositive (>1:64) at 10-17 DPI, remained seropositive through 87-123 DPI, but thereafter became seronegative (<1:64) except for 1 deer had a second rise in antibody titer beginning 207 DPI. Based on polymerase chain reaction (PCR) and tissue culture, each of the 4 inoculated deer was intermittently rickettsemic through at least 123 DPI even though morulae were not detected in Giemsa stained blood films. Two sham-inoculated control deer monitored through 87 DPI were consistently negative by serology, PCR, and culture. None of the animals became clinically ill. These findings confirm that deer can be persistently infected with *E. chaffeensis*.

(92) Detection of Ehrlichia chaffeensis, the Agent of Human
Monocytotropic Ehrlichiosis, in Archived Tissue of
White-Tailed Deer (*Odocoileus virginianus*) by PCR

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White-tailed deer in the southeastern United States are commonly infected with Ehrlichia chaffeensis, the causative agent of human monocytotropic ehrlichiosis. A polymerase chain reaction (PCR)-based diagnostic test is routinely used to detect ehrlichiosis agents in whole blood samples from white-tailed deer. We attempted to adapt this assay for use on archived white-tailed deer tissue and serum samples held by the Southeastern Cooperative Wildlife Disease Study. Sections of lymph node and bone marrow from a white-tailed deer from 1985 which had unidentified rickettsia in circulating monocytes and in macrophages from lymph node impression smears at the time of the necropsy examination were tested by PCR and were found to contain 16S rRNA gene fragments characteristic of Ehrlichia chaffeensis, but not evidence of the agent of human granulocytic ehrlichiosis or of a novel Ehrlichia-like organism commonly found in deer. Assay of archived or fresh deer serum samples by PCR for E. chaffeensis infection in a wild white-tailed deer one year prior to discovery of the index case of human monocytotropic ehrlichiosis, and suggests that assay of archived deer tissue samples may provide an alternate means of retrospective study into the natural history of this organism.

(93) Lack of Seroreactivity to *Ehrlichia chaffeensis* in
Southeastern Rodent Populations

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The significance of different species of rodents collected from the southeastern United States in the epidemiology of *Ehrlichia chaffeensis* (causative agent of human monocytic ehrlichiosis) was investigated. Serum samples (N=281) representing nine species of rodents collected between 1973 and 1993 were evaluated using an indirect fluorescent antibody test. All samples, screened at a dilution of 1:32, were negative for antibodies to *E. chaffeensis*. Sixty-three percent of the rodents tested were from areas where *E. chaffeensis* has been confirmed or is strongly suspected to be endemic. When considered in light of suspicions that rodents may play a significant role in the epidemiology of human granulocytic ehrlichiosis, these data suggest limited or no involvement of rodents in the epidemiology of *E. chaffeensis*.

(94) Serologic Survey for Antibodies to *Borrelia burgdorferi*
in White-Tailed Deer in Southern Ontario, Canada

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Serum samples collected from 623 white-tailed deer (*Odocoileus virginianus*) in southern Ontario from 1985 to 1989 were tested for antibodies to *Borrelia burgdorferi* using an indirect fluorescent antibody (IFA) staining method. Samples from 150 of the deer were also tested using an enzyme-linked immunosorbent assay (ELISA). At IFA titers of 1:64 and 1:128, reactors to *B. burgdorferi* appeared to be widespread throughout southern Ontario, with an apparent prevalence ranging from 3 to 47%. At IFA titres =1:256 and ELISA titres =1:160 reactors to *B. burgdorferi* were present only on Long Point, the only known endemic focus of *Ixodes scapularis*, the primary vector for *B. burgdorferi*, in southern Ontario. At these titres the apparent prevalence of reactors to *B. burgdorferi* on Long Point was only 5 to 7%, even though the mean intensity of infestation of adult *I. scapularis* on deer was >180, and 60% of the adult ticks are infected with *B. burgdorferi*. Based on these results, white-tailed deer do not appear to be a good sentinel species for serologic evaluation of the distribution of *B. burgdorferi* among wildlife.

(95) An Unusual Syndrome in Black-Tail Deer

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We are currently investigating an unusual disease syndrome in Black-tailed deer (*Odocoileus hemionus*) in Western Washington. This condition has become widespread in less than a year. Yearling deer are most severely affected, and there is anecdotal evidence that many yearlings are dying. The deer are noted to be emaciated, alopecic, unwilling to jump and have diarrhea. Five deer were euthanized by gunshot for diagnostic purposes. Skin scrapings for mites were negative for all deer. Hemograms were within normal limits except for one deer which was leukopenic. Serum chemistries were within normal limits. Antibody titers were not present in any of the deer for bovine viral diarrhea (BVD), bovine respiratory syncytial virus, infectious rhinotracheitis virus and blue tongue virus. The animals were negative for malignant catarrhal fever by polymerase chain reaction (PCR) on blood. Evidence of pestivirus infection with homology to BVD virus was detected by PCR from blood of the one deer tested. Viral isolation attempts from lymph node and spleen of one deer were negative. Gross lesions included emaciation patchy alopecia, leukoderma, enlarged tonsils and generalized lymphadenomegaly in all deer. Three deer had lice. Microscopic examination revealed hypersensitivity dermatitis, lymphoid hyperplasia, tonsillar hyperplasia, verminous pneumonia (*Parelaphostrongylus odocoilei* larvae), atrophy of fat, and *Sarcocystis* sp. muscle cysts in all deer; lymphoplasmacytic, eosinophilic enteritis, and portal lymphocytic hepatitis in 4 deer; mild lymphocytic myocarditis, in three deer; mild perivascular lymphocytic encephalitis, and mild to severe lymphocytic nephritis in two deer. One deer had syncytial cells in the tonsil, and one deer had syncytial cells in one lymph node. Additional diagnostic procedures, including immunohistochemistry for BVD, and additional viral isolation attempts are in progress.

(96) Adenovirus Hemorrhagic Disease in Deer: Natural and
Experimental Infection

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An apparently novel adenovirus was identified as the cause of a high mortality event which occurred in mule deer (*Odocoileus hemionus*) in California during the latter half of 1993. Two disease manifestations of adenovirus infection occurred during the outbreak. A systemic vasculitis with subsequent pulmonary edema and hemorrhagic enteropathy was seen in deer necropsied during the event, and a localized vasculitis with ulceration and abscessation in the upper alimentary tract was seen in an equal number of deer. Gross and microscopic changes seen in deer that were necropsied during the outbreak of natural disease were reproduced in deer using purified adenovirus isolated from animals that died during the event. Adenovirus was reisolated from the tissues of animals that died of experimental disease thus fulfilling Koch's postulates. In addition, age and species susceptibility and mode of transmission were studied. Results of experimental studies demonstrated that adenovirus causes acute systemic vasculitis in black-tailed deer and white-tailed deer and necrotizing ulcers in the oral cavity and forestomachs in black-tailed deer. Transmission is by direct contact and the route of inoculation does not affect the outcome of the disease in black-tailed deer. Natural and experimental infection suggests that fawns are more susceptible to infection resulting in severe clinical disease, than are juveniles and adults. Deer adenovirus is endotheliotropic. In the systemic form, endothelial cells in arteries, arterioles, veins, and capillaries in several organs are infected with the virus, but vascular damage is most frequently seen in the lungs and intestines. Vascular damage is likely the pathophysiologic event of the acute systemic form of the disease. Local vascular thrombosis and vasculitis in the upper alimentary tract results in ischemic necrosis with subsequent ulceration and secondary bacterial invasion in the localized form of the disease. And ELISA test is currently being developed for future epidemiologic studies. Molecular characterization of

the virus will soon begin to determine the relationship between the deer adenovirus and adenovirus currently recognized in domestic livestock.

(97) Heart Rate as an Indicator of Stress in Bighorn Sheep

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Because animals' behavioral responses to disturbance do not always correspond with their physiological responses, biologists and veterinarians require alternate methods to identify and measure stress imposed on animals and to assess the efficacy of techniques aimed at alleviating stress. The objective of this study was to develop a technique to remotely monitor the physiological response of bighorn sheep (*Ovis canadensis*) to disturbance. Specifically, we evaluated heart rate as a potential predictor of serum cortisol levels. Initial evaluation was performed using 15 captive, adult bighorn sheep. We first developed a safe and humane surgical technique to implant Telonics model HR400 heart rate transmitters on the animal's lateral thorax. The transmitters accurately monitored heart rate, life expectancy was excellent (12 of 15 continued to function >1 year after implantation), and line of sight range for a standing bighorn sheep was >800 m. Next, we exposed bighorn sheep to a range of acute stressors in controlled experiments to examine the relationship between heart rate and serum cortisol. A positive linear relationship ($P < 0.0001$; $r^2 = 0.57$) was found between heart rate and serum cortisol level. Furthermore, our data suggest a threshold level in heart rate (>125 bpm in response to a disturbance) corresponding to serum cortisol values (>20 ng/ml) that may be cause for concern. These studies with captive animals suggest that heart rate telemetry offers a promising new technique for monitoring stress in free-ranging bighorn sheep and potentially in other wildlife species.

(98) The Potential for False-Positive Diagnosis of Meningeal

Worm Infection by Extraction of Larvae from Feces

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In the past, any cervid passing dorsal-spined nematode infection. Although the Baermann method, and other related methods, are valuable techniques for collecting protostrongylid larvae from fecal samples, specific diagnosis of infection is virtually impossible. We investigated the potential of larvae to survive passage through the alimentary canal of non-infected mammals. For this “free passage”, we utilized two red deer (*Cervus elaphus*) and four lab rats (*rattus norvegicus*) which were each fed non-infective first-stage larvae (L1) of *Parelaphostrongylus tenuis*. The entire daily fecal output of each animal was collected and examined for presence of *P. tenuis* L1. Larvae were recovered, intact and alive, from the fecal samples of all six animals. Larvae of *P. tenuis*, and probably other related species, can survive passage through the alimentary canal of non-infected mammals and they can be collected using the Baermann method. These findings raise the possibility of ingestion and passage of L1 originating in the environment. This potential for false-positive diagnosis of infection in live animals necessitates careful interpretation of a hosts infection status. Such a finding reinforces the need for a reliable method of diagnosing infections in live animals.

(99) Chronic Wasting Disease in White-Tailed Deer

(*Odocoileus virginianus*) from Wyoming

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Chronic wasting disease (CWD) is a spongiform encephalopathy of captive and free-ranging deer (*Odocoileus* spp.) and Rocky Mountain elk (*Cervus elaphus nelsoni*) in southeastern Wyoming and northcentral Colorado. Four (two male and two female) captive and four (all female) free-ranging white-tailed deer were diagnosed with clinical CWD in Wyoming. The first case occurred in 1990, but due to the degree of autolysis was only recently identified by retrospective examination of the brain using immunohistochemistry for PrPres. The other cases were diagnosed from 1993-1996; deaths occurred throughout the year. Ages ranged from 17 months to one old adult, but the majority were prime aged animals. Three of the free-ranging deer were from one ranch. Clinical signs were similar to those reported for mule deer (*O. hemionus*) and included emaciation, increased salivation, polydipsia, polyuria, and behavioral alterations. Gross lesions reflected the clinical signs. Significant microscopic lesions were confined to the central nervous system and were characterized as a spongiform encephalopathy. Spongiform lesions tended to be more severe and more widely distributed in the brain than what is typical of CWD in mule deer and elk. In addition, amyloid plaques were prominent in most cases. Abundant PrPres deposits were detected by immunohistochemistry and scrapie associated fibrils were identified by negative stain electron microscopy. The susceptibility of white-tailed deer to CWD is of concern to wildlife managers because they are the most widespread big game species in North America.

(100) An Update on Bovine Tuberculosis in Free-Ranging

White-Tailed Deer in Michigan

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A 4.5 yr-old male white-tailed deer (*Odocoileus virginianus*) killed by a hunter during the 1994 firearm season in northeastern Michigan (USA) had lesions suggestive of tuberculosis and was positive on culture for *Mycobacterium bovis* (*M. bovis*) the causative agent for bovine tuberculosis. In response to the 1994 tuberculosis positive deer, a multi-agency cooperative team consisting of representatives from the Michigan Department of Natural Resources (MDNR), Michigan Department of Agriculture (MDA), Michigan Department of Community Health (MDCH), Michigan State University, and the United States Department of Agriculture was formed. Subsequently, surveys in 1995 and 1996 of 4,556 white-tailed deer heads from a 5-county area surrounding the 1994 tuberculosis positive deer resulted in 69 *M. bovis* culture positive deer. A survey of 51 possible spill-over hosts, (8 coyote [*Canis latrans*], 4 red fox [*Vulpes vulpes*], 8 opossum [*Didelphis virginiana*], 28 raccoon [*Procyon lotor*], 2 badger [*Taxidea taxus*], and 1 bobcat [*Felis rufus*]), resulted in one *M. bovis* culture positive coyote. Additional surveillance of over 3,300 free-ranging white-tailed deer from all counties in Michigan outside the affected 5-county area were negative. Additional monitoring of 79 free-ranging elk from the nearby elk range and 3,732 head of livestock (3,608 cattle, 64 goats, 54 pigs, and 6 llamas) within

a five mile radius of the location of each bovine tuberculosis infected deer revealed no cases of tuberculosis. This appears to be the first time *M. bovis* has been maintained in free-ranging cervidae in North America without involvement of infected livestock. A combination of environmental (high deer density and poor quality habitat) and management-related factors (extensive supplemental feeding) may be responsible for this epizootic. Spring deer densities in the northeastern lower peninsula of Michigan have been maintained at 19-23 per km² for many years. Focal concentrations of deer at feeding sites can result in much higher densities (100-200 per km²). While overall deer densities are moderately high for Michigan, it is the unnatural concentration of deer at supplemental feeding sites and subsequent prolonged close contact between deer which is thought to play a major role in the transmission of tuberculosis between animals. The multi-agency bovine tuberculosis committee, as well as involved stakeholders (farmers, hunters, landowners, and the public), have developed recommendations for the Directors of the MDNR, MDA, and MDCH with the goal of eliminating bovine tuberculosis from Michigan's wild deer population. The proposed management strategy involves surveying wildlife populations, testing livestock, eliminating supplemental feeding of deer, reducing the deer density in the area through hunting, and educating the public.

(101) Chemical Immobilization of Large Numbers of
Pronghorn in an Urban Environment Using Carfentanil
and Xylazine

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Forty-three pronghorn were chemically immobilized at F. E. Warren Air Force Base in southeastern Wyoming with 0.047 mg/kg carfentanil and 1.15 mg/kg xylazine. Pronghorn could be approached closely by a vehicle containing shooters armed with range-adjusting dart guns. Mean induction time (n = 40) was 7.1 (1.5 min (median = 4 min; range 1-43 min). Once immobilized, pronghorn were transported to a base camp where they were fitted with radio collars, treated prophylactically, had the immobilizing drugs antagonized, and were placed into a trailer for shipment. Two adult females died after capture (mortality rate = 4.6%) and one was released without shipment. Forty pronghorn were translocated to Oklahoma; five animals (12.5%) died en route and one moribund pronghorn was euthanized within a week of release. As of March, 1997, 23 animals were known to be alive, all of which were within 4.8 km of the release site. The use of carfentanil and xylazine was a safe, efficacious, and economical method for capturing a large number of pronghorn in an urban area. Also, this operation provided evidence that translocation of pronghorn accustomed to a restricted environment may not migrate long distances from a release site.

(102) Evaluation of the Vaccine Efficacy of *Brucella abortus*

Strain Rb51 in Elk

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A stable rough variant of *Brucella abortus*, strain RB51, has been associated with limited or no pathogenesis and provides protection against virulent *B. abortus* challenge in experimentally infected mice, goats, swine, and cattle. Domestic ungulates vaccinated orally with RB51 are protected against challenge with *B. abortus* strain 2308. Pregnant female elk were used in this study to determine whether RB51 could be used as an oral vaccine in wild ungulates. The animals were divided into 2 groups; group 1 received saline and group 2 received 2×10^{10} colony forming units of RB51 placed into their mouths following scarification with a float. At mid-gestation, all of the animals were challenged conjunctively with 1×10^7 colony forming units of virulent strain 2308. The animals were monitored for abortions, and weak or live births. All of the calves were necropsied soon after birth, and tissues were collected for culture. The adult females were necropsied, and tissues were cultured for *Brucella*. No healthy, live births were observed in the non-vaccinated saline controls (0/15) whereas 4 live births were recorded in the RB51 vaccinates (4/8). These results suggest that RB51 administered orally to female elk provided partial protection against virulent *B. abortus* challenge compared to non-vaccinated controls as demonstrated by abortion and colonization.

(103) Pathogenicity of Intramuscularly Injected *Brucella*

abortus Strain RB51 in Male Elk Calves

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Some elk in the Greater Yellowstone Area are infected with bovine brucellosis and may represent a risk to domestic cattle of the area. *Brucella abortus* strain RB51 vaccine has potential to control *Brucella* induced abortions in elk. However, the most probable vaccination strategy would also result in some male elk being inoculated. Thus, it is important to insure that the vaccine is not pathogenic in male elk. Ten male elk were captured as calves on the National Elk Refuge, Jackson, Wyoming, tested for *Brucella* antibodies, and intramuscularly inoculated with 1.0×10^9 colony forming units of *B. abortus* strain RB51. Elk were bled for hemoculture and serology every 2 weeks after inoculation. Beginning 4 months postinoculation and continuing until 10 months postinoculation elk were serially euthanized, necropsied, and tissues collected for culture and histopathology. These elk cleared the organism from the blood within 6 weeks and from all tissues by 10 months postvaccination. No lesions attributable to *Brucella* were found grossly; mild lymphocytic epididymitis occurred in several animals. This study suggests that *B. abortus* strain RB51 is safe in male elk inoculated as calves.

(104) RB51 Vaccine Update: Safety study of RB51 Vaccine in
Pregnant Bison from Yellowstone National Park

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The thirty-five pregnant, Brucellosis Card Tested negative, bison from Yellowstone National Park were randomly divided into three groups for this vaccine safety study. One group of 12 animals served as controls and was injected with sterile saline. One group of 12 animals was vaccinated with 1×10^7 CFU's of RB51, while a third group of 11 animals was vaccinated with 1×10^9 CFU's of RB51. Control and vaccine injections were administered only during the last trimester of pregnancy. Because of preliminary research on RB51 possibly causing abortion and sheading in bison, both of the RB51 vaccine dosages were lower than the recommended cattle vaccination dose. Data from this ongoing RB51 vaccination safety study will be presented. Recommendations for proceeding with an efficacy study based on the data will be discussed. Management of captive wild bison will also be addressed during this presentation. This research project is a positive step taken by all collaborators toward a solution for the potential disease interactions between wildlife and livestock in the Greater Yellowstone Ecosystem.

(105) Biosafety of Parenteral *Brucella abortus* Strain RB51

Vaccine in Bison (*Bison bison*) Calves

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During routine handling and vaccination of the Ft. Niobrara National Wildlife Refuge bison herd, 37 approximately 5 month old calves were subcutaneously vaccinated with 1.5×10^{10} CFU *B. abortus* strain RB51 vaccine, lot #1039. We assessed the persistence, pathology, shedding and transmission of RB51 in these bison calves by serial necropsy of calves and dot-blot seroassays of non-vaccinated adult females in the herd. Necropsy followed the Greater Yellowstone Interagency Brucellosis Committee guidelines established to detect brucellae in bison. Four calves were sacrificed at 4 week intervals. In calves, standard tube agglutination for brucella remained negative, RB51 dot-blot assays were positive at 1:40 to 1:80 dilutions from week 14 through week 26 postvaccination. RB51 was cultured from lymph nodes in 4/4, 4/4, 1/4, 3/4 and 0/4 calves at 14, 18, 22, 26 and 30 weeks postvaccination (PV), respectively. No gross lesions were observed and histologic changes consisted of occasional mild neutrophil or histiocytic infiltrates in the draining lymph node early in sampling times. No significant histologic lesions were noted in liver, lung, kidney, cardiac muscle or spleen. Adverse clinical effects were not observed in vaccinates. Swabs of nasopharynx, conjunctiva, rectum and vagina were uniformly negative. Dot-blot assays of 10 cows were negative at 26 weeks PV. Our results suggest that RB51 persists longer in bison calves, however bison apparently clear vaccine infection without shedding and have no clinically significant adverse reactions. RB51 distributes widely in bison to a variety of lymph nodes. The distribution of vaccine infection in cattle is unknown.

(106) Wildlife Forensics Seminar

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Law Enforcement is a necessary tool of wildlife management. Forensics science supports wildlife law enforcement officers at the state and federal levels. Training in the pathological examination of illegally killed wildlife is presented to enhance the recognition of lesions of law enforcement significance, to handle evidence in a manner to preserve its value in a judicial setting and to give effective court room testimony based on the examination of evidence.

Evidence handling may be the weakest point in a scientific investigation which may be attacked by a defense attorney. A "chain of evidence" which clearly documents the exchange of critical evidence and the distribution of samples derived from that evidence is essential. Proper transfer documentation, sealing and storage of evidence is demonstrated. Procedures for the examination of evidence must be recognized by the court as "scientifically acceptable" within the discipline represented and that the examination was done according to approved protocols with appropriate quality control procedures. Evidence must be handled such that the defense equal access to the samples for scientific testing by a third party if they so choose.

Documentation of pathological findings is essential. Photographs which accurately portray the evidence and assist the judge or jury in understanding what the pathologist has observed and its significance are essential. Visual aids necessary for court presentation may be created and used to convey accurately complex scientific concepts or findings.

Wildlife forensic cases primarily involve gunshot, trauma, poisoning and more recently environmental contaminant related deaths of protected species. Understanding the mechanisms of gunshot wounds is essential to provide the investigating officer with useful information. Recognition of differences in

wounds caused by high velocity rifle bullets, low velocity bullets, shotgun slugs, black powder rifle bullets and arrows is presented. Evaluation of wounds to determine trajectory and lethality are important aspects of gunshot wound evaluation. Illegal use of lead shot for waterfowl can be differentiated legal steel shot with radiographs. Proper handling of bullets, bullet fragments and pellets recovered from the carcass is illustrated.

Poisoning of wildlife is a major legal issue. The diagnosis of pesticide poisoning for legal purposes requires the demonstration of significant amounts of the pesticide in the tissues or gut contents of the victim. Information on the types of pesticides and the methods which are commonly used to poison wildlife, particularly eagles are presented based on the investigations conducted by the US Fish and Wildlife Special Agents. Documentation of the source of pesticide through the identification of digestive track contents is stressed. Differentiation of primary “intentional” poisonings verses secondary or accidental poisoning due to the illegal or careless use of pesticides is necessary for the pursuit of criminal activity.

Trauma deaths include intentional, accidental and natural sources of trauma. Trauma deaths may be related to trapping, vehicular or stationary object collisions, or predators. Of particular importance is the differentiation of pre and post mortem animal induced trauma to a carcass.

The limitations of time of death determinations are discussed. The recognition, collection and handling of “trace evidence” derived from the examination of the carcass along with available scientific techniques to process such evidence are illustrated.

An expert witness is defined as any one who by reason of education, training or experience has special knowledge not held by the public. An expert witness may give an “opinion” as part of his/her testimony. Qualification and preparation techniques used to be an effective expert witness in a court of law are presented. Working with an investigating field officer and/or an attorney handling a case must be timely and must consider the practical restraints of field conditions, level of understanding of

basic scientific principles and the objectives of the court presentations.

References for additional training and resources are available but must be adapted for use in veterinary forensics from the experiences of human medical examiners. “Let the body speak”, is a quote from a prominent human forensic pathologist. It may reveal the manner cause manner and manner of death. The objective of this presentation is to train the forensic examiner of wildlife to listen carefully and translate what he hears to the submitting agent and finally to the judge or jury.