51ST ANNUAL WILDLIFE DISEASE ASSOCIATION CONFERENCE

PROGRAM AND ABSTRACTS
OF PAPERS PRESENTED

JULY 28 – AUGUST 1, 2002

HOSTED BY:

HUMBOLDT STATE UNIVERSITY

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51ST ANNUAL
WILDLIFE DISEASE ASSOCIATION CONFERENCE

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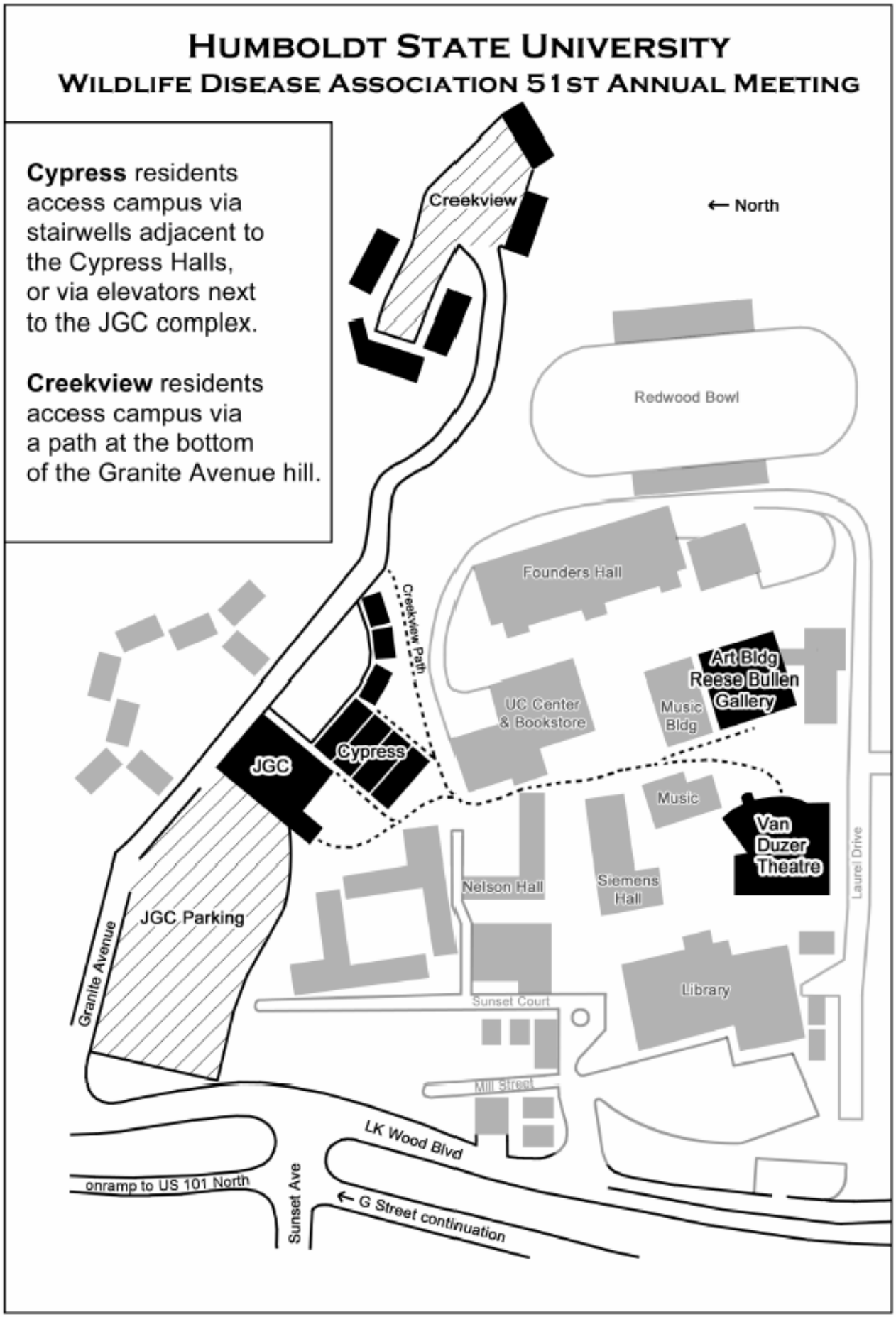
WILDLIFE DISEASE ASSOCIATION 51ST ANNUAL MEETING



51ST ANNUAL WILDLIFE DISEASE ASSOCIATION CONFERENCE

SUNDAY JULY 28th

7:30 – 8:00 am, 1:00 - 7:00 pm	Registration (2nd floor, Jolly Giant Commons)
8:00 – 12:00 noon	JWD Editorial Board Mtg (Mad River Room, 2nd floor, Jolly Giant Commons)
12:00 – 1:00 pm	Lunch for Editorial Board and Council (3rd floor, Jolly Giant Commons)
1:00 – 5:00 pm	WDA Council Meeting (Mad River Room, Jolly Giant Commons)
6:00 – 7:00 pm	Student reception (1st floor, Jolly Giant Commons)
7:00 – 9:00 pm	General reception (1st floor, Jolly Giant Commons)

MONDAY JULY 29th

7:00 – 2:00 pm	Check in/Registration (Van Duzer Theatre)
8:00 – 8:30 am	Greetings and Opening address (Van Duzer Theatre)
8:30 – 11:50 am	Symposium on Emerging Diseases of Wildlife (Van Duzer Theatre)
11:50 – 1:00 pm	Announcements and Lunch
1:00 – 3:30 pm	Environmental/Ecosystem Health (Van Duzer Theatre)
3:45 – 4:15 pm	AAWV Cutting Edge Speaker (Van Duzer Theatre)
4:15 – 5:30 pm	Aquatic 1 Session (Van Duzer Theatre)
6:30 – 9:00 pm	Picnic at Camp Bauer: Bus pick up begins at 6:00 pm. Food served from 7:00–8:30

TUESDAY JULY 30th

7:30 – 9:00 am, 1:15 – 2:30 pm	Registration (Van Duzer Theatre)
8:00 – 8:30 am	Student Research Recognition Award (Van Duzer Theatre)
8:30 – 12:00 pm	Student Presentations (Van Duzer Theatre)
12:00 – 1:15 pm	Lunch
1:15 – 2:00 pm	Student Presentations (Van Duzer Theatre)
2:00 – 3:00, 3:15 – 5:00 pm	Carnivore 1 & Avian 1 Sessions (Van Duzer Theatre)
5:00 – 6:00 pm	Auction Items on display In Mad River and Klamath Rooms, Jolly Giant Commons
5:00 – 6:00 pm	AAWV Business Meeting (Van Duzer Theatre) and Sections meetings (TBA)
7:00 – 11:00 pm	WDA Auction (1st floor, Jolly Giant Commons): Bar opens at 1900; auction at 1930

WEDNESDAY JULY 31st

7:00 – 8:00 am	Prayer Breakfast (Agate A & B rooms, Jolly Giant Commons; Speaker: Dr. Lori Walker, "A Vastly Ugandan Experience")
7:30 – 9:00 am, 1:00 – 2:30 pm	Registration (Van Duzer Theatre)
8:00 –9:55 am	Symposium of Diseases of Wild Sheep (Van Duzer Theatre)
9:55 – 10:45 am	Poster Session break (Reese Bullen Gallery, Art Building)
10:45 – 11:45 am	Ungulate 1 Session (Van Duzer Theatre)
11:45 – 1:00 pm	Lunch
1:00 – 2:30 pm	Avian 2 Session (Van Duzer Theatre)
2:30 – 3:00 pm	Poster Session break (Reese Bullen Gallery, Art Building)
3:00 – 4:15 pm	Carnivore 2 Session (Van Duzer Theatre)
4:30 – 5:30 pm	WDA Business Meeting (Van Duzer Theatre)
6:30 – 11:00 pm	Awards Banquet (Arcata Community Center). Social hour at 1830; banquet at 1930

THURSDAY AUGUST 1st

8:00 – 8:30 am	Preview of WDA 2003 meeting (Van Duzer Theatre)
8:30 – 10:30 am	Chronic Wasting Disease Symposium (Van Duzer Theatre)
10:45 – 11:45 am	Aquatic 2 Session (Van Duzer Theatre)
11:45 – 1:00 pm	Lunch
1:00 – 2:15, 2:30 – 4:45 pm	Aquatic 2 & Ungulate 2 Sessions (Van Duzer Theatre)
4:45 – 5:00 pm	Final announcements and adjourn

FRIDAY AUGUST 2nd

9:00 – 12:00 pm	WWHC Meeting (Agate Beach Rooms A & B, Jolly Giant Commons)

PRESENTATION SCHEDULE

MONDAY JULY 29th

8:00-8:30 **WELCOME AND OPENING COMMENTS**
- Rick Botzler and Rick Brown, Conference Co-Chairs
- Jim Howard, Dean of the College of Natural Resources and Sciences
- Rollin Richmond, President of Humboldt State University

SYMPOSIUM ON EMERGING DISEASES OF WILDLIFE
Moderator: Robert G. McLean

8:30-8:50 **(1)** **Investigating Wildlife EIDs – Lessons from Chytridiomycosis and Nipah Virus**
Peter Daszak

8:50-9:10 **(2)** **Estimating the Basic Reproductive Number, R0, for a Recently Introduced or Emerging Pathogen**
Andy Dobson and Ottar Bjornstad

9:10-9:30 **(3)** **Does Winter Climate Influence Prevalence of Mycoplasmal Conjunctivitis in Wisconsin House Finches?**
Barry K. Hartup, Sonia Altizer, Wesley M. Hochachka and Andre A. Dhondt

9:30-9:50 **(4)** **West Nile Virus, an Emerging Disease of North American Birds**
Robert G. McLean

9:50-10:10 **BREAK**

10:1010:30 **(5)** **Linking Emerging Disease and Degradation of Marine Ecosystems**
Jonna A.K. Mazet, Kirsten V.K. Gilardi, Christine Kreuder, Patricia A. Conrad, Michael H. Ziccardi, David A. Jessup, Melissa A. Miller, and M. Tim Tinker

10:30-10:50 **(6)** **The Quicksand Surrounding So-Called Emerging and Re-Emerging Human and Animal Diseases in Africa: Political, Cultural, and Institutional Factors that Influence Disease Management, Ecosystem Health, and Human Livelihoods**
Michael D. Kock and Richard A. Kock

10:50-11:10 **(7)** **Pathogens and Predators: Using Viruses to Track Wildlife Populations**
Mary Poss, Roman Biek and Chuck Anderson

11:10-11:30 **(8)** **Use of Oral Vaccination, Trap-Vaccinate-Release and Population Reduction to Control Raccoon Rabies in Ontario, Canada**
Rick Rosatte

11:30-11:50 **(9)** **Legislative Issues on Emergent Wildlife Diseases and Bioterrorism Threats**
Mark J. Abdy

11:50-1:00 **LUNCH**

ENVIRONMENTAL AND ECOSYSTEM HEALTH SESSION
Moderator: Kirsten Gilardi

1:00-1:15 **(10)** **Protozoal Problems Emerging at the Human-Wildlife-Domestic Animal Interface**
Patricia A. Conrad, Melissa Miller, Anne Kjemtrup, Ian Gardner, Christine Kreuder, Woutrina Smith, Rob Atwill and Dave Jessup

1:15-1:30 **(11)** **Pathobiological Changes in White Storks (*Ciconia ciconia*) After a Mine Tailings Disaster in Southwestern Spain**
Judit E. Smits, Gary R. Bortolotti, Raquel Baos, Julio Blas, Jose Tella and Fernando Hiraldo

1:30-1:45 **(12)** **Low Prevalence of Chytridiomycosis in Larval Red-Legged Frogs (*Rana aurora aurora*) in Redwood National Park**
Nathan C. Nieto and John O. Reiss

1:45-2:00 **(13)** **The Risk of Avian Botulism Outbreaks from Avicide DRC-1339 in North Dakota Wetlands**
Michael D. Samuel, Diana R. Goldberg, Tonie E. Rocke and Kevin M. Johnson

2:00-2:15 **(14)** **Medical Survey of the Local Human Population to Determine Possible Health Risks to the Mountain Gorillas (*Gorilla gorilla beringei*) of Bwindi Impenetrable Forest National Park, Uganda**
Jonathan M. Sleeman, William Guerrera, Jasper B. Ssebide, Lonny B. Pace, Travers Y. Ichinose and John S. Reif

2:15-2:30 **(15)** **Test of an Environmental Model: Northern Red-Legged Frogs (*Rana aurora aurora*) as Bioindicators of Endocrine-Disrupting Contaminants Along California's Northwest Coast**
James B. Bettaso, Hartwell H. Welsh and Brent D. Palmer

2:30-2:45 **(16)** **Patterns of Illegal Wildlife Poisoning in the United States - A Review of Cases Submitted to the National Wildlife Forensic Laboratory (1990 - 2001)**
Richard K. Stroud, Rhoda R. Ralston, Mark Kirms, Pamela McClure and Shelly O'Connell

2:45-3:00 **(17)** **Invasive Pathogens: Detections on National Wildlife Refuges and Potential Risks to Additional Refuges**
Grace S. McLaughlin and F. Joshua Dein

3:00-3:15 **(18)** **Patagonia Seabirds as Indicators of Marine Ecosystem Health**
Marcela M. Uhart, Flavio Quintana, Esteban Frere, Patricia Gandini, William B. Karesh and Robert A. Cook

3:15-3:30 **(19)** **Marine Ecosystem Health and Sentinel Species: Adding an Ecological Element to the Proverbial "Canary in the Mineshaft"**
A. Alonso Aguirre And Gary M. Tabor

3:30-3:45 **BREAK**

AMERICAN ASSOCIATION OF WILDLIFE VETERINARIANS CUTTING EDGE SPEAKER

3:45-4:15 **(20)** **Will Technology Benefit Wildlife Disease Evaluations?**
Joe Bielitzki

AQUATIC I SESSION
Moderator: A. Alonso Aguirre

4:15-4:30 **(21)** **Bacteremia in Free-Ranging Hawaiian Green Turtles with Fibropapillomatosis**
Thierry Work, George Balazs, Mark Wolcott and Robert Morris

4:30-4:45 **(22)** **Infection of Hatchling Alligators (*Alligator mississippiensis*) with *Mycoplasma alligatoris***
Lauren J. Richey, Trenton R. Schoeb and Mary B. Brown

4:45-5:00 **(23)** **Gross and Histopathologic Features and Parasites of Migrating Wild Adult Fall Chum Salmon (*Oncorynchus keta*) in the Yukon River, Alaska**
Kathy Burek and Tevis J. Underwood

5:00-5:15 **(24)** **Spatial, Demographic and Environmental Risk Factors for *Toxoplasma gondii* Exposure in Southern Sea Otters**
Melissa Miller, Ian Gardner, Christine Kreuder, David Paradies, Karen Worcester, David Jessup, Erin Dodd, Mike Harris, Jack Ames, Andrea Packham and Patricia Conrad

5:15-5:30 **(25)** **Population Ecology of Pinniped Herpesvirus Infections and Cancer in California Sea Lions**
Andy Dobson, Linda Lowenstine and Frances Gulland

TUESDAY JULY 30th

8:00-8:10 **ANNOUNCEMENTS**

STUDENT RESEARCH RECOGNITION AWARD
Moderator: Thierry Work

8:10-8:30 **(26)** **West Nile Virus Detection in the Organs of Naturally-Infected Blue Jays (*Cyanocitta cristata*)**
Samantha E. J. Gibbs, Daniel G. Mead, Angela E. Ellis, Andrew B. Allison, Elizabeth W. Howerth and David E. Stallknecht

TERRY ADMUNSON STUDENT PRESENTATION COMPETITION
Moderator: Thierry Work

8:30-8:45 **(27)** **Immune Response in Black-Tailed Prairie Dogs (*Cynomys ludovicianus*) to Voluntary Consumption of RCN-F1 Plague Vaccine Incorporated into a Palatable Bait**
Jordan Mencher, Tonie Rocke and Susan Smith

8:45-9:00 **(28)** **Evaluation of a Sentinel System for *Ehrlichia chaffeensis* and *E. ewingii* Using White-Tailed Deer as Indicators**
Michael J. Yabsley, Vivien G. Dugan, Cynthia M. Tate, David E. Stallknecht, Susan E. Little, Andrea S. Varela and William R. Davidson

9:00-9:15 **(29)** **Evidence of *Ehrlichia* spp. in Raccoons (*Procyon lotor*) from Georgia**
Vivien G. Dugan, William R. Davidson, Joseph K. Gaydos, Michael J. Yabsley, Susan E. Little, Ashley D. Beall and Colin C. Hurd

9:15-9:30 **(30)** **Morbidity and Mortality of Reptiles Admitted to the Wildlife Center of Virginia, 1991 to 2000**
Justin D. Brown and Jonathan M. Sleeman

9:30-9:45 **(31)** **A Comparison of Immunohistochemistry and Virus Isolation in Diagnosis of West Nile Virus**
Angela E. Ellis, Daniel G. Mead, Andrew B. Allison, Samantha E.J. Gibbs, Nicole L. Gottdenker, David E. Stallknecht and Elizabeth W. Howerth

9:45-10:00 **(32)** **Factors Affecting Survival of Juvenile Franklin's Gulls (*Larus pipixcan*): The Role of Hatching Asynchrony, Immune Function, and Disease**
Catherine Soos, Gary R. Bortolotti and Gary Wobeser

10:00-10:15 **BREAK**

10:15-10:30 **(33)** **Phocine Herpesvirus-1 Infections in North American Harbor Seals (*Phoca vitulina*)**
Tracey Goldstein, Frances M.D. Gulland, Brian M. Aldridge, James T. Harvey, Teri Rowles, Jeffery L. Stott and Jonna A.K. Mazet

10:30-10:45 **(34)** **Recent Patterns of Mortality in Southern Sea Otters**
Christine Kreuder, Melissa A. Miller, David A. Jessup, Linda J. Lowenstine, Michael D. Harris, Jack A. Ames, Tim Carpenter, Patricia A. Conrad and Jonna A.K. Mazet

10:45-11:00 **(35)** **Prevalence of Zoonotic Bacteria in Seabirds Undergoing Rehabilitation and Associated Risks to Rehabilitators in the Pacific Northwest, USA**
Christine M. Steele, Richard N. Brown and Richard G. Botzler

11:00-11:15 **(36)** **Is Avian Influenza in Shorebirds Species and Location Specific?**
Britta A. Hanson, David E. Swayne, Dennis A. Senne, Joan Beck and David E. Stallknecht

11:15-11:30 **(37)** **Coyotes (*Canis latrans*) as an Experimental Host for the Oklahoma Isolate of *Babesia gibsoni***
Holly V. Evers, A. Alan Kocan and James H. Meinkoth

11:30-11:45 **(38)** **Evidence of Pathogen Pollution: Shellfish as Bioindicators of Fecal Borne Pathogenic Protozoa and Bacteria in the California Nearshore Marine Ecosystem**
Woutrina A. Smith, Melissa A. Miller, Ian A. Gardner, Edward R. Atwill, Spencer Jang, David A. Jessup, Christian M. Leutenegger, Ann Melli and Patricia A. Conrad

11:45-12:00 **(39)** **Aggressive Anaplastic Sarcomas in Juvenile California Sea Lions (*Zalophus californianus*), an Immunohistochemical and PCR Analysis of Three Cases**
Elizabeth L. Buckles, Frances M.D. Gulland, Martin Haulena, Diane Naydan and Linda J. Lowenstine

12:00-1:15 **LUNCH**

1:15-1:30 **(40)** **Experimental Transmission and Isolation of a Novel *Ehrlichia* sp. From White-Tailed Deer**
Cynthia Tate, Ulrike Munderloh, Page Luttrell, Daniel Mead, Elizabeth Howerth, Vivien Dugan, Michael Yabsley and William Davidson

1:30-1:45 **(41)** **Sequence Variation of the Internal Transcribed Spacer 2 rRNA Gene of Two Spatially Distinct Populations of *Amblyomma americanum* (Acari: Ixodidae)**
Mason V. Reichard, A. Alan Kocan and Ron A. Van Den Bussche

1:45-2:00 **(42)** **Risk Factors for Viral Infections in Spotted Hyenas (*Crocuta crocuta*) from the Masai Mara National Reserve, Kenya**
Tara M. Harrison, Jonna A.K. Mazet, Kay E. Holekamp, Edward Dubovi, Anne L. Engh, Keith Nelson, Russell C. Van Horn and Linda Munson

CARNIVORE I SESSION
Moderator: Colin Gillin

2:00-2:15 **(43)** **Endoparasites of Island Foxes (*Urocyon littoralis*) and their Geographic Distribution among the Channel Islands, California**
Linda Munson, Sharon Patton, Charles Faulkner, Eileen Johnson, Elizabeth L. Buckles, Steven F. Timm, Grace Smith, David Garcelon, Mark Willett and Timothy Coonan

2:15-2:30 **(44)** **Colonic Spirocercosis in Channel Island Foxes (*Urocyon littoralis*)**
Elizabeth L. Buckles, Linda Munson, Sharon Patton, Eileen Johnson, Grace Smith, David Garcelon and Timothy Coonan

2:30-2:45 **(45)** **Spatial Epidemiology of *Bartonella vinsonii* subsp. *berkhoffii* and *Yersinia pestis* in California Coyotes**
B.R. Hoar, B.B. Chomel, C.C. Chang and T.E. Carpenter

2:45-3:00 **(46)** **Immunization of Black-Footed Ferrets (*Mustela nigripes*) Against Sylvatic Plague (*Yersinia pestis*)**
Tonie E. Rocke, Jordan Mencher, Arthur Friedlander and Gerard P. Andrews

3:00-3:15 **BREAK**

AVIAN I SESSION
Moderator: John Fischer

3:15-3:30 **(47)** **The Epizootiology of Type C Botulism at the Salton Sea**
Pauline Nol, Judy L. Williamson, Douglas L. Berndt, Jodie A. Bayerl and Tonie E. Rocke

3:30-3:45 **(48)** **Type E Botulism in Fish-Eating Birds On Lake Huron and Lake Erie, 1998-2001**
G. Douglas Campbell, Jeffrey Robinson, Tim Johnson, Brian Locke and Ian K. Barker

3:45-4:00 **(49)** **The Effectiveness of Carcass Removal in Reducing Losses due to Avian Botulism**
Trent Bollinger and Dan Evelsizer

4:00-4:15 **(50)** **The Role of Lesser Snow Geese as Carriers of Avian Cholera in the Playa Lakes Region**
Michael D. Samuel, William P. Johnson, Daniel J. Shadduck and Diana R. Goldberg

4:15-4:30 **(51)** **Antibody Response of Four Bird Species After Vaccination with a Killed West Nile Virus Vaccine**
Danelle M. Okeson, Shirley Yeo Llizo, Chriss Miller and Amy L. Glaser

4:30-4:45 **(52)** **What Lurks in Yonder Forest? - Miscellaneous Viruses Isolated From Wild Birds During West Nile Virus Surveillance in Georgia**
E. W. Howerth, A. Ellis, N. Gottdenker, D.G. Mead, S. Gibbs and D.E. Stallknecht

4:45-5:00 **(53)** **Enhanced Passive Surveillance for West Nile Virus in Dead Wild Birds in Canada – 2001**
Ian K. Barker, Michael Drebot, Harvey Artsob, L. Robbin Lindsay, Paul Sockett, Peter Buck, Robert Meyers, Frederick Leighton, Ronald Templeman, André Dallaire, Pierre-Yves Daoust and Hugh Whitney

WEDNESDAY JULY 31st

8:00-8:10 **ANNOUNCEMENTS**

SYMPOSIUM ON THE DISEASES OF WILD SHEEP
Moderator: Ben Gonzales

8:10-8:40 **(54)** **Bighorn Pasteurellosis: Do We Understand its Epidemiology Well Enough to Prevent or Manage Epidemics?**
Michael W. Miller

8:40-9:10 **(55)** **Diversity of Bacteria in the *Pasteurellaceae* Family and Factors Associated with Disease Potentials**
Alton C. S. Ward and Glen C. Weiser

9:10-9:25 **(56)** **Ecology of Disease-Related Mortality of Bighorn Sheep in Hells Canyon, Idaho, Oregon, and Washington, USA**
E. Frances Cassirer, Wendy M. Lammers and A.R.E. Sinclair

9:25-9:40 **(57)** **Long Term Monitoring of Bighorn Sheep (*Ovis canadensis*) that have been in Contact with Domestic Livestock**
Mark L. Drew

9:40-9:55 **(58)** **Disease Risk Assessment for Bighorn Sheep Captive Breeding Programs**
Holly Ernest

9:55-10:45 **POSTER BREAK**

(59) **Low-Grade Fibrosarcomas in Green Turtles (*Chelonia mydas*) with Fibropapillomatosis**
A. Alonso Aguirre, Terry R. Spraker, Robert A. Morris, Barbara Powers and B. Zimmerman

(60) **Investigation into the Etiology of "Hairless" (Alopecic) Ringed Seals (*Phoca hispida*) in the Bering Sea**
Kathy A. Burek, Kimberlee B. Beckmen, John E. Blake, Shannon Atkinson, Pamela Tuomi and Robert J. Small

(61) **A Survey of Raptor Mortality in Georgia**
Angela E. Ellis, Elizabeth W. Howerth, David E. Stallknecht and Daniel G. Mead

(62) **Tissue Distribution of Liver Enzymes in California Sea Lions and Harbor Seals**
Deborah A. Fauquier, Frances M. Gulland, Terry R. Spraker, Mary M. Christopher and Jonna A.K. Mazet

(63) **The Yellowstone to Yukon Conservation Medicine Program: Measuring and Modeling Wildlife Disease**
Colin M. Gillin, Erik Lindquist, Jim Else, Peter Daszak, and Gary M. Tabor

(64) **Oiling of Waterbirds Due to Exposure to Fish Oils**
Robert C. Hosea and David Jessup

(65) **Characterization and Clinical Manifestations of *Arcanobacterium phocae* Infections in Marine Mammals Stranded along the Central California Coast**
Shawn Johnson, Frances Gulland, Judy Lawrence, Spencer Jang, Juliet Herrera, Melissa Miller and David Casper

(66) **Acute West Nile Virus Infection of Reptiles and Amphibians**
Kaci Klenk and Nicholas Komar

(67) ***Parelaphostrongylus odocoilei*** **in Thinhorn Sheep (*Ovis dalli*) - Distribution, Life Cycle, and Significance**
Emily J. Jenkins, Alasdair M. Veitch, Eric P. Hoberg, Susan J. Kutz, Brett T. Elkin and Lydden Polley

(68) **Investigation of Heterophil to Lymphocyte Ratios and Granulocyte to Agranulocyte Ratios in Relation to Condition in Northern Saw-Whet Owls (*Aegolius acadicus*)**
Lynda L. Leppert, Alfred M. Dufty and Sarah L. Hamilton

(69) **Hemoparasite Survey From Imperial Eagles (*Aquila heliaca*), Steppe Eagles (*Aquila nipalensis*), and White-Tailed Sea Eagles (*Haliaeetus albicilla*) in Kazakhstan**
Lynda L. Leppert, Todd Katzner, Evgeny A. Bragin and Seth Layman

(70) **Characterization of *Campylobacter* Species Isolated from Northern Elephant Seals on the California Coast**
S. Joy Milan, Spencer S. Jang, Janet Foley, Judy Lawrence and Frances M.D. Gulland

(71) **Immune Responses of Elk (*Cervus elaphus*) to *Brucella abortus* Strain 19 and RB51 Vaccines**
Steven C. Olsen, Mitch V. Palmer. W. Ray Waters, Sasha J. Fach and Randy E. Sacco

(72) **Analysis of Interferon-G Production by *Mycobacterium bovis* Infected White-Tailed Deer (*Odocoileus virginianus*) using an In-Vitro Blood Based Assay**
Mitchell V. Palmer, W. Ray Waters, Diana L. Whipple, Ralph E. Slaughter and Stephen L. Jones

(73) **Horsfall Bauer Units used for Pathogenicity Testing in Chickens**
Jacquelyn Parker

(74) **Preliminary Investigation of Squirrels As a Possible Reservoir for Deer Adenovirus**
J. Parker, L.W. Woods, H.D. Lehmkuhl and M.H. Stillian

(75) **Of Mice and Math: A Model for Hantavirus Transmission in Deermice (*Peromyscus maniculatus*)**
Jessica Pearce and Fred Adler

(76) **Ceruminous Gland Adenocarcinoma in Channel Island Foxes (*Urocyon littoralis catalinae*) from Santa Catalina Island**
Brian A. Stacy, Steven F. Timm, Deana Fritcher, David K. Garcelon and Linda Munson

(77) **Case Report: Subcutaneous *Taenia crassiceps* Cysticercosis in an Arctic Fox (*Alopex lagopus*)**
Brent Wagner, Eric Hoberg and Trent Bollinger

(78) **Analysis of Lymphocytes Isolated From White-Tailed Deer (*Odocoileus virginianus*) Fawns**
Sasha J. Fach, W. Ray Waters, Mitchell V. Palmer, William C. Davis and Randy E. Sacco

(79) ***Mycobacterium bovis*-Infected White-Tailed Deer (*Odocoileus virginianus*): Detection of Immunoglobulin Specific to Crude Mycobacterial Antigens**
W. Ray Waters, Theresa E. Rahner, Mitchell V. Palmer and Diana L. Whipple

(80) **Diagnosis of Mycoplasmal Upper Respiratory Tract Disease in Chelonians**
Lori Wendland, Diane Duke, Daniel Brown, Elliott Jacobson, Paul Klein and Mary Brown

(81) ***Eimeria auritusi* n. sp. in the Kidneys of Double-Crested Cormorants (*Phalacrocorax auritus*): Species Description and Lesions**
Michael J. Yabsley, Nicole L. Gottdenker and John R. Fischer

UNGULATE I SESSION
Moderator: Todd Cornish

10:45-11:00 **(82)** **Paratuberculosis and Repercussions for Management of Free-Ranging Tule Elk at Point Reyes National Seashore**
Natalie B. Gates and Elizabeth J.B. Manning

11:00-11:15 **(83)** **Validation of Protein G-Based ELISAs for Johne's Disease Surveillance in Elk**
Elizabeth J. B. Manning and Joely Kramsky

11:15-11:30 **(84)** **Milk Replacer Containing *Mycobacterium bovis* as a Source of Infection for White-Tailed Deer Fawns (*Odocoileus virginianus*)**
Mitchell V. Palmer, W. Ray Waters and Diana L. Whipple

11:30-11:45 **(85)** **Immune Responses of Elk (*Cervus elaphus*) to *Mycobacterium bovis* Bacille Calmette Guerin (BCG) Vaccination**
W. Ray Waters, Mitchell V. Palmer, Steven C. Olsen, Randy E. Sacco and Diana L. Whipple

11:45-1:00 **LUNCH**

AVIAN II SESSION
Moderator: Tonie Rocke

1:00-1:15 **(86)** **Experimental Vacuolar Myelinopathy in Red-Tailed Hawks**
John R. Fischer, Lynn A. Lewis and Cynthia M. Tate

1:15-1:30 **(87)** **Avian Influenza Virus, Avian Paramyxovirus-1 and Circovirus Infections of Ring-Billed Gulls (*Larus delawarensis*) in Southern Ontario, Canada**
Roser Velarde, Ian K. Barker, Èva Nagy, Bruce Hunter, Scott McEwen and Chip Weseloh

1:30-1:45 **(88)** **Levels of Fecal Corticosterone in Sandhill and Whooping Cranes During Preparation for Human-Led Migration**
Barry K. Hartup, Nancy M. Czekala, Glenn H. Olsen and Julia A. Langenberg

1:45-2:00 **(89)** **Lead Levels, Ectoparasites, and Morphometric Symmetry of Turkey Vultures Captured in Humboldt County, California**
Patricia C. Halpin and Richard G. Botzler

2:00-2:15 **(90)** **Differential Susceptibility of House Finches (*Carpodacus mexicanus*) to Three Doses of *Mycoplasma gallisepticum***
Kristy L. Farmer, Geoffrey E. Hill and Sharon R. Roberts

2:15-2:30 **(91)** **Development of Enteric Antibiotic Resistance in Avian Patients at the Tufts Wildlife Clinic**
Florina Tseng, Katheryn Ziegerer and Mark Pokras

2:30-3:00 **POSTER BREAK**

CARNIVORE II SESSION
Moderator: Kristen Charlton

3:00-3:15 **(92)** **A Novel Epizootic of Insectivorous Bat Variant Rabies Virus in Skunks**
Mira J. Leslie, Jean Smith, Cathleen Hanlon, Rodney Rohde, Ron Cheshier and Charles E. Rupprecht

3:15-3:30 **(93)** **Health Evaluation of Wild Siberian Tigers (*Panther tigris altaica*) and Amur Leopards (*Panthera pardus orientalis*) in the Russian Far East**
Kathleen S. Quigley, Douglas L. Armstrong, Dale G. Miquelle, John M. Goodrich and Howard B. Quigley

3:30-3:45 **(94)** **Feral Cat Altering Programs (FCAP): What's wrong with them? What can be done about it?**
David A. Jessup

3:45-4:00 **(95)** **Evidence of Exposure of American Black Bears to the Agent Causing Human Granulocytic Ehrlichiosis, *Anaplasma phagocytophila*, in Northern Humboldt County, California**
Richard N. Brown, Janet E. Foley, Jaime L. Sajecki and J. Mark Higley

4:00-4:15 **(96)** **Orphaned Black Bear Cub Rehabilitation in Southern Colorado**
Herman F. Dieterich and Susan B. Dieterich

THURSDAY AUGUST 1st

8:00-8:10 **ANNOUNCEMENTS**

8:10-8:30 **PREVIEW OF THE 2003 WDA CONFERENCE**

SYMPOSIUM ON CHRONIC WASTING DISEASE
Moderator: Mike Miller

8:30-8:45 **(97)** **Chronic Wasting Disease in Captive and Wild Cervids in Saskatchewan**
Trent K. Bollinger, Ronald E. Lind, Keith West and Richard M. Jobin

8:45-9:00 **(98)** **Chronic Wasting Disease in Wisconsin Wild White-Tailed Deer: Surveillance Program, Detection and Future Disease Management Plans**
Kerry A. Beheler, Julie A. Langenberg and Barb Walser

9:00-9:15 **(99)** **Emergence of Chronic Wasting Disease in Wisconsin White-Tailed Deer: Information from the Field and Preliminary Analysis**
Damien O. Joly, Julie A. Langenberg, Michael D. Samuel, Robert E. Rolley, Tim Van Deelen, Kerry Beheler and Christine A. Ribic

9:15-9:30 **(100)** **Evaluation of Tonsillar Biopsy Data for Estimating Chronic Wasting Disease Prevalence in Free-Ranging Mule Deer**
Lisa L. Wolfe, Mary M. Conner, Thomas H. Baker, Victoria J. Dreitz, Kenneth P. Burnham, Elizabeth S. Williams, N. Thompson Hobbs and Michael W. Miller

9:30-9:45 **(101)** **Land-Use Impacts on the Prevalence of Chronic Wasting Disease in Colorado Mule Deer Populations**
Victoria J. Dreitz, N. T. Hobbs, Thomas H. Baker, Lisa L. Wolfe, Kenneth P. Burnham, Mary M. Conner and Michael W. Miller

9:45-10:00 **(102) Estimating the Relationship Between Chronic Wasting Disease Prevalence and Mule Deer Density in Northcentral Colorado**
Matthew L. Farnsworth, Robin M. Reich, N. Thompson Hobbs and Michael W. Miller

10:00-10:15 **(103) Mule Deer (*Odocoileus hemionus*) Movements in Relation to Spatial Patterns of Chronic Wasting Disease Prevalence in Northcentral Colorado**
Mary M. Conner, Thomas H. Baker, Lisa L. Wolfe and Michael W. Miller

10:15-10:30 **(104) Interstate Management Plan for the Control of Chronic Wasting Disease**
Terry J. Kreeger, Michael W. Miller and Bruce Morrison

10:30-10:45 **BREAK**

AQUATIC II SESSION
Moderator: David Jessup

10:45-11:00 **(105) Immunoglobulin G Responses of Northern Elephant (*Mirounga angustirostris*) and Pacific Harbor (*Phoca vitulina richardsi*) Seals Naturally Infected with *Otostrongylus circumlitus***
Jocelyn G. Elson-Riggins, Frances M.D. Gulland and Edward G. Platzer

11:00-11:15 **(106) Cancer in Free-Ranging California Sea Lions (*Zalophus californianus*): Investigations into the Role of a Gamma Herpesvirus, Environmental Contaminants and Other Co-Factors**
Linda J. Lowenstine, Elizabeth L. Buckles, Donald P. King, Gina Ylitalo, John E. Stein, Robert Delong, Sharon Melin, Jeffry L. Stott, Brian M. Aldridge and Frances M.D. Gulland

11:15-11:30 **(107) Clinical Pathology of Harp (*Phoca groenlandica*) and Hooded Seals (*Cystophora cristata*) during the Breeding Season**
France Boily, Sandra Beaudoin and Lena Measures

11:30-11:45 **(108) Biology, Movements and Health Assessment of Free-Ranging Manatees in Belize**
A. Alonso Aguirre, Robert K. Bonde and James A. Powell

11:45-1:00 **LUNCH**

1:00-1:15 **(109) Southern Sea Otters and Pathogen Pollution: A Preliminary Study of Exposure to Fecal Pathogens**
David A. Jessup, Melissa A. Miller, Nancy Christian, Erin Dodd, Spencer Jang and Michael Murray

1:15-1:30 **(110) A Program to Protect Captive Southern Sea Otters (*Enhydra lutris nereis*) from Morbillivirus Exposure at a Veterinary Care and Research Facility Adjacent to Wildlife Habitat**
Christine M. Arnold, David A. Jessup, David Casper, Michael J. Murray, Brett M. Long, Jennifer Gafney, Traci L. Fink and Terrie M. Williams

1:30-1:45 **(111) A Review of Potential Infectious Disease Threats to Southern Resident Killer Whales (*Orcinus orca*)**
Joseph K. Gaydos, Leslie A. Dierauf, Kenneth C. Balcomb III and Richard W. Osborne

1:45-2:00 **(112) Classification of Epidermal Lesions: Potential Etiologies, Characterization and Significance in the Bowhead Whale (*Balaena mysticetus*)**
Cheryl Rosa, John E. Blake and Todd M. O'Hara

2:00-2:15 **(113) Increased Gray Whale (*Eschrichtius robustus*) Strandings in 1999 and 2000 – Was Malnutrition the Cause?**
Frances Gulland, Linda Lowenstine, Elizabeth Buckles, Teri Rowles, Janet Whaley, Jonna A.K. Mazet and Christine Kreuder

2:15-2:30 **BREAK**

UNGULATE II SESSION
Moderator: Joe Gaydos

2:30-2:45 **(114) Tolazoline Reversal of Xylazine in Bison (*Bison bison*): Mitigation of Adverse Effects**
Thomas J. Roffe and Steven J. Sweeney

2:45-3:00 **(115) An Update of Adenoviral Hemorrhagic Disease in Mule Deer in California**
Pamela K. Swift, Leslie W. Woods, Howard D. Lehmkuhl and Karen R. Jones

3:00-3:15 **(116) Survey of Diseases Diagnosed in California Black-Tailed Deer at the California Animal Health and Food Safety Laboratory, 1992-2002**
Leslie W. Woods, Pamela Swift and Deryck Read

3:15-3:30 **(117) Epizootic of Hemorrhagic Disease in Mule Deer in Arizona**
Shelli A. Dubay, James C. Devos, Jr., Ted H. Noon and Sue Boe

3:30-3:45 **(118) Ocular Disease in Moose (*Alces alces*) Associated with Carotid Artery Worms (*Elaeophora schneideri*) in Idaho**
Mark L. Drew and Bill Foreyt

3:45-4:00 **(119) Epidemiology of Abomasal Parasites of Muskoxen on Banks Island, Northwest Territories, Canada**
Susan Kutz, John Nagy, Brett Elkin, Eric Hoberg, Louis Gasbarre, Brent Wagner and Lydden Polley

4:00-4:15 **(120) Effectiveness of *Brucella abortus* Strain 19 Single Calfhood Vaccination in Elk (*Cervus elaphus*)**
Thomas J. Roffe, Lee C. Jones, Kenneth Coffin, Steven J. Sweeney, Mark Drew, Phil Elzer and Donald Davis

4:15-4:30 **(121) Analysis of Lectins As Oral Vaccine Adjuvants Conjugated to *Brucella abortus* Strain RB51, in BALB/C Mice**
Jenny G. Powers, Paul B. Nash, Lowell A. Miller, Jack C. Rhyan, Jennifer Flood and Steven C. Olsen

4:30-4:45 **(122) Risk Management at Yellowstone National Park: Bison and Brucellosis**
Rick Wallen and Glenn Plumb

16:45 **CLOSING**

(1) INVESTIGATING WILDLIFE EIDS – LESSONS FROM CHYTRIDIOMYCOSIS AND NIPAH VIRUS

PETER DASZAK, Consortium for Conservation Medicine, Lamont-Doherty Earth Observatory of Columbia University, 61 Rt. 9W, Palisades, NY 10964

Wildlife EIDs, like human EIDs, are almost entirely driven by anthropogenic environmental changes such as pathogen introduction, encroachment, fragmentation, human migration, deforestation urban sprawl and others. Understanding emergence therefore requires an understanding of these complex and often under-researched drivers. In this talk I demonstrate how multidisciplinary teams of investigators are tackling two key EIDs: amphibian chytridiomycosis, and two emerging zoonotic pathogens - Nipah and Hendra virus. For chytridiomycosis, I present new DNA sequence data that strongly suggest the causative agent has been disseminated internationally via globalized trade in amphibians. This anthropogenic introduction of a pathogen to new geographic regions ("pathogen pollution") is a seriously underestimated form of anthropogenic environmental change and the most commonly cited driver of wildlife EIDs. For Nipah and Hendra virus, preliminary data suggest that climate oscillations, deforestation and dramatic changes to agricultural practice drove their emergence. Further complexity arises from the multiple reservoir hosts of these pathogens and their apparent dependence on amplifier hosts for emergence. It is increasingly clear that to move towards prediction and prevention of emerging diseases will involve more vigorous examination of the factors that drive emergence. This will require significantly improved communication between veterinary, medical, ecological, conservation and other communities and vets who understand how to investigate environmental change.

(2) ESTIMATING THE BASIC REPRODUCTIVE NUMBER, R0, FOR A RECENTLY INTRODUCED OR EMERGING PATHOGEN

ANDY DOBSON, EEB, Eno Hall, Princeton University, Princeton, NJ 08544-1003; OTTAR BJORNSTAD, Penn State University, College Park, PA 17837

Determining the short-term population dynamic trajectory of a novel pathogen is a central problem in both bio-terrorism and conservation biology. Both are crisis disciplines, where policy decisions have to be made swiftly using crucial analysis of minimal sets of data. We develop a new technique for estimating the basic reproductive number of a pathogen, R0, using data obtained in the initial stages of an infectious disease outbreak. The method is relatively unbiased and quickly produces estimates of R0 that can be used to determine if the outbreak will continue to spread (R0>1), or whether it will quickly die out (R0<<1). We illustrate the utility of the technique by applying it to data sets for pathogen outbreaks in human and animal populations: anthrax, Ebola virus, small pox, foot and mouth disease, hog cholera, and phocine distemper.

(3) DOES WINTER CLIMATE INFLUENCE PREVALENCE OF MYCOPLASMAL CONJUNCTIVITIS IN WISCONSIN HOUSE FINCHES?

BARRY K. HARTUP, International Crane Foundation, E-11376 Shady Lane Road, Baraboo, WI 53913; SONIA ALTIZER, Department of Environmental Studies, Emory University, 1715 N. Decatur Road, Atlanta, GA 30322; WESLEY M. HOCHACHKA and ANDRE A. DHONDT, Cornell Laboratory of Ornithology, 159 Sapsucker Woods Road, Ithaca, NY, 14853

The House Finch Disease Survey (HFDS) of the Cornell Laboratory of Ornithology has been instrumental in monitoring the range expansion of mycoplasmal conjunctivitis in house finches (*Carpodacus mexicanus*) across North America since 1994. A secondary benefit of the survey has been the ability to construct an index of disease prevalence based on the proportion of days diseased birds were observed in a given month, which has allowed investigations into seasonal variability in disease frequency and host population effects in various regions. We reviewed 1587 monthly HFDS records from Wisconsin-based observers received between November 1994 and February 2002 to more clearly understand the seasonal dynamics of the disease in this area. Epidemic conjunctivitis first occurred among Wisconsin house finches during the fall of 1996 and winter of 1996-97, a full 3 years after the disease was first identified in Maryland and Virginia. Seasonal fluctuations in disease prevalence in Wisconsin mirror well-documented variability in other northern areas, including minimal disease prevalence in summer (July) and two peaks of disease, one repeatable increase in late fall (October-November) and a less predictable increase in early spring (March-April). Close inspection of the HFDS data revealed a dichotomy of disease prevalence across years during the winter months of December-February following the epidemic winter of 1996-97: high disease prevalence in 1997-98 (13.2%) and 2000-01 (10.1%), and low disease prevalence in 1998-99 (1.9%), 1999-2000 (2.3%) and 2001-02 (<1%). A recent study suggests that maximal declines in house finch numbers may not occur for at least 2 years after the initial invasion of the disease into an area with high host density. This finding may partly explain the high disease prevalence that was sustained throughout the 1997-98 winter, despite an El Nino event that moderated winter temperatures. Each of the successive four winter periods, however, included temperature anomalies that may have influenced host susceptibility or exposure to mycoplasmal disease. Each winter period of low disease prevalence was characterized by unusually warm temperatures and low December precipitation; the 2000-01 winter with high disease prevalence was characterized by low temperatures and above normal December snowfall. The difference in disease frequency from the last 2 winters was confirmed by field data from south-central Wisconsin. Lower counts of house finches at feeders, though nearing 20% less than pre-epidemic levels, do not appear consistently related to the low prevalence estimates. We propose that lower cold stress and changes in flocking behavior may be responsible for the observed variation in disease prevalence during warmer winters in Wisconsin.

(4) WEST NILE VIRUS - AN EMERGING DISEASE OF NORTH AMERICAN BIRDS

ROBERT G. MCLEAN, USGS National Wildlife Health Center, Madison, WI 53711 (new address, National Wildlife Research Center, WS/APHIS/USDA, Fort Collins, CO 80521)

The introduction of a virulent strain of West Nile virus (WNV) into New York City (NYC), New York, in 1999 initiated an epizootic in local birds, particularly in American crows, and was the beginning of a rapidly expanding disease in free-ranging wild birds of the United States and Canada. West Nile virus became established in the NYC area and has persisted through the temperate climates of the northeastern U.S. for 3 years despite no continuous mosquito activity during the dormant winter months. Dissemination of WNV to the southern warmer climates of Florida and other Gulf Coast states in 2001 where there is an extended period of mosquito activity has further enhanced the survival of WNV. These southern foci of WNV activity likely served as a source of virus for migrating birds in the spring to seed the virus into northern locations. Migratory birds subsequently moved the virus southward during fall migration from northern foci of transmission to new locations along their migratory pathway in the Mississippi flyway. This pattern of expansion will eventually disseminate the virus throughout North America. The American crow and blue jay have been the most susceptible species so far and are experiencing high mortality rates. However, nearly 100 species of birds have been found virus positive, including more than 60 free-ranging species of native birds. The rapid geographical expansion of WNV in the eastern U.S. and Canada and the rapid increase in infection and mortality rates in birds during the last 3 years reflect the virulent nature of this introduced virus strain and indicate the emergence of an epizootic disease of major importance to North American birds.

(5) LINKING EMERGING DISEASE AND DEGRADATION OF MARINE ECOSYSTEMS

JONNA A.K. MAZET, KIRSTEN V.K. GILARDI, CHRISTINE KREUDER, PATRICIA A. CONRAD, and MICHAEL H. ZICCARDI, Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, CA 95616; DAVID A. JESSUP and MELISSA A. MILLER, CDFG Marine Wildlife Veterinary Care and Research Center, 1451 Shaffer Road, Santa Cruz, CA 95060; M. TIM TINKER, 316A Earth and Marine Sciences Bldg., University of California, Santa Cruz, CA 95064

Emerging diseases have been a major focus of attention in human and veterinary medicine over the past two decades, but emerging diseases of wildlife are now also being recognized as an important threat to biodiversity and conservation. Diseases emerge in populations when normal host-pathogen ecology is disrupted, and the ever-increasing human population and the pervasive nature of our existence are frequently to blame. Environmental stressors such as habitat degradation, pollutants, municipal runoff, global climate change, and harvest of marine resources are largely suspected to negatively impact marine animal populations, yet this assumption has rarely been rigorously examined. Disease emergence has been detected in a range of taxonomic groups along the Atlantic coast of North America and has been clustered both geographically and temporally, particularly during El Nino Southern Oscillation events, suggesting possible associations with coastal pollution and abrupt climactic change. However, direct associations with these underlying system-level processes remain untested.

Anthropogenic influence may be linked to outbreaks of disease in marine ecosystems through complex processes. For example, the appearance of land-based pathogens as important causes of mortality in southern sea otters and adult harbor seals raises concern over the potential for sewage outfalls and runoff to transport these pathogens from land to sea, causing high levels of exposure in immunologically ill-prepared species. Habitat degradation in coastal estuaries that historically filtered watersheds may further contribute to the appearance of these pathogens in marine systems. Municipal and industrial runoff may also transport contaminants, some of which have been directly linked to immunosuppression in marine mammals. Abrupt climate change may not only cause shifts in species range, patterns of habitat use, interspecific interactions and prey composition, thereby increasing the spread of pathogens, but may also have direct impacts on immune system function and susceptibility to disease. Naturally, these factors have enormous potential to interact within an ecosystem and may have additive or even multiplicative effects on the occurrence of disease in any single species.

The association of specific environmental stressors with pathogen exposure and emerging disease across a range of marine animal indicators can be evaluated using epidemiologic risk factor analysis. A successful analysis will temporally and geographically match environmental stressor data, like water quality (nutrients, presence of coliforms, salinity, temperature), proximity to high human population density, economic development, and watershed land use to pathogen exposure and disease across trophic levels. Specific marine indicators can be chosen for analysis in order to focus investigations and increase the likelihood of finding specific associations in a comprehensive framework including the fields of wildlife diseases, demographics, oceanography, molecular biology, and water quality. For example, such a

multidisciplinary approach is currently being used by scientists studying sea otters in California to link foraging ecology, habitat use, and environmental contamination to disease in order to establish the current and potential demographic consequences for this threatened population. We hope this type of cooperation will be the next logical step in rigorously evaluating the potential associations between a degraded marine environment and the occurrence of disease in the sea otter population – potentially documenting pathways by which human activity and ongoing natural processes affect marine ecosystem health.

(6) THE QUICKSAND SURROUNDING SO CALLED EMERGING AND RE-EMERGING HUMAN AND ANIMAL DISEASES IN AFRICA: POLITICAL, CULTURAL, AND INSTITUTIONAL FACTORS THAT INFLUENCE DISEASE MANAGEMENT, ECOSYSTEM HEALTH, AND HUMAN LIVELIHOODS

MICHAEL D. KOCK, Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, CA 95616; RICHARD A. KOCK, OAU-PARC IBAR, Nairobi, Kenya

In Africa, political machinations, civil strife and widespread environmental changes have negatively affected ecosystem health and human livelihoods. Habitat loss and fragmentation, droughts and water issues, so called emerging diseases, impacts of existing and re-emerging diseases and human population expansion have all contributed to increasing morbidity and mortality. Many "new" diseases (affecting humans, their domestic animals, and wildlife) have had a negative influence on human health. Animal diseases such as Rinderpest and Foot-and-Mouth have indirectly affected human and ecosystem health through livestock and production losses, and have had direct impacts on natural resource areas, their biodiversity and on natural resource users. Human diseases, such as HIV, have had huge socioeconomic impacts on the African continent, and both treatment and preventive measures have been compromised by behavioral, social and political problems.

It is clear that the issues are not simply the diseases themselves but how political and cultural factors, and prejudice impact on the ability of individuals or groups to manage, control and prevent both human and animal diseases, and compromise the ability and desire of rural communities to utilize resources.

(7) PATHOGENS AND PREDATORS: USING VIRUSES TO TRACK WILDLIFE POPULATIONS

MARY POSS and ROMAN BIEK, Wildlife Biology Program and Division of Biological Sciences, University of Montana, Missoula MT 59812; CHUCK ANDERSON, Wyoming Cooperative Fish and Wildlife Research Unit, Box 3166, University Station, Laramie, WY 82071

Emergent viral infections most commonly arise when viruses enzootic in one population emerge as virulent pathogens in a susceptible population. Because the enzootic area for disease is defined by the geographic distribution of the carrier species, it follows that changes in reservoir population demographics may have significant impact on zoonotic and epizootic infection. Many factors that affect host population dynamics are recent in origin, making detection of changes in reservoir population demographics difficult to assess by standard genetic means. Members of the virus family *Retroviridae* are obligate intracellular parasites that integrate into the genome of the infected host, thus becoming a proxy host "gene". Significantly, the evolutionary rate of a retrovirus is one million times that of a eukaryotic host. Because of rapid accumulation of genetic change in retroviral genes it may be possible to use them as an indicator of recent changes in population structure and dynamics of a host population. We will present data to support the utility of this methodology using a model of cougars (*Felis concolor*) infected with the retrovirus, feline immunodeficiency virus.

(8) USE OF ORAL VACCINATION, TRAP-VACCINATE-RELEASE AND POPULATION REDUCTION TO CONTROL RACCOON RABIES IN ONTARIO, CANADA

RICK ROSATTE, Ontario Ministry of Natural Resources, Wildlife Research and Development Section, Trent University, Science Complex, P.O. Box 4840, Peterborough, Ontario, Canada, K9J 8N8

The raccoon strain of rabies virus was first reported in Ontario, Canada, during July 1999. Since that time, the Ontario Ministry of Natural Resources (OMNR) has implemented a massive program to contain and eradicate this disease. Three different tactics are being used to control raccoon rabies in Ontario: (1) aerial distribution of oral vaccine baits (2) Trap-Vaccinate-Release (TVR) (3) population reduction. Between July 1999 and November 2001, more than 1.5 million baits containing Vaccinia-Rabies Glycoprotein (V-RG) oral rabies vaccine have been distributed over an 8,000 sq km area of eastern Ontario. Using TVR methodologies about 17,000 raccoons and 2,500 skunks were live-captured, vaccinated by injection and released. To prevent the spread of rabies by animals that were clinical or incubating the disease (vaccination will not work on those animals), about 8,700 raccoons and 1,500 skunks were humanely euthanized. To date (June 2002), 101 cases (99 raccoons, 2 skunks) of raccoon strain of rabies have been reported in Ontario. The cost of the program to date is $5 million Cdn. The disease is currently contained to a 400 sq km area of eastern Ontario and has only spread 49 km in 3 years. Raccoon rabies spread in the U.S. where control was not implemented was about 40-60 km/yr. During 2002, plans are to continue to control and eliminate raccoon rabies in Ontario utilizing the three control tactics discussed above.

(9) LEGISLATIVE ISSUES ON EMERGENT WILDLIFE DISEASES AND BIOTERRORISM THREATS

MARK J. ABDY, Division of Vaccines and Related Product Applications, Office of Vaccines Research and Review, Food and Drug Administration, HFM 475, Rockville, MD 20852

During recent months, the U.S. Congress has held hearings, introduced legislation and voted on various public and animal health issues that will have a direct and indirect impact on management of wildlife. This is in part due to the terrorism attacks in the late summer/early fall of 2001, but also the Foot and Mouth Disease outbreak in Europe during 2001, the ongoing Bovine Spongiform Encephalopathy outbreak, the emergence of West Nile Virus in North America in 1999 and the spread of Chronic Wasting Disease in the United States. This paper will highlight the Bioterrorism Preparedness Act of 2001, various Chronic Wasting Disease bills, the Marine Mammal Protection Act and the Multinational Species Conservation Fund.

(10) PROTOZOAL PROBLEMS EMERGING AT THE HUMAN-WILDLIFE-DOMESTIC ANIMAL INTERFACE

PATRICIA A. CONRAD, MELISSA MILLER, ANNE KJEMTRUP, IAN GARDNER, CHRISTINE KREUDER, WOUTRINA SMITH, and ROB ATWILL, School of Veterinary Medicine, University of California, Davis, CA 95616; DAVE JESSUP, California Department of Fish & Game, Santa Cruz, CA, 95060

This talk will focus on the potential for exciting parasitological discoveries to be made where wildlife, humans and domestic animals interface. Two examples of "emerging" protozoal diseases illustrate this potential: protozoal myeloencephalitis of marine mammals and human babesiosis. Increasing evidence in the past decade shows the emergence of two protozoan parasites, *Toxoplasma gondii* and *Sarcocystis neurona*, as important contributors to mortality in California marine mammals. A recent pathological study revealed that 36% of southern sea otters (*Enhydra lutris nereis*) recovered freshly dead from the California coast between 1998 and 2001 had brain infections of *T. gondii* and 4% had *S. neurona* brain infections. The results of a recently completed epidemiologic study provided insights as to the demographic and environmental risk factors for *T. gondii* infection in sea otters. The impact of pollution with zoonotic protozoa, such as *T. gondii* and *Cryptosporidium* spp., on the coastal marine ecosystem and its human and wildlife inhabitants will be highlighted. Human babesiosis is another example of an emerging protozoal disease, with increasing numbers of cases of *Babesia microti* reported in the northeastern and midwestern U.S.A. and the discovery of a new babesial species in the West. Wildlife reservoir hosts are important to the maintenance of piroplasm parasites globally. Molecular characterization of piroplasm isolates from humans, dogs and wildlife in the western U.S.A. has provided important clues to the relationship of these parasites which form a distinct clade, separate from other piroplasms found worldwide. Molecular phylogenetic data suggest that large ungulates are potential reservoirs for this new human babesial parasite.

(11) PATHOBIOLOGICAL CHANGES IN WHITE STORKS (*CICONIA CICONIA*) AFTER A MINE TAILINGS DISASTER IN SOUTHWESTERN SPAIN

JUDIT E. SMITS, Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N 5B4; GARY R. BORTOLOTTI, Department of Biology, University of Saskatchewan, Saskatoon, SK. S7N 5B3; RAQUEL BAOS, JULIO BLAS, JOSE TELLA, and FERNANDO HIRALDO, Doñana Biological Station, Seville, Spain

The Doñana National Park and Reserve in southwestern Spain has been a protected sanctuary for wildlife since 1969. This area is used extensively as a breeding site for thousands of resident and migratory birds. In April 1998, an upstream dam containing toxic acid sludge waste from a pyrite ore mine collapsed, causing the inundation of approximately 4,500 ha of adjacent river valley. The spill, estimated at 5 million cubic meters of toxic sludge, reached to the borders of the Doñana preserve, but it also affected feeding grounds for birds breeding in the reserve. Metals and metalloids released in this spill included lead, cadmium, copper, arsenic and zinc which have passed into some parts of the food chain.

The largest colony of white storks (Ciconia ciconia) in the western Palearctic is less than 1 km from the area covered by the spill. Storks feed on aquatic organisms such as crayfish, which were contaminated. Our work with storks is one component of a multi-year project to define biological effects as the spill site matures. We examine biological effects of mine contaminants on stork nestlings being raised on this colony, two and three years after the accident (2000, 2001). Brood survival, occurrence of disease, and tissue residues have been examined. Blood samples have been collected to assess physiological (hormonal and immunological) information as well as to determine contaminant levels.

Offspring produced on the colony at the spill margin were compared with those from a distant reference area. Deformities of the legs and bills of the contaminated nestlings have been observed with increasing frequency over the years since the spill. Pathological examinations were carried out on 10 deformed juveniles from the contaminated area and 11 reference area juveniles. Physiological variables compromised by contaminant exposure included corticosterone and thyroid hormone levels, as well as the antibody-mediated immune response. The corticosterone stress response was significantly delayed in the spill-exposed nestlings. Analysis of long bones, liver and kidney provide data on the toxic metal burden in the young storks. Cross-sectional analysis of the bones allows the spatial distribution of the mine contaminants to be elucidated. From this, the temporal exposure to the different metals through the diet can be extrapolated for the young growing storks. Results from biochemical and histopathological studies will be presented / discussed in relation to the observed skeletal deformities.

(12) LOW PREVALENCE OF CHYTRIDIOMYCOSIS IN LARVAL RED-LEGGED FROGS (*RANA AURORA AURORA*) IN REDWOOD NATIONAL PARK

NATHAN C. NIETO and JOHN O. REISS, Department of Biological Sciences, Humboldt State University, Arcata, CA 95521

Chytridiomycosis is an emerging infectious fungal disease of amphibians. It has been responsible for mass mortality events and has been implicated as a primary cause of decline in many localities. In tadpoles, chytridiomycosis is not believed to cause mortality; instead this stage is hypothesized to provide a reservoir for the pathogen. We are conducting a field study to determine the extent of this disease in lowland populations of northern red-legged frogs (*Rana aurora aurora*) located in and adjacent to Redwood National Park, California. 3600 red-legged frog tadpoles from 12 breeding localities were examined with a dissecting microscope for the loss of oral pigmentation associated with chytrid infection; nine such animals were collected. In addition, 5 randomly selected individuals from each breeding site were collected to search for cryptic infection. Keratinized mouthparts of collected animals were examined histologically for *Batrachochytrium dendrobatidis*; animals were also examined for ectoparasites and intestinal parasites. The intestinal fauna contained both the trematode *Megalodiscus microphagus* and the nematode *Gyrinicola batrachiensis*. The ectoparasite *Trichodina sp*. was also observed. One of the tadpoles that showed loss of mouthpart pigmentation had abundant zoosporangia of *B. dendrobatidis* in the anterior beak. The locality where chytridiomycosis was identified has a previous history of infection in very low prevalence. In another location a mass mortality event occurred (>500 moribund or dead larval red-legged frogs), but this was not associated with chytridiomycosis. This study represents a naturally occurring situation in which chytridiomycosis exists in extremely low prevalence and has apparently not been associated with any mass mortality events.

(13) THE RISK OF AVIAN BOTULISM OUTBREAKS FROM AVICIDE DRC-1339 IN NORTH DAKOTA WETLANDS

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Blackbirds (Family Icterinae) are responsible for damage to ripening sunflower crops in the prairie pothole region of the United States. The avicide 3-chloro-p-toluidine hydrochloride (DRC-1339) has been studied as a means of lethal control to reduce blackbird depredation on the crop. Affected birds frequently seek water and often die within 1-3 days in or near wetlands where they are roosting. Decomposing vertebrate carcasses can provide an optimal growth medium for production of *Clostridium botulinum* toxin. Toxic maggots can be ingested by waterbirds, especially waterfowl, causing additional mortality and initiating an outbreak of avian botulism through the carcass-maggot cycle. During 2000 and 2001, we investigated the role of blackbird carcasses as a potential source of *C. botulinum* toxin in North Dakota wetlands. To simulate the affects of DRC-1339 control actions, blackbird carcasses were seeded (artificially placed) in a number of wetlands and monitored periodically to determine disappearance rates and to collect carcasses for laboratory analysis. Carcass remains and associated maggots were screened for the presence of *C. botulinum* type C toxin by ELISA and the amount of toxin in positive samples was quantified using a standard mouse inoculation test. Results were used to estimate the amount of toxin that might be produced from blackbirds killed in wetlands with DRC-1339 and to assess the risk of avian botulism outbreaks occurring after blackbird control efforts.

(14) MEDICAL SURVEY OF THE LOCAL HUMAN POPULATION TO DETERMINE POSSIBLE HEALTH RISKS TO THE MOUNTAIN GORILLAS (*GORILLA GORILLA BERINGEI*) OF BWINDI IMPENETRABLE FOREST NATIONAL PARK, UGANDA

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Recently, there has been increasing contact between mountain gorillas (*Gorilla gorilla beringei*) and the human population surrounding Bwindi Impenetrable Forest National Park (BIFNP) in Uganda. Due to the close taxonomic relationship between humans and gorillas the potential for disease transmission between the species exists. Preventing the introduction or spread of transmissible diseases to the gorillas is essential for protecting this endangered species. We interviewed 301 villagers living in close proximity to BIFNP with a medical questionnaire in July, 2000. We collected information on demographics, vaccination and health history, and human/gorilla interaction. Our objectives were to estimate the prevalence of several diseases in the human population, and to evaluate the risk for anthropozoonotic transmission (from humans to gorillas). We found a high prevalence of disease symptoms such as coughing (72.1%) and fever (56.1%) compatible with acute infectious diseases; over half of the respondents (59.1%) had a specific disease diagnosis within the six months preceding the study. Using a Chi Square test we compared villagers who had visual contact with gorillas in the six months preceding the study (53.5%) to villagers who had no visual contact (46.5%). Men were 2.3 times more likely to have visual contact with gorillas than women. In addition, individuals aged 41-59, those living in Buhoma or Bujengwe, or with the occupation of peasant or trader were more likely to have had visual contact with gorillas compared with other demographic groups. In general, the frequency of disease history and symptoms was similar for people with and without contact. The high prevalence of acute infectious diseases in the population surrounding BIFNP and the high rate of contact with gorillas creates the potential for anthropozoonotic disease transmission. Interventions and educational efforts should be directed at increasing the understanding of inter-species disease transmission, and promoting behaviors designed to minimize risk such as burial of wastes. Improvements in public health infrastructure would benefit the villagers as well as the mountain gorillas.

(15) TEST OF AN ENVIRONMENTAL MODEL: NORTHERN RED-LEGGED FROGS (*RANA AURORA AURORA*) AS BIOINDICATORS OF ENDOCRINE-DISRUPTING CONTAMINANTS ALONG CALIFORNIA'S NORTHWEST COAST

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We examined the use of *Rana aurora aurora* as a bioindicator of ecosystem stress resulting from endocrine-disrupting contaminants (EDCs). Vitellogenin is a lipoglycophospho-protein that is normally produced by the liver of only female oviparous vertebrates under the control of estrogen. The presence of vitellogenin in male or subadults can indicate the affect of EDCs in the environment on an organism. We sought to determine whether the female protein vitellogenin could be detected in serum of male and subadult red-legged frogs (*Rana aurora aurora*) in quantities sufficient such that it could be used as an indicator of contamination by estrogen-mimicking compounds in NW California. In 1999 and 2000, we located a total of 14 populations of *R. a. aurora* during the spring breeding season (January to May). Male and subadult *R. a. aurora* were captured, anesthetized, blood was drawn for serum separation. Bioassays were conducted on the serum to determine presence/absence of vitellogenin. Analyses showed 67% (4 of 6) of the populations with vitellogenin in the 1999 surveys, and 56% (5 of 9) of the populations with vitellogenin in the 2000 surveys. For the two years of the study, 9 of 13 sites (69%) had male or subadult frogs with vitellogenin. These results indicate that red-legged frogs can function as bio-indicators of EDCs and that an exogenous source or sources of estrogen exists in the environment of the north coast of California.

(16) PATTERNS OF ILLEGAL WILDLIFE POISONING IN THE UNITED STATES - A REVIEW OF CASES SUBMITTED TO THE NATIONAL WILDLIFE FORENSIC LABORATORY (1990 - 2001)

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Intentional poisoning of wildlife is illegal yet wide spread in the United States. Law enforcement agents of the US Fish and Wildlife Service investigate and prosecute suspected intentional poisoning cases. These prosecutions can lead to substantial fines and even incarceration of individuals convicted. Wildlife, especially eagles, are submitted to the Wildlife Forensic Laboratory for examination and analysis in support of these investigations. Determinations are made based on the demonstration of significant amounts of pesticide residue in upper gastro-intestinal tract contents or in organ tissues. The identification of recently consumed food items may indicate the source of the poison and provide information as to either primary or secondary poisoning.

The use of carbamate and organophosphate pesticides to kill targeted species as well as non targeted species is the most commonly encountered poisoning scenario. Because these pesticides generally kill rapidly, the victims are more likely to be found near the site of poison distribution. Of 1037 poisoning cases diagnosed at the laboratory, the following cholinesterase inhibiting pesticides were encountered: carbofuran (40%), aldicarb (9%), diazinon (8%), famphur (12%), fenthion (7%), terbufos (2%), and other organophosphate and carbamate pesticides (3%). Other toxic substances included strychnine (8%), avitrol (5%), pentobarbital (2%), starlacide (2%), and other miscellaneous poisons (2%).

Considering a total of 1071 bald and golden eagles examined, pesticide poisoning was confirmed in 191 deaths (18%). Pesticides found in eagle poisonings were carbofuran (46%), aldicarb (20%), diazinon (0%), famphur (14%), fenthion (6%), terbufos (1%), and methiocarb (2%). The barbiturate pentobarbital accounted for 17 eagle deaths (9%) and zinc phosphide in 5 (2%).

Specific patterns of pesticide misuse are evident in wildlife poisoning cases. Carbofuran and aldicarb are frequently placed in carcasses of deer, cattle, sheep, or poultry to poison birds of prey such as eagles, red tailed hawks, and vultures that feed on carrion. Grain soaked in famphur may be used to control pest birds around dairies, feed lots, or other agricultural facilities. This practice has resulted in many predatory hawks and eagles being poisoned when they consume the target species such as starlings, blackbirds, and grackles. Strychnine impregnated grain improperly used in rock dove (pigeon) and rodent control operations also leads to secondary raptor poisoning.

(17) INVASIVE PATHOGENS: DETECTIONS ON NATIONAL WILDLIFE REFUGES AND POTENTIAL RISKS TO ADDITIONAL REFUGES

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In 2001, the USGS Biological Resources Discipline's Mid-continent Ecological Science Center initiated a project to identify invasive plants, fish, birds, mammals and pathogenic organisms on National Wildlife Refuges (NWR), using the definition of invasive species set forth in Executive Order 13112 (3 February 1999). The goals of the project are to determine the current extent of invasive species problems on NWR, identify potential problems, and to determine additional information needs. These data will assist Refuges and other management agencies in developing management strategies for existing invasive species problems, and contingency plans for possible future problems. Through literature searches and surveys of wildlife disease professionals, we developed an initial list of approximately 45 viruses, fungi, bacteria, and helminths that are potentially invasive species. Through the evaluation of database records of 25,320 submissions to the National Wildlife Health Center (NWHC) from NWR for 1975-1999, we identified 18 potentially invasive species, only 10 of which were on our initial list. We then searched the refuge submissions to determine on which refuges those species had been identified. We identified 83 refuges or refuge complexes from which we had received 1710 submissions that resulted in the identification of a possibly invasive pathogen. We examined the spatial and temporal distributions of those species by mapping, and compared those distributions to non-Refuge sites (>65,000 records) for the same time period. Targeted lists of potentially invasive species for which monitoring programs could be developed are being prepared for the Refuges.

(18) PATAGONIA SEABIRDS AS INDICATORS OF MARINE ECOSYSTEM HEALTH

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Long-term health monitoring is instrumental in the interpretation of the present status of wildlife populations and the prediction of future trends. Additionally, surveillance of specific sentinel populations allows for the indirect evaluation of the health of the ecosystem in which they live. Amongst key sentinel species, seabirds are good indicators of marine environmental health. They forage in specific areas and on specific prey, reflecting the condition of marine resources. Also, because of their colonial nature, they are particularly susceptible to catastrophic events and disease epidemics.

The marine ecosystem off the Patagonian shores is being subjected to significant human modifications as evidenced by increased coastal fishing activities, tourism, oil exploration and drilling, mining, industrial establishment, urban development, etc. However, baseline health parameters are still limited for most coastal wildlife species, restricting our capacity to predict environmental changes reflected in these biological indicators.

In an effort to understand the effects of long-term environmental stressors, we began monitoring seabird health along the Patagonia coastline in 1993. Surveyed species include imperial cormorants *(Phalacrocorax atriceps*) (n=83), rock shags (*P. magellanicus*) (n=65) and southern giant petrels (*Macronectes giganteus*) (n=25) from 1999-2001 and Magellanic penguins (*Spheniscus Magellanicus*) (n=430) between 1993-2001. Selected baseline hematology values, serum chemistries and minerals and metals will be presented for each species. Results on serological evidence of infectious disease exposure to infectious laryngotracheitis, avian encephalomyelitis, avian influenza, avian reovirus, infectious bursal disease, infectious bronchitis virus, paramyxovirus-1, -2, and –3, avian adenovirus, Salmonellosis, Chlamydiosis and Aspergillosis will be presented. Spatial and temporal variations in pathogen exposure will be discussed. The analysis of this information will set the basis for future evaluations required to understand disease processes in these wild populations, react accordingly to mass mortalities or disease epidemics, and predict population trends and overall ecosystem impact.

(19) MARINE ECOSYSTEM HEALTH AND SENTINEL SPECIES: ADDING AN ECOLOGICAL ELEMENT TO THE PROVERBIAL "CANARY IN THE MINESHAFT"

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Methods to assess marine ecosystem health are grossly lacking and a system to monitor and gauge marine health threats linked to conservation and management policies is needed. Emerging infectious diseases, mass mortality events, harmful algal blooms, and anomalous changes in selected marine species abundance and composition - occurrences which can be defined as major marine ecological disturbances - may signal a decline in ecosystem health. There is currently an effort by many scientists to examine the systemic health threats to marine vertebrate species, including marine mammals, as they relate to marine environmental health. Unprecedented number of emerging and re-emerging diseases such as brucellosis in dolphins, aspergillosis in coral reefs and morbillivirus infections linked to large-scale marine mammal die-offs have occurred in recent times. Marine turtles are facing a worldwide epidemic of fibropapillomatosis and Florida manatees have been identified harboring skin tumors caused by a papillomavirus with unknown long-term impacts to these endangered species.

One proactive method of trying to get a handle on this large-scale problem of disease emergence and resurgence is by surveying sentinel species. Sentinel species are the proverbial "canaries in the mineshaft". They serve as indicators of their environment and may reflect the quality of health in marine ecosystems. The single species approach may provide a series of "snap shots" of environmental changes to determine if animal, human or ecosystem health may be affected. Marine vertebrates are good integrators of changes over space and time and represent excellent sentinels of ecosystem health. By moving in and out of infected/polluted areas, they can spread pathogens and contaminants geographically as well as throughout the food chain. The sentinel species concept can be useful for providing an "early warning" system of emerging diseases or for monitoring the course of disease related activities requiring prevention, remediation or control. We have identified a number of critical research needs and opportunities for transdisciplinary collaboration that could help advance the use of sentinel species in ecosystem health and monitoring of disease emergence.

(20) WILL TECHNOLOGY BENEFIT WILDLIFE DISEASE EVALUATIONS?

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(21) BACTEREMIA IN FREE-RANGING HAWAIIAN GREEN TURTLES WITH FIBROPAPILLOMATOSIS

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Past studies of free-ranging green turtles with fibropapillomatosis have shown that animals become immunosuppressed with increasing severity of disease. Additionally, pilot studies have revealed that some animals that strand with severe FP also have circulating bacteria in their blood stream (bacteremia). To test the hypothesis that in addition to being immunosuppressed, turtles with FP are also bacteremic. We captured free-ranging green turtles from the Kona coast, Hawaii where FP is absent and from Kaneohe Bay, Oahu where FP is endemic. Each turtle was given an FP severity score ranging from 0 (non-tumored) to 3 (severely tumored). A fifth category included turtles that were stranded on land. We found that percent of turtles with blood cultures positive for bacteria increased with severity of FP and that the majority of bacteria cultured were *Vibrio* sp. These data continue to support the hypothesis that immunosuppression is a sequela to FP rather than a pre-requisite and that debilitated turtles offer a permissive environment for bacterial growth in their blood.

(22) INFECTION OF HATCHLING ALLIGATORS (*ALLIGATOR MISSISSIPPIENSIS*) WITH *MYCOPLASMA ALLIGATORIS*

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Mycoplasma alligatoris is one of the few infectious agents known to cause disease in alligators. However, little is known about the susceptibility to, and significance of, this disease in hatchling alligators. The objective of this study was to study the clinical signs, histologic lesions, and colony forming units (CFU)/ml of tissue in hatchling alligators exposed to *M. alligatoris*. Hatchling alligators were inoculated via intratracheal administration with 0, 101, 102, 103, 104, 105, 106, and 107 CFU of *M. alligatoris* isolated from the joint of a captive alligator with septic fatal mycoplasmosis. Animals were sacrificed at 2 or 4 weeks after inoculation. Quantitative cultures of lung, brain, blood, and elbow and knee joint swabs were performed. A second experiment was performed using intravenous inoculation of 102, 104, 106, or 108 CFU of M. alligatoris. At 4 wk post infection or at time of euthanasia, blood, brain, and joint fluids were cultured for M. alligatoris. In the intratracheal study, the only observed clinical signs were anorexia and weight loss and rare mild joint swelling. Mycoplasma was isolated from blood, brain, and/or lung of animals receiving ≥ 104 CFU. Hatchlings infected intravenously had significant mortality. All of the hatchlings receiving 102 CFU survived, but 100% mortality was seen by day 14 post infection in hatchlings receiving 108 CFU. The survival patterns for hatchlings receiving 104 or 106 CFU were similar. Initial mortality (25%) was observed at day 9 post infection, with less than 50% survival by day 20 post infection. Gross lesions included fibrinous polyarthritis, edema of the limbs and joints, fibrinous epicarditis and pericarditis, fibrinous pleuritis, and pneumonia. The experimental infection of hatchling alligators with M. alligatoris produced similar clinical, gross, and histological results as reported in the natural disease outbreak in adults and in an experimental infection of juveniles.

(23) GROSS AND HISTOPATHOLOGIC FEATURES AND PARASITES OF MIGRATING WILD ADULT FALL CHUM SALMON (*ONCORYNCHUS KETA*) IN THE YUKON RIVER, ALASKA

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Two problems have been occurring in the Yukon River chum salmon. First, excessive mortality of tagged fish has been occurring in a mark-recapture study using fish wheels and spaghetti tags. Second, there has been a significant drop in the overall population of these fish, resulting in hardships for the subsistence community. In a pilot study, we described and established a grading system for gross and histologic changes in migrating chum salmon and surveyed for parasites and potential pathogens.

The Yukon River salmon are remarkable in having the longest migration, over 2700 km. Establishing a consistent method for histopathology as an initial step was important because there are many histologic features in these animals that are either normal for the species or degenerative changes associated with the physiological stresses associated with the fasting and physical exertion of the migration. Such histologic descriptions specific to migrating Pacific salmon are not present in the literature in a cohesive form. Familiarity with these "background" changes could aid in determining lesions of significance in future work in migrating chum salmon. A survey of the parasites present could direct future work for determining the possible effects of these organisms.

Full necropsies were performed on 60 animals. This included describing gross lesions, taking the weight and length, performing hematology, bacteriology, ELISA for bacterial kidney disease antigen, and semiquantitative histopathology. The gross and histopathologic findings and disease agent work will be presented here. A single untagged fish had a systemic infection of *Aeromonas salmonicida*. There was one weak positive for an antigen of the bacterial kidney disease agent indicating that this disease was not a significant factor in morbidity. Blood smears were negative for viral erythrocytic necrosis inclusion bodies. Lesions typical of these migrating salmon included skin ulcers, fin reddening, and fin fraying, somatic and gastrointestinal skeletal muscle degeneration, contraction band necrosis in the heart, fibrin thrombi in the heart and gills, apoptosis of the gastrointestinal epithelial cells, bone remodeling, ulcers, vacuolar change in the islets of Langerhans and zymogen granule depletion in the pancreas, and mesenteric fat atrophy.

Our chum salmon had several significant gender-related differences. Males weighed more than females, but female liver weight was greater than in males. Males had extensive vacuolar change due to a combination of glycogen and fat while the females had significantly fewer vacuoles in the liver.

Features present in almost all animals that were not degenerative were considered to be normal for the species of this age group. These included a mild amount of inflammatory cells within the epicardial fat, small numbers of lymphocyte aggregates within the heart, lipofuscin accumulation within the cells lining the space of Disse in the liver and within macrophages of the meninges, a mild glomerulonephritis, and a low-number of inflammatory cells within the lamina propria of the stomach and intestines (gastritis and enteritis). Melanomacrophages were

typically scattered throughout the liver, spleen and kidney as individual cells. In the kidneys, primordial structures were very common in all fish with a slight tendency for more of these structures to occur in the downstream fish.

Parasites included *Ichthyophonus hoferi*, *Loma salmonae* in the gill and intra-arterial, an unidentified microsporidian in the kidney, a myxosporidian *Parvicapsula sp.* in the kidney and intestine, nematode larvae within the walls of the stomach and intestine, intestinal cestodes, and gastric trematodes. This represents an extension of the described range of *Loma salmonae*.

Future research opportunities would include sampling fish along the course of the Yukon to determine the histologic changes throughout the course of the migration from salt water to the spawning grounds. Comparing tagged versus untagged fish may help determine causes of mortality. It would be useful to do a similar study in animals with healthy returns to help determine what factors may be acting to cause a decline. Since we don't know at what stage of development the population of decline is occurring, looking at fry, and outmigrating smolts and possibly ocean phase animals would be needed.

(24) SPATIAL, DEMOGRAPHIC AND ENVIRONMENTAL RISK FACTORS FOR *TOXOPLASMA GONDII* EXPOSURE IN SOUTHERN SEA OTTERS

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Two parasites, *Toxoplasma gondii* and *Sarcocystis neurona* have been associated with fatal meningoencephalitis in sea otters. Molecular and antigenic characterization of isolated parasites have revealed no significant differences from similar protozoa affecting terrestrial animals and humans. Infective parasite oocysts or sporocysts are shed in the feces of cats (*T. gondii*) or opossums (*S. neurona*). Because of their apparent terrestrial origin, we expected to detect few natural protozoal infections when screening necropsied sea otters via parasite isolation in cell culture, histopathology and brain immunohistochemistry. However *T. gondii* and *S. neurona* infections were detected in 36% and 4% of fresh dead otters, respectively. Sera from live, free-ranging otters from California, Washington and Alaska were screened for *T. gondii* exposure using an immunofluorescent antibody test (IFAT) that had been previously validated for sea otters using sera from 77 sea otters with necropsy-confirmed positive or negative *T. gondii* infection status. Thirty-six percent of California otters were found to be seropositive, compared to 38% of Washington otters and 0% of Alaskan otters.

To investigate factors contributing to the apparent marine emergence of *T. gondii*, we compiled demographic, spatial and environmental data from 223 live or necropsied California sea otters sampled between 1997 and 2001. Demographic variables that were assessed include sea otter gender, age class, sampling date, sampling location and live-dead status at the time of sampling. Environmental variables that were assessed include human population density at the sample or stranding location, relative exposure to municipal sewage effluent and relative exposure to major points of freshwater runoff. These data were examined for associations with *T. gondii* seropositivity on IFAT, with the ultimate goal of identifying risk factors for *T. gondii* exposure. The *T. gondii* seroprevalence was 42% (49/116) for live otters, and 62% (66/107) for necropsied otters. Demographic risk factors that were positively correlated with *T. gondii* seropositivity included male gender and older age class. Spatial analysis revealed clusters of seropositive otters at two coastal locations, and one site with lower than expected *T. gondii* seroprevalence. Otters sampled from the vicinity of Cayucas/ Morro Bay, California were 9 times more likely to be seropositive for *T. gondii* than otters sampled at all other locations. Finally, otters sampled near locations of high freshwater runoff were 2.9 times more likely to be seropositive to *T. gondii* than otters sampled from low-flow areas. This study provides specific evidence of contamination of the coastal marine environment with the terrestrial pathogen, *Toxoplasma gondii*. It provides statistical evidence implicating land-based surface runoff as a source of sea otter exposure and is a convincing illustration of pathogen pollution in the marine ecosystem.

(25) POPULATION ECOLOGY OF PINNIPED HERPESVIRUS INFECTIONS AND CANCER IN CALIFORNIA SEA LIONS

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Herpes virus are endemic in California sea lions, while infection with these pathogens is by itself harmless, the presence of organic pollutants leads to aggressive carcinomas. In this talk we will present an age- and sex-structured model for California sea lions that incorporates both herpes transmission and the presence of environmental pollutant. As female sea lions forage in more polluted waters than males they carry higher doses of the pollutant and this interacts with the herpes virus to increase their risk of mortality. The model is used to examine the long-term impact of organic pollutants on the sea lion population. The expected mortalities are compared with the observed patterns along the coast of California.

(26) WEST NILE VIRUS DETECTION IN THE ORGANS OF NATURALLY INFECTED BLUE JAYS (*CYANOCITTA CRISTATA*)

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Blue jays (*Cyanocitta cristata)* are highly susceptible to West Nile Virus (WNV) and are routinely included in WNV surveillance programs. The tissue tropism of WNV in this species and the sensitivity of routine virus detection techniques as applied to this species are undefined. In order to explore this, gross pathology, virus titration, immunohistochemistry, and RT-nPCR techniques were used on 20 positive and 5 negative blue jays. Gross pathology results were consistent with previous findings in corvids. Maximum virus titers for tissues were: brain$10^{7.6}$ $TCID_{50}$/0.5 ml, heart $10^{8.0}$ $TCID_{50}$/0.5 ml, lung $10^{8.8}$ $TCID_{50}$/0.5 ml, liver $10^{6.7}$ $TCID_{50}$/0.5 ml, kidney $10^{7.7}$ $TCID_{50}$/0.5 ml, spleen $10^{7.1}$ $TCID_{50}$/0.5 ml, muscle $10^{7.0}$ $TCID_{50}$/0.5 ml, bursa $10^{5.9}$ $TCID_{50}$/0.5 ml. Significant differences in virus titer were present between two groups of tissues: brain/heart/lung and kidney/liver/muscle. Immunohistochemistry results correlated well with RT-nRCR and with the exception of two tissue samples, all tissues with a detectable virus titer showed positive RT-nPCR results. The brain was least sensitive for detecting WNV antigen by immunohistochemistry. Tissue tropism for WNV in blue jays appears to be greatest in brain, heart, and lung tissue based on titer. These results may be used to streamline WNV surveillance efforts that use dead bird submissions by suggesting optimum tissues for the method of diagnosis being applied.

(27) IMMUNE RESPONSE IN BLACK-TAILED PRAIRIE DOGS (*CYNOMYS LUDOVICIANUS*) TO VOLUNTARY CONSUMPTION OF RCN-F1 PLAGUE VACCINE INCORPORATED INTO A PALATABLE BAIT

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Sylvatic plague (infection with the bacterium *Yersinia pestis*) can have a dramatic effect on local and regional population dynamics of prairie dog species (*Cynomys* sp.), often killing greater than 95% of individuals in a colony undergoing an epizootic. As well as threatening the viability of the prairie dog populations themselves, the resultant instability in prairie dog population dynamics can have an effect on other species whose natural histories are linked to the prairie dog, such as the highly endangered Black-footed ferret (*Mustela nigripes*). Parenteral vaccination of large free-ranging prairie dog populations against *Yersinia pestis* infection presents substantial logistical difficulties; however, recent development of the virally-vectored recombinant RCN-F1 vaccine has provided a means of potentially eliciting a protective immune response against plague infection via *oral* vaccine administration. Large-scale field vaccination could then be accomplished by incorporating vaccine into baits and distributing those baits on prairie dog colonies for voluntary consumption. The purpose of this laboratory study is to determine if a significant protective immune response against *Yersinia pestis* infection can be elicited by voluntary consumption of a palatable vaccine-laden bait.

Black-tailed prairie dogs (*Cynomys ludovicianus*) were offered sweet potato/gelatin baits into which had been incorporated RCN-F1 vaccine (1×10^7 PFU). Immune response to vaccine was evaluated by semi-quantitative assessment of anti-F1 antibody production by use of an enzyme-linked immunosorbent assay (ELISA) and by response to post-vaccination challenge with virulent *Yersinia pestis*. Results suggest a significant difference in survivorship between vaccinates (10/18, or 55.56%) and negative controls (2/18, or 11.11%; p=0.006, Fisher Exact Test). Although assessment of antibody response is not complete, preliminary results suggest a significant difference in antibody titer between experimental groups after both initial and booster vaccination.

Future work for this project will include: modification of vaccine formulation to maximize protective immune response; optimizing the dosing regimen to maximize protection while minimizing administration cost and effort; designing a palatable carrier bait which can withstand environmental degradation without affecting vaccine efficacy; and testing of vaccine efficacy in other prairie dog species.

(28) EVALUATION OF A SENTINEL SYSTEM FOR *EHRLICHIA CHAFFEENSIS* AND *E. EWINGII* USING WHITE-TAILED DEER AS INDICATORS

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Two closely related zoonotic ehrlichiae, *Ehrlichia chaffeensis* and *E. ewingii*, are known to be transmitted by *Amblyomma americanum*, the lone star tick. White-tailed deer (WTD; *Odocoileus virginianus*) are critical hosts for all mobile stages of *A. americanum* and are important vertebrate reservoirs of *E. chaffeensis*. Whether *E. ewingii*, which also causes canine granulocytic ehrlichiosis, is maintained in vertebrate reservoirs other than dogs is unknown. The relationship between WTD and lone star ticks raises the possibility that *E. ewingii* might also occur in deer. The current study utilized an indirect immunofluorescent antibody (IFA) serological test, polymerase chain reaction (PCR), and culture to delineate the geographic distribution of *E. chaffeensis*. Because *E. ewingii* has never been isolated in culture, antigen was not available for *E. ewingii* specific IFA testing. Consequently, inoculation of naïve fawns with whole blood from deer infested with *A. americanum* and nested polymerase chain reaction (PCR) were used to test WTD for *E. ewingii* infections.

IFA testing revealed that 962 (48.1%) of 1,999 WTD serum samples collected from 17 states from 1981-2002 were positive for *E. chaffeensis*-reactive antibodies (≥1:128). Seropositive deer were absent from several locations where *A. americanum* is rare or absent including West Virginia, western Virginia, eastern Tennessee, southern Florida, eastern Kentucky, southern Louisiana, and northwestern Georgia. Seventy-four of 76 (97.4%) tick-positive locations contained seropositive deer, while only 4 of 39 (10.3%) tick-negative locations contained seropositive deer. Serologic data was confirmed through positive PCR and/or culture results from 8 locations in Arkansas, Georgia, Kansas, and North Carolina. PCR testing of whole blood from 111 WTD revealed 7 (6.3%) and 6 (5.4%) were positive for *E. chaffeensis* and *E. ewingii* DNA, respectively. In addition, natural *E. ewingii* infections in two deer were confirmed through successful infection of captive deer with blood from wild deer infested with *A. americanum*. This is the first report of *E. ewingii* from WTD and expands the known geographic distribution of *E. ewingii* to include areas of Kentucky, Georgia, and South Carolina. These results suggest, similar to the epidemiology of *E. chaffeensis*, that WTD may serve as vertebrate reservoirs for *E. ewingii*. Further, these results indicate that WTD are frequently exposed to *Ehrlichia* spp. in *A. americanum* endemic areas and are useful as sentinels for these tick-borne human pathogens.

(29) EVIDENCE OF *EHRLICHIA* SPP. IN RACCOONS (*PROCYON LOTOR*) FROM GEORGIA

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A total of 60 raccoons acquired from six contiguous counties in the Piedmont physiographic region of Georgia from February to June, 1999, were investigated for their role in the epidemiology of *Ehrlichia* spp. Sera samples were tested by indirect fluorescent antibody (IFA) assay for the presence of antibodies reactive to *Ehrlichia chaffeensis*, *Ehrlichia canis*, and *Anaplasma phagocytophila* (HGE agent). Nested polymerase chain reaction (PCR) assay was used to test whole blood for the presence of *Ehrlichia* 16S rRNA gene fragments. In addition, ticks were collected and identified from 30 of 60 raccoons. Twenty-three of 60 raccoons (38.3%) had *E. chaffeensis*-reactive antibodies (≥1:64), 13 of 60 raccoons (21.7%) had *E. canis*-reactive antibodies, and 1 of 60 raccoons (1.7%) had *A. phagocytophila*-reactive antibodies. Using species-specific nested primers for *E. chaffeensis*, *E. canis*, and *A. phagocytophila*, amplicons were generated from 1 of 60, 2 of 60, and 8 of 34 raccoons, respectively. However, the single *E. chaffeensis* PCR product, one *E. canis* PCR product, and one *A. phagocytophila* product were larger than the expected 389-411 base pair gene segments when visualized by gel electrophoresis. Sequence analysis of the amplicon generated by *A. phagocytophila* nested primers revealed DNA of an organism most closely related to an *Ehrlichia*-like species, recently identified in *Ixodes* ticks in Europe. Four tick species including *Dermacentor variabilis* (28/30 infested), *Amblyomma americanum* (16/30), *Ixodes texanus* (12/30), and *Ixodes cookei* (7/30) were identified from these raccoons and represent potential vectors for the ehrlichiae detected. Additional molecular testing of whole blood and tissue samples for other ehrlichial organisms is necessary to fully understand the role raccoons play in the natural cycle of disease. This study suggests that raccoons and ticks that parasitize them may be involved in the epidemiology of one or more ehrlichial organisms of public health and veterinary importance.

(30) MORBIDITY AND MORTALITY OF REPTILES ADMITTED TO THE WILDLIFE CENTER OF VIRGINIA, 1991 TO 2000

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Medical records from 694 reptiles admitted to the Wildlife Center of Virginia (WCV) from 1991 to 2000 were reviewed to determine causes of morbidity and mortality. Eighteen species were represented but the majority of cases consisted of four species; eastern box turtle (*Terrapene carolina*), eastern painted turtle (*Chrysemys picta*), common snapping turtle (*Chelydra serpentina*), and rat snake (*Elaphe* sp.). There was a significant increase in reptile cases during the study period both in absolute number and in proportion to the total caseload. Trauma (74.2%) was the most frequent cause of morbidity and mortality followed by unknown or undetermined (13.3%), aural abscessation (7.2%), captivity (3.0%), infectious diseases (2.2%), and nutritional disorder (0.1%). Causes of morbidity and mortality differed between the four most numerous species. Impact with a motor vehicle was most frequent cause of trauma for eastern box turtles, eastern painted turtles, and common snapping turtles; however, garden trauma was the most frequent cause for rat snakes. Aural abscessation was only seen in eastern box turtles and may be due to exposure to organochlorine (OC) pesticides. This condition in eastern box turtles may be a good indicator of OC environmental contamination. Eighty percent of cases occurred between May and September and 65% occurred within the five most proximal counties to the WCV. The vast majority of morbidity and mortality was the result of human activities. With the expanding human population in Virginia it is likely that humans will continue to have an impact on the health of wild reptile populations.

(31) A COMPARISON OF IMMUNOHISTOCHEMISTRY AND VIRUS ISOLATION IN DIAGNOSIS OF WEST NILE VIRUS

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West Nile virus has spread rapidly throughout the eastern United States since its appearance in 1999 and has caused high mortality in wild birds. As part of the West Nile Surveillance effort, we tested birds from Georgia that had died or were euthanized and were suspected to have West Nile virus. Immunohistochemistry and virus isolation were performed on 1,055 birds. Virus isolates were identified using virus specific RT-PCR or microneutralization. On initial screening, 355 birds were found to be West Nile virus positive by immunohistochemistry, virus isolation, or both. Of these, 269 were positive by both methods. Immunohistochemistry and virus isolation results were in disagreement in only 86/1,055 cases (8%). Of these, 42 were virus isolation positive but IHC negative while 44 were virus isolation negative but IHC positive. These results indicate that virus isolation and immunohistochemistry are approximately equal in their ability to detect West Nile virus. Therefore, either method would be appropriate for use in surveillance for West Nile virus.

(32) FACTORS AFFECTING SURVIVAL OF JUVENILE FRANKLIN'S GULLS (*LARUS PIPIXCAN*): THE ROLE OF HATCHING ASYNCHRONY, IMMUNE FUNCTION, AND DISEASE

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Extensive mortality of juvenile Franklin's gulls (*Larus pipixcan*) occurs each summer at Eyebrow Lake, Saskatchewan. In 1999 and 2000, causes of mortality included starvation, trauma, and conditions commonly associated with immunosuppression. In a pilot study, hatch sequence and egg size were important factors affecting chick survival. Hatching asynchrony, caused by incubation prior to clutch completion, creates an age and size hierarchy that places last-hatched chicks at a competitive disadvantage. In gulls, this can be exacerbated by the smaller size of third-laid eggs, and has been termed the "third chick disadvantage." One main objective of this study was to identify factors that affect Franklin's gull chick condition and prefledgling survival, and, in particular, to examine the relationships among hatching asynchrony, egg sequence, stress response, immune function, disease, and survival. In 2001, chicks of 102 nests were monitored for condition, immune function, and survival. To separate the effects of egg sequence and hatch sequence, eggs were exchanged among nests to create 24 asynchronous clutches each of A-eggs only, B-eggs only, and C-eggs only, and 30 control nests. Cell-mediated immunity of chicks was evaluated using the phytohemagglutinin (PHA) test at 0-2 and 14 days of age, and humoral immunity was evaluated using response to sheep red blood cell (SRBC) vaccination at 14 days. Hatch sequence was a significant factor for chick survival, while egg sequence had no effect. Almost all first-hatched chicks survived, while the majority of third-hatched chicks died, regardless of original egg sequence. PHA response at 0-2 days of age was highest in third-hatched chicks, possibly due to immunostimulant effects of acute stress. At 2 weeks, PHA response was lowest in third-hatched chicks, reflecting the immunosuppressive effects of chronic stress. PHA response of chicks that died was significantly lower than that of chicks that survived. Antibody response to SRBC was not affected by hatch sequence or egg sequence, but was significantly correlated with egg size and female quality. Hatching asynchrony appears to have considerable consequences for prefledgling Franklin's gull survival. Third-hatched chicks are at a competitive disadvantage which can lead to starvation, immunosuppression, infectious disease, and mortality. This is the first study to separate the effects of hatch sequence from egg sequence, determine the significance of hatch sequence in terms of survival and cell-mediated immunity, and determine the significance of egg size and female quality on chick humoral immunity.

(33) PHOCINE HERPESVIRUS-1 INFECTIONS IN NORTH AMERICAN HARBOR SEALS (*PHOCA VITULINA*)

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Phocine herpesvirus type-1 (PhHV-1) has been associated with morbidity and high mortality in neonatal harbor seals (*Phoca vitulina*) in California, USA and in Northern Europe. Despite this high mortality, there is little information available regarding the epidemiology and pathogenesis of PhHV-1 infection. The objectives for this study were to determine prevalence of exposure to PhHV-1 in harbor seal populations around North America, to identify modes of transmission of the virus, and to examine temporal changes in viral shedding and antibody responses in infected and susceptible seals. For the prevalence survey, serum samples were collected from harbor seals from three age classes [preweaned pups (n=269), weaned pups (n=176) and subadults/adults (n=421)] from four geographical areas around North America (Alaska, northeast, northwest and southwest) and assayed for PhHV-1 specific antibodies by ELISA. To identify potential routes of virus transmission between individuals, blood samples and mucosal swabs (oral, nasal, ocular, rectal and vaginal) were collected from harbor seals at The Marine Mammal Center, a rehabilitation facility on the central California coast (n=52), as well as from free ranging harbor seals in Monterey Bay, San Francisco Bay, Point Reyes National Seashores and Humboldt Bay (n=225). Temporal changes in viral shedding and antibody responses were determined using blood, serum and swab samples, collected from animals upon admission to The Marine Mammal Center, and then sequentially until release or death. The presence of viral nucleic acid in mucosal swabs and leukocyte samples was detected by PCR and presence of PhHV-1 specific antibodies was determined by ELISA.

The prevalence of exposure to PhHV-1 increased with age, with 37.5% of pre-weaned pups testing positive, compared to 87.6% post-weaned pups, and 99% subadults/adults positive, respectively. PhHV-1 specific antibody levels were low in neonatal harbor seals, but rapidly increased around the time of weaning, and remained high throughout subadult and adult life, suggesting recurrent exposure to this virus is common. Studies at The Marine Mammal Center showed the infection spread rapidly between harbor seals following exposure to a shedding pup, and that seroconversion occurred within two weeks of the detection of virus by PCR in clinical samples. Seals that seroconverted after exposure did not develop clinical disease. Similar aged free ranging and rehabilitating harbor seals were found to be shedding virus at similar times of the year. These data indicate that PhHV-1 is endemic in North American harbor seal populations, and that transmission is followed by viral shedding and seroconversion but not commonly by clinical disease.

(34) RECENT PATTERNS OF MORTALITY IN SOUTHERN SEA OTTERS (*ENHYDRA LUTRIS NEREIS*)

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Detailed postmortem examination of southern sea otters (*Enhydra lutris nereis*) found along the California coast has provided an exceptional opportunity to understand the factors influencing survival in a threatened marine mammal species. In order to evaluate recent trends in causes of mortality, the demographic and geographic distribution of causes of death in 105 freshly deceased beachcast sea otters necropsied from 1998 through June, 2001 were evaluated. Encephalitis due to *Toxoplasma gondii* (16%), acanthocephalan infection (16%), shark attack (13%), and cardiac disease (13%) were identified as the leading causes of death in sea otters examined. Encephalitis, caused by *Sarcocystis neurona*, was the primary cause of death identified in another 7% of otters and all *S. neurona* cases were detected during spring months (March through May). The proportionate mortality attributed to protozoal encephalitis caused by *T. gondii* and *S. neurona* in recent years is nearly 3 times the prevalence detected in otters examined from 1992 - 1995. Spatial clusters of cause-specific mortality were detected for *T. gondii* encephalitis (in southern Estero Bay), acanthocephalan peritonitis (in southern Monterey Bay), and shark attack (from Santa Cruz to Point Ano Nuevo). Otters with fatal shark bites were over 7 times more likely to have concurrent encephalitis suggesting that this long-recognized source of mortality may have been modified by an emerging disease. Cardiac disease is a newly recognized cause of mortality affecting adult females primarily, and *T. gondii* encephalitis was significantly associated with this condition. Infectious diseases dominate the pattern of mortality observed in sea otters recently, with parasites or bacteria causing death in 45% of otters examined and contributing to death in another 26% of otters. Infectious disease, as a primary or contributing cause of death, was more common in juveniles (58%) and prime-aged adults (62%) than in aged adults (39%), and 15% of the otters examined actually had two distinct infectious diseases contributing to death. The epidemiology of disease in southern sea otters has some concerning implications for the overall health and recovery of this population.

(35) PREVALENCE OF ZOONOTIC BACTERIA IN SEABIRDS UNDERGOING REHABILITATION AND ASSOCIATED RISKS TO REHABILITATORS IN THE PACIFIC NORTHWEST, USA

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Large numbers of seabirds are rehabilitated annually by wildlife rehabilitation centers located in the Pacific Northwest (USA). Although various strains of zoonotic bacteria have been isolated from seabirds, the risk to rehabilitators has not been well documented. Additionally, seabirds carry the spirochetes that cause Lyme disease (*Borrelia burgdorferi* sensu lato) in northern Europe, but the role of seabirds in North American cycles, and the associated risks to rehabilitators, has not been assessed. Our purpose was to survey the Gram-negative bacteria of seabirds in rehabilitation centers, as well as to investigate the potential of a marine cycle of *B. burgdorferi* s.l. transmission on the Pacific Northwest coast. Using cloacal swabs, we determined the prevalence of detectable enteric fauna by isolation and characterization of Gram-negative bacteria of common murres (*Uria aalge*), gulls (*Larus spp.*), and other common seabirds encountered by rehabilitators in northern California, Oregon, and Washington (USA). In addition, we attempted to culture Lyme disease spirochetes from these same birds. Among 42 seabirds sampled to date, bacterial isolates have been tentatively identified as multiple strains of *Escherichia coli*, *Enterobacter cloacae, Citrobacter freundii,* and *Klebsiella pneumoniae*, among others. These data will be used to generate guidelines for minimizing risk to rehabilitators as well as to better understand the relationship between seabirds and their pathogenic bacteria.

(36) IS AVIAN INFLUENZA IN SHOREBIRDS SPECIES AND LOCATION SPECIFIC?

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Since the initial isolation from common terns (*Sterna hirundo*) in 1961, avian influenza viruses (AIV) have been isolated from more than 90 species of wild birds. Most isolates were obtained from the Anseriformes but AIVs have been reported from additional orders. Extensive surveys of AIVs in shorebirds (Families Charadriidae and Scolopacidae) are few with only one study reporting AIV isolations from birds migrating through North America. Results of that survey suggested that the predominant hemagglutinin (H) subtypes were H9 and H13 (44% of all isolates) while the 6 neuraminidase (N) subtypes isolated were distributed fairly evenly. In this study, shorebirds migrating along the Atlantic and Gulf coasts of North America were sampled during spring and fall migration April 2000 - May 2001. More than 4,700 shorebirds were sampled and 73 AIVs have been isolated to date (1.5%). Most AIVs (78.3%) were isolated from ruddy turnstones (*Arenaria interpres*) caught during spring migration in the Delaware Bay region. In contrast to the previous study, the H10 and H12 subtypes comprised >70% of all isolates and no H9 or H13 subtypes were detected. The predominant N subtype, N7, was isolated > 50% of the time. These results suggest that AIV transmission may be localized and species specific. In addition, the H and N predominance of AIVs in North American shorebirds appears to be extremely variable over time.

(37) COYOTES (*CANIS LATRANS*) AS AN EXPERIMENTAL HOST FOR THE OKLAHOMA ISOLATE OF BABESIA GIBSONI

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Babesia species are tick-transmitted Apicomplexan hemoprotozoan parasites that are known to infect a wide variety of animals throughout the world. Babesiosis results in hemolytic anemia, depression and anorexia and sometimes hemoglobinuria and multi-organ dysfunction causing severe disease and occasionally death. Genomic comparisons have recently documented the existence of 3 distinct "small" *Babesia* sp. infecting dogs worldwide, 2 of which occur in North America. Canine isolates from several Midwestern and Eastern states have been almost exclusively found in American Staffordshire Terriers and are genetically indistinguishable from the *B. gibsoni* isolates from Japan. Because little is known of the significance of this parasite in free-ranging canids, and because of an apparent increase in prevalence of the organism in domestic dogs, this study was initiated to evaluate the clinical response of coyotes (*Canis latrans*) to experimental infection. Captive coyotes were inoculated with whole blood from a domestic dog naturally infected with the Oklahoma isolate of *B. gibsoni* and then complete blood counts (CBC) with differentials, reticulocyte counts, and evaluation of 21 biochemistry values were performed until 20 weeks post inoculation. Exposures resulted in 100% infection without mortality. Regenerative anemia, thrombocytopenia, splenomegaly, and hemoglobinuria occurred, as well as mild depression and inappetance. The high level (11%) and long duration (20 weeks) of parasitemia indicate that coyotes may serve as a significant wildlife reservoir for *B. gibsoni*.

(38) EVIDENCE OF PATHOGEN POLLUTION: SHELLFISH AS BIOINDICATORS OF FECAL BORNE PATHOGENIC PROTOZOA AND BACTERIA IN THE CALIFORNIA NEARSHORE MARINE ECOSYSTEM

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Pathogen pollution into the marine environment may be a significant threat to wildlife and human health. Bivalves (shellfish such as *Mytilus* spp.) have been shown to filter and retain pathogenic protozoa, viruses, and bacteria, so they may be useful bioindicators of pathogen pollution and water quality. Bivalves filter large amounts of water, up to 2.5 L/bivalve/hour, allowing them to concentrate pathogens from their environment. The nearshore marine environment along the California coast serves as habitat for many wildlife species, including the threatened southern sea otter (*Enhydra lutris nereis*). Sea otters consume approximately 25% of their body weight each day, and a major part of their diet is shellfish. The link between pathogen pollution and sea otter mortality has yet to be proven; however, the California data to date provide support for this hypothesis. Recent isolation of fecal pathogens such as *Cryptosporidium*, *Giardia*, *Sarcocystis*, *Toxoplasma*, *Clostridium perfringens, Salmonella*, and *Vibrio* spp. from southern sea otters raises the question as to the sources of these pathogens.

A study was designed to investigate pathogen pollution in the California nearshore marine environment. This study tests the hypothesis that mussels collected from sites near freshwater runoff or human sewage outfalls are more likely to contain pathogenic protozoa and bacteria than mussels collected further away from these sites, and that the risk of infection will be greatest during the storm season when runoff into the nearshore marine environment is high. On a quarterly basis we are monitoring mussels at sites designated as 'higher risk' and 'lower risk', based on known sources of fecal inputs to the marine ecosystem and historic sea otter infections with protozoal parasites. Mussel tissues sampled include hemolymph, gill and digestive gland. For protozoal parasites we are using conventional and TaqMan PCR techniques, as well as direct fluorescent antibody assays. For bacteria, we are culturing and confirming identification with biochemical and DNA sequencing methods.

In our first dry and wet season collections we have identified pathogenic protozoa and bacteria in wild mussels harvested along the central California coast. *Cryptosporidium* spp. have been found in wild mussels from 'higher risk' sites but not in mussels from 'lower risk' sites. *Vibrio cholerae, V. parahaemolyticus, V. damsela, V. metschnikovii,* and *V. ordalli* have only been found at 'higher risk' sites. *Salmonella* was isolated from an innkeeper worm at a 'higher risk' site. *Clostridium perfringens* and *V. alginolyticus* have been found at 'higher risk' and 'lower risk' sites. No *Campylobacter, E. coli 0157:H7,* or *Plesiomonas shigelloides* has been isolated to date.

With continued monitoring, this study will provide critical information to evaluate the health of the nearshore marine environment and the pathogen load that sea otters and other wildlife species may be ingesting via contaminated food and water. Further investigation may provide isolates of parasites and bacteria that will help trace the sources of pathogen pollution. Evaluating sources of fecal contamination and using management strategies to mitigate them will help reduce pathogen pollution into the nearshore marine environment, an invaluable ecosystem and resource for people and animals, both wild and domestic.

(39) AGGRESSIVE ANAPLASTIC SARCOMAS in JUVENILE CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*), AN IMMUNOHISTOCHEMICAL AND PCR ANALYSIS OF THREE CASES

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California sea lions (*Zalophus californianus*) have a high incidence of neoplasia. The most commonly encountered tumor is a highly aggressive urogenital carcinoma occurring in sexually mature animals. These tumors have been associated with infection by Otariine herpesvirus 1 (OtHV-1). During an ongoing study to investigate the role of OtHV-1 in neoplasia development, we identified a distinct subset of non-urogenital tumors occurring in juvenile and subadult animals. Clinically, these tumors grew rapidly, leading to severe debilitation and ultimate euthanasia. Of the three such tumors examined to date, one arose in the retrobulbar space, another in the thymus and the third in the perirenal tissue. Light microscopic analysis revealed these tumors to be composed of densely pack pleomorphic spindle shaped or round cells with a high degree of anisocytosis and anisokaryosis. Distant metastasis and intravascular thrombi were also noted. Immunohistochemistry confirmed the diagnoses of anaplastic sarcomas. Polymerase chain reaction (PCR) analysis, using primers specific for OtHV-1 DNA polymerase gene, was performed on tumor tissue from both the retrobulbar and perirenal sarcoma and were negative. Thus, though the sites of origin of these sarcomas differed, these cases indicate that juvenile, as well as adult, sea lions are prone to development of aggressive tumors, which likely arise due to a combination of environmental, genetic and viral factors.

(40) EXPERIMENTAL TRANSMISSION AND ISOLATION OF A NOVEL *EHRLICHIA* SP. FROM WHITE-TAILED DEER

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Previous research has established the presence of at least three zoonotic *Ehrlichia* in white-tailed deer. In addition, an undescribed *Ehrlichia* sp. of white-tailed deer has been detected by PCR only. Infection with this novel *Ehrlichia* sp. in deer has been associated with parasitism by lone star ticks (*Amblyomma americanum*) and thus potentially represents an additional zoonotic risk. The objectives of this study were to experimentally infect deer with this novel *Ehrlichia* sp. and to isolate the organism in cell culture. Two captive white-tailed deer fawns were inoculated (ID, SQ, IV and IP routes) with blood from naturally infected wild deer. Both fawns (#76 and #81) became positive by reverse-transcriptase nested polymerase chain reaction (RT-nPCR) for the novel *Ehrlichia* sp. within 5 days post-inoculation (DPI) and maintained persistent infections through DPI 250 and 117, respectively. The novel *Ehrlichia* sp. was isolated twice from blood of the infected fawns. Isolations were made in ISE6 tick cells and the organism has been maintained since in continuous culture. An ISE6 tick cell culture isolate from Fawn #76 was used to successfully infect (IV, SQ, ID and IP routes) a naïve fawn (#86). Fawn #86 first became RT-nPCR positive on DPI 19. An initial attempt at tick transmission of the organism from Fawn #76 to a naïve fawn (#82), using laboratory-raised *A. americanum*, was unsuccessful; however, further attempts at tick transmission are currently underway. Establishment of donor animals and in vitro culture of this novel *Ehrlichia* sp. provides opportunity for further characterization of this organism.

(41) SEQUENCE VARIATION OF THE INTERNAL TRANSCRIBED SPACER 2 rRNA GENE OF TWO SPATIALLY DISTINCT POPULATIONS OF *AMBLYOMMA AMERICANUM* (ACARI: IXODIDAE)

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Sequence analysis of the internal transcribed spacer 2 (ITS 2) rRNA gene of two spatially distinct populations of *Amblyomma americanum* (Lone Star tick) revealed intra-species specific variation. Nucleotide sequences from multiple-independent DNA extractions and PCR amplifications of eggs from mixed-parentage from samples of each Lone Star tick (LST) population identified that 12 of 1144 (1.0%) sites were variable. Six of the 12 sites of variation were explicit between the two LST populations and correspond to a rate of 0.5%. We speculate that if LST populations become genetically distinct, alteration in their genome could be indicative of their ability to act as competent vectors of infectious agents. To test the preceding speculation, we plan to qualitatively and quantitatively assess the aptitude (i.e., vector competence) of the two genetically characterized LST populations to acquire, maintain, and transmit the protozoan parasite *Theileria cervi* to white-tailed deer (*Odocoileus virginianus*). Empirical analysis of both the genetic differentiation and vector competency of the same tick species may provide insights to the role these arthropods play in the occurrence and establishment of vector-borne diseases.

(42) RISK FACTORS FOR VIRAL INFECTIONS IN SPOTTED HYENAS (*CROCUTA CROCUTA*) FROM THE MASAI MARA NATIONAL RESERVE, KENYA

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Spotted hyenas (*Crocuta crocuta*) are one of the most abundant predators in the Serengeti ecosystem and interact regularly with lions and domestic animals in ways that may encourage disease transmission. Hyenas have large home ranges that overlap with territories of sympatric species and human habitations, this overlap may increase the potential for viral disease exposure and transmission. Hyenas have unique hierarchical status in clans. Male spotted hyenas have a social rank lower than female hyenas and therefore, hunt in less optimal hunting habitats which include domestic animal habitation. Female hyenas hunt in prime habitats and interact more extensively with lions, cheetahs, and African wild dogs. If susceptible to infection by domestic feline and canine viruses, hyenas could succumb to infection or transport these viruses to lions, wild dogs, and cheetahs throughout the ecosystem. In preliminary studies, antibodies to canine distemper virus (CDV), canine parvovirus/feline panleukopenia virus (CPV/FPLV), feline immunodeficiency virus (FIV), feline corona virus (FECV/FIPV), feline calici virus (FCV), and feline herpes virus 1 (FHV1) were detected in a population of hyenas that had been intensively studied in the Masai Mara. The present study evaluated whether home range or social rank were risk factors for infection with these viruses. The risk of infection by age class, sex, location in the Masai Mara and social rank were evaluated in a logistic model. Seroprevalence differed among age groups, social rank, and by year sampled. Age was a risk factor for FIV and FECV/FIP and social rank was a risk factor for FIV. Low risk spatial clusters of seroprevalence for CDV, FIV, and FECV/FIPV were located near human habitation; while a high risk cluster of seroprevalence was found for CPV/FPLV near human habitation. These results indicate that hyenas may play a role in the ecology of these viruses in this ecosystem.

(43) ENDOPARASITES OF ISLAND FOXES (*UROCYON LITTORALIS*) AND THEIR GEOGRAPHIC DISTRIBUTION AMONG THE CHANNEL ISLANDS, CALIFORNIA

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The island fox (*Urocyon littoralis*), the largest native terrestrial mammal on the Channel Islands, has evolved as distinct subspecies on the 6 largest islands. Because populations have declined precipitously since the mid 1990s, four subspecies [on San Miguel Island (SMI), Santa Rosa Island (SRI), Santa Cruz Island (SCrI), and Santa Catalina Island (SCaI)] have been proposed to be added to the Federal list of endangered and threatened species by the U.S. Fish and Wildlife Service. Because disease could be contributing to the species decline, a comprehensive pathology investigation was initiated in 1998 to define the types and prevalence of diseases in this species. Deceased foxes from all 6 islands have been necropsied for this study, although sample sizes from SRI and SCrI to date are small. Necropsies have disclosed that foxes on some islands had unusual parasites not typically found in canids, and these parasites caused considerable tissue damage in some animals. Large occlusive plaques associated with a *Spirocerca* were found in the colons of foxes on SMI, SRI, SCrI, and San Nicholas Island (SNI), but not in foxes from SCaI or San Clemente Island (SClI). *Angiocaulus gubernactulatus*, a parasite typically found in mustelid hosts, was detected in pulmonary granulomas, heart blood, and feces of SMI foxes, but not in foxes from other islands. *Uncinaria* infestations of the small intestines also were found only in SMI foxes. Abundant *Mesocestoides* were present without associated enteric or visceral lesions in foxes from SMI, SNI, SClI, and SCaI. No *Dirofilaria* were detected in more than 120 necropsied foxes, despite previous reports of high antigen seroprevalence in this population. These results indicate that the parasitic fauna differs among island fox subspecies, a factor that should be considered if animal translocations are planned during recovery efforts. Although unlikely to have been a significant factor in the recent decline, parasitism was associated with some morbidity. Ongoing studies aim to better characterize these parasites by molecular speciation and to assess the health risks of parasitism in animals held in reserves as part of the recovery program.

(44) COLONIC SPIROCERCOSIS IN CHANNEL ISLAND FOXES (*UROCYON LITTORALIS*)

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During an ongoing study to determine the cause of a population decline in the Channel island fox (*Urocyon littoralis*), necropsies were performed on 83 animals and revealed unusual colonic, small intestinal and mesenteric lesions attributable to a spirurid nematode of the genus *Spirocerca*. Thirty-five of the 83 juvenile and adult animals (42%) were affected. Most colonic lesions consisted of fibrous mural nodules and plaques encasing adult *Spirocerca* sp. The plaques extended into the intestinal lumen of all foxes but only obstructed the ileo-ceco-colonic junction in two foxes. Intestinal perforations and mesenteric lesions were rare. No esophageal or aortic spirocercosis was present. The high prevalence of subclinical infection and patency of infection suggests that the fox is the definitive host for this parasite. Whereas morphologic features of the parasites were consistent with the genus *Spirocerca*, the colonic localization and lack of esophageal lesions were not typical of the well-described *S. lupi*. Additional molecular studies are currently underway to establish if the parasite in the Channel Island fox is *S. lupi* or another closely related spirurid.

(45) SPATIAL EPIDEMIOLOGY OF *BARTONELLA VINSONII* SUBSP. *BERKHOFFII* AND *YERSINIA PESTIS* IN CALIFORNIA COYOTES

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As human society continues to encroach upon wildlife habitats, the opportunity for exposure to bacterial zoonotic agents increases. Coyotes serve as a reservoir of infection for the agent of plague, *Yersinia pestis*, and recent investigations have shown that coyotes are also capable of being infected with another potential zoonotic agent, *Bartonella vinsonii* subsp. *berkhoffii.* While the natural history of sylvatic plague infection is well described, that of *Bartonella vinsonii* subsp. *berkhoffii* is not. To determine whether the two agents have a similar mode of disease transmission, we evaluated results of serologic testing of 863 samples from coyotes throughout California from 1994 to 1998 for evidence of exposure to these two agents. There was spatial and temporal clustering of seropositivity to both agents, however the clusters were located in geographically disparate areas. Coyotes from coastal areas of the state were more likely to test positive for evidence of *Bartonella vinsonii* subsp. *berkhoffii* exposure, while coyotes from the Modoc plateau and Kern County were more likely to test positive for evidence of *Yersinia pestis* exposure. We interpret these results as indication that the two agents have different transmission dynamics, likely a consequence of having different insect vectors.

(46) IMMUNIZATION OF BLACK-FOOTED FERRETS (*MUSTELA NIGRIPES*) AGAINST SYLVATIC PLAGUE (*YERSINIA PESTIS*)

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Black-footed ferrets (*Mustela nigripes*) are highly susceptible to sylvatic plague, caused by the bacterium *Yersinia pestis*, and this disease has severely hampered efforts to re-establish ferrets to their historic range. A study was conducted to assess the efficacy of vaccination of black-footed ferrets against plague using a recombinant protein vaccine, designated F1-V, that was developed by personnel at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID). Seven ferrets were immunized with the vaccine, followed by 2 booster immunizations on days 22 and 143; control animals received a placebo. After the second immunization, antibody titers to F1 antigen were found to be significantly higher in vaccinates than controls. Upon challenge with virulent plague by subcutaneous injection, the 3 control animals died within 3 days, but 6/7 vaccinates survived with no ill effects. In a second experiment, 6 ferrets were immunized with the vaccine, followed by one booster immunization on day 29; 3 controls received a placebo. All 6 vaccinates developed antibody to F1 antigen, but only 2/5 showed evidence of a boost in titer after the second immunization (1 animal died from an unrelated cause). The controls remained antibody negative. Upon challenge with virulent plague via consumption of a mouse that died from plague infection, the only survivors to challenge were the 2 animals that were successfully boosted. These results indicate that ferrets can be immunized to plague and can survive challenge by subcutaneous injection (similar to flea bite) or by consumption of infected meat.

(47) THE EPIZOOTIOLOGY OF TYPE C BOTULISM AT THE SALTON SEA

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In recent years the Salton Sea has been the site of massive mortality events involving pelicans and other fish-eating birds. During the summer of 1996, type C avian botulism killed nearly 20,000 birds, nearly half of which were western white pelicans (*Pelecanus erythrorhynchos*) and close to 1,200 being endangered California brown pelicans (*Pelecanus occidentalis californicus*). Smaller botulism die-offs have occurred every year thereafter. Three years ago, NWHC launched a comprehensive study to investigate the ecology of avian botulism at the Sea. In 1999, we conducted a yearlong survey to assess prevalence of *Clostridium botulinum* type C in the sediments around the Sea at different times of the year. In 1999, 2000 and 2001, we collected tilapia (*Oreochromis mossambicus*) at various sites during botulism outbreaks in order to determine the presence of toxin-producing bacteria in their intestines, as well as to look for the presence of type C botulinum toxin in their blood and intestines. We found that toxin-producing cells were present in the sediments during winter and spring and are detected mostly in agricultural drains and at the river deltas. This suggests that the sediments are not the primary source of toxin during botulism outbreaks, which occur during the summer. Within the tilapia population we noted differences among the years in regard to prevalence of the active bacteria and toxin. Tilapia captured in 2000 had significantly higher prevalences of toxin and toxin-producing bacteria than in the other years. These differences correspond to the increased severity of the 2000 outbreak in pelicans compared to 1999 and 2001. This information, in conjunction with spatial data obtained from affected pelicans, as well as population data from tilapia and pelicans, will provide insight into the dynamics of this unique disease system.

(48) TYPE E BOTULISM IN FISH-EATING BIRDS ON LAKE HURON AND LAKE ERIE, 1998-2001

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Type E botulism was the cause of episodic die-offs of fish-eating birds, particularly common loons on Lake Michigan during the 1960's and 1980's but has not been reported from any of the other Great Lakes. In November, 1998 botulism (not typed) was diagnosed in a small number of Common Loons from Pinery Provincial Park in the southeastern corner of Lake Huron. In October and November, 1999 a large scale die-off, estimated in the thousands of birds, of Common Loons and Red-Breasted Mergansers, with lesser numbers of other species, including gulls and diving ducks, occurred in the Pinery Park area on Lake Huron and in the western basin of Lake Erie, adjacent to Rondeau Provincial Park and Point Pelee National Park. In 2000 and 2001, type E botulism was confirmed in birds from Lake Erie only, with the geographic centre of activity gradually shifting eastward, reaching Port Colborne in the eastern basin in 2001. There is an apparent pattern of sporadic mortality in gulls and shorebirds during late summer, followed by more intense epizootics, primarily involving loons and mergansers, during fall migration. The source of toxin has not been determined. Identification of stomach contents of birds (N=85) found that 37% contained gobies (*Neogobius* spp.), an exotic species of fish that has been introduced into the Great Lakes within the past 10 years. Other fish species, including alewife, smelt, cyprinids, sheepshead and gizzard shad were present in 32%. Other food items identified included zebra or quagga mussels and mudpuppies.

(49) THE EFFECTIVENESS OF CARCASS REMOVAL IN REDUCING LOSSES DUE TO AVIAN BOTULISM

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Type C avian botulism is one of the most significant diseases of waterfowl in North America. Total annual losses to avian botulism are unknown and vary from year to year but in some years documented losses can exceed a million birds. In 1994 through to 1999 approximately 1 million water-birds died annually of avian botulism on the Canadian Prairies alone. Carcass removal, to prevent transfer of toxin via maggots on rotting carcasses, is the primary technique used to control botulism outbreaks. Although carcass removal is a logical response to this disease, and has been shown to improve survival rates under experimental conditions, under field conditions carcass removal does not significantly improve survival rates of waterfowl. Failure to affect survival rates is primarily due to an inability to find and retrieve enough carcasses on heavily vegetated wetlands to prevent transfer of toxin to susceptible birds.

During the last 4 years we have monitored carcass cleanup operations by using randomly marked carcasses and found that between 7% and 42% of carcasses are retrieved during botulism outbreaks. The lower numbers correspond to large heavily vegetated wetlands while better carcass retrieval rates occur on small, less vegetated wetlands. We also compared survival rates of radio-marked molting mallards during botulism outbreaks on similar sized, paired, wetlands in which carcass removal occurred at one lake and not at the other and found no difference in survival rates. Comparisons of survival rates were made on 5 pairs of wetlands over three years and in all cases carcass cleanup did not significantly improve survival rates of molting mallards. Survival rates of molting mallards varied from approximately 5% to 100%. The lower survival rates occurred on large lakes where hundreds of thousand of birds have died of botulism annually in the past.

(50) THE ROLE OF LESSER SNOW GEESE AS CARRIERS OF AVIAN CHOLERA IN THE PLAYA LAKES REGION

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We conducted laboratory challenge trials using mallard ducks (*Anas platyrhynchos*) infected with *Pasteurella multocida* to develop and validate methods for detecting carriers of avian cholera in the wild population. We found that swab samples (oral, cloacal, nasal, eye, and leg joint) stored in liquid nitrogen and processed using selective broth were more effective than other tissues for detecting carrier birds up to 14 weeks post infection. These methods were applied to wild geese collected in the Playa Lakes Region during the winter of 2000-01 and 2001-02. We found that a small proportion of the wild birds (<10%) were carriers of *P. multocida* and, based on serology, an additional group (< 10%) were survivors of recent avian cholera infection. These results confirm the suspicion that wild waterfowl are carriers of avian cholera and add support for the hypothesis that wild birds are the reservoir for this disease. In concert with other research studies, this work indicates that enzootic infection with avian cholera occurs in snow goose populations throughout their annual cycle. Even in the absence of disease outbreaks, low level disease transmission and infection are apparently occurring in these highly gregarious birds.

(51) ANTIBODY RESPONSE OF FOUR BIRD SPECIES AFTER VACCINATION WITH A KILLED WEST NILE VIRUS VACCINE

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West Nile virus has been associated with numerous bird mortalities in the United States since 1999. Four avian species at three zoological parks were selected for vaccination for West Nile virus and serology to assess antibody response. Black-footed penguins (*Spheniscus demericus)*, little blue penguins (*Eudyptula minor*), American flamingos (*Phoenicopterus ruber*), and Attwater's prairie chickens (*Tympanuchus cupido attwateri*) were chosen because of West Nile virus associated morbidity or mortality within their family or genus at other zoos, or because of their high conservation or economic value. Birds were vaccinated with a killed West Nile virus vaccine two to three times at three- to four- week intervals. Blood samples were collected to evaluate antibody titers to West Nile virus at pre-vaccination, booster vaccinations, and at three- to four- weeks following the final booster.

(52) WHAT LURKS IN YONDER FOREST? - MISCELLANEOUS VIRUSES ISOLATED FROM WILD BIRDS DURING WEST NILE VIRUS SURVEILLANCE IN GEORGIA

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Virus isolation attempts were made from 1566 wild birds during the 2001 West Nile virus surveillance program in Georgia. Viruses were isolated from 332 birds. Although West Nile virus was by far the most common virus isolated, other viruses were isolated from 10 birds. Eastern equine encephalitis (EEE) virus was isolated from 7 birds, Highlands J virus from 1, and currently unidentified viruses from 2 birds. Various tissues were available for histologic and immunohistochemical evaluation from 4 birds (an egret, a crow, a mourning dove, and an orchard oriole) from which EEE virus was isolated. Significant changes included massive hepatic necrosis, necrosis of the sheathed arterioles in spleen, renal tubular degeneration, mild nonsuppurative encephalitis, and myocardial degeneration. EEE virus antigen was detected in the sheathed arterioles of an egret and in a small numbers of neurons in two birds with encephalitis. Despite massive necrosis in the liver, virus antigen was not readily detected in these livers. EEE virus was most likely the cause of death in these 4 birds. A barred owl from which Highlands J virus was isolated had degeneration of the sheathed arterioles in spleen, but, unlike the birds with EEE virus infection, there was no evidence of hepatic necrosis. It is unclear if viral infection in this bird was clinically significant. A Red-tailed hawk from which an unidentified virus was isolated had myocardial degeneration and lymphoplasmacytic portal hepatitis. A rock dove from which an unidentified virus was isolated had foci of gliosis in brain and, unlike the EEE virus infected birds, had multifocal necrosis in liver and nonsuppurative interstitial nephritis. The relationship between viral infection and microscopic lesions in these two birds is unknown at present. While West Nile virus is probably the most significant viral cause of wild bird mortality in the Southeastern U.S., the impact of other viral infections, particularly EEE virus, on wild bird populations needs to be better defined.

(53) ENHANCED PASSIVE SURVEILLANCE FOR WEST NILE VIRUS IN DEAD WILD BIRDS IN CANADA - 2001

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The Canadian Cooperative Wildlife Health Centre (CCWHC), under contract to and in partnership with Health Canada, acted as a clearing house to organize the collection, processing and submission to the National Microbiology Laboratory (NML), Health Canada, Winnipeg, of tissues from wild birds as part of the West Nile virus (WNV) surveillance programme in Canada in 2001. Surveillance was focussed on members of the Corvidae (American crow, blue jay, common raven, gray jay, and black-billed magpie in the west), since among wild birds they seem exceptionally susceptible to mortality caused by WNV. WNV RNA was detected in tissue specimens using a real time RT-PCR system (TaqMan) that employed primer-probe sets specific for 5' and 3' portions of the WNV genome. Initial cases detected in a local health jurisdiction were confirmed by inoculation of tissue homogenates into cell culture and confirmation of viral isolation by immunofluorescence assays. In addition, the CCWHC collected sightings of dead wild birds from the public, and established and maintained a data base for tracking specimens, recording and reporting results, and mapping and epidemiologic analysis of events.

In all provinces from Saskatchewan eastward, systems customized to the province or region were put in place to promote awareness of the surveillance programme, and to collect and transmit to the four CCWHC Regional Centres carcasses of dead birds and information on sightings. They utilized various combinations of local public health authorities, and provincial health ministries, agriculture ministries and wildlife agencies.

Over the West Nile Virus season, approximately 15 May to 31 October, 2001, CCWHC accessioned 3911 carcasses of wild birds (of which specimens from 2807 were suitable for submission to the NML for WNV detection and 2804 were tested), and data on an additional 2923 sightings of dead birds, a total of 6834 records in the data base. Ontario/Nunavut Region

CCWHC handled over 66% of the workload; Quebec Region ~15%; Atlantic Region ~10%, and Western Region the remainder.

A total of 3860 crows (1959 carcasses, 1901 sightings); 2208 blue jays (1408 carcasses, 800 sightings) ; 129 ravens (85 carcasses, 44 sightings); 30 black-billed magpies (17 carcasses, 13 sightings); 10 gray jay sightings; and 597 other species (442 carcasses, 155 sightings) were accessioned.

West Nile Virus was detected only in southern Ontario. A total of 128 WNV-positive birds were detected in 12 Health Units, from Windsor in the southwest to Durham and York regions, east and north of Toronto, in south-central Ontario. The date of first pickup of a WNV positive bird, the species, and the total wild birds WNV positive for each Health Unit, are as follows: Windsor-Essex, 8 August, crow, 20; Halton, 13 August, blue jay, 7; Peel, 14 August, crow & blue jay, 17; Toronto, 15 August, crow, 41; Hamilton-Wentworth, 23 August, crow, 4; Chatham-Kent, 24 August, blue jay, 3; York, 25 August, blue jay, 25; Durham, 29 August, crow, 5; London-Middlesex, 29 August, crow, 3; Lambton, 30 August, crow, 1; Niagara, 11 September, blue jay, 1; Waterloo, 24 September, blue jay, 1. The last WNV positive birds were picked up on 29 October in York Region, north of Toronto.

Twenty one percent of 190 crows tested in August, 31% of 151 crows tested in September, and 14% of 100 crows tested in October were infected with West Nile virus. The corresponding figures for blue jays were 3% of 196, 6% of 224, and 0.6% of 162.

The WNV incursion into southern Ontario in 2001 reflected a more widely disseminated but apparently disjunct distribution of the virus in the eastern half of North America, perhaps reflecting introduction of the virus by spring migrant birds which originated in or had passed through the focus of WNV activity that became apparent in the southeastern USA in spring/summer 2001.

(54) BIGHORN PASTEURELLOSIS: DO WE UNDERSTAND ITS EPIDEMIOLOGY WELL ENOUGH TO PREVENT OR MANAGE EPIDEMICS?

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Pasteurellosis epidemics have plagued North America's free-ranging bighorn sheep populations since at least the early 1900s. Although a variety of pathogens and other factors may contribute to individual respiratory disease outbreaks in wild sheep, infection with *Pasteurella* spp. and related bacteria seem to be a common denominator of most epidemics studied. Because field investigations of pasteurellosis epidemics tend to be conducted *post hoc*, our understanding of the inciting conditions and cause(s) is often incomplete. Both introduced and endemic *Pasteurella* spp. strains have been associated with epidemics, but determining the ultimate source of responsible pathogens is rarely accomplished with certainty. Consequently, it has been difficult to predict or prevent epidemics in most cases. Moreover, effective tools for managing pasteurellosis epidemics in free-ranging bighorns remain elusive. Population and habitat management may not prevent epidemics. Treatment of affected populations tends to be unrewarding. Some vaccines have shown promise in preventing or diminishing losses during epidemics, but inability to accomplish population-level delivery remains a significant obstacle to field application. Until practical vaccination or other intervention tools become available, managers are left with conservative population management and segregation as the most viable strategies for preventing pasteurellosis epidemics in wild sheep.

(55) DIVERSITY OF BACTERIA IN THE *PASTEURELLACEAE* FAMILY AND FACTORS ASSOCIATED WITH DISEASE POTENTIALS

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Bacteria in the *Pasteurellaceae* family are obligate parasites of the mucosa of the upper respiratory and distal reproductive tracts of a broad range of vertebrates. The taxonomy of these bacteria is highly artificial and subject to continual change due to their great diversity. Organisms of greatest concern as potential pathogens for ruminants have been identified in the genus, *Pasteurella*, a portion of which have been reclassified in a new genus *Mannheimia*. We are currently differentiating these isolates into multiple biovariant groups based on biochemical utilization tests, comparing isolates within biovariants by similarity coefficients of DNA restriction fragment length polymorphism (RFLP) profiles, and conducting polymerase chain reactions (PCR) for genes associated with disease potentials. *Pasteurella (Mannheimia) haemolytica* and *trehalosi* isolates from wild ruminants have been differentiated into more than 80 biovariants which serve as a first step in epidemiological surveys. Comparing similarity coefficients of RFLP profiles serves as a second step and has been applied to demonstrate another level of diversity within biovariant groups as well as being used to demonstrate sharing and transmission of specific strains. PCR has been used to screen isolates for the *lktA* and *sodC* structural genes responsible for leukotoxin and superoxide dismutase production associated with virulence. Tests currently being conducted in our laboratory are also demonstrating variances of the *lktA* and *sodC* genes which may provide greater understanding of disease potentials of the organisms and how these may interact with host susceptibility.

(56) ECOLOGY OF DISEASE-RELATED MORTALITY OF BIGHORN SHEEP IN HELLS CANYON, IDAHO, OREGON, AND WASHINGTON, USA

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Disease-related mortality is a limiting factor for bighorn sheep populations throughout much of the U.S. and Canada. Factors contributing to this mortality are poorly understood, but are critical to implementing appropriate management. We tested the hypotheses that population density and nutrition were critical factors in precipitating disease outbreaks in bighorn sheep (*Ovis canadensis*). We monitored movements and survival of 121 radio-marked ewes and 46 radio-marked rams at least biweekly in 9 herds in Hells Canyon over the period 1997 - 2001. During this period, annual adult survival rates varied from 40 to 100% and seasonal lamb survival varied from 14% to 100%. Disease was the cause of 32% of ewe mortality and 42% of ram mortality. Causes of disease-related mortality were bacterial pneumonia (Pasteurellosis) and hypothermia due to severe scabies (*Psoroptes ovis*) infection. Most disease-related adult mortality occurred November – January 2000 – 2001 in 5 of the 9 study herds. We used home range analysis and an interaction index to estimate seasonal herd densities and potential contact rates. We also collected pellets monthly and analyzed % fecal nitrogen. We used a general linear model to evaluate the effect of population density and nutrition on survival. Results and implications for management are discussed.

(57) LONG TERM MONITORING OF BIGHORN SHEEP (*OVIS CANADENSIS*) THAT HAVE BEEN IN CONTACT WITH DOMESTIC LIVESTOCK

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Pneumonia is bighorn sheep (*Ovis canadensis*) is commonly associated with *Pasteurella* spp. although the epidemiology of disease outbreaks is often unclear. Contact between bighorns and domestic sheep or goats is often considered to be the source of disease agents that lead to die-offs of bighorns. The Idaho Department of Fish and Game Wildlife Health Laboratory has housed or examined 10 bighorns that have had contact with domestic livestock. Five of these animals were maintained in captivity for up to three years for repeated sampling and clinical observation. Sampling included collection of blood for serology, feces for parasitology, and pharyngeal swabs for bacteriology. Samples were collected on the day of collection, 30 days after collection and approximately 3-4 month intervals. A variety of *Pasteurella* spp. has been isolated from these sheep, but none have developed clinical illness and none died of respiratory disease. A clear definition of contact between domestic livestock and bighorns is needed to better define the risk factor associated with contact.

(58) DISEASE RISK ASSESSMENT FOR BIGHORN SHEEP CAPTIVE BREEDING PROGRAMS

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A key to success in captive breeding programs is to recognize that disease will occur. Disease outbreaks in a breeding facility can endanger not only the captive herd, but also the wild populations that are to be augmented through captive breeding, other wildlife populations, and livestock herds. Detrimental impacts of disease occurrence to the captive herd and the, will be minimized by carefully assessing the risks and planning steps toward mitigation early in the program development. A disease risk assessment was conducted as part of a comprehensive contingency captive breeding plan written for endangered populations of Sierra Nevada bighorn sheep in California. Bluetongue virus and *Pasteurella* spp. pneumonia were among the diseases that were considered most likely to endanger a captive herd in the Sierra Nevada. Factors that may exacerbate disease outbreaks in a captive herd include climate, habitat quality and quantity, herd density, herd immune status, abundance and distribution of disease vectors, and proximity to domestic animals, humans, and wildlife including wild bighorn sheep. Management actions, such as maintenance of low captive herd density, elimination of disease vectors, and exclusion of all other animals from the captive facility will reduce risks. Captive breeding implemented for populations in other regions will present different and unique sets of factors that influence disease risks, therefore detailed risk assessments should be done for each situation.

(59) LOW-GRADE FIBROSARCOMAS IN GREEN TURTLES (*CHELONIA MYDAS*) WITH FIBROPAPILLOMATOSIS

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The green turtle (*Chelonia mydas*) is protected under the U.S. Endangered Species Act and the Wildlife Laws of the State of Hawaii. Fibropapillomatosis (FP) is a disease of marine turtles characterized by multiple cutaneous masses ranging from 0.1 to more than 30 cm in diameter that has primarily affected green turtles. The disease has a worldwide distribution and has been observed in all major oceans and in most species of marine turtles that are considered endangered of extinction. Where present, prevalence of the disease varies among locations, ranging from as low as 1% to as high as 90%. Although several viruses have been identified associated with the tumors, including herpesviruses, a retrovirus and a papilloma-like virus, the primary etiological agent remains to be isolated and identified. Concurrent infections of FP and cardiovascular trematodiasis have been recognized as the most important mortality factors of Hawaiian green turtles considerably reducing the survival of the species. The neoplastic processes observed in our previous studies and more recently during gross and histopathologic examination of 14 turtles with FP suggest a synergistic effect of cardiovascular trematodes and the primary agent of FP.

Most tumors of integument and internal organs were characteristic of fibropapillomas, fibromas and myxomas. Herein we describe the histopathology of cutaneous and internal spindle cell tumors found in green turtles and present histopathological and molecular evidence for the presence of low-grade fibrosarcomas. Histologically, approximately 3% of tumors of the nasopharynx, mouth, temporomandibular tissues, lungs and intestine appear to have an aggressive, invasive behavior. These masses are well demarcated from adjacent tissues but demonstrate infiltration of surrounding stroma and bone lysis. Although there is no evidence of vascular invasion or high mitotic activity, the tumors have been classified as low-grade fibrosarcomas. We are in the process of determining the biological behavior and molecular characterization of these tumors. Current retrospective and prospective studies will determine the implications of these novel findings.

(60) INVESTIGATION INTO THE ETIOLOGY OF "HAIRLESS" (ALOPECIC) RINGED SEALS (*PHOCA HISPIDA*) IN THE BERING SEA

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During the last several years, Alaskan native subsistence hunters have reported "hairless" ringed seals (*Phoca hispida*) taken along the Alaska Bering Sea coast. Pathologic findings of four cases of a patchy alopecia syndrome are presented. Case 1 was diagnosed as a possible endocrine disorder based on histopathology of a previously frozen carcass from the native subsistence harvest. Case 2 examined skin samples from a subsistence-harvested seal. On histopathology, the hair follicles appeared to be in an inactive state consistent with a non-molting animal. Additionally, there was a bacterial folliculitis at the intersection of haired versus unhaired skin. An unusual lesion was observed in both case- 3 (a subsistence-harvested seal) and case 4 (a live-captured juvenile seal). In both animals, hair shafts within and around the affected areas were colonized by organisms that destroyed the shaft, most likely resulting in shaft breakage. The lesions were accompanied by a moderate folliculitis and perifollicular dermatitis. Dermatophytes are the most common organisms to specifically colonize hair, although dermatophyte colonies usually have a different appearance than the organisms observed in cases 3 and 4. *Trichophyton shoenleini* and an unidentified fungus were cultured from the case 3 and a possible *Madurella grisa* was isolated from case 4. The animal representing case 4 was encountered during live-capture/release studies in Bristol Bay. This animal was transported to a rehabilitation center and treated for fungal dermatitis with oral itraconazole and topical povidone iodine applications. After six weeks of treatment, a repeat biopsy demonstrated marked reduction in the skin inflammation, consistent with a positive treatment response. Serum chemistry values were within expected ranges throughout the rehabilitation period, but complete blood cell counts indicated a mild eosinophilia during the first four weeks of treatment. Thyroid hormone levels were lower than similarly-aged phocids but reached an acceptable range prior to release. Challenges with thyroid-stimulating hormone (TSH) and adrenocorticotropic hormone (ACTH) were performed to further assess endocrine responses. Serum retinol and trace minerals concentrations were measured and found to be higher than published values for similar species but as with thyroid hormones, no reference ranges for ringed seals were available. Organochlorine contaminant concentrations in blubber from two cases (1 and 4) subjected to analysis were lower than sympatric pinnipeds examined by the same laboratory. Electron microscopy of the hair shafts on select cases was not enlightening. This occurrence of alopecia is of particular interest because it may be an emerging problem and could indicate either the introduction of a new organism, increased susceptibility to an endemic organism, or the possible range extension of an uncharacterized organism due to an environmental change. Additionally, there are important subsistence food concerns for native people who handle and consume these animals. There is also the potential for wastage due to carcass rejection both due to concerns about the safety of the food and also the quality of the pelt.

(61) A SURVEY OF RAPTOR MORTALITY IN GEORGIA

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A prospective study on mortality in raptors in Georgia was performed on birds submitted to the Southeastern Cooperative Wildlife Disease Study for West Nile Virus testing from August 2001 to April 2002. The study included 88 raptors of 11 species. With one exception, all birds were submitted post mortem. By far, the most common cause of mortality was trauma (60%), followed by poor nutrition (18%), infectious disease (7%), degenerative disease (1%), and neoplasia (1%). In 13% of cases, no cause of death could be identified. In several of the trauma cases, other significant lesions were identified, indicating that these deaths were probably multifactorial even though trauma was the ultimate cause of death.

(62) TISSUE DISTRIBUTION OF LIVER ENZYMES IN CALIFORNIA SEA LIONS AND HARBOR SEALS

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In domestic animal medicine, changes in serum enzyme levels are routinely used as diagnostic tools to help detect liver disease. Although hepatic disease has been identified in pinnipeds stranding along the California coast, limited data are available on the relationship between changes in serum enzyme levels and specific organ disease, and on the tissue distribution of liver enzymes in marine mammals. In ringed (*Phoca hispida*), harp (*Phoca groenlandica*), and grey (*Halichoerus grypus*) seals, St. Aubin and Geraci found ALT and SDH activities to be highest in the liver. We determined the tissue distribution of seven enzymes (ALT, AST, ALKP, GGT, LDH, SDH and CK) in California sea lions (*Zalophus californianus*; n=5), and harbor seals (*Phoca vitulina*; n=5) that stranded and then died at The Marine Mammal Center, Sausalito, California, USA. Tissue samples were collected from liver, kidney, skeletal muscle, cardiac muscle, spleen, pancreas, intestine, adrenal, lymph node, and lung. Serum and red blood cells also were analyzed. Tissue homogenates were prepared according to the technique of Herzfeld and Greengard, and enzyme analysis was performed on a Hitachi 912 chemistry analyzer using standard methods and reagents. Enzyme activity was expressed as U/mg wet tissue. In California sea lions, cardiac muscle and liver had the highest level of ALT activity (1.00 ± 0.11, 0.81 ± 0.15) of all tissues tested. Muscle tissues had the highest CK (203.51 ± 24.56) and AST (4.71 ± 1.56) activity, but liver also had high AST activity (4.23 ± 0.87). GGT activity was highest in the kidney (1.45 ± 0.93), while ALKP activity was highest in the adrenal gland (4.61 ± 1.89). Skeletal muscle had the highest LDH activity (10.17 ± 2.00), but cardiac muscle, liver and kidney also had appreciable LDH activity. Liver had the highest SDH activity (0.67 ± 0.22). Harbor seals had similar patterns of enzyme distribution as California sea lions except for ALT activity. Liver and skeletal muscle had the highest activity of ALT (1.05 ± 0.87, 0.43 ± 0.10) in harbor seals. This pattern of tissue distribution of enzymes in pinnipeds suggests diagnosis of liver disease depends upon interpretation of changes in a combination of serum enzyme levels. Currently we are researching the association of these serum enzymes with lesions suggestive of specific liver diseases.

(63) THE YELLOWSTONE TO YUKON CONSERVATION MEDICINE PROGRAM: MEASURING AND MODELING WILDLIFE DISEASE

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In the Yellowstone to Yukon bioregion (Y2Y), the Tufts Center for Conservation Medicine (Tufts CCM) and the Consortium for Conservation Medicine (CCM) are focused on issues that pertain to the linkage between wildlife, disease and the establishment of ecological connectivity in this increasingly fragmented landscape. Since 1998, this collaborative effort has collated map layers of important diseases including brucellosis, bovine tuberculosis, chronic wasting disease, plague and hantavirus infection. Using geographical information system mapping technology, we are bringing together varied jurisdictional data of important wildlife and human diseases throughout the Y2Y bioregion. With this format, we aim to provide wildlife and public health agencies, universities and the public, with information for policy decisions. The occurrence and distribution of disease on a bioregional scale provides an important indicator of ecosystem health and has important implications for management involving translocation of species or for re-establishing habitat corridors between isolated populations. Our program is part of a much larger effort known as the Y2Y Conservation Initiative. This continental initiative is made up of over 300 conservation groups committed to maintaining biodiversity and restoring habitat connectivity throughout the 1.2 million-km^2 Y2Y bioregion.

We can link information on disease distribution, pathophysiology and transmission to other metadata such as animal distribution and human demographics to answer questions addressing how habitat corridor design will affect disease transmission between reconnected naive populations of similar and susceptible species. As we develop these answers, we should be able to predict the benefits and consequences of habitat corridors in relation to disease and its effects on humans, wildlife and domestic animals. This poster presentation was presented in June 2002 at the International Society for Ecosystem Health, Healthy Ecosystems-Healthy People Conference in Washington, DC.

(64) OILING OF WATERBIRDS DUE TO EXPOSURE TO FISH OILS

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The South Wilbur storm water retention basin in Kings County California is used to store winter runoff water for use in irrigating surrounding cropland during the summer and fall months. The water is pumped from the basin into the adjacent Homeland Canal and from there into various feeder water supply canals in Kings and Tulare Counties. In September 1997, a large fish kill occurred in the South Wilbur and an adjacent 18 miles of the canal. An estimated 2 million fish were killed. Within two to three days of the fish kill, adult and juvenile Western Grebes (*Aechmophorus occidentalis*) and Clark's Grebes (*A. clarkia*) were found beached along the shoreline of the South Wilbur. Ultimately an estimated 1,600 birds may have been impacted. The birds exhibited evidence of excessive preening, self mutilation, on the neck and shoulders, listlessness, and little fright response to approach by humans. All of the beached birds appeared to have lost their water repellency and were soaked through. Many of the early birds were severely emaciated by the time they were recovered. Initial tissue analyses following necropsy indicated very high levels of chlorinated hydrocarbons including Toxaphene, PCB 1260, and pp-DDE. This led to the inaccurate conclusion that a banned chlorinated pesticide may have been applied to adjacent cropland and drifted into the canal and stormwater basin. Later analyses of feathers from the dead birds and comparative analyses of decomposing fish from the site demonstrated that the birds had in fact been coated with fish oil and the elevated tissue levels of the chlorinated compounds was due to mobilization of fat reserves to try and maintain body temperature.

In October and November of 1997 approximately 500 birds, primarily Western and Clark's grebes, common loon (*Gavia imer*), surf scoter, and brown pelican, *(Pelicanus occidentalis*) became fouled by fish oil in a small section of central Monterey Bay. The vast majority of these birds were picked up in a three day period and all showed signs of loss of waterproofing, hypothermia, and hypoglycemia. The source was believed to be a boat load of decomposing anchovies that had been illegally dumped. Many of these birds had recently migrated from the arctic and were in very poor body condition. Many also suffered from Salmonellosis and died or were euthanized due to poor prognosis. Twice in the last decade significant numbers of brown pelicans in Monterey harbor have become oiled subsequent to localized anchovy mortality events. Although not particularly toxic in the classical sense, fish oil exposure can be very debilitating to birds and may result in significant mortality events and may also have a bacterial component.

(65) CHARACTERIZATION AND CLINICAL MANIFESTATIONS OF *ARCANOBACTERIUM PHOCAE* INFECTIONS IN MARINE MAMMALS STRANDED ALONG THE CENTRAL CALIFORNIA COAST

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Between 1994 and 2000, 141 *Arcanobacterium phocae* isolates were recovered from marine mammals that had stranded along the central California coast. *Arcanobacterium phocae* was cultured from tissue sites with abnormal discharge or evidence of inflammation in 66 California sea lions (*Zalophus californianus*), 50 Pacific harbor seals (*Phoca vitulina richardii*), 19 northern elephant seals (*Mirounga angustirostris*), five southern sea otters (*Enhydra lutris nereis*), and one common dolphin (*Delphinus delphis*). The overall prevalence of *A. phocae* among cultured stranded marine mammals was 8 %. Although common in this study, *A. phocae* has not previously been reported from the Pacific Ocean. Sequence analysis of a portion of the 16S ribosomal RNA gene confirmed recent isolates as *A. phocae*[1]. All *A. phocae* isolates were non-motile, catalase-positive, gram-positive coccobacilli or short rods that were beta-hemolytic on blood agar within 24 hours of inoculation. A reverse CAMP reaction resulted with *Staphylococcus aureus* and a positive CAMP reaction with *Rhodococcus equi*. Prior to phylogenetic testing and the routine use of the esculin hydrolysis and motility tests, *A. phocae* isolates had been misidentified as *Listeria ivanovii*. *Arcanobacterium phocae* was commonly isolated from superficial abscesses but occasionally was associated with systemic infections. Isolates were often present in mixed bacterial infections and were susceptible to all antimicrobial agents tested. *Arcanobacterium phocae* is most likely an opportunistic pathogen that can cause severe infection in animals with wounds or other pre-existing disease.

(66) ACUTE WEST NILE VIRUS INFECTION OF REPTILES AND AMPHIBIANS

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Because West Nile (WN) virus primarily cycles between mosquitoes and birds, few studies have looked at the possibility of reptiles or amphibians as competent reservoir hosts of WN virus. It has previously been shown that some reptiles are competent reservoir hosts of other arboviruses and one European study successfully demonstrated that an amphibian species, *Rana ridibunda*, could produce high viremic titers of WN virus capable of re-infecting feeding mosquitoes.

This present study focuses on acute WN virus infection of two species of reptiles and one species of amphibian. The representative species include: *Iguana iguana* (green iguana), *Thamnophis sirtalis parietalis* (northern garter snake), and *Rana catesbeiana* (North American bullfrog). After inoculation with WN virus (NY99), some green iguana and bullfrog individuals showed detectable levels of viremia (peaks of 3.3 and 2.7 log pfu/ml serum, respectively). No viremia was detected in the garter snakes. Results from tissue harvests and oral and cloacal swabs will also be discussed in this poster.

(67) *PARELAPHOSTRONGYLUS ODOCOILEI* IN THINHORN SHEEP (*OVIS DALLI*) - DISTRIBUTION, LIFE CYCLE, AND SIGNIFICANCE

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In 2000, the muscleworm *Parelaphostrongylus odocoilei* was identified in Dall's sheep (*Ovis dalli dalli*) of the Mackenzie Mountains, Northwest Territories (NWT). Because *P. odocoilei* had not previously been reported in wild sheep (only in cervids and mountain goats), we began investigating the geographic distribution, patterns of larval shedding, life cycle, and effects of this parasite in thinhorn sheep.

Fecal surveys and molecular work suggest that *P. odocoilei* is present in thinhorn sheep (*Ovis dalli*) in two "metapopulations", Eastern (the Mackenzie and Selwyn Mountains, NWT and the Yukon Territory) and Western (the central Alaska and Wrangel-St. Elias ranges, and northern British Columbia), but absent in the more northern thinhorn sheep populations (Brooks Range and Richardson Mountains) and in bighorn sheep (*Ovis canadensis*) near Hinton, Alberta. Preliminary DNA sequencing of the ITS-2 region indicates that larvae from the two positive metapopulations are genetically similar (99.6-99.8%), and that *P. odocoilei* larvae from thinhorn sheep are genetically similar (98.2-98.6%) to those from mule deer near Hinton, Alberta.

We also examined seasonal patterns of larval shedding in a naturally infected Dall's sheep population in the northern Mackenzie Mountains. The prevalence of infection with *P. odocoilei* ranged from 87-100% throughout 2000 and 2001. The pattern of larval shedding was similar for both years, with the highest levels in March/April/May, a decline through summer until August, followed by an increase in October/November to relatively high levels that were maintained over winter.

In 2001, we completed the life cycle of *P. odocoilei* in an experimentally infected captive Stone's sheep (*Ovis dalli stonei*). The life cycle, pre-patent period (72 days), patterns of larval shedding [peak at ~10,000 larvae per gram of feces (LPG) at 5 weeks post-patency, and plateau at ~6,000 LPG from 6-15 weeks post-patency], and effects (weight loss, chronic pulmonary hemorrhage, and granulomatous interstitial pneumonia) were similar to those described in experimentally and naturally infected mule deer. We continue to monitor the effects of *P. odocoilei* on body weight, blood and pulmonary wash cytology and chemistry, blood gases, pulmonary function, and lung structure in two more experimentally infected Stone's sheep.

(68) INVESTIGATION OF HETEROPHIL TO LYMPHOCYTE RATIOS AND GRANULOCYTE TO AGRANULOCYTE RATIOS IN RELATION TO CONDITION IN NORTHERN SAW-WHET OWLS (*AEGOLIUS ACADICUS*)

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Recently, the ratio of two white blood cells types, heterophils and lymphocytes (H:L), have been used as a measure chronic stress. We investigated the relationship between H:L ratios and body condition in northern saw-whet owls (*Aegolius acadicus*). In an effort to avoid a bias from only selecting two white blood cell types, we also investigated the relationship of the granulocyte to agranulocyte ratio in relation to condition. Previous studies in birds have shown increased H:L ratios with decreased body condition. We captured northern saw-whet owls at the Idaho Bird Observatory during fall migration in 1999 and 2000. The owls were captured in mist-nets using audio lures. Several physical measurements and blood smears were taken from each bird. Preliminary analysis showed a significant negative relationship between H:L ratio and body condition but only explained ~17% of the total variability in body condition. Indeed, there was little variability in body condition, perhaps because migrating birds must achieve a certain threshold level of body condition before they will migrate. This might account for the lack of explanatory power of the results. These results suggest that the utility of H:L ratios in migrants needs further study.

(69) HEMOPARASITE SURVEY FROM IMPERIAL EAGLES (*AQUILA HELIACA*), STEPPE EAGLES (*AQUILA NIPALENSIS*), AND WHITE-TAILED SEA EAGLES (HALIAEETUS ALBICILLA) IN KAZAKHSTAN

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Hemoparasites have been linked to decreased body condition in many avian species during the breeding season. The first steps towards investigating the link between hemoparasites and body condition are initial surveys of the hemoparasites normally found in a species. Blood smears were collected from 46 nestling Imperial Eagles (*Aquila heliaca*), 5 Steppe Eagles (*Aquila nipalensis*) and 14 nestling White-tailed Sea Eagles (*Haliaeetus albicilla*) at the Naurzum Zapovenik [National Nature Reserve] (51 degrees N, 64 degrees E) in Kazakhstan during summers of 1999 and 2000. In 1999, five of 65 Imperial Eagles (8%) were infected with *Leucocytozoon toddi*. Two of 17 Imperial Eagles (12%) and one of 14 White-tailed Sea Eagle (7%) were infected with *Leucocytozoon toddi* in the year 2000. Steppe Eagles had no parasites found in either year. No birds had simultaneous infections by multiple species of parasites. These data are important because few hematological studies of these eagle species have been conducted, and we know of no such research from this region of Asia.

(70) CHARACTERIZATION OF *CAMPYLOBACTER* SPECIES ISOLATED FROM NORTHERN ELEPHANT SEALS ON THE CALIFORNIA COAST

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Campylobacter-like organisms were isolated from emaciated and dehydrated Northern Elephant Seals on nine beaches in California stretching from Bodega Bay to Santa Cruz during the months of March through May in 2001. The organisms resembled C*ampylobacter* species in several ways, including being microaerophilic, Gram-negative, catalase positive and oxidase positive. Electron microscopy revealed pleomorphic helices and bipolar flagella. Analysis of cloned 16S rRNA gene sequences showed a similarity of 98% to *C. jejuni*. However, analysis of the glyA gene showed 83% similarity to *C. lari* and an 80% similarity to *C. jejuni*. Based on these findings this new strain constitutes a previously undescribed species, whose nearest phylogenetic neighbors are *Campylobacter jejuni* and *Campylobacter lari.*

(71) IMMUNE RESPONSES OF ELK (*CERVUS ELAPHUS*) TO *BRUCELLA ABORTUS* STRAIN 19 AND RB51 VACCINES

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The prevalence of *Brucella abortus* infection in elk in the Greater Yellowstone Area is a potential reservoir for reinfection of cattle. Data from other studies have suggested that vaccination of elk with *B. abortus* strain RB51 (SRB51) does not induce protection against challenge with a virulent strain of *B. abortus* during pregnancy. In the studies reported here, the immunologic responses of elk to two brucellosis vaccines were characterized. In the first study, elk were SQ vaccinated at approximately 5 to 7 months of age with saline or 10^{10} CFU of SRB51. In the second study, elk were SQ vaccinated with saline, or 10^{10} CFU of SRB51 or *B. abortus* strain 19 (S19). In study 1, SRB51 was recovered from the blood of all elk at 2 weeks, and 3 of 6 vaccinates at 4 weeks after vaccination. In both studies, S19 and SRB51 vaccinates had greater ($P<0.05$) antibody responses when compared to responses of nonvaccinated elk. Antibody responses of elk to SRB51 were greater than responses of bison and cattle in comparable studies. Neutrophil iodination was reduced ($P<0.05$) in elk when compared to bison and cattle. Proliferative responses of elk to S19 or SRB51 were delayed in both studies when compared to vaccination responses of cattle or bison. Flow cytometry data suggested that elk B cells were responsible for proliferative responses detected by thymidine incorporation. Our data suggests immunologic responses of elk differ from responses of bison and cattle. Our data also suggest that immunologic responses of elk to vaccination with RB51 and S19 are predominantly humoral.

(72) ANALYSIS OF INTERFERON-γ PRODUCTION BY *MYCOBACTERIUM BOVIS* INFECTED WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) USING AN IN-VITRO BLOOD BASED ASSAY

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Tuberculosis due to *Mycobacterium bovis* in captive Cervidae was identified as an important disease in the US in 1990 and prompted the addition of captive Cervidae to the USDA uniform methods and rules for the eradication of bovine tuberculosis. As well, *M. bovis* infection was identified in free-ranging white-tailed deer in northeast Michigan in 1995. Tuberculosis in both captive and free-ranging Cervidae represents a serious challenge to eradication of *M. bovis* from the US. Currently, the only approved antemortem tests for tuberculosis in Cervidae are intradermal tuberculin skin testing and the blood tuberculosis test (BTB). The BTB is presently unavailable in North America. Tuberculin skin testing of Cervidae is time consuming and involves repeated handling and risk of injury to animals and humans. A blood based assay for tuberculosis in Cervidae would decrease animal handling, stress, and losses due to injury. Additionally a blood based assay could provide a more rapid diagnosis. Twenty, 6-9 month old, white-tailed deer, male and female, were experimentally inoculated by instillation of 300 CFU of *M. bovis* in the tonsillar crypts. Seven, age-matched uninfected deer served as controls. Blood was collected on days 90, 126, 180, 210, 238, 263 and 307 after inoculation and analyzed for the production of interferon-γ (IFN-γ) in response to incubation with *M. bovis* PPD, *M. avium* PPD, pokeweed mitogen (PWM), or media alone. Production of IFN-γ was significantly greater ($P<0.05$) at all time points in samples from *M. bovis* infected deer as compared to uninfected control deer while IFN-γ production to PWM did not differ significantly between infected and control deer. Measurement of IFN-γ production to *M. bovis* PPD may serve as a useful assay for the antemortem diagnosis of tuberculosis in Cervidae.

(73) HORSFALL BAUER UNITS USED FOR PATHOGENICITY TESTING IN CHICKENS

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Many domestic and wild avian species can be infected with influenza viruses. These viruses vary widely in their ability to cause disease (pathogenicity) and their ability to spread among birds. When an influenza virus of moderate to high pathogenicity is identified and isolated at any of the CAHFS laboratories, the Horsfall Bauer room may be used to test the virulence of the isolate. The Davis animal vivarium contains thirty-two individual isolators that under filtered air with negative pressure can house up to 10 birds per cage. Chickens are inoculated, monitored up to 10 days to develop clinical signs, then bled and necropsied. This poster depicts the step by step process of a pathogenicity test using the Horsfall Bauer isolators.

(74) PRELIMINARY INVESTIGATION OF SQUIRRELS AS A POSSIBLE RESERVOIR FOR DEER ADENOVIRUS

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A novel adenovirus was the cause of an epizootic that caused high mortality in deer in California in 1993-1994. Retrospective analysis demonstrated adenovirus in tissues from deer that died during a similar epizootic in 1987. It is unknown whether there is a reservoir species that harbors the virus between epizootics or whether latent infection occurs in deer, and shedding with subsequent clinical disease triggered by stress. Observation of the interface between captive deer and free ranging squirrels prompted a preliminary experimental inoculation study to determine if squirrels are capable of carrying and shedding the deer adenovirus. Six squirrels were inoculated via intramuscular, intraocular, and oral routes with deer adenovirus and observed for six weeks. No clinical signs were observed during the study. Squirrels were necropsied six weeks post-inoculation and tissues were collected for histopathologic examination, immunohistochemistry and virus isolation. Serum virus neutralization test results demonstrated high antibody titers to adenovirus in all six squirrels. Serum virus neutralization was performed on two free-ranging squirrels and no antibody was detected to deer adenovirus. Results of this study will be illustrated.

(75) OF MICE AND MATH: A MODEL FOR HANTAVIRUS TRANSMISSION IN DEERMICE (*PEROMYSCUS MANICULATUS*)

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The deer mouse (*Peromyscus maniculatus*) is the reservoir host for Sin Nombre virus, a hantavirus that causes acute pulmonary disease in humans. Although many field surveys have detailed SNV prevalence patterns found in natural populations of deer mice, a unified framework for explaining and predicting these patterns is lacking. A model was constructed to provide a cohesive description of virus transmission in mice. It includes a distinction between winter and summer and male and female transmission. The model's predictions were then compared to prevalence patterns described in the literature. The model can successfully account for patterns in deer mouse prevalence across gender, time, and habitat type. The model suggests that different prevalence patterns are largely due to differences in movement rates and hence contact rates of individual mice. Contact rates are dependent on the sex of the individual, the time of year, and the vegetative structure of the environment. However, contact velocity is not necessarily dependent on density. These results suggest that researchers should focus on measuring movement patterns rather than density in efforts to better understand prevalence in natural populations. The fit between the model and the empirical data suggests the model can serve as a valuable tool for both focusing future research and identifying critical host population parameters to predict human risk.

(76) CERUMINOUS GLAND ADENOCARCINOMA IN CHANNEL ISLAND FOXES (*UROCYON LITTORALIS CATALINAE*) FROM SANTA CATALINA ISLAND

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The Channel Island fox (*Urocyon littoralis*), a diminutive relative of the gray fox (*Urocyon cinereoargenteus*), is divided into six subspecies among the six Channel Islands off of the coast of southern California. Populations on four islands have been in decline since the mid-90's, including loss of close to 80% of the foxes on Santa Catalina in less than one year. The current Santa Catalina population is estimated as 300 individuals. From 1999 to 2002, five foxes trapped during routine census or for captive breeding were evaluated for morbidity associated with aural pathology, including three cases of neoplasia and two cases of extensive inflammatory disease. All of these animals were captured from different locations on the island. Biopsy and subsequent necropsy confirmed three cases of ceruminous gland adenocarcinoma and one case of an inflammatory aural polyp. Foxes with cancers included a young adult (2-4 years old) male and female and a mature adult male (3-6 years old). Two affected animals kept under observation had a rapid clinical course resulting in death. Necropsy findings included locally extensive invasion of periauricular tissues by the carcinoma and regional metastases, as well as exudative otitis externa. Histologic examination revealed adenocarcinoma of the ceruminous glands, chronic neutrophilic and lymphoplasmacytic otitis and ceruminous gland hyperplasia and dysplasia. One proposed pathogeneses for aural neoplasms in other species is neoplastic transformation in chronically inflamed hyperplastic epithelium. Chronic otitis media associated with acariasis has been observed in multiple foxes on Santa Catalina and other islands. This unusual clustering of an uncommon tumor suggests either a genetic predisposition or environmental carcinogen. More in depth surveillance for this disease on other islands is in progress.

(77) CASE REPORT: SUBCUTANEOUS *TAENIA CRASSICEPS* CYSTICERCOSIS IN AN ARCTIC FOX (*ALOPEX LAGOPUS*)

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A trapper from Arctic Bay, Nunavut, Canada, submitted a mature female arctic fox (*Alopex lagopus*) for post mortem after observing several subcutaneous lesions while skinning the animal. At necropsy, numerous fluid-filled cysts measuring from <1 cm to 7 cm in diameter, were found scattered through the subcutis and fascia of the shoulder, neck and axillary regions. Similar cysts were also found deep in the musculature. These cysts contained many ovoid cysticerci ranging from 1.5 – 6 mm in length. Otherwise the animal appeared normal. The cysticerci examined (n=10) contained 25 – 42 hooks (mean 33) in two rows; small hooks measured 84.2 – 131 μm (mean 115.6 μm) in length, and large hooks 119.5-179.3 μm (mean 155.2 μm). Most of the cysticerci had small internal or external buds. These measurements are consistent with Taenia *crassiceps*, normally found as a strobilate adult in canid definitive hosts, including arctic foxes, in North America. Although there have been cases of this infection in domestic dogs in various parts of the world, this is the first report of *T. crassiceps* cysticercosis in a wild canid in North America. The development of the parasite in an atypical intermediate host may explain the large variation in hook size and number compared to other published data.

(78) ANALYSIS OF LYMPHOCYTES ISOLATED FROM WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) FAWNS

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Peripheral blood mononuclear cells (PBMC) were isolated from female white-tailed deer fawns at 48 hours of age and every two weeks there after until the fawns were three months of age. Lymphocytes were phenotyped to examine the expression of specific surface receptors as the fawns aged. Three-color flow cytometric analysis of leukocytes was performed using a panel of monoclonal antibodies (mAb). Adult control deer were also phenotyped using the same monoclonal antibody panel. Included in the panel were mAbs recognizing WC1, the gamma chain and delta chains, CD4, CD8, CD62L, CD44, CD21, IL-2R, MHC Class II, B-B1, B-B2 and B-B4. Analysis revealed dynamic changes in the expression of specific surface receptors associated with development and maturation of lymphocyte subpopulations. WC1+ gamma delta T cells were predominant in neonatal fawns and decreased with age. In contrast, percentages of CD4 and CD8 populations were observed to increase over time. B cell surface IgM expression was heterogeneous at two days, but became more discrete as the fawns matured. Interestingly, B-B2, a putative B cell lineage marker expressed on lymphocytes at 24-48 hours was down modulated by two weeks. However, expression of another B cell lineage marker, B-B4, was consistently expressed throughout the fawns' development. Mononuclear cells isolated from bone marrow aspirates revealed phenotypically distinct expression of surface receptors as compared to PBMCs. Mononuclear cells co-expressing B-B2 and surface IgM were a unique population found in the bone marrow and not in the peripheral blood. B-B4 was expressed on a small percentage of bone marrow mononuclear cells. Proliferative responses of the isolated PBMC's to pokeweed mitogen (PWM), Concanavalin A (ConA) and Mannheimia (Pasteurella) haemolytica LPS were also examined. Cells isolated from fawns proliferated in response to PWM and ConA, but not in response to LPS stimulation. Data obtained in the present study provides baseline information regarding lymphocyte subpopulations in white-tailed deer fawns.

(79) *MYCOBACTERIUM BOVIS*-INFECTED WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*): DETECTION OF IMMUNOGLOBULIN SPECIFIC TO CRUDE MYCOBACTERIAL ANTIGENS

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White-tailed deer (*Odocoileus virginianus*) have recently emerged as a source of *Mycobacterium bovis* infection for cattle within North America. The objective of this study was to evaluate the antibody response of *M. bovis*-infected deer to crude mycobacterial antigens. Deer were experimentally inoculated with *M. bovis* strain 1315 either by intratonsilar instillation or by exposure to *M. bovis*-infected (i.e., in contact) deer. To determine the time course of the response including effects of antigen administration for comparative cervical skin testing, serum was collected periodically and evaluated by ELISA for immunoglobulin (i.e., IgG heavy and light chains) reactivity to mycobacterial antigens. Reactivity to *M. bovis* purified protein derivative (PPDb) exceeded ($p < 0.05$) the reactivity to *M. avium* PPD (PPDa) only after in vivo administration of PPDa and PPDb for comparative cervical testing of infected deer. The mean immunoglobulin response, as measured by ELISA, of intratonsilar-inoculated deer to a proteinase K-digested whole cell sonicate (WCS) of *M. bovis* strain 1315 exceeded ($p < 0.05$) that of pre-challenge responses to this antigen at approximately 1 month post inoculation and throughout the remainder of the study (i.e., ~ 11 months). This response also exceeded ($p < 0.05$) that of non-infected deer. Western blot analysis of serum from infected deer revealed several strong bands of reactivity (~ 22 to 30 kilodaltons) to the *M. bovis* WCS. Specific reactivity to the proteinase K-digested WCS was not detected by Western blot analysis, likely due to nature of the antigen (i.e., carbohydrate and lipid). While encouraging, further studies are necessary to validate the usage of proteinase K-digested *M. bovis* antigens in antibody-based assays of tuberculosis.

(80) DIAGNOSIS OF MYCOPLASMAL UPPER RESPIRATORY TRACT DISEASE IN CHELONIANS

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Current diagnostic methods available for upper respiratory tract disease (URTD) caused by *Mycoplasma agassizii* in tortoises include polymerase chain reaction (PCR) performed on nasal lavage or culture samples, and enzyme-linked immunosorbent assay (ELISA) performed on serum or plasma. Since 1992, over 10,000 samples from 30 different chelonian species have been analyzed by ELISA. The majority of these samples were obtained from free-ranging gopher tortoises *(Gopherus polyphemus)* and desert tortoises (*Gopherus agassizii*), the two species for which the test has been validated. A large number of samples representing tortoises from private and zoological collections, as well as confiscated animals, have additionally been screened. This study reports results for species that had a minimum of 10 animals sampled. Specific antibody to *M. agassizii* was detected in the Russian spurred (*Testudo graeca nikolski;* 5% seropositive, n=58), elongated (*Indotestudo elongata;* 6%, n=18), Galapagos (*Geochelone elephantopus;* 9% seropositive, n=55), aldabra (*Geochelone gigantea;* 15% seropositive, n=41), Herman's (*Testudo hermanni;* 19% seropositive, n=239), radiated (*Geochelone radiata;* 21% seropositive, n=103), African spurred (*Geochelone sulcata;* 48% seropositive, n=31), red-footed (*Geochelone carbonaria;* 48%, n=27), Texas (*Gopherus berlanderi;* 49% seropositive, n=67), spur-thighed (*Testudo graeca;* 52% seropositive, n=135), Indian star (*Geochelone elegans;* 65% seropositive, n=92), Egyptian (*Testudo kleinmanni;* 66% seropositive, n=50), and leopard (*Geochelone pardalis;* 83% seropositive, n=35) tortoises. Box turtles (*Terrapene carolina;* 23% seropositive, n=163), bog turtles (*Clemmys muhlenbergii;* 16 % seropositive, n=19) and spotted turtles (*Clemmys guttata;* 3% seropositive, n= 37) also tested positive. No seropositive animals were detected in spider tortoises (*Pyxis arachnoids;* n=24) or pancake tortoises (*Malacochersus tornieri;* n=11). From over 6,000 gopher *(G. polyphemus)* and desert (*G. agassizii*) tortoise samples received, 25% of desert and 39% of gopher tortoises had positive results.

The PCR analysis was designed to screen for the presence of any mycoplasma species in a tortoise nasal lavage or culture sample. The sensitivity of this assay is highly variable but the specificity is very high and therefore, there is a high degree of confidence in a positive result. PCR analysis has been performed on more than 1000 samples from 27 chelonian species, with positive results in 12 different species. The species for which at least one positive PCR result has been obtained include African-spurred (*G. sulcata*), desert (*G. agassizii*), flat-tailed (*Pyxis planicauda*), gopher *(G. polyphemus)*, Indian star (*G. elegans*), leopard (*G. pardalis*), radiated (*G. radiata*), spider (*P. arachnoides*), spur-thighed (*T. graeca*), and Texas (*G. berlanderi*) tortoises, as well as box turtles (*T. carolina*). Restriction fragment length polymorphism (RFLP)

analysis of these positive samples have revealed the presence of *Mycoplasma agassizii*, a second recognized but un-named mycoplasma species, and at least one other, and possibly several other, unknown mycoplasmas. Genetic analyses of some of these unknown isolates are pending.

Sixty-two of 74 PCR-positive animals (84%) also had concurrent ELISA analysis of serum or plasma. Thirty-five of 38 tortoises (92%) found to be infected with *M. agassizii* by PCR also tested positive by ELISA. The remaining 24 tortoises were infected with unknown or other mycoplasmas, and only 8 of these 24 tortoises (33%) tested ELISA positive. This suggests that the ELISA assay detects antibodies produced in response to infection by *M. agassizii* but not necessarily to other mycoplasmal species. The assay detected antibodies in approximately 1/3 of the tortoises infected with other mycoplasmas, indicating that some mycoplasmal species have shared antigenic epitopes resulting in cross-reaction with the assay. These results also suggest that the ELISA assay, although specifically designed for desert and gopher tortoises, appears to work well with several other tortoise species. However, controlled infection studies would be required to quantitatively assess the accuracy of the assay in these alternate species. Therefore, positive ELISA results in species other than gopher and desert tortoises should be interpreted cautiously, taking into consideration the clinical status of the animal, as well as other diagnostic assays.

The results of these surveys indicate that *M. agassizii* is capable of colonizing and infecting many turtle and tortoise species. Although clinical history was not available on all of these animals, several had documented clinical signs compatible with mycoplasmosis. Therefore it is important that individuals or zoological collections exercise caution and practice good management and quarantine procedures to prevent spread of disease among captive species. Further, investigators conducting studies with free-ranging turtles and tortoises should follow appropriate disinfection protocol between animals to prevent cross-contamination with infectious diseases.

(81) *EIMERIA AURITUSI* N. SP. IN THE KIDNEYS OF DOUBLE-CRESTED CORMORANTS (*PHALACROCORAX AURITUS*): SPECIES DESCRIPTION AND LESIONS

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Since 1984, several outbreaks of renal coccidiosis involving over 1,200 double-crested cormorants (DCC; *Phalacrocorax auritus*) have been documented in several states in the Midwestern and southeastern United States. In this report, we describe *Eimeria auritusi* n. sp., a cause of renal coccidiosis in DCC collected from Skidaway Island, Chatham County, Georgia, USA. Samples of kidney tissue were placed in 2.5% (wt/v) aqueous potassium dichromate (K2Cr7O2) or 10% buffered formalin. Sporulated oocysts were spherical to subspherical and measured 16.1 × 16.5 (13.8-18 × 14-19) μm, with an average length: width ratio of 1:1.02. Oocyst wall was thin (1 μm), greenish, and pitted on the outer surface. A micropyle, micropylar cap, Stieda body, and polar bodies were absent. A small oocyst residuum (4-8 granules) was usually absent but occasionally present. Sporocysts were oval and measured 6.6 × 9.3 (6-7 × 8-10.5) μm with an average length: width ratio of 1:1.4. A sporocyst residuum was present, located in between sporozoites, and was composed of numerous granules of unequal size. A small refractile body was present in each sporozoite. Collecting duct and distal renal tubular epithelial cells were distended by large oocysts in their cytoplasm and many oocysts were present in the lumen of ectatic tubules. Various stages of meronts, gamonts, and developing oocysts were present in other renal tubular epithelial cells. Multiple infections of parasitized cells were frequently observed, with cells containing up to 12 gamonts or developing oocysts. Interestingly, pulmonary capillaries in several DCC contained unsporulated oocysts suggesting that oocysts entered the circulatory system through damaged kidney tissue or were inhaled. A fragment of the 18S rRNA gene was successfully amplified from kidney tissue using *Eimeria* conserved primers. Sequencing revealed that *E. auritusi* is most closely related to *E. tropidura*, a parasite of the Hood island lizard. The importance of *E. auritusi* to DCC populations is not known; however, in this case significant mortality and lesions were associated with infection.

(82) PARATUBERCULOSIS AND REPERCUSSIONS FOR MANAGEMENT OF FREE-RANGING TULE ELK AT POINT REYES NATIONAL SEASHORE

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Paratuberculosis, or Johne's disease, presents different challenges to wildlife managers than it does to livestock owners. As a case study, the translocation of tule elk at Point Reyes National Seashore will be discussed. Forty-five adult tule elk (*Cervus elaphus nannodes*) were moved from a fenced population to a holding pen 6 months prior to release in an unfenced region of the park. All translocated elk were apparently healthy throughout the study. Because infection with *Mycobacterium avium* ssp. *paratuberculosis* had been reported in the source population since the 1980s, translocated elk underwent extensive testing for this infection. Twenty-one animals were test-positive on at least one assay and were euthanatized and necropsied. Since their release, although the new herd has remained apparently asymptomatic, 5 additional animals have been culled because of positive fecal cultures. Paratuberculosis continues to present a diagnostic challenge in wild ungulates. Differences in presentation, pathobiology and diagnosis in elk versus domestic livestock are important considerations for managers, especially in light of additional attention being given to nationwide paratuberculosis surveillance in livestock. Management of potentially infected free-ranging elk will be discussed in the context of the policy constraints and philosophy of the National Park Service.

(83) VALIDATION OF PROTEIN G-BASED ELISAS FOR JOHNE'S DISEASE SURVEILLANCE IN ELK

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The prevalence of Johne's disease (an emaciating gastrointestinal mycobacterial infection) in wild and domestic agriculture species is increasing. In addition, a spill-over of the infection from cattle to rabbits *(Oryctolagus cuniculus)* to non-ruminant species (e.g. fox, stoat, weasel, crow, rook, rat, badger) preying on the infected rabbits has been reported. The finding has increased concerns that wildlife reservoirs of the infection may threaten Johne's disease management and control programs for domestic agriculture. Cervid species are susceptible to this infectious disease and share range with domestic agricultural species in many parts of the country. No serologic assays for Johne's disease have yet been validated for deer or elk. To investigate the possibility of developing a valid serologic method of diagnosing the infection in cervids, the binding characteristics of a non-species specific conjugate (protein G) were determined for purified serum immunoglobulin from three cervid species (elk, white tail deer and muntjac). The conjugate was found capable of detecting antibody produced by all three species but the binding patterns differed. This means the conjugate offers the potential for detecting antibody in an ELISA assay for these species (for Johne's disease or any other disease eliciting antibody), but that different interpretation algorithms by species may need to be developed. Two commercial protein G conjugate-based ELISAs were then assessed for their ability to detect antibody in elk with Johne's disease. The assays were evaluated in three free-ranging populations with minimal exposure to cattle or cattle ranges (N = 480) and in 46 well-characterized Johne's disease cases from three farm-raised populations. The preliminary sensitivity and specificity of the serologic assays were as follows:

Assay	Sensitivity	Specificity
IDEXX	75.5%	97.6%
Synbiotics*	58.7%	96.5%
Synbiotics**	82.6%	83.9%
AGID	27.3%	na

* Synbiotics "suspect" range considered negative
** Synbiotics "suspect" range considered positive

In summary, ELISAs based on the protein G conjugate were shown to be effective tools for detecting *M. a. paratuberculosis* infection in populations of elk. The AGID is not recommended for this purpose. The strengths, weaknesses and appropriate use of these assays in cervid species will be discussed.

(84) MILK REPLACER CONTAINING *MYCOBACTERIUM BOVIS* AS A SOURCE OF INFECTION FOR WHITE-TAILED DEER FAWNS (*ODOCOILEUS VIRGINIANUS*)

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White-tailed deer represent the first wildlife reservoir of *Mycobacterium bovis* in the US. The behavior of does with nursing fawns provides several potential mechanisms for disease transmission. Little information exists concerning transmission between doe and fawn, specifically transmammary transmission. To examine the potential for such transmission, seventeen, 21 day-old, white-tailed deer fawns were inoculated orally with 2×10^8 CFU (high dose, n=5), 2.5×10^5 to 2.5×10^6 CFU (medium dose, n=5), and 1×10^4 CFU (low dose, n=5) of *M. bovis* in milk replacer over a five day period. Positive control fawns (n=2) received 1×10^5 CFU of *M. bovis* instilled in the tonsillar crypts. All fawns in the tonsillar, high oral and medium oral dose groups developed generalized tuberculosis involving numerous organs and tissues by 35-84 days after inoculation. Three of five fawns in the low dose oral group had tuberculous lesions in the mandibular lymph node, and 1 of 5 had lesions in the medial retropharyngeal lymph node when examined 115 days after inoculation. This study demonstrates that white-tailed deer fawns can become infected through oral exposure to *M. bovis*. Therefore, the potential exists for fawns to acquire *M. bovis* while nursing tuberculous does.

(85) IMMUNE RESPONSES OF ELK (*CERVUS ELAPHUS*) TO *MYCOBACTERIUM BOVIS* BACILLE CALMETTE GUERIN (BCG) VACCINATION

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Detection of *Mycobacterium bovis* infection of captive or free-ranging elk (*Cervus elaphus*), although rare, elicits serious concern due to regulatory and zoonotic implications. As captive Cervids are frequently moved between herds, the development of improved diagnostic capabilities and vaccines for tuberculosis would be particularly desirable for this industry. In the present study, elk were vaccinated with live *Mycobacterium bovis* bacillus Calmette Guerin (BCG, Pasteur strain) for determination of immune responsiveness to this attenuated live vaccine. Mononuclear cells obtained from the peripheral blood of vaccinated elk proliferated in response to stimulation with a crude soluble mycobacterial antigen preparation (i.e., *M. bovis* purified protein derivative, PPDb). Greater ($p<0.05$) numbers of IgM^+ cells (i.e., B cells) proliferated in response to PPDb than did either $CD4^+$, TCR^+ or $CD8^+$ cells. As a measurement of *in vivo* reactivity to mycobacterial antigens, PPDb and *M. avium* PPD (i.e., PPDa) were intradermally administered in the cervical region of control elk, BCG-vaccinated elk, and BCG-vaccinated cattle; and, responses (i.e., measurement of skin induration and edema) measured after 24, 48, and 72 hours. The response to PPDb by vaccinated elk exceeded ($p<0.05$) that of both non-vaccinated elk and BCG-vaccinated cattle at each time point. Responses to PPDb by vaccinated elk diminished ($p<0.05$) after 72 hours as compared to responses at 24 and 48 hours. Histologic evaluation of biopsy sites revealed mononuclear cell infiltrates that increased from 48-72 hrs, consistent with a delayed type hypersensitive response. Edema, however, was most pronounced at 24 hrs and progressively decreased after 48 and 72 hrs. Serum was also collected periodically and evaluated by ELISA for immunoglobulin (i.e., IgG heavy and light chains) reactivity to mycobacterial antigens. Beginning 2-weeks post vaccination, serum antibody reactivity to PPDb and to a proteinase K-digested whole cell sonicate of BCG exceeded ($p<0.05$) the reactivity of serum from non-vaccinated elk. Administration of mycobacterial antigens for skin testing significantly boosted ($p<0.05$) this antibody response. These findings demonstrate that vaccination of elk with an attenuated live *M. bovis* vaccine induces: 1) an early serum antibody response specific for *M. bovis* antigens, 2) a modest *in vitro* proliferative (predominantly B cell) response, and 3) *in vivo* trafficking of mononuclear cells to sites of mycobacterial antigen administration (i.e., delayed type hypersensitivity).

(86) EXPERIMENTAL VACUOLAR MYELINOPATHY IN RED-TAILED HAWKS

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Avian vacuolar myelinopathy (AVM) is an emerging disease confirmed or suspected of killing at least 90 bald eagles (*Haliaeetus leucocephalus*) in four southeastern states. Lesions in birds with AVM consist of vacuolization of central nervous system white matter. The cause of AVM and its source remain undetermined despite extensive diagnostic and research investigations; however, a natural or manmade neurotoxicant is suspected. Avian vacuolar myelinopathy was recognized in 1994 as a cause of wild bird mortality when 29 bald eagles succumbed to the disease in southwestern Arkansas. In 1996, when AVM killed 26 eagles in the same area, it became apparent that American coots (*Fulica americana*) had identical neurologic signs and lesions, and it was hypothesized that eagles acquired AVM via ingestion of affected coots.

In order to test this hypothesis, we fed coot tissues (brain, liver, kidney, muscle, fat, and gastrointestinal tract) to rehabilitated, non-releasable red-tailed hawks (*Buteo jamaicensis*). Five hawks received tissues from coots with confirmed AVM lesions, and one hawk received tissues from coots without brain lesions that had been collected at a site where AVM never has been documented. All hawks received 12-70g/day (average = 38g/day) of coot tissues for 28 days. Neurological examinations were conducted on the hawks and blood samples were drawn weekly. All birds remained clinically normal during the study and clinical pathological abnormalities were not apparent.

The birds were euthanatized and necropsied on day 29. Significant gross lesions were not apparent in any of the birds. Microscopic examination of brain and spinal cord sections from birds receiving tissues of affected coots revealed moderate vacuolization of white matter, with lesions most pronounced in the optic lobe. Transmission electron microscopy of affected white matter revealed splitting of myelin laminae at the intraperiod line, a lesion characteristic of intramyelinic edema that is consistently present in birds with AVM. Lesions were not apparent in the hawk that received tissues from unaffected coots.

This marks the first time that AVM has been produced in birds under laboratory conditions and proves that raptors or scavengers may acquire AVM via ingestion of tissues from affected birds.

(87) AVIAN INFLUENZA VIRUS, AVIAN PARAMYXOVIRUS-1 AND CIRCOVIRUS INFECTIONS OF RING-BILLED GULLS (*LARUS DELAWARENSIS*) IN SOUTHERN ONTARIO, CANADA

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A survey of ring-billed gulls (*Larus delawarensis*) was conducted at three rookeries in Southern Ontario (Canada) to determine the prevalence of antibody in serum, and subtypes of avian influenza virus (AIV) and avian paramyxovirus (APMV) in cloacal swabs. Lesions possibly associated with such infections were sought at autopsy and microscopically. Histologic evidence of circovirus infection also was studied in these populations, as well as in cases submitted to the Canadian Cooperative Wildlife Health Center (CCWHC) Ontario Region since 1970. From the middle of May to the middle of July 2000, 360 birds were sampled, 120 in each rookery, of which 40 were adults, 40 were three weeks old, and 40 were five weeks old. Hemagglutinating agents, all of which were avian influenza A virus, were isolated by chicken embryo inoculation from the cloacal swabs/tissue samples of 53 birds. Isolates were characterized by hemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests as H13N6. Juvenile age was identified as the primary risk factor for AIV recovery. Avian paramyxoviruses were not isolated from any of the birds, but serological evidence of infection with an AVPMV-1 was present in 90% of the population. *Circovirus* infection, based on the presence of characteristic inclusion bodies in cells in lymphoid tissue, and particles typical of *Circovirus* by electron microscopy, was only detected in juvenile birds, with a prevalence varying between 0% and 43% among colonies. The first evidence of *Circovirus* infection in the retrospective study was in a juvenile ring-billed gull accessioned in 1973. *Cirvovirus*-like inclusions also were seen in juvenile herring gulls (*Larus argentatus*) and a great black-backed gull (*Larus marinus*).

The H13N6 avian influenza A subtype is associated with gulls, in which it has low virulence. High prevalence of avian influenza virus infection in breeding colonies of ring-billed gulls was confirmed, and is of the magnitude reported in waterfowl. Infection with an APMV-1, previously rare in gulls on the lower Great Lakes, was common based on the serologic findings, but its clinical significance is unknown. The significance of circovirus infection, which occurred commonly only in juveniles in some colonies, also is unknown.

(88) LEVELS OF FECAL CORTICOSTERONE IN SANDHILL AND WHOOPING CRANES DURING PREPARATION FOR HUMAN-LED MIGRATION

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The use of fecal corticosterone assays in North American cranes has been limited to controlled laboratory trials performed with sandhill cranes (*Grus canadensis*). We chose to apply this assay to a specific release project where the ability to handle birds repeatedly for health evaluations was limited, and supplementary diagnostic information was needed to fully assess stress induced by the methods used to rear the cranes prior to release. We documented and compared 3 month trends in fecal corticosterone (FC) concentrations in cohorts of greater sandhill cranes (*G. canadensis tabida*) and whooping cranes (*G. americana*) raised for release to the wild. During summer 2000, sandhill cranes (n=14) reared in isolation from humans were transferred from Maryland to Wisconsin and trained to fly after ultralight aircraft as a prelude to an aircraft-guided 1250-mile migration to Florida. During 2001, the same methods were used with whooping cranes (n=10), with the goal of establishing a new migratory flock of this endangered species (Whooping Crane Eastern Partnership; www.bringbackthecranes.org). Fresh feces were collected from individuals at arrival in Wisconsin, and then anonymously from their pens until migration in early fall. All samples were subject to radioimmunoassay for determination of corticosterone levels; levels >200 ng/g were considered increased over baseline. Increased FC levels were noted in both sandhill (median 292 ng/g, range 60-651 ng/g, n=12) and whooping cranes (median 711 ng/g, range 273-1130 ng/g, n=9) upon arrival in Wisconsin, likely reflecting the stress of shipment. The lower FC levels observed in some cranes may have been due to sampling of feces produced prior to a hormonal increase. FC levels in both groups of cranes returned to baseline levels within 7 days and were sustained throughout the remainder of the study (sandhill crane median 93 ng/g, range 61-264 ng/g, n=32; whooping crane median 63 ng/g, range 22-190 ng/g, n=133). There were large deviations from baseline FC levels for up to 96 hours in individual whooping cranes, however, related to a pre-migration handling event 4 weeks prior to departure (median 176 ng/g, range 116-553 ng/g, n=19). Significant disturbances, such as shipment to the release site, and restraint for banding, radiotransmitter placement or health examinations, appear most linked to acute increases in FC in sandhill and whooping cranes. The methods used to train cranes to follow ultralight aircraft, including manipulations of social groups prior to migration, do not appear to result in acute or chronic stress that may alter performance or survival during migration.

(89) LEAD LEVELS, ECTOPARASITES, AND MORPHOMETRIC SYMMETRY OF TURKEY VULTURES CAPTURED IN HUMBOLDT COUNTY, CALIFORNIA

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The health and vigor of both wild and captive birds of prey can be adversely affected by ingested lead and a wide variety of parasitic organisms, including arthropods, hematozoa, and protozoa. Yet there is limited information on the role of lead and parasites in raptors. Morphometric symmetry is one measure of health; there is no available information on the relations of lead or parasites to morphometric symmetry in most raptors. Turkey vultures *(Cathartes aura)* were live-captured between June and September, in both 2000 and 2001 in Humboldt County, California (USA). Forty-two individuals and 3 re-captures were evaluated for blood lead levels, hematozoa, arthropod parasites, and asymmetry of facial features, wing surface area, and tarsus and wing lengths. No hematozoa were found in any sampled birds. Blood lead levels were detected in all vulture blood samples by the atomic absorption spectrometry method, with values ranging from 0.02 to 2.1ppm and a mean (± SD) of 0.14 (±0.33) ppm. A total of 218 arthropod parasites were collected from 45 captured vultures. Mallophaga (Suborder Amblycera, Family Laemobothriidae) were the most commonly represented arthropod with 65 individuals taken from 12 of 42 birds; intensity ranged from 1 to 16 lice with a mean (± SD) of 1.67 (±3.86) individuals per infected bird. Sixteen Oribatid mites (Family Humerbates) were collected from 12 of 42 birds; intensity ranged from 1 to 3 mites per bird. Several species of ectoparasitic Diptera also were collected. Five morphometric features of vultures were evaluated for asymmetry. Mean (± SD) tarsus length was 7.4 mm (±0.25) with a mean (± SD) difference of 11(±0.78) mm. Mean wing length was 520 mm (±0.15) with a mean difference of 9.8 (±0.13) mm. Mean wing surface area was 15,867 (±333.63) mm^2 with a mean difference of 2,549 (±1428.97) mm2. Mean eye-nare distance was 33 (±3.06) mm with a mean difference of 2.7 (±1.88) mm. Mean eye-mouth distance was 12.9 (+1.81) mm with a mean difference of 1.4 (±1.76) mm. Tarsus length difference was found correlated to the number of ectoparasites (p=0.07, 95%CI) by Stepwise Regression. And wing surface area difference was found correlated to the number of ectoparasites (p= 0.03, 95%CI) by Stepwise Regression. No significant relations were observed between blood lead levels and extent of morphometric asymmetry or ectoparasites. These findings support recent literature suggesting that asymmetry of morphometric features may reveal aspects of individual fitness value, specifically parasitism.

(90) DIFFERENTIAL SUSCEPTIBILITY OF HOUSE FINCHES (*CARPODACUS MEXICANUS*) TO THREE DOSES OF *MYCOPLASMA GALLISEPTICUM*

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We have been monitoring the mycoplasmal conjunctivitis outbreak in the Auburn Alabama house finch (*Carpodacus mexicanus*) population since 1995 when this new host parasite interaction was first observed in this population. Many basic questions regarding this new host-pathogen relationship remain to be answered. The objective of this study was to analyze the dose response of house finches to MG. Thirty finches were captured on the Auburn University campus and quarantined for four weeks at the Auburn University aviary. All of the birds were negative for antibodies to MG by serum plate agglutination assay and MG could not be detected in any bird by PCR at the end of quarantine. After quarantine birds were randomly assigned to one of three flocks or eight or one flock of six. The three flocks of eight finches were infected with either 10^2, 10^4, or 10^6 CCU/mL of MG by dropping 10µL into each eye. The flock of six birds served as a negative control and was given sterile SP4 media. Finches inoculated with the lowest dose of MG (10^2CCU/mL) developed a short-lived antibody response, but none of the birds developed conjunctivitis and MG could never be detected by PCR. All sixteen of the finches inoculated with the medium and high doses developed antibodies to MG and we could detect MG by PCR at some time during the infection in all of these birds. However, in each of these two groups, only six of the eight finches developed conjunctivitis. This study demonstrates a dose response to inoculation with MG in house finches and suggests that careful consideration should be given to the dose in future experimental infection studies of this host-pathogen interaction.

(91) DEVELOPMENT OF ENTERIC ANTIBIOTIC RESISTANCE IN AVIAN PATIENTS AT THE TUFTS WILDLIFE CLINIC

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Antibiotic resistance is a growing problem worldwide. It has been demonstrated that antibiotic use in agriculture leads to selection for antibiotic resistant strains of bacteria, including zoonotic pathogens. Direct contact with animals, as well as their feces, has been suggested as a major pathway for the transmission of these pathogens from agricultural animals to humans. Although antibiotics are used extensively in wildlife rehabilitation, very few studies have evaluated the effects of this on the health of the animals involved, rehabilitators, or the environment. The objective of this study was to examine the level of antibiotic resistance within the enteric bacteria of birds entering Tufts Wildlife Clinic and any changes in resistance after hospitalization. The antibiotic resistance of some environmental samples within the Clinic was also evaluated. This study is a work in progress.

Within 24 hr of presentation to the Clinic and prior to the administration of any antibiotics, cloacal samples were obtained using BDL™ CultureSwabs™ (Liquid Stuart Medium; BD Biosciences: Sparks, MD 21152 USA). Samples were cultured, identified, and evaluated for antibiotic resistance by IDEXX Veterinary Services (North Grafton, MA 01536 USA). Exit cloacal samples were obtained in the same manner immediately prior to the resolution of some of the cases.

Cultures were obtained from 45 birds admitted to the Clinic between 24 May -18 August 2000 and 5 June - 25 October 2001. Species sampled included Canada geese, mallard ducks, American crows, and several species of hawks and owls. Entrance swabs were obtained from 43 birds and exit swabs were obtained from 14 birds. The entrance swabs yielded 99 isolates consisting predominately of *Escherichia coli* and *Enterococcus* sp. Thirty six isolates (36%) were not resistant to any antibiotics. Thirty isolates (30%) were resistant to only one antibiotic. Seven isolates (7%) were resistant to three antibiotics. Ten isolates (10%) were resistant to four or more antibiotics. The average antibiotic resistance per entrance isolate was 1.52 antibiotics, with a range of 0-13. Exit swabs yielded 32 isolates. These consisted primarily of *E. coli*, *Enterococcus* sp. and *Enterobacter* sp. Three isolates (10%) were resistant to only one antibiotic. Three isolates (10%) were resistant to two antibiotics. Four isolates (13%) were resistant to three antibiotics. Sixteen isolates (53%) were resistant to four or more antibiotics. The average antibiotic resistance per exit isolate was 4.31 antibiotics, with a range of 0-13. Resistance to aminoglycosides, fluoroquinolones, cephalosporins, tetracyclines, trimethoprim-sulfa, and penicillins was higher in the exit isolates than in the entrance isolates.

We are continuing to collect cultures from animals within the Clinic and are in the process of gaining approval to sample the staff members of the Clinic in order to compare the resistance patterns of their enteric bacteria with that of animals leaving the Clinic. These studies should further illustrate trends in development of antibiotic resistance in animals presented to wildlife rehabilitation clinics.

(92) A NOVEL EPIZOOTIC OF INSECTIVOROUS BAT VARIANT RABIES VIRUS IN SKUNKS

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Between January and July 2001, nineteen rabid striped skunks (*Mephistis mephistis*) were identified in Flagstaff, Arizona. Historically, neither the city of Flagstaff nor the surrounding counties were considered to have enzootic rabies virus activity in any species except bats. Regional and national reference laboratories identified a rabies virus variant associated with insectivorous bats from the brain tissue of all of the skunks and the salivary glands of a sample of the skunks.

A multi-phased campaign in response to this epizootic included active case surveillance, public awareness and education, low cost pet vaccination clinics, and a trap, vaccinate and release (TVR) program. As of November 15, 2001, more than 217 skunks were trapped, parenterally vaccinated, ear tagged and released. Surveillance efforts continue to delineate the extent of the epizootic as well as to assess the effectiveness of the TVR program.

This represents the first time that sustained transmission of a bat rabies virus variant has been documented among terrestrial mammals. This outbreak highlights the role of public health in recognizing and collaboratively responding to unusual animal disease events, and the need to advance new strategies to control rabies in wildlife.

(93) HEALTH EVALUATION OF WILD SIBERIAN TIGERS (*PANTHER TIGRIS ALTAICA*) AND AMUR LEOPARDS (*PANTHERA PARDUS ORIENTALIS)* IN THE RUSSIAN FAR EAST

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From 1992 through 1997, twenty wild Siberian tigers (*Panthera tigris altaica*) and seven wild Amur leopards (*Panthera pardus orientalis*) were captured, darted, and immobilized thirty-six times and ten times, respectively, for radio-telemetry studies in the Russian Far East. Initially, all 27 animals were captured with Aldrich spring-activated foot snares (Margo Suppliers, High River, Alberta, Canada), then immobilized and radio-collared. Subsequently, six of the twenty radio-collared tigers were re-darted and immobilized from a helicopter for re-collaring.

Tigers and leopards were darted for immobilization using 3 ml plastic darts and 1.5mm x 38 mm collared needles projected from a CO_2 powered rifle (Telinject USA, Saugus, CA 91350 USA). Both species were immobilized with an initial dose of 6.6 mg/kg ketamine (Fort Dodge Laboratories, Inc. Fort Dodge, IA 50501 USA) mixed with 0.66 mg/kg xylazine (Bayer Corporation, Shawnee Mission, KS 66201 USA). Anesthesia was maintained with supplements of 1.1 mg/kg ketamine. Seizures during immobilization were observed twenty-two times in fifteen tigers, and once in one leopard, and were managed with 0.07 – 0.2 mg/kg diazepam i.v. (Roche Pharmaceuticals, Nutley, NJ 07110 USA). Anesthesia was reversed in both species with 0.04 - 0.13 mg/kg yohimbine (Lloyds Laboratory, Shenandoah, IA 51601 USA). Blood and tissue samples were taken during each immobilization. Fecal samples were collected sporadically, and yielded no evidence of gastrointestinal parasites. Ticks (*Dermacentor* and *Ixodes* spp) were found on six tigers and two leopards.

Packed cell volumes were determined in the field using microhematocrit samples spun in a bench top centrifuge. Serum samples were frozen, and transported to the United States for analysis using an automated analyzer (Hitachi 717 Automatic Analyzer, Roche Diagnostics, Indianapolis, IN 46250 USA). Serum chemistries for wild tigers were compared to results from captive tigers. Sixteen of the twenty blood parameters tested between wild and captive tiger populations were significantly ($P \leq 0.05$) different. Serum chemistries for wild leopards were compared to results from captive leopards. Eight of the twenty blood parameters tested between wild and captive leopard populations were significantly ($P \leq 0.05$) different.

Noticeable differences in hematocrit values between the wild and captive populations may be due to a number of things including the type of centrifuge used, centrifuge speed and cycle variations due to the use of a generator for wild samples, and environmental differences between captive and wild cats. In particular this difference may reflect that most captures were done in the winter when there is limited water availability for the wild populations as compared to the captive population which always has water freely available.

Infectious disease serology tests were performed (Washington Area Disease and Diagnostic Laboratory, Pullman, Washington 99165 USA) for feline leukemia virus antigen (ELISA), and antibodies to feline enteric Coronavirus/feline infectious peritonitis (FECV/FIP; IFA), feline immunodeficiency (ELISA), canine distemper virus (CDV; virus neutralization), and feline panleukopenia (IFA) viruses. Serology antibody tests for *Bartonella henselae* (Western Blot) were also performed (National Veterinary Laboratory, Inc., Franklin Lakes, NJ 07417 USA).

These data supply the first baseline information on free-ranging animals for comparison to captive populations of two highly endangered felids. Contrasts in serum chemistry results, or seroprevalence of disease may be indicators of potential conservation threats from human impacts requiring further investigation. The antibody titers to CDV, FECV/FIP, *Bartonella henselae*, and panleukopenia detected in these cats may reflect the endemic presence of these viruses in the wild population, or may reflect exposure through predation on infected domestic animals. In either case these viruses may pose a significant threat to the wild populations, and the presence of antibodies warrants further investigation.

(94) FERAL CAT ALTERING PROGRAMS (FCAP): WHAT'S WRONG WITH THEM? WHAT CAN BE DONE ABOUT IT?

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FCAP's are of questionable legal status on both humane grounds and due to clear and premeditated impacts on wildlife, and do not provide protection, shelter or companionship for cats. FCAPs result in well documented negative ecological impacts on wildlife because: 1) they do not remove feral cats (a non-native, invasive, subsidized predator), but re-abandon and replace cats into potential wildlife habitats, 2) they may prolong the lives and thus the environmental damage done by program cats, 3) many FCAPs do not attempt to avoid impacts on sensitive habitats or to educate either participating veterinarians or their clients about wildlife issues, 4) concentration of feral cats can create sources of disease organisms that may be detrimental to human and animal health, and 5) they send the "wrong message" about feral cats and feral cats colonies which may lead to a social acceptance of this ecologically destructive avocation and thus its proliferation. The majority of evidence indicates that it is extremely difficult to eliminate feral cat populations unless: 1) the population is "closed" and new animals are not added, 2) a very high percentage of animals within the population are neutered quickly, and 3) the program continues for many years (10 or more). Practically speaking these conditions are seldom met and there is little documentation of FCAP programs actually achieving elimination of feral cat populations. Similarly, reductions in feral cat population size are difficult to achieve or sustain. The price of FCAP's to veterinary and non-profit organization, as some have been operated, may ultimately be a serious loss of credibility with conservationists and the public, particularly as it comes to light that veterinarians paid themselves to operate and promote programs that prolonged suffering of feral cats, exacerbated rather than abated destruction of native species, were operated under questionable legal premises, and ultimately failed to reduce the number of feral cats in California or the United States. Suggestions will be made for both countering negative aspects of and improving FCAP's.

(95) EVIDENCE OF EXPOSURE OF AMERICAN BLACK BEARS TO THE AGENT CAUSING HUMAN GRANULOCYTIC EHRLICHIOSIS, *ANAPLASMA PHAGOCYTOPHILA,* IN NORTHERN HUMBOLDT COUNTY, CALIFORNIA

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Human granulocytic ehrlichiosis (HGE) is a new and emerging tick-borne disease of the Northeastern US, the Northern Midwest, and California. Fewer human cases have been identified in California than from the other regions (seven human cases have been confirmed in CA of which three were clustered in Southern Humboldt County). In contrast, granulocytic ehrlichiosis has been well documented in dogs and horses in this state. An enzootic cycle involving dusky-footed woodrats, *Neotoma fuscipes*, as putative reservoir hosts and western-black-legged ticks, *Ixodes pacificus*, as likely vectors to people has been reported. We report here that 88% (70 of 80) of American black bears, *Ursus americanus,* trapped in the Hoopa Valley during the summer of 2001 were considered seropositive (based on an indirect immunofluorescence assay) to *Anaplasma phagocytophila*. In contrast, only one (~1%) of the bears was mildly seropostive for the related species *Ehrlichia canis*. We were unable to amplify rickettsial DNA from any of these samples. In our region, adult *Ixodes pacificus* are most active during the winter and spring and nymphs are most active somewhat later during the spring and early summer. The bears in this area are fed upon by large numbers of ticks, and the lack of bacteremia during summer suggests that *A. phagocytophila* infections in bears may be of short duration. Several authors have discussed the use of carnivores as sentinels of HGE in the West. However, this represents the highest seroprevalence reported from a well-sampled species from the region. Our findings suggest that local people, at least those of the Hoopa Valley, may be exposed to the agent more commonly than might have been previously expected.

(96) ORPHANED BLACK BEAR CUB REHABILITATION IN SOUTHERN COLORADO

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During the summer and fall of 2001, a total of thirty-five (35) Black Bear (*Ursus americanus*) cubs were received at the Center, a full service(all species) wildlife hospital and care facility. The cubs weighed from 10- 35 lbs. and were found in human habitation in Southern Colorado. Most (23) were found along the Southern Front Range. Cause of the large number of cubs was thought to be a late freeze (May) in the high country which destroyed the hard mast and berry crops. Additionally, there had been a two year drought throughout the entire southern part of the state. Sows with cubs wandered into towns searching for food and had to be trapped and relocated, destroyed or in some cases, sows abandoned their cubs. The cubs were captured by Colorado Division of Wildlife Officers and relayed to the Center. Facilities existed at the Center to care for these cubs. A few of the cubs (6) were injured or severely malnourished and required special nutritional, medical and/or surgical care. Food, water, husbandry and other needed care was provided by the authors. Cubs were housed in chain-link runs (8 'x l4' and 12' x 24') with attached exterior dens. In most cases they were placed two or three compatible cubs to a run and in all cases known siblings were housed together. During November, the cubs were sedated (Ketamine-3mg/kg and Xylazine-l-2mg/kg), weighed, chest girth measured, given a thorough physical examination and then ear tagged. Cubs had been admitted between July 15 and November 7, and weighed from 70-143 lbs. at the time of sedation for ear tagging. The smaller cubs were kept on food longer and continued to gain weight to an estimated 90 lbs. Colorado Division of Wildlife Biologists and Officers met at the Center on November 7 and reviewed the cubs and the information obtained and decided on release locations and dates for the cubs to be transported to the winter den sites. Food was withdrawn at least two weeks prior to planned denning and the cubs became very lethargic and in most cases went into a state of hibernation but could be easily aroused. Denning dates ranged from December 10 - January 14 depending on conditions in the high country. Smaller bears that had been admitted later were kept on food and released later. All cubs demonstrated, favorable body fat percentage and good to excellent general condition. Disturbances were kept to a minimum and on the pre-determined date, the dens with the enclosed cubs were transported to the denning site, covered with bales of straw, tarps, conifer limbs and snow and all transport personnel left the site. Several days later, one of the officers returned and quietly opened the den doors and locked it open securely, placed two bales of straw in the opening which could easily be pushed aside by the emerging cubs and left. Past experience (1993-2000) with a total of 29 bears has given us a high percentage of success using these methods.

(97) CHRONIC WASTING DISEASE IN CAPTIVE AND WILD CERVIDS IN SASKATCHEWAN

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Chronic wasting disease (CWD) was first diagnosed in a single captive elk in Saskatchewan in 1996. By 2002 infected elk had been detected on approximately 40 herds and approximately 8,000 captive elk have been destroyed in order to eradicate the disease in captive elk. It is believed that the disease was introduced into Saskatchewan by importation of elk in the late 1980s from a ranch in South Dakota subsequently found to be infected with the disease. CWD also has been found in 2 wild mule deer, one in the fall of 2000 and the other in the spring of 2001. Both deer were males between 3 and 4 years of age and appear to be full siblings based on genetic analysis. The deer were shot approximately 5 km apart and were less than 10 km from the Alberta-Saskatchewan border. Both deer appeared healthy when shot and the diagnosis was based on positive immunohistochemical staining of abnormal prion protein in the vagal nucleus of the brain. Approximately 5,000 wild deer and elk have been tested for CWD in Saskatchewan and to date only 2 have tested positive. Results of the ongoing CWD survey in the province will be reviewed.

(98) CHRONIC WASTING DISEASE IN WISCONSIN WILD WHITE-TAILED DEER: SURVEILLANCE PROGRAM, DETECTION AND FUTURE DISEASE MANAGEMENT PLANS

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Chronic wasting disease (CWD) is a progressively degenerative and fatal neurologic disease in deer (*Odocoileus* spp.) and elk (*Cervus elaphus*), believed to be caused by a transmissible protease-resistant prion. CWD is a disease threat to North American deer populations. Prior to 2001, CWD had only been diagnosed in wild free-ranging deer and elk in Colorado, Wyoming, Nebraska, South Dakota, and Saskatchewan. The Wisconsin Department of Natural Resources (WDNR) has had a CWD surveillance program for wild white-tailed deer (*O. virginianus*) since 1999. This program utilizes targeted sampling of deer 18 months of age or older who show clinical signs compatible with CWD, such as poor body condition and abnormal behavior. Additional CWD sampling is performed on deer 18 months of age or older from hunter harvested or sharpshot overabundant municipal deer. Hunter harvested deer were sampled from areas of Wisconsin based on presence of captive elk farms which imported elk from CWD infected Nebraska or Colorado farms, high density of both captive cervid farms and wild deer, or areas considered for establishment of new wild elk herds. Brainstem samples were collected from voluntarily submitted hunter harvested deer at registration stations, and submitted to the National Veterinary Services Laboratory (Ames, IA) for immunohistochemistry assay for CWD protease-resistant prion. Nearly 1000 deer were tested statewide 199-2001. Unexpectedly, 3 hunter harvested deer shot within three miles of one another during the 2001 hunting season tested positive for CWD. One buck had clinical signs compatible with CWD. Upon notification of these index cases, WDNR began immediate sampling of wild deer within an 11 mile radius, and identified 15 additional positive animals. Identification of CWD in these free-ranging Wisconsin deer is the first detection of this disease east of the Mississippi River. CWD is of great concern in Wisconsin and neighboring states due to high deer densities, agricultural and land use patterns, and recreational uses of this important wildlife resource. Many unknown parameters associated with CWD in white-tailed deer include agent transmission and transmission rates, latency period, genetic susceptibility, and population impacts. WDNR will expand CWD monitoring efforts statewide, and attempt to elucidate further information concerning CWD in white-tailed deer.

(99) EMERGENCE OF CHRONIC WASTING DISEASE IN WISCONSIN WHITE-TAILED DEER: INFORMATION FROM THE FIELD AND PRELIMINARY ANALYSIS

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Chronic wasting disease (CWD) is a progressively degenerative and ultimately fatal condition in deer (*Odocoileus* spp.) and elk (*Cervus elaphus*) associated with transmissible protease resistant prion proteins. Chronic wasting disease was previously only known to be present in wild cervids in Colorado, Wyoming, Nebraska, South Dakota, and Saskatchewan. Since 1999, the Wisconsin Department of Natural Resources has tested over 1000 white-tailed deer (*O. virginianus*) for the presence of CWD. Three of 345 deer harvested and tested state-wide during the 2001 hunting season were positive for CWD. These three cases were males, 2.5-3 years of age, shot within three adjacent square miles in south-central Wisconsin. A subsequent sample of 476 deer within an 11 mile radius of these index cases revealed a further 15 deer positive on testing of testing of both obex and retropharyngeal lymph node samples. Prevalence of CWD in a three mile radius of the harmonic mean location of positive deer was estimated to be 13% (95% CI 6-23%), and declined to 3% (95% CI 1-8%) and 2% (95% CI 0.2-7%), 3-6 and 6-9 miles from this center, respectively. Prevalence did not vary among males (prevalence 1.6%, 95% CI 0-6%) and females (2.6%, 95% CI 0.6% - 4.6%), but older females had higher than expected prevalence of CWD. This outbreak represents a significant expansion of the range of CWD, and evokes particular concern as very high deer densities in southern Wisconsin may facilitate rapid transmission of the agent and may have significant negative impacts on this important wildlife resource.

(100) EVALUATION OF TONSILLAR BIOPSY DATA FOR ESTIMATING CHRONIC WASTING DISEASE PREVALENCE IN FREE-RANGING MULE DEER

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We conducted a field study to evaluate tonsillar biopsy immunohistochemistry (IHC) as a tool for diagnosing chronic wasting disease (CWD) in live, free-ranging mule deer (*Odocoileus hemionus*) and estimating CWD prevalence. Using a simple mouth gag and a 6 mm biopsy forceps, and taking the biopsy starting at the rostral rim of the tonsillar sinus, 155/161 (96%) samples yielded ≥1 lymphoid follicle. To assure that biopsy-based prevalence estimates would not substantially underestimate "true" prevalence, we calculated CWD prevalence (p_b) using tonsillar biopsies from 161 free-ranging mule deer and compared this to prevalence (p_h) estimated from tonsil samples from 161 deer harvested or culled in spatial and temporal proximity to our study areas; we considered the latter a close approximation of "true" prevalence. Biopsy-based prevalence estimates exceeded prevalence estimated by tonsillar IHC of samples from harvested or culled deer. Although 95% confidence intervals (CIs) for p_h - p_b included 0 for area-specific estimates, biopsy-based estimates were ≥3 times higher than harvest-based estimates in both study areas. Moreover, when data from both study areas were combined p_h (= 0.025) was lower than p_b (= 0.081) and the 95% CI for $p_h - p_b$ (-0.104 – -0.007) did not include 0. Observed differences in prevalence most likely reflected spatial or temporal variation in populations (or subpopulations) of deer sampled. Tonsillar biopsy IHC appears to be reliable for detecting CWD infections in free-ranging mule deer and estimating prevalence in affected populations. Based on our findings, tonsillar biopsy is now being used in both surveillance and experimental "test-and-cull" CWD management programs in a few parts of northeastern Colorado where harvest or large-scale culling is not feasible.

(101) LAND-USE IMPACTS ON THE PREVALENCE OF CHRONIC WASTING DISEASE IN COLORADO MULE DEER POPULATIONS

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Anthropogenic changes on land cover can influence wildlife populations and their diseases both directly and indirectly. Over the last two decades, the Front Range of the Rocky Mountains in northern Colorado has experienced rapid growth in human populations. Land-use types have been converted from native areas and agricultural lands into residential developments, and regional projections of human population growth suggest that such trends will likely to continue. This particular region overlaps the southern portion of the geographic area where an epidemic of chronic wasting disease (CWD) has persisted in free-ranging cervids for over two decades. We have hypothesized that land-use changes may be shaping the spatial and temporal dynamics of CWD as infected deer and elk populations respond to both subtle and profound alterations in their native habitats. Our initial objective was to examine differences in CWD prevalence in free-ranging mule deer associated with two different land-use types. We broadly categorized land-use areas by housing densities: urban areas contained $\geq$1 housing unit/4 ha (we set a buffer of 1-2 km around such areas), and non-urban areas contained ranch, state, and federal lands with <1 housing unit/4 ha. We replicated sampling in three separate areas where urban and non-urban areas were juxtaposed. Field samples were collected via harvest/culling and tonsillar biopsy. Our preliminary analyses suggest that CWD prevalence was higher in sampled urban areas (11.9%; 95% CI = 6.8-17.0%) than in nearby non-urban areas (5.3%; 95% CI =3.5 - 7.1%). It follows that urbanization may be playing an undesirable role in exacerbating transmission and perhaps spread of CWD in mule deer in northeastern Colorado.

(102) ESTIMATING THE RELATIONSHIP BETWEEN CHRONIC WASTING DISEASE PREVALENCE AND MULE DEER DENSITY IN NORTHCENTRAL COLORADO

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Elucidating the relationship between chronic wasting disease (CWD) prevalence and mule deer (*Odocoileus hemionus*) population densities at the landscape scale is important in understanding and forecasting epidemic dynamics and spread across a large geographic area. It follows that estimating spatial distribution of mule deer across endemic landscapes is a necessary first step in evaluating strategies for controlling the spatial distribution of CWD. Although it is difficult to produce accurate estimates of the spatial distribution of mobile animals, mule deer are relatively faithful to historical wintering grounds, often returning to the same general location for generations. We used 3 yrs of aerial count data collected across roughly 150 km^2 of northcentral Colorado to develop a surface of estimated mule deer density based on observed spatial variability in mean deer counts. We also independently estimated CWD prevalence by sampling deer harvested in this same geographic area during the same years as inventories were conducted. Unfortunately, distributions of these two data sets rarely coincided on the landscape when examined at a biologically-meaningful spatial resolution. Consequently, we developed an approach for exploring this relationship based on models that estimated both population density and disease prevalence in locations where these two data sets did not coincide. Our preliminary results suggest that a significant amount of the variation in CWD prevalence observed across the studied landscape can be attributed to variations in estimated density of mule deer. Based on our initial findings, further exploration of the relationship between mule deer density and CWD prevalence is clearly warranted.

(103) MULE DEER (*ODOCOILEUS HEMIONUS*) MOVEMENTS IN RELATION TO SPATIAL PATTERNS OF CHRONIC WASTING DISEASE PREVALENCE IN NORTHCENTRAL COLORADO

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Chronic wasting disease (CWD) is endemic in northeastern Colorado and southeastern Wyoming. Prevalence in mule deer (*Odocoileus hemionus*) varies widely among different spatial areas but tends to follow biologically-relevant patterns: lower elevation foothills populations of mule deer at the core of this endemic area show highest prevalence, and around this core area prevalence declines to varying degrees in all directions. We have hypothesized that migration and dispersal movements of deer contribute to the observed spatial patterns of CWD prevalence. The primary goals of our study were to describe patterns of mule deer movement and determine whether deer movements to and/or from areas of high CWD prevalence were related to levels of CWD prevalence in deer populations residing in adjacent areas. From December 1999 to March 2002, we radiocollared 230 mule deer from representative populations throughout Larimer and northern Boulder Counties in northcentral Colorado. Radiocollared deer were located every 1–2 mo during January 2000–May 2002. We found little evidence to suggest that dispersal contributed to distant spread of the disease; only 1 of 98 radiocollared deer for which we had ≥1 yr of data dispersed from their original home ranges. However, migratory movements appeared to be related to CWD prevalence patterns. Populations in which prevalence was high on their winter range tended to migrate in summer to areas with equally high prevalence; in contrast, populations with lower prevalence on winter range migrated and summered in areas of even lower prevalence. We also observed that some populations with separate winter ranges had overlapping summer ranges. It follows that seasonal altitudinal migration patterns of some local deer mule populations may be responsible for the southern spread of CWD in northern Colorado. Our observations suggest that well-established historical migration patterns of mule deer may partially explain spatial patterns of CWD prevalence in northcentral Colorado and southcentral Wyoming.

(104) INTERSTATE MANAGEMENT PLAN FOR THE CONTROL OF CHRONIC WASTING DISEASE

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Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy of deer and elk endemic in southeastern Wyoming, northeastern Colorado, and perhaps southwestern Nebraska. Wildlife management agencies from these three states have formed a working group to develop a joint management plan for the control of CWD. Two goals to consider in such a plan would be to (1) stop the spread of CWD and (2) decrease the prevalence of CWD within the endemic area. These goals may be accomplished by taking some, or all, of the following actions: (1) continued surveillance within the endemic area; (2) intensive surveillance around new cases outside of the endemic area; (3) removal of clinically affected animals; (4) no translocation of deer or elk from the endemic area; (5) monitoring of captive cervid facilities; (6) restriction on movement of whole carcasses from the endemic area; (7) intensive population management and development of herd management plans; (8) restriction of artificial feeding or congregation of cervids; (9) habitat development to decrease crowding; (10) continued research and application of new techniques; (11) training of wildlife management professionals; and (12) full disclosure to the public on all aspects of CWD and encouragement of public participation in the efforts to control CWD.

(105) IMMUNOGLOBULIN G RESPONSES OF NORTHERN ELEPHANT (*MIROUNGA ANGUSTIROSTRIS*) AND PACIFIC HARBOR (*PHOCA VITULINA RICHARDSI*) SEALS NATURALLY INFECTED WITH *OTOSTRONGYLUS CIRCUMLITUS*

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Otostrongylus circumlitus (Nematoda:Metastrongyloidea) is a significant cause of mortality in Pacific harbor seals (PHS), *Phoca vitulina richardsi*, and northern elephant seals (NES), *Mirounga angustirostris*, along the central California coast. The adult nematode is principally a lungworm of seals under one year of age. The characteristics of infection in PHS are typical of those in other host species. Some NES however, have a more severe reaction to the parasite. The signs and symptoms are non-specific in this host, and occur during the pre-patent period of infection, so that the presence of larvae in feces cannot be used for diagnosis. The IgG responses of PHS and NES to tissues of adult *O. circumlitus* were examined by immunoblotting. Each seal that was positive at necropsy for *O. circumlitus* responded to all tissues of the nematode. Northern elephant seals of one year and older and the majority of PHS had an increased response to the excretory-secretory (ES) glands that was not seen in 2-9 month old NES. All young positive NES and a proportion of the older NES and PHS also responded to a 28 kDa band that was dominant in the female reproductive tract of the nematode. The potential of this band as a diagnostic marker was further investigated. The IgG responses of NES and PHS to adult *Parafilaroides* sp. (collected from PHS, NES, and California sea lions, *Zalophus californianus*), larval *Pseudoterranova* sp. and larval and adult *Anisakis* sp. were examined for cross-reactivity with *O. circumlitus* bands. Although a faint band of 28 kDa was present in *Parafilaroides, O. circumlitus* could be distinguished from this and the other genera tested using serum from both host species. The results suggest that *O. circumlitus* nematodes most likely die and disintegrate in these hosts and that NES of one year and older and most PHS respond differently to the ES glands than NES of 2-9 months. The 28 kDa band has the potential for a differential diagnostic test for *O. circumlitus* in young NES if used in conjunction with a fecal exam for *Parafilaroides* larvae.

(106) CANCER IN FREE-RANGING CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*): INVESTIGATIONS INTO THE ROLE OF A GAMMA HERPESVIRUS, ENVIRONMENTAL CONTAMINANTS AND OTHER CO-FACTORS

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For several years now it has been apparent that neoplasia is common in California sea lions (CSL) that strand along the California coast, accounting for about 20% of subadult and adult mortality. In a recent review of the literature, both captive and stranded CSL were over-represented in case reports of tumors in marine mammals. Additionally, subclincal tumors were found in about 6% of adult female CSL dying acutely of domoic acid intoxication. Both benign and malignant tumors are seen in CSL, but the most common (about 85%) are aggressive carcinomas of urogential epithelial origin that are found equally in males and females. As high level predators, CSL bioaccumulate a variety of contaminants including potentially oncogenic chemicals such at DDE's and PCB's. Naturally occurring marine toxins such as okadaic acid are also potential carcinogens. Recently completed analyses confirm preliminary data suggesting that total PCBs and DDEs as well as chlordanes are significantly elevated in CSL with neoplasms when compared to matched CSL without tumors that died of trauma.

Recent work in two different laboratories has also identified the presence (by PCR) of a potentially oncogenic sea lion gamma herpesvirus in 100% the tumors examined. Preliminary comparisons using PCR amplified and cloned sequences from two genes (DNA polymerase and terminase) show that this herpesvirus (proposed name Otarine herpesvirus-1, OtHV-1) is most closely related to the human Kaposi's sarcoma herpesvirus (HHV-8). Since carcinogenesis is a multi-step process requiring at least 2 mutational and promotional events, it is probable that cancers in CSL are multifactorial with both chemicals and infectious agents as likely co-factors.

Because the majority of cancers of CSL are of urogenital origin we hypothesize that OtHV-1 is a sexually transmitted virus. To test this hypothesis we are using PCR of oropharyngeal, vaginal or prepucial swabs and peripheral blood lymphocytes to determine presence of OtHV-1. We are also using PCR to locate the virus in multiple tissues taken from animals at necropsy. To date viral genome has been detected in 10/20 prepucial swabs, but no pharyngeal swabs or leukocytes collected from 20 free-ranging male CSL. In female CSL examined by PCR post mortem none of 9 females without neoplasia had viral genome in the lower genital track.

By comparison with sexually transmitted viruses in other species, we also hypothesize that OtHV-1 infection may be facilitated by abnormal genital flora as well as by the genetic background of individual animals. A large multidisciplinary study is underway to investigate the prevalence of the gamma herpesvirus infection in CSL and to examine for risk factors such as intercurrent viral and bacterial infections, reproductive activity, and genetics (MHC) in the development of cancers in CSL.

(107) CLINICAL PATHOLOGY OF HARP (*PHOCA GROENLANDICA*) AND HOODED SEALS (*CYSTOPHORA CRISTATA*) DURING THE BREEDING SEASON

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Each year, harp (*Phoca groenlandica*) and hooded seals (*Cystophora cristata*) migrate to NE Newfoundland and southern Gulf of St. Lawrence, Canada to breed in March. Because these species breed on remote offshore ice and have pelagic habits, few hematological and serum chemistry studies have been done. Whole blood was collected in K3 EDTA Vacutainer™ tubes from seals shot or live-captured during a scientific sampling of adult males and mother-pup pairs for various research projects. Whole blood was analyzed within 24 hours of collection at a local human hospital and sub-samples were sent to a certified veterinary laboratory for validation. Standard hematological parameters were determined for 28 harp seals and 22 hooded seals, and serum biochemistry values for 28 harp and 19 hooded seals. Significant inter-laboratory differences were found for some blood parameters. Adult female harp and hooded seals, and hooded seal pups had similar haematocrit (HCT) and hemoglobin levels (HB). Harp seal pups had the lowest HCT, HB, mean cell hemoglobin concentration (MCHC) and leucocytes counts, and showed a significantly higher proportion of nucleated red blood cells (NRBC) than other seals sampled. Hooded seal pups had relatively few NRBC. These differences between harp and hooded seal pups are likely due to the precocious development of hooded seals (lactation ≤ 4 days) as opposed to harp seals (lactation of about 12 days) – an adaptation for rapidly deteriorating ice conditions. Significant variations in biochemistry and hematology values occurred more frequently between age groups than between species.

(108) BIOLOGY, MOVEMENTS AND HEALTH ASSESSMENT OF FREE-RANGING MANATEES IN BELIZE

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Although relatively little information is known about West Indian manatees (*Trichechus manatus*) outside Florida, studies in other regions offer opportunities to better understand the behavioral ecology, life history and health of the species where they are less affected by anthropogenic factors and cold weather. We have studied Antillean manatees (*T. m. manatus*) in Southern Lagoon, Belize since 1997. Manatees were captured using a boat and a net set in open water 1-1.5 m deep. Fifteen individuals were captured 35 times between November 1997 and March 2002. Out-of-water holding time ranged from 24 to 140 minutes with an average duration of 90 minutes. Most animals were tagged with VHF and satellite radio tags connected to belts fitted around the tailstock and passive integrated transponder (PIT) chips were inserted to facilitate re-identification. Most of the tagged manatees were recaptured biannually to replace the tags or batteries. Health assessments were conducted based on clinical exams, ultrasonic fat measurements, hematology, blood biochemistry, and urine and fecal analyses. Morphometrics and skin tissue specimens for genetics were also collected. Data were also collected on seagrasses and environmental parameters such as salinity, water turbidity and temperature. Aerial surveys by helicopter were conducted twice a year to monitor population numbers.

Tagged females and few males stayed in Southern Lagoon, whereas some males roamed along the coast. The calving interval for three females was longer relative to Florida manatees (*T. m. latirostris*). Results of urine analyses revealed a new species of diplogasterid nematode. Fecal samples were not pathologic but did allow for identification of local vegetation types. Blood values for hematology and serum chemistry profiles where normal when compared to manatees previously examined in Florida and Puerto Rico. Duplicate samples were collected from six animals and analyzed to check for laboratory quality control. Variables examined to address potential affects of capture stress included the serum enzymes aspartate aminotransferase (range, 9.4-60.6 U/L), creatinine kinase (10.1-287 U/L), and lactate dehydrogenase (0-260 U/L); as well as biochemical indicators such as BUN (2.0-11.6 mg/dL), creatinine (0.76-3.0 mg/dL) and potassium (3.6-6.42 mmol/L). Higher-than-normal elevations of serum enzymes were not detected in 12 individuals sampled 20 times. Dugongs (*Dugong dugon*), a related species, have been prone to capture myopathy and typically display elevated serum enzymes and biological indicators of tissue damage when handled. Florida manatees have been documented to tolerate capture and handling activities without susceptibility to capture stress. No adverse effects of capture stress were detected post-release in the manatees handled in Belize. Future studies include a serologic survey for selected pathogens, bacteriologic and virologic analysis of samples and hormonal profiles. Prospective epidemiological studies comparing condition indices, hematological and serum biochemistry parameters within and among individuals, seasons and subpopulations will serve as indicators of health of this brackish lagoons ecosystem.

(109) SOUTHERN SEA OTTERS AND PATHOGEN POLLUTION: A PRELIMINARY STUDY OF EXPOSURE TO FECAL PATHOGENS

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The southern sea otter (*Enhydra lutris nereis*) is a federally listed threatened species found only in central California coastal waters. Previous postmortem examinations have implicated infectious agents such as bacteria and parasites in the death of up to 40% of fresh dead otters. Our preliminary data suggested that some sea otter deaths were caused by protozoa and bacteria which are also pathogenic to humans and domestic animals and could be forms of "pathogen pollution"coming from sewage or runoff. Fresh feces were obtained from 40 free-ranging sea otters and selective media were used to screen for presence of *Campylobacter* sp., *Clostridium perfringens, E. coli* 0157:H7, *Pleisomonas shigelloides, Salmonella* sp., and *Vibrio* sp. Fecal flotation and direct immunofluorescent antibody testing (DFAT) were used to screen for presence of *Giardia* and *Cryptosporidium*. Most of the 40 samples were obtained from apparently healthy animals which had hauled out or were captured for research purposes. Eight were from freshly dead sea otters presented for pathologic examination. Twelve additional fecal samples obtained from live stranded otters were screened for protozoal parasites, but not bacteria, as most had been treated with antibiotics. The majority of samples came from the greater Monterey Bay region. All of the major groups of bacteria and protozoa were detected in sea otter feces except for *E. coli* 0157:H7. In two animals these intestinal pathogens contributed to mortality. Fourteen of the 40 samples were positive for more than one organism. This is the first report of isolation of *Cryptosporidium* and *Giardia* from sea otters. We feel these preliminary findings have significant implications for southern sea otter recovery, for human health, and for waste water management in California's central coast region. From a policy perspective, it is interesting to note that this investigation was paid for by fines money levied against a municipality for repeated sewage spills.

(110) A PROGRAM TO PROTECT CAPTIVE SOUTHERN SEA OTTERS (*ENHYDRA LUTRIS NEREIS*) FROM MORBILLIVIRUS EXPOSURE AT A VETERINARY CARE AND RESEARCH FACILITY ADJACENT TO WILDLIFE HABITAT

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Prior to 1988, the genus morbillivirus included only 4 viruses: canine distemper virus (CDV), which infects primarily terrestrial carnivores; rinderpest virus (RPV), which infects cattle and other large ruminants; peste des petits ruminants virus (PPRV), which infects small ruminants and the measles virus (MV), which causes human measles. Morbillivirus in marine mammals was first reported in the spring of 1988 on Anholt, a Danish island in Western Europe. Aborted harbor seal pups were the index cases for a morbillivirus die-off eventually affecting over 18,000 seals. Subsequently, three morbilliviruses were identified in marine mammals: porpoise morbillivirus (PMV), dolphin morbillivirus (DMV) and phocine distemper virus (PDV). Phocine distemper virus was identified as the cause of the Anholt seal colony die-off. Prior to their identification in 1988, morbilliviruses were suspected as the cause of many marine mammal die-off events, including Antarctic crabeater seals (*Lobodon carcinophagus*) in 1955 and Bottlenose dolphins (*Tursiops truncatus*) in 1982, 1987 and 1988. In 1987, thousands of Baikal Seals (*Phoca sibirica*) died of distemper and the die-off was attributed to CDV, thought to originate from a terrestrial carnivore.

Morbilliviruses are transmitted through contact, by respiratory route and by excrement and body fluids. The symptoms can include fever, vomiting, watery hemorrhagic diarrhea, neurological symptoms, abortion and death. Treatment for Morbillivirus infection is palliative. It is currently prevented in humans and domestic animals by vaccination. Past attempts to vaccinate wildlife with modified-live vaccines have sometimes resulted in fatalities. Recent advances in vaccine technology have led to the development of the Merial Purevax ™ Ferret Distemper Vaccine, a live recombinant canarypox vector expressing the HA and F glycoproteins of canine distemper. This vaccine may be appropriate for certain high-risk captive wildlife. Grey foxes and black-footed ferrets, which previously have contracted distemper from modified live vaccines, have been safely vaccinated with this new vaccine. Merial Purevax™ Ferret Distemper Vaccine has also been used safely in several species of captive animals at risk for contracting distemper including domestic ferrets, red foxes, red pandas and large cats. There is no information available on the susceptibility of sea otters, but other mustelids such as the black-footed ferret have been reported to have a high susceptibility to CDV. We now report the vaccination of captive Southern sea otters (*Enhydra lutris nereis*), also a mustelid, using this "ferret-safe" vaccine.

The California Department of Fish and Game, Marine Wildlife Veterinary Care and Research Center (CDFG-MWVCRC) currently houses two male Southern sea otters. These animals are part of an ongoing research project at the University of California, Santa Cruz, Long Marine Laboratory where they are trained to voluntarily participate in research. Captive marine mammals are kept in environments where all reasonable measures are taken to protect them from disease. However, it is impossible to remove all risk of exposure from feral, companion and wild animal species known to harbor morbilliviruses. Shortly after CDFG-MWVCRC was completed it became apparent that wildlife from adjacent agricultural fields, the Younger Lagoon Ecological Reserve, Natural Bridges State Park and suburban and rural areas were gaining access to the grounds and interior compound. These animals include raccoons, bobcats, resident and migratory birds, sea birds and other species. Of these species, raccoons are the most likely to transmit disease to captive sea otters. Locally, raccoons have been reported to have a high incidence of mortality due to CDV. The facility has a series of physical barriers and fencing modifications that were made to exclude wildlife, but raccoons were able to gain access to the animal care areas despite efforts to bar their entry. Veterinarians from the University of California, Santa Cruz, CDFG-MWVCRC and the Monterey Bay Aquarium concluded that the risk of morbillivirus warranted vaccination. Following the manufactures' recommended protocol, a series of three vaccines given three weeks apart were administered subcutaneously to each animal. Blood was drawn prior to vaccination and at post vaccine intervals. Serum samples were evaluated for CDV titers using a serum neutralization method. Serum neutralization is not thought to be species-specific; hence, it is thought to be valid for use in sea otters.

The animals were monitored closely following the initial vaccinations and for each additional administration of the vaccines. There were no observed reactions. Their activity, attitude and behavior remained normal following vaccination. The animal care staff also reported that both animals continued to behave and eat normally throughout the vaccine series.

The incidence of vaccine reaction in ferrets reported for the Merial vaccine is 0.3% (3 in 1,085 ferrets vaccinated had serious adverse side effects described as anaphylaxis and all survived with treatment). At the time of each vaccination, a protocol was followed that included the presence of a veterinarian, as well as the availability of oxygen, epinephrine (a fast-acting vasoconstrictor), diphenhydramine (an antihistamine) and Solu-Delta-Cortef^{R} (a rapid-acting glucocorticoid). Uneventful vaccination of two animals does not rule out the possibility of future vaccine reactions. Future plans for a vaccine protocol include a veterinary exam every three months, a yearly booster, the presence of veterinarian at the time of vaccination, anti-anaphylaxis drugs, oxygen and close monitoring for booster vaccinations and follow-up antibody serum titers.

(111) A REVIEW OF POTENTIAL INFECTIOUS DISEASE THREATS TO SOUTHERN RESIDENT KILLER WHALES (*ORCINUS ORCA*)

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The southern resident stock of killer whales (*Orcinus orca*) is declining and now numbers only 78 individuals. This population, also called the eastern North Pacific southern resident stock of killer whales, resides in the shared coastal waters of Washington State and the Province of British Columbia. The Canadian Committee for the Status on Endangered Wildlife in Canada (COSEWIC) recently listed this stock as endangered. The U.S. National Oceanic & Atmospheric Administration Fisheries (NOAA Fisheries) has formally initiated a status review for southern resident killer whales under the U.S. Federal Endangered Species Act. The Center for Whale Research (Friday Harbor, WA) has taken an annual census of the southern resident killer whale population since 1976. Reasons for the decline in this population since 1996 are unknown, but high tissue contaminant levels, decreased prey abundance, and increased vessel traffic have been suggested. Information about the role that infectious diseases may play in this population's decline is not available, nor is information regarding the threat that infectious diseases may play in the long-term viability of this small population. Infectious agents that have been reported in free-ranging killer whales and captive killer whales were identified using available databases and reference literature. Also reviewed were infectious agents not specifically reported in killer whales, but reported in free-ranging and captive odontocetes sympatric to southern resident killer whales, particularly common dolphins (*Delphinus delphis*), Dall's porpoises (*Phocoenoides dalli*), false killer whales (*Pseudorca crassidens*), harbor porpoises (*Phocoena phocoena*), northern right whale dolphins (*Lissodelphis borealis*), Pacific bottlenose dolphins *(Tursiops truncatus*), Pacific white-sided dolphins (*Lagenorhynchus obliquidens*), Risso's dolphins (*Grampus griseus*), short-finned pilot whales (*Globicephala macrorhynchus*), and striped dolphins (*Stenella coeruleoalba*). Some of the pathogens reviewed could be responsible for reduced recruitment within the southern resident killer whale population. Others identified have the potential to impact the long-term viability of this population. The potential impacts of pathogens such as marine *Brucella* spp., cetacean poxvirus, herpesviruses, and morbilliviruses will be discussed. Efforts need to continue in order to learn more about infectious diseases of free-ranging southern resident killer whales and sympatric odontocetes. For this reason, it is imperative that when possible, complete postmortem evaluations (including analyses to detect presence/absence of infectious agents) need to be performed on all odontocetes stranded or accidentally caught in fishing gear throughout the range of the southern resident killer whale population.

(112) CLASSIFICATION OF EPIDERMAL LESIONS: POTENTIAL ETIOLOGIES, CHARACTERIZATION AND SIGNIFICANCE IN THE BOWHEAD WHALE (*BALAENA MYSTICETUS*)

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Cetacean skin is a highly specialized organ with diverse functions. The reported causes of cetacean epidermal lesions include aberrant or anomalous sloughing; direct trauma; bacterial, fungal and viral infection; and parasitism. Bowhead whale epidermal lesions found during the 1970's and early 1980's were classified into five groups using gross description only: circular depression, sloughing type, raised roughened areas, roughened flat areas and depressed irregularly shaped areas (Philo, 1993). These epidermal lesions were considered benign and self-limiting (J.T. Haldiman, unpublished data), though few were characterized histologically and their cause undetermined. We reviewed samples of additional epidermal lesions collected from bowhead whales (*Balaena mysticetus*) subsistence-harvested from areas at or near Point Barrow, Alaska. Lesions have been described at a gross level and when possible under light microscopy. In this study, we assess the various epidermal lesions in conjunction with information gathered as part of a recent bowhead whale health assessment program. Included is a discussion of functional and pathological aspects of cetacean skin and the potential impact of different causative agents on mysticete populations.

(113) INCREASED GRAY WHALE (ESCHRICHTIUS ROBUSTUS) STRANDINGS IN 1999 AND 2000 – WAS MALNUTRITION THE CAUSE?

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In 1999 and 2000, the number of gray whale (*Eschrichtius robustus*) strandings along the west coast of North America increased to seven times the mean annual stranding of 52 animals between 1995 and 1998. By 2001 strandings decreased again to 21 whales in total. The majority of the dead whales occurred in the breeding lagoons in Baja California, Mexico. In the U.S., mortality occurred throughout the migration route, with the most dramatic increase in mortality occurring in Alaska and a cluster of animals (about 30) found floating dead in San Francisco Bay in April and May 2000. Carcass examination was limited because of inaccessibility or stage of decomposition of the carcasses. Only three animals that stranded in the U.S. received complete post mortem examinations. These three animals stranded alive in California, and were euthanized due to poor prognosis based upon their emaciated condition and prolonged stranding. All three were young animals, one had a granulomatous enteritis associated with *Bulbosoma balanae*; one had histological changes in the cerebrum suggestive of viral encephalitis and was seropositive to western, eastern and Venezualan equine encephalitis but no virus was isolated from cultured brain tissue; the third had the biotoxin, domoic acid, in blood, urine and feces, suggesting intoxication, as well as transmural abscesses in the gut-associated lymphoid tissue. Whales that were found dead in San Francisco Bay were mostly observed in or adjacent to the main shipping channels. One of these animals that beached had propeller wounds along its dorsum. A second whale was reported to have been hit by a tug-boat, but the carcass was not recovered. It is likely that malnutrition was an important predisposing factor in the mortality of each of these cases. The ultimate cause of the malnutrition of gray whales is unexplained, although changes in distribution of sea ice, El Niño, the Pacific Decadal Oscillation, global warming and a population at carrying capacity have all been suggested as ultimate causes. Detailed examination of stranded animals, coupled with long term monitoring of gray whale populations and their prey base are needed to determine the ultimate cause of the increased strandings in 1999 and 2000.

(114) TOLAZOLINE REVERSAL OF XYLAZINE IN BISON (*BISON BISON*): MITIGATION OF ADVERSE EFFECTS

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Tolazoline is a mixed alpha-1 and -2 adrenergic antagonist used to reverse the sedative, analgesic and muscle-relaxing effects of xylazine, a potent alpha adrenergic agonist. Tolazoline has been used in cattle and is superior to yohimbine, another alpha adrenergic antagonist, in this species. In white-tailed deer, tolazoline shortened recovery times and reversed xylazine-induced bradycardia, respiratory depression, and bloat following xylazine-ketamine anesthesia (Kreeger et al. 1986). We have used it for a number of years in moose without any detected adverse reactions. Caulkett et al. (2000) used tolazoline in wood bison to reverse the xylazine-induced effects of xylazine-tiletamine/zolazepam anesthesia and did not report any ill effects. However, the reported side effects of tolazoline in horses (species for which the drug was developed and is labeled) include abdominal discomfort, gastrointestinal hypermotility, diarrhea, tachycardia, ventricular dysrhythmia, hypertension and apprehensiveness.

During field studies of bison in the greater Yellowstone area, we observed several instances of unusual behavior in bison 1-2 days after anesthetic antagonism. These clinical behaviors included nervousness, pacing and aggression, repeated standing and sternal recumbency, continuous tail swishing, abdominal spasms with head bobbing, and repeatedly rocking to the side during sternal recumbency. Because we first observed this behavior after we included tolazoline in our anesthetic regimen, we hypothesized that bison were responding to the gastrointestinal stimulatory effects of tolazoline. However, in the few bison we have reversed with atipamezole, a much more selective and potent alpha-2 antagonist, we have occasionally seen similar reactions. Other components of our anesthetic protocol are unlikely to cause such signs. Signs are not consistent with renarcotization, and neither carfentanil nor xylazine is known to produce similar symptomology. To test our hypothesis and rule out other aspects of our bison anesthesia, we conducted two experiments under controlled and field conditions.

In the controlled experiments, we used 29 adult cow bison at Ft. Niobrara National Wildlife Refuge. We randomly assigned the bison to one of three treatment groups: Group I received high dose tolazoline (1.6 mg/kg IV); Group II received low dose tolazoline (0.7 mg/kg IM); and Group III received saline (2ml, 0.9% saline, IM). We immobilized all bison with carfentanil (10 µg/kg body weight) and xylazine (110 µg/kg body weight) by hand injection into the left thigh while restrained in a chute. Five minutes after induction, we reversed the anesthetics with naltrexone (125mg/mg carfentanil, IM and SC) and the assigned dose of tolazoline. We allowed bison to spontaneously recover and monitored their behavior over the next 3 days in a 1 ha (day 1) and 40 ha (days 2 and 3) enclosures. In the field trials conducted during a research project on the National Elk Refuge, we immobilized 18 free-ranging, fed, tagged bison with carfentanil and xylazine remotely by dart gun. We used 4.8mg carfentanil combined with 50mg xylazine, and antagonized with 300mg tolazoline IM and 600mg naltrexone IM and SC for the first 7 adult bison captures. The last 11 bison were captured using 5.1mg carfentanil and 20-30mg xylazine (adults), or 4.2mg carfentanil and 10mg xylazine (yearlings) and antagonized with naltrexone

alone. We visually examined bison the following morning and periodically during operations of that day.

In the controlled experiment, both positive and negative effects of tolazoline in bison were observed. Group III (control) had a mean recovery time of 5.37 minutes compared to Group I (high dose tolazoline) and Group II (low dose tolazoline) mean recovery of 3.35 and 4.86 minutes, respectively. The high dose, IV route, provided the fastest recovery and was significantly different from low dose and control groups. We observed sedation and incoordination, lasting up to 7 hours, only in the control group, an indication that tolazoline effectively reversed the lingering effects of xylazine. Although somewhat slower to induce recovery, the IM low dose tolazoline was just as effective as the IV high dose at reversing xylazine-induced sedation. However, both high and low dose groups suffered from adverse effects of tolazoline. Tail swishing and agitation were common in both tolazoline groups and absent in the control group. These adverse effects occurred in 10-100% of bison in each group at any one particular observation time. Although the effects lasted as long as 44 hours, we did not see the severe signs (abdominal spasms with head bobbing, thrashing, repeated rising and bedding) we observed in the field.

In our field experiment, negative effects of tolazoline were much more pronounced. All 7 bison that received tolazoline were agitated, nervous, twitching, pacing, and tail swishing by the following morning. We observed 6 of 7 to repeatedly rise and recline. One animal was severely affected, repeatedly thrashing to its side while in sternal recumbency, and drew the attention of several bulls in the herd. We saw these same adverse responses in only 1 of 11 bison that did not receive tolazoline. This one bison recovered normally and showed no adverse effects the afternoon of capture, but the following morning was highly agitated, tail swishing and occasionally flailing to her side. This animal had 20-22 cm long gore wound in the center of a patch of reddened hairless skin in her lower left flank (the side to which she persistently rolled in the snow). All other bison behaved normally, were on feed lines, and interacted with herd mates in a calm manner.

Our data indicate tolazoline can have severe adverse effects in bison, likely secondary to gastrointestinal spasms. The response of bison may be more generalized to any potent alpha-adrenergic antagonist based on the limited few similar responses we have seen with atipamezole in this species. The one field bison not treated with tolazoline that showed adverse recovery signs had behavior referable to abdominal pain on the left side, the location of significant trauma. Likely her clinical behavior was related to her injury rather than to drugs. Xylazine-induced sedation in bison is effectively antagonized by tolazoline, however the adverse effects are so pronounced that we no longer recommend its use in this species. Instead we get the same anesthetic effect by increasing the narcotic component of our anesthesia and decreasing xylazine to a level where no antagonism is needed. We don't know why Yellowstone area bison under field capture regimens show a much more severe reaction to tolazoline compared to bison kept at Ft. Niobrara, Nebraska, but this phenomenon may be related to genetics and/or nutrition.

(115) AN UPDATE OF ADENOVIRAL HEMORRHAGIC DISEASE IN MULE DEER IN CALIFORNIA

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In the summer and fall of 1993 a newly recognized disease, adenoviral hemorrhagic disease, caused widespread mortality in black-tailed (*Odocoileus hemionus columbianus*) and California mule deer (*Odocoileus hemionus californicus*) in northern California. Greater than a thousand deer were estimated to have died as a result of this epizootic in 17 counties extending from the Oregon border to Yosemite National Park. Fawns appeared to be more susceptible to adenoviral infection, but all ages of deer suffered from this infection. Clinical disease, when observed, was reported to follow a rapid course. The most prominent gross lesions noted were consistently found in the lung and alimentary tract (pulmonary edema and congestion and hemorrhage in the jejunum). In some deer, mucosal erosions and/or ulcerations were seen in the oral cavity and esophagus. Since 1993, only sporadic small epizootics have been documented. The adenovirus associated with the clinical disease was isolated and characterized, and clinical disease has been reproduced experimentally. A serum-virus neutralization test was developed using newborn deer lung cells. Adenovirus antibody titers were determined on deer sera collected from 1980 to1999 in areas experiencing recurrent disease outbreaks and in areas with no history of disease outbreaks. The results of this serologic survey will be discussed.

(116) SURVEY OF DISEASES DIAGNOSED IN CALIFORNIA BLACK-TAILED DEER AT THE CALIFORNIA ANIMAL HEALTH AND FOOD SAFETY LABORATORY, 1992-2002

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Case records of the California Animal Health and Food Safety Laboratory System were reviewed for causes of mortality in deer submitted between July 1992 and May 2002 to determine disease trends in California Black-tailed deer (*Odocoileus hemionus columbianus*). During this period, newly emerging diseases included adenovirus hemorrhagic disease and systemic Neosporosis. Epizootic hemorrhage disease was associated with high mortality that occurred in Black-tailed deer in southern California in 2000. A syndrome of unknown etiology was apparently associated with herd mortality in four northern California counties in 2001-2002. Rumenitis and nephrosis were changes seen in four deer examined from affected counties.

(117) EPIZOOTIC OF HEMORRHAGIC DISEASE IN MULE DEER IN ARIZONA

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Epizootic hemorrhagic disease (EHDv) and bluetongue virus (BTv) cause clinical hemorrhagic disease (HD) in several free-ranging and captive ungulate species in North America. Hemorrhagic disease was first diagnosed in deer in Arizona in 1993, and epizootics of HD among wild ungulates in Arizona seem to be unusual despite some serologic evidence suggesting that infection by the causative viruses may be common. The epizootiology and pathogenesis of HD in animals in the desert southwest remains largely unknown. Our goal in this work was to document the causative agents and investigate the epizootiology of HD in mule deer (*Odocoileus hemionus*) during an epizootic that occurred in Arizona in 2001. We investigated two male mule deer mortalities that occurred near Prescott, AZ in September 2001. Based on extensive hemorrhaging to internal organs, HD was the preliminary diagnosis. Bluetongue and epizootic hemorrhagic disease (EHD) viruses were identified via polymerase chain reaction (PCR), but attempts to isolate either virus were unsuccessful. To determine geographic range and viral serotypes involved, we collected blood samples from hunter-harvested male mule deer in three game management units near Prescott, AZ in October 2001. Forty-one blood samples were collected and antibodies against EHDv and/or BTv were found in 21/41 (51 %) samples. Of those 21, evidence of exposure to EHDv serotype 1 was found in 8/21 (38 %) and serotype 2 was identified from 17/21 (81 %). BTv serotype 2 was identified from 20/21 (95 %), serotype 10 was found in 18/21 (86 %), bluetongue serotype 11 was identified in 19/21 (90 %), serotype 13 was identified in 21/21 (100 %), and serotype 17 was found in 3/21 (14 %). Serologic evidence of exposure to multiple serotypes of both BTv and EHDv was found in mule deer sera from all three game management units sampled. Although EHDv and BTv are common in other areas in North America, their detection in Arizona is unique as HD has been diagnosed in only five clinical cases in Arizona. We will discuss possible reasons for the low prevalence and recent detection of this disease.

(118) OCULAR DISEASE IN MOOSE (*ALCES ALCES*) ASSOCIATED WITH CAROTID ARTERY WORMS (*ELAEOPHORA SCHNEIDERI*) IN IDAHO

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The carotid artery worm (*Elaeophora schneideri)* is a relatively common parasite in mule deer (*Odocoileus hemionus*) in the western United States. Clinical problems including necrosis of the ears, muzzle and blindness have been reported in elk (*Cervus elaphus*) and in one moose (*Alces alces*). Over the past several years, numerous moose in southeastern Idaho have been reported to be blind, disorientated and behaving abnormally. Diagnostic efforts including necropsy, histopathology, and bacteriology have been done on 5 moose with these clinical signs. Carotid artery worms have been found in 4 of these animals, all of which had ocular lesions. Case summaries and lesions in these animals will be described as these cases may represent a new problem in moose populations that are rapidly expanding into areas that overlap with mule deer.

(119) EPIDEMIOLOGY OF ABOMASAL PARASITES OF MUSKOXEN ON BANKS ISLAND, NORTHWEST TERRITORIES, CANADA

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As part of a broader effort to define the biodiversity and effects of mammalian parasites in northern ecosystems, we investigated abomasal parasites in muskoxen from Banks Island, Northwest Territories. Objectives were to determine: (1) parasite distribution among age (calves, yearlings, sub-adults, and adults) and sex classes; (2) the pathology associated with larval nematodes; (3) the relationship between serum pepsinogen and intensity of infection; and (4) seasonal patterns of nematode egg production. We quantified parasites in feces and abomasa from 113 and 40 muskoxen collected in November 1999 and from March to May 2001, respectively, and approximately 54 fecal samples collected each month from May 2001 to August 2002. We also determined serum pepsinogen levels in the muskoxen collected in the spring.

Teladorsagia boreoarcticus and *Marshallagia marshalli* were the most common abomasal nematodes. In November, yearlings had the highest prevalence and intensity of adult nematodes in the abomasum and eggs in the feces, and the highest ratio of adult to larval nematodes. In adult cows in the spring, the number of larvae in the abomasal lumen relative to the mucosa, and the total number of immature adults increased from March to May. The severity of mucosal lesions, weight of the abomasa, and serum pepsinogen levels also increased during this time period. Serum pepsinogen levels were positively correlated with the total number of larvae in March, but not in April or May.

The seasonal abundance of *Marshallagia* eggs in feces was similar to the pattern described for this parasite in Svalbard reindeer: prevalence of eggs in feces was highest in November and April, and the number of eggs per gram (epg) of feces peaked in December and April. In contrast, the prevalence of eggs of *Teladorsagia* was highest from August through October with a very small rise from January through May; epg of feces peaked in August with a small increase in May.

This study has helped to elucidate the life history and effects of the abomasal parasites *Marshallagia* and *Teladorsagia* in muskoxen. Results indicate that arrested development of abomasal nematodes occurs during the winter and subsequent larval development in April and May results in severe abomasal pathology that may cause clinical disease. Abomasal parasitism may be an important regulating factor in this muskox population.

(120) EFFECTIVENESS OF *BRUCELLA ABORTUS* STRAIN 19 SINGLE CALFHOOD VACCINATION IN ELK (*CERVUS ELAPHUS*)

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Brucellosis in Greater Yellowstone Area (GYA) bison and elk has been a source of controversy and focus of the Greater Yellowstone Interagency Brucellosis Committee (GYIBC) for years. Brucellosis has been eradicated from cattle in the 3 states of Wyoming, Montana, and Idaho and all three states currently are classified as "brucellosis free" with regard to livestock. Yet free-ranging elk that attend feedgrounds in the GYA, and bison in Yellowstone and Grand Teton National Parks, still have high seroprevalence to the disease and are viewed as a threat to the state-federal cooperative national brucellosis eradication program. Recently, cattle in eastern Idaho were found infected with brucellosis and transmission was apparently from fed elk. The GYIBC, formed of state and federal agencies involved in wildlife and livestock management in the 3 states, has committed to eventual elimination of the disease from wildlife. Management tools to control or eliminate the disease are limited; however, wildlife vaccination is one of the methods currently employed. Effective wildlife vaccination depends on dose efficacy, deliverability, and safety to non-targeted species. We commenced a single-dose efficacy study of vaccine *Brucella abortus* strain 19 (S19) in elk in 1999.

We included a control and vaccinate group from two different year cohorts in the study design. Approximately 25 elk were in each group of control and vaccinate elk, for each year cohort, resulting in about 100 elk in the study. Elk were bred as 3.5 and 2.5 year olds for those captured in 1999 and 2000, respectively, and pregnant elk were challenged midgestation. Evaluation criteria for vaccine efficacy will be differences in abortion rate and cow infection rate between control and vaccinated elk.

All elk were captured as calves by netgun during February of their respective years from southwestern Montana (75) and northeast Idaho (25) in areas previously surveyed and known *Brucella* free. Each was bled, ear tagged and transported to the Wildlife Health Laboratory in Caldwell, ID where they were kept in age and treatment segregated enclosures. Enclosures were separated by a minimum 10 ft gap between any enclosure and adjacent pens. Animals were allowed to acclimate for 3-5 weeks, then bled again and vaccinated intramuscularly with S19 or saline (2ml) for vaccinates and controls, respectively, in March of their capture year. Vaccine was procured from and titrated by Colorado Serum Company and consisted of 4.42×10^9 colony-forming-units (CFU) and 8.58×10^9 CFU per dose for 1999 and 2000 vaccinations, respectively. Periodic assessments of *Brucella* serology were conducted throughout the study and included Card, Buffered Acidified Plate Antigen, Standard Plate, Standard Tube, Rivanol, and Complement Fixation (CF). We placed bulls with cow elk in the fall of 2001 for breeding and determined successful pregnancy by pregnancy specific protein B (PSPB) and ultrasound January 2002. PSPB and transrectal ultrasound results at challenge were used as the final

determination of pregnancy status entering the challenge phase of the experiment. Elk were considered pregnant when both tests concurred. Based on these data, 89 elk entered the challenge phase as pregnant adults (47 3-year-olds, 42 2-year-olds). We challenged elk with 1 x 10^7 CFU of pathogenic *Brucella abortus* strain 2308 by bilateral intraconjunctival sac instillation on February 28, 2002. Following challenge, elk were placed in 3 pens of approximately 30 elk each containing equal numbers of control, vaccinate, 2 and 3 year olds. We used this arrangement to control for any potential secondary *Brucella* exposure following abortions or for pen effects. We collected abortions daily and froze the fetuses for later *Brucella* culture. Following abortion or calving, cows were euthanatized, necropsied and tissues frozen for *Brucella* culture. Live born calves will be allowed to survive 5 days, then euthanatized and tissues cultured. Genetic markers have been detected for all cows and bulls to permit determination of maternity for those abortions or calves for which maternity could not be determined by observation.

All elk were seronegative at capture and before vaccination. Results to date indicate all vaccinates responded strongly to S19 vaccination. 100% of vaccinates seroconverted on all 5 serologic tests, with rapidly rising and high titers (maximum geometric mean CF titers of 1:147 and 1:110 for 1999 and 2000 cohorts, respectively) within 1 month of vaccination. This titer fell rapidly over the next 2 months. By one year post-vaccination, seropositive reactions occurred in less than 15% of vaccinates and were generally negative on CF. Most positive reactors were positive on only 2 or 3 tests. Controls remained seronegative throughout the study until challenged in February 2002. At this writing (April 2002) abortions are underway in all 3 pens. We will present preliminary data on abortion rates and serologic response to challenge in control and vaccinated elk. Bacteriology will not be completed by conference time.

(121) ANALYSIS OF LECTINS AS ORAL VACCINE ADJUVANTS BOUND TO *BRUCELLA ABORTUS* STRAIN RB51, IN BALB/C MICE

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The final stages of *Brucella abortus* eradication in the United States have been thwarted by the continued maintenance of this disease in elk and bison of the Greater Yellowstone Area (GYA). Current disease management options for GYA bison include remote vaccination with *Brucella abortus* Strain RB51. SRB51 is the approved *Brucella* vaccine in cattle, and preliminary studies indicate it is effective in bison when parenterally delivered. Oral delivery of SRB51, if safe and efficacious, would offer the opportunity for more widespread and cost effective vaccination, as well as the potential for increased mucosal immunity. A study was undertaken to assess lectin modification of SRB51 for its effects on oral immunization.

Plant lectins and other lectin like proteins may assist in increasing mucosal uptake of orally administered vaccines. Lectins are non-immunoglobulin proteins capable of binding to complex carbohydrates. They may be soluble or membrane bound. Examples include: concavalin A (Con A), lentil, peanut, potato and soybean lectins, K88, K99, and p987 *E. coli* pili, viral hemagglutinin, cholera toxin, and *E. coli* heat labile toxin. Lectins bind to glycolipids and glycoproteins on the intestinal mucosa and are transported across the intestinal mucosa into the systemic circulation. In this study Con A and soybean lectin were combined with SRB51 and given orally. Bacterial uptake, colonization, and induction of humoral and cell mediated immune responses were measured.

The first phase of the study was a determination of bacterial penetration in lymph nodes (head and mesentery), intestine and spleen at 1, 3, 8, and 24 hours post oral vaccination. The second phase measured *Brucella* colonization of the spleen, head and mesenteric lymph nodes, at 3 days post oral or intraperitoneal vaccination. In the third phase, colonization of spleen and lymph nodes, as well as humoral and cellular immune responses were measured at 2, 4, and 8 weeks after the last immunization. *Brucella abortus* strain 19, SRB51, and modifications of SRB51 were tested.

(122) RISK MANAGEMENT AT YELLOWSTONE NATIONAL PARK: BISON AND BRUCELLOSIS

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In December 2000, Yellowstone National Park agreed to implement a long-term interagency risk-management program that conserves the wild, free-ranging bison population while also reducing the risk of transmission of brucellosis to livestock in Montana. In 1917, brucellosis was detected in Yellowstone bison and the present population is considered 40-50% seropositive. During the early and mid-20th century, bison management at Yellowstone NP varied between intensive animal husbandry to increase the population, intensive population reduction for range condition and brucellosis eradication, and the present natural regulation paradigm. Since the late 1980s, there has been considerable dissension between state and federal agencies about what the predominant disease management direction should be. Agricultural interests have maintained that near-term eradication is the appropriate solution for the chronically infected Yellowstone bison population. The National Park Service has consistently maintained that the appropriate direction is long-term risk management. The Interagency Bison Management Plan (IBMP) focuses primarily on temporal and spatial separation of bison and livestock outside the park and vaccination. The plan includes a multi-step adaptive management framework that also requires new research that answers important management questions.

During FY2002-2005, the IBMP includes development and implementation of a practical, safe and effective program for delivery of a safe and effective brucellosis vaccine to vaccination eligible free-ranging bison inside the park. In 2004-05, the park expects to complete legal compliance (National Environmental Policy Act) on remote vaccination of bison. The park then expects to begin vaccinating free-ranging bison calves and yearlings with a remote ballistic delivery system. The expected specifications of a bona fide remote vaccination system for bison inside the park include that the delivery system must be:

- Simple and practical (e.g. can be transported by foot or horse and operated by a two-person team in a wilderness setting under all weather conditions).
- Capable of being operated annually for an expected 20-year life span with measurable improvements in delivery effectiveness during the first five years.
- Designed and implemented so as to curtail to the degree possible the intrusiveness into the natural and human environments.

The expected issues that determine the effectiveness of a remote delivery system include:

- In all likelihood, the program will take the form of remote ballistic delivery (e.g. with modified conventional smokeless powder or compressed air firearm) of a bio-absorbable polymer “bio-bullet” impregnated with the vaccine to individual target animals.
- A practical remote vaccine delivery system will need to account for biological and mechanical constraints that will likely include a) tolerance of targeted bison for multiple vaccine dosages, b) whether marking of bison is required to avoid multiple doses and c) effective range of delivery.

- The efficacy of remote vaccination will be the product of the effectiveness of the vaccine and the effectiveness of the delivery system. Example: If a remote vaccination system is targeted at an estimated 80% of vaccination eligible bison and effectively delivers a biobullet containing a 75% effective vaccine to 75% of targeted animals, then the system will achieve approximately 45% vaccination efficacy of all vaccination eligible bison (0.80 x 0.75 x 0.75 = 0.45).

The expected issues that influence the intrusiveness into the natural and human environment include:

- The delivery system will need to achieve an effective range of delivery so as to optimize vaccine delivery efficacy while concurrently minimizing resultant individual, group and herd aggressive, flight or otherwise abnormal bison behaviors.
- The delivery system will need to achieve an effective delivery described above while also being reasonably cost-effective.
- The delivery system will need to be able to be implemented at a range and in a manner that will not put field crew(s) at unmitigated risk.

Vaccination of large free-ranging ungulates without capture is unprecedented. In order to develop and test the safety and efficacy of a remote vaccine delivery system, Yellowstone National Park is working to coordinate with state, federal and private-sector expertise to improve existing technologies including bio-absorbable polymers, frangible armaments, vaccine encapsulation and animal health and welfare.

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