#### PRESENTATION SCHEDULE

MONDAY, August 9	
8:00-8:20	WELCOME AND OPENING COMMENTS  Keith Prasse, Dean Susan Little and Charlotte Quist, Conference Co-Chairs John Fischer, Scientific Program Chair
	INTERNATIONAL RABIES SYMPOSIUM (8:20 – 12:00) Moderator: Charles Rupprecht
8:20-8:50	(1) THE RABIES PARADOX: WILL CHANCE STILL FAVOR THE PREPARED MIND?  CHARLES E. RUPPRECHT
8:50-9:20	(2) RABIES VIRUS: REFLECTIONS ON ITS EVOLUTION.  ALEXANDER I. WANDELER
9:20-9:50	(3) AUSTRALIAN BAT LYSSAVIRUS – CURRENT SITUATION AND SOME EXPERIMENTAL STUDIES. K. A. McCOLLI, R. A. LUNT, P. T. HOOPER
9:50-10:15	BREAK
10:15-10:45	(4) ECOLOGY & POPULATION BIOLOGY OF BAT RABIES IN THE UNITED STATES.  SHARON L. MESSENGER
10:45-11:15	(5) RABIES IN LATIN AMERICA: IT'S A JUNGLE OUT THERE!  CECILIA C. DeMATTOS, MYRIAM FAVI, VERÓNICA YUNG, SALVANA FAVORETTO, NELIO MORAIS, ELIZABETH LOZA-RUBIO, ALVARO AGUILAR-SETIÉN, CARLOS A. DeMATTOS
11:15-11:30	(6) A PROPOSED MIDDLE EAST REGIONAL RABIES CONTROL PROGRAM.  S.B. LINHART, M. AMARIN, R. de ROOIJ, A. el-TAWEEL, M. HASSUNAH, A. SHIMSHONY
11:30-12:00	(7) SUCCESS AND SETBACKS OF THE ORAL VACCINATION OF RED FOXES IN EUROPE.  M. AUBERT, V. BRUYERE, J. BARRAT, F. CLIQUET
12:00-1:30	LUNCH

# RABIES II-CONTRIBUTED PAPERS (1:30-2:45) Moderator: Joseph Corn

1:30-1:45	(8) RABIES VIRUS (GENOTYPE 1): GENETIC DIVERSITY AND INSIGHT INTO THE EPIDEMIOLOGY OF VIVERRID RABIES IN SOUTHERN AFRICA.  J. P. LOWINGS, E. BLACK, J. SMITH, L. M. McELHINNEY, J. H. A. BOWEN-DAVIES, A. KING, P. JOHNSTONE, J. E. WHITBY, P. HEATON
1:45-2:00	(9) AN OUTBREAK OF RABIES IN CENTRAL SAUDI ARABIA: HAS IT AFFECTED HOST POPULATION DEMOGRAPHY? STEPHANIE OSTROWSKI, DANIEL M. LENAIN
2:00-2:15	(10) ORAL WILDLIFE RABIES VACCINATION: BIOMARKER (TETRACYCLINE) ASSESSMENT.  CATHLEEN A. HANLON, MICHAEL NIEZGODA, CHARLES E. RUPPRECHT
2:15-2:30	(11) RABIES VACCINE, LIVE VACCINIA VECTOR: BIOSIMILARITY COMPARISON BETWEEN THREE V-RG VACCINE DELIVERY SYSTEMS BY SEROLOGIC EVALUATION IN RACCOONS, PROCYON LOTOR.  LAURI MOTES-KREIMEYER, JOHN C. WLODLOWSKI, PATRICK A. TANNER, GUILLERMO GALLO, JENNIFER CONLON, MARC MACKOWIAK
2:30-2:45	(12) RABIES VACCINE, LIVE VACCINIA VECTOR ORAL VACCINATION OF COYOTES, CANIS LATRANS, IN SOUTH TEXAS USING AN EXPERIMENTAL COATED SACHET VACCINE DELIVERY SYSTEM.  SAMUEL B. LINHART, JOHN C. WLODKOWSKI, LAURI MOTES-KREIMEYER, M. G. FEARNEYHOUGH, RICK SRAMEK, JOHN McCONNELL
2:45-3:15	BREAK
3:15-3:45	AMERICAN ASSOCIATION OF WILDLIFE VETERINARIANS: CUTTING EDGE SPEAKER  (13) DEATH IN THE PLEISTOCENE: DID EMERGING INFECTIOUS DISEASES CAUSE QUATERNARY MEGAFAUNAL EXTINCTIONS? ROSS D. E. MacPHEE

#### **DISEASES AND EXTINCTION (3:45-4:30)**

Moderator: Walter Cook

3:45-4:00	(14) NUCLEAR DNA FROM THE WOOLLY MAMMOTH (MAMMUTHUS PRIMIGENIUS): THE SEARCH FOR PLEISTOCENE VIRUSES.  ALEX D. GREENWOOD, CLARE FLEMMING, ROBERT DESALLE, PRESTON A. MARX, ROSS D. E. MacPHEE
4:00-4:15	(15) WILDLIFE DISEASES & GLOBAL EXTINCTION: LESSONS FROM THE <i>PARTULA</i> SNAIL CONSERVATION PROGRAM. <u>PETER DASZAK</u> , ANDREW A. CUNNINGHAM
4:15-4:30	(16) PREDATORS, PATHOGENS, AND `ALALA: THE TRAVAILS OF ENDANGERED SPECIES REPATRIATION IN HAWAII. THIERRY M. WORK, J. GREGORY MASSEY, DAVID LEDIG, BRUCE RIDEOUT, J. P. DUBEY
4:30-4:40	GEORGIA MOMENTS- SOUTHERN FOODS GARY DOSTER, SCWDS

TUESDAY morning, AUGUST 10

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

8:10-8:30

1999 WDA STUDENT RESEARCH RECOGNITION AWARD
(17) DETECTION OF THE CAUSATIVE AGENT OF DISSEMINATED
VISCERAL COCCIDIOSIS (EIMERIA SP.) IN SANDHILL CRANES
(GRUS CANADENSIS) AND WHOOPING CRANES (GRUS AMERICANA)
BY POLYMERASE CHAIN REACTION AMPLIFICATION OF
18S rDNA.
SCOTT P. TERRELL, SUSAN E. LITTLE, MARILYN G. SPALDING,

<u>SCOTT P. TERRELL</u>, SUSAN E. LITTLE, MARILYN G. SPALDING, CALVIN M. JOHNSON

#### TERRY AMUNDSEN STUDENT PRESENTATION COMPETITION (8:30 – 11:55)

Moderator: Ellis Greiner

8:30-8:45 (18) FLUOROQUINOLONE RESISTANT MYCOPLASMA
GALLISEPTICUM FROM ENROFLOXACIN-TREATED HOUSE
FINCHES (CARPODACUS MEXICANUS).

JAMES F. X. WELLEHAN, PAUL J. FUSCO, ALONGKORN AMONSIN,
MARIA CALSAMIGLIA, VIVEK KAPUR

8:45-9:00	(19) THE PREVALENCE OF LEPTOSPIRA POMONA IN CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS) ALONG THE CALIFORNIA COAST, 1996.  ANGELA M. COLAGROSS-SCHOUTEN, JONNA MAZET, MELISSA CHECHOWITZ, FRANCES GULLAND, SHARON HIETALA
9:00-9:15	(20) FIELD INVESTIGATION OF CONJUNCTIVITIS AND MYCOPLASMA GALLISEPTICUM INFECTIONS IN SONGBIRDS FROM NEW YORK. BARRY K. HARTUP, GEORGE V. KOLLIAS, DAVID H. LEY
9:15-9:30	(21) WILDLIFE IN SOUTH CAROLINA AS RESERVOIR HOSTS FOR TRYPANOSOMA CRUZI.  MICHAEL J. YABSLEY, GAYLE PITTMAN NOBLET
9:30-9:40	GEORGIA MOMENT – HUNTING DOGS OF THE SOUTHEAST VICTOR NETTLES, SCWDS
9:40-10:10	BREAK
10:10-10:25	(22) ASSOCIATIONS BETWEEN WATER CHARACTERISTICS AND AVIAN CHOLERA AT THE SACRAMENTO NATIONAL WILDLIFE REFUGE, CALIFORNIA.  MARGARET A. LEHR, RICHARD G. BOTZLER, MICHAEL D. SAMUEL, DANIEL J. SHADDUCK
10:25-10:40	(23) ORAL TRANSMISSION AND EARLY LYMPHOID TROPISM OF CHRONIC WASTING DISEASE PrPres IN MULE DEER FAWNS.  CHRISTINA J. SIGURDSON, ELIZABETH S. WILLIAMS, MICHAEL W.  MILLER, TERRY R. SPRAKER, KATHERINE I. O'ROURKE, EDWARD A. HOOVER
10:40-10:55	(24) PRELIMINARY DISCUSSIONS ON THE EPIDEMIOLOGY OF BRUCELLOSIS IN WOOD BUFFALO NATIONAL PARK, CANADA. DAMIEN O. JOLY, FRANÇOIS MESSIER
10:55-11:10	(25) GENETIC VARIATION OF EPIZOOTIC HEMORRHAGIC DISEASE VIRUS ISOLATES: SPATIAL AND TEMPORAL INFLUENCES.  M. D. MURPHY, N. J. MACLACHLAN, E. W. HOWERTH, D. E. STALLKNECHT

11:10-11:25 (26) THYROID LESIONS IN BELUGA WHALES (DELPHINAPTERUS LEUCAS) FROM THE ST. LAWRENCE ESTUARY AND HUDSON BAY, QUEBEC, CANADA.

PHILIPPE LABELLE, IGOR MIKAELIAN, SYLVAIN DE GUISE, DANIEL MARTINEAU

11:25-11:40 (27) SEROLOGIC AND PCR SURVEY FOR EHRLICHIAL ORGANISMS IN RACCOONS AND OPOSSUMS FROM AN EHRLICHIA CHAFFEENSIS ENDEMIC AREA.

JOSEPH K. GAYDOS, DAVID E. STALLKNECHT, WILLIAM R. DAVIDSON, SUSAN E. LITTLE, ASHLEY D. BEALL

11:40-11:55 **(28) SAFETY OF BRUCELLA ABORTUS STRAIN RB51 IN COLLARED LEMMINGS.**MATTHEW EDMONDS, JULIA BEVINS, GERHARDT SCHURIG, SUE HAGIUS, FRED ENRIGHT, TODD FULTON, PHILIP ELZER

11:55-1:20 **LUNCH** 

TUESDAY afternoon, August 10

1:20-1:40

1997 WDA STUDENT RESEARCH RECOGNITION AWARD

(29) LAKE WHITEFISH, COREGONUS CLUPEAFORMIS, FROM THE ST. LAWRENCE RIVER, QUEBEC, CANADA; A BIOINDICATOR OF ENVIRONMENTAL CONTAMINATION.

IGOR MIKAELIAN, DANIEL MARTINEAU, YVES DE LAFONTAINE CHANTAL MENARD, JOHN C. HARSHBARGER

#### **STUDENT PRESENTATION COMPETITION (1:40 – 3:25)**

Moderator: Sarah Shapiro Hurley

1:40-1:55 (30) HEALTH EVALUATION OF FREE-RANGING ASIAN ELEPHANTS (ELEPHAS MAXIMUS) REQUIRING TRANSLOCATION IN SABAH, MALAYSIA.

A. M. KILBOURN, E. J. BOSI, M. ANDAU, E. S. DIERENFELD, R. A COOK, W. B. KARESH

1:55-2:10

(31) PRELIMINARY REPORT ON ADULT AND KITTEN SURVIVAL
TIME OF FERAL CATS IN MANAGED COLONIES IN RANDOLPH
COUNTY, NORTH CAROLINA.
FELICIA B. NUTTER, JAY F. LEVINE, MICHAEL K. STOSKOPF

- 2:10-2:25 (32) HELICOBACTER-LIKE ORGANISMS IN FREE-RANGING BOBCATS (FELIS RUFUS) IN THE SOUTHEASTERN UNITED STATES.
  RENE MEISNER, W. RANDY DAVIDSON, LESLIE D. BAUER, MOLLY MURPHY, DEBORAH E. PERZAK, ELIZABETH W. HOWERTH
- 2:25-2:40 **(33) AUSTRALIAN BAT LYSSAVIRUS IN QUEENSLAND.**JANINE BARRETT, PETER YOUNG, HUME FIELD, BARRY RODWELL,
  GREG SMITH
- 2:40-2:55

  (34) IMMUNOHISTOCHEMICAL DIAGNOSIS OF CHRONIC
  WASTING DISEASE IN PRECLINICALLY AFFECTED ELK FROM A
  CAPTIVE HERD.

  JEANINE PETERS, JANICE M. MILLER, ALLEN L. JENNY, TERRY L.
  PETERSON, K. PAIGE CARMICHAEL
- 2:55-3:10 (35) THE DEER MOUSE (PEROMYSCUS MANICULATUS) AS A POTENTIAL AMPLIFYING HOST FOR VESICULAR STOMATITIS VIRUS NEW JERSEY SEROTYPE.

  TODD E. CORNISH, ELIZABETH W. HOWERTH, DAVID E. STALLKNECHT
- 3:10-3:25 (36) DEVELOPMENT AND USE OF A RECOMBINANT CELL CULTURE BIOASSAY SYSTEM FOR THE DETECTION OF PETROLEUM EXPOSURE IN SEA OTTERS (ENHYDRA LUTRIS SPP.).

  M. H. ZICCARDI, I. A. GARDNER, J. A. K. MAZET, B. A. BALLACHEY, D. A. JESSUP, M. S. DENISON
- 3:25-3:45 BREAK AND ADJOURN FOR THE DAY

WEDNESDAY, August 11

POSTER SESSION – Authors available during breaks Wednesday morning through noon Thursday. Posters on display Wednesday 8:00 am – 4:30 pm and Thursday 8:00 am – noon in Hill Atrium outside Masters Hall.

- (37) VACUOLAR MYELINOPATHY IN WILD BIRDS FROM AN IMPOUNDMENT IN THE NORTH CAROLINA SANDHILLS. TOM AUGSPURGER, KIMBERLI J. G. MILLER, JOHN R. FISCHER
- (38) LEUCOCYTOZOONOSIS IN NESTLING BALD EAGLES, HALIAEETUS LEUCOCEPHALUS, IN MICHIGAN AND MINNESOTA. JOHN N. STUHT, WILLIAM W. BOWERMAN, DAVID A. BEST

(39) AN INVESTIGATION OF SHELL LESIONS IN A POPULATION OF TURTLES IN A MARYLAND LAKE.

<u>CINDY P. DRISCOLL</u>, SUSAN KNOWLES, BRENDA KIBLER, BRETT COAKLEY

(40) IMMOBILIZATION OF FREE-ROAMING RACCOONS (PROCYON LOTOR) AND OPOSSUMS (DIDELPHIS VIRGINIANA) WITH MEDETOMIDINE-KETAMINE AND REVERSAL WITH ATIPAMEZOLE.

TROY D. EVERSON, RAMIRO ISAZA, MELISA MINTO, MICHAEL W. DRYDEN

(41) DESCRIPTION AND CLASSIFICATION OF MALFORMATIONS IN THE NORTHERN LEOPARD FROG.

<u>CAROL METEYER</u>, KATHRYN CONVERSE, JUDY HELGEN, JIM BURKHART, RICHARD LEVY, SUSAN KERSTEN, LAURA EATON-POOLE

(42) ORAL DELIVERY OF SEDATIVE AGENTS TO CAPTIVE WILDLIFE.

EDWARD C. RAMSAY, DANIEL GROVE, JUERGEN SCHUMACHER, KAREN KEARNS

(43) BEETLES, BIRDS AND BATS: CHLORDANE POISONING IN NEW JERSEY.

WILLIAM STANSLEY, <u>DOUGLAS E. ROSCOE</u>, ELIZABETH HAWTHORNE, JANE E. HUFFMAN

(44) CAPTURE, HEALTH, AND MORPHOLOGICAL ASSESSMENT OF FREE-RANGING MANTLED HOWLER MONKEYS (*ALOUTTA PALLIATA*) IN NICARAGUA.

GREGORY K. PETER, <u>REX SOHN</u>, LINDA A. WINKLER

(45) MORBIDITY AND MORTALITY OF WEDDELL SEALS, LEPTONYCHOTES WEDDELLII, IN McMURDO SOUND, ANTARCTICA. PAMELA K. YOCHEM, BRENT S. STEWART, THOMAS S. GELATT, DONALD B. SINIFF

#### WEDNESDAY morning, August 11

#### 8:00-8:10 ANNOUNCEMENTS 8:10-8:45 **UNIVERSITY OF GEORGIA SPECIAL PRESENTATION: HUMANITY'S TWO HOUSES.** EUGENE P. ODUM, Founder and Director Emeritus of Georgia Institute of Ecology; Author of Fundamentals of Ecology, and regarded as "The Father of Modern Ecology" **TUBERCULOSIS IN WILDLIFE (8:40 – 9:25)** Moderator: John Fischer (46) THE HOOK LAKE WOOD BISON RECOVERY PROJECT: AN 8:45-9:00 ATTEMPT TO ERADICATE BOVINE TUBERCULOSIS AND BRUCELLOSIS FROM A WOOD BISON HERD IN NORTHERN CANADA. JOHN S. NISHI, BRETT ELKIN, TROY R. ELLSWORTH, DON BALSILLIE, C. CORMACK GATES 9:00-9:15 (47) BOVINE TUBERCULOSIS IN MICHIGAN. STEPHEN M. SCHMITT, SCOTT D. FITZGERALD, COLLEEN S. BRUNING-FANN, NATHAN ZAUEL, DALE E. BERRY 9:15-9:30 (48) NATURALLY OCCURRING TUBERCULOSIS IN WHITE-TAILED DEER. MITCHELL V. PALMER, DIANA L. WHIPPLE, DAVID P. ALT, KEVIN J. ESCH, JANET B. PAYEUR, COLLEEN S. BRUNING-FANN **BREAK and POSTER SESSION** 9:30-10:30 **CERVIDS** (10:30 - 12:00)Moderator: Scott Terrell (49) IDENTIFICATION OF A PROTEIN IMPORTANT IN 10:30-10:45 IMMUNODIAGNOSIS OF PARELAPHOSTRONGYLUS TENUIS INFECTIONS IN RED DEER. MICHAEL S. DUFFY, MICHAEL D. B. BURT (50) XYLAZINE-INDUCED ASPIRATION PNEUMONIA IN SHIRA'S 10:45-11:00 MOOSE.

TERRY J. KREEGER

11:00-11:15	(51) REDUCING THE RISK OF TRANSMISSION OF BRUCELLOSIS FROM ELK TO CATTLE.  WALTER E. COOK, MICHAEL W. MILLER, ELIZABETH S. WILLIAMS
11:15-11:30	(52) DIAGNOSIS OF CHRONIC WASTING DISEASE OF DEER AND ELK. E. S. WILLIAMS, M. W. MILLER, T. R. SPRAKER, C. SIGURDSON, A.
	JENNY, J. MILLER, M. HALL
11:30-11:45	(53) CHRONIC WASTING DISEASE SURVEILLANCE IN ARIZONA USING HUNTER-KILLED DEER AND ELK.  JAMES C. DE VOS, DEBBIE BROWN, LORRAINE AVENETTI
11:45-12:00	(54) SURVEY FOR SELECTED DISEASES IN HUNTER-KILLED ELK FROM ARKANSAS.  TODD E. CORNISH, JOSEPH L. CORN, MICHAEL E. CARTWRIGHT
12:00-1:20	LUNCH
WEDNESDA	AY afternoon, August 11
	FISH, AMPHIBIANS AND REPTILES (1:20 – 2:35) Moderator: Todd Cornish
1:20-1:35	(55) EVALUATION OF THE USE OF A SURROGATE SPECIES (NOTROPIS SCEPTICUS) FOR CAPE FEAR SHINER (NOTROPIS MEKISTOCHOLAS) RECOVERY PROGRAM HABITAT HEALTH RISK ASSESSMENT.
	MICHAEL K. STOSKOPF, BETH CHITTICK, MAC LAW, TOM AUGSPURGER, NORM HEIL
1:35-1:50	(56) A DIAGNOSTIC EVALUATION OF THREE SALAMANDER MORTALITY EVENTS ASSOCIATED WITH AN IRIDOVIRUS AND SUBSEQUENT GENOMIC COMPARISON OF THE VIRUS ISOLATES OBTAINED.
	DOUGLAS DOCHERTY, V. GREGORY CHINCHAR, CAROL METEYER, ROGER BRANNIAN, WALLACE HANSEN, JUN WANG, JINGHE MAO
1:50-2:05	(57) CHYTRIDIOMYCOSIS OF AMPHIBIANS: INVESTIGATING AN

DAVID PORTER, CORRIE C. BROWN

<u>PETER DASZAK</u>, ANDREW A. CUNNINGHAM, ALEX D. HYATT, LEE BERGER, DAVID EARL GREEN, RICK SPEARE, MARK A. FARMER,

2:05-2:20	(58) IMMUNOLOGY OF GREEN TURTLE FIBROPAPILLOMATOSIS IN HAWAII.  THIERRY M. WORK, ROBERT A. RAMEYER, GEORGE H. BALAZS, SANDRA P. CHANG, CAROLYN CRAY, JOHN BERESTECKY
2:20-2:35	(59) CAPTIVE REARING BLANDING'S TURTLES (EMYDOIDEA BLANDINGII) AS PART OF A MANAGEMENT PROGRAM OF A THREATENED POPULATION. DANIEL R. LUDWIG, TED ANCHOR, CATHERINE M. BROWN
2:35-2:45	GEORGIA MOMENT – MOUNTAINS OF NORTH GEORGIA PAGE LUTTRELL, SCWDS
2:45-3:15	BREAK
ECTOPARASITES (3:15 – 4:15) Moderator: Susan Little	
3:15-3:30	(60) EXPERIMENTAL TRANSMISSION OF A DOG-ORIGIN ISOLATE OF HEPATOZOON AMERICANUM TO COYOTES BY THE GULF COAST TICK, AMBLYOMMA MACULATUM.  ALAN KOCAN, C. A. CUMMINGS, R. J. PANCIERA, J. S. MATHEW, S. A. EWING, R.W. BARKER
3:30-3:45	(61) ADAPTATIONS OF WINTER TICKS ( <i>DERMACENTOR ALBIPICTUS</i> ) TO INVADE MOOSE AND MOOSE TO EVADE TICKS. <u>BILL SAMUEL</u>
3:45-4:00	(62) WILDLIFE SURVEILLANCE FOR THE SOUTH AFRICAN TORTOISE TICK (AMBLYOMMA MARMOREUM) IN FLORIDA. JOSEPH L. CORN, LEROY M. COFFMAN, JAMES W. MERTINS
4:00-4:15	(63) TROMBIDIOSIS IN THE FLORIDA BLACK BEAR (URSUS AMERICANUS FLORIDANUS).  MARK W. CUNNINGHAM, LYNETTE A. PHILLIPS, PAMELA E. GINN, JUDY S. SMITH, DAVID S. MAEHR, DONALD J. FORRESTER

#### THURSDAY morning, August 12

#### PREVIEW OF YEAR 2000 WDA MEETING – TERRY KREEGER 8:00-8:15 **INTERNATIONAL WILDLIFE HEALTH (8:15 – 9:45)** Moderator: Mark Abdy (64) THE CENTER FOR CONSERVATION MEDICINE: LINKING 8:15-8:30 ECOSYSTEM HEALTH TO HUMAN AND ANIMAL WELL-BEING. A. ALONSO AGUIRRE, GARY TABOR, COLIN GILLIN 8:30-8:45 (65) HEALTH ASSESSMENT OF A MONITORED POPULATION OF FREE-RANGING BAIRD'S TAPIRS (TAPIRUS BAIRDII) IN CORCOVADO NATIONAL PARK, COSTA RICA. SONIA H. FOERSTER, CHARLES R. FOERSTER 8:45-9:00 (66) HEALTH EVALUATION OF PAMPAS DEER (OZOTOCERUS BEZOARCTICUS CELER) IN ARGENTINA. MARCELA M. UHART, ALFREDO BALCARCE, ALEJANDRO R. VILA, MARIO S. BEADE, WILLIAM B. KARESH 9:00-9:15 (67) CALF RECRUITMENT AND SURVIVAL IN A POPULATION OF BLACK RHINOCEROS, DICEROS BICORNIS, IN ZIMBABWE FOLLOWING IMMOBILIZATION AND DEHORNING. MARK W. ATKINSON, MICHAEL D. KOCK 9:15-9:30 (68) SERO-SURVEILLANCE OF MALAYSIAN BATS FOR EVIDENCE OF NIPAH VIRUS INFECTION. HUME FIELD, JOHARA MOHD YOB, CHRIS MORRISSY, PAUL SELLECK 9:30-9:45 (69) AN EPIDEMIC OF BLINDNESS IN KANGAROOS CAUSED BY A VIRUS. P. T. HOOPER GEORGIA MOMENT – BIRD CONSERVATION IN THE SOUTHEAST 9:45-9:55 E. J. WILLIAMS, GEORGIA DEPARTMENT OF NATURAL RESOURCES 9:55-10:15 **BREAK**

#### **WILDLIFE TOXICOLOGY (10:15 – 11:45)**

Moderator: Lauren Richey

10:15-10:30	(70) DOMOIC ACID TOXICITY IN CALIFORNIA SEA LIONS
	(ZALOPHUS CALIFORNIANUS) STRANDED ALONG THE CENTRAL
	CALIFORNIA COAST.
	FRANCES M. D. GULLAND, MARTY HAULENA, LINDA J. LOWENSTINE
	K. LEFEBVRE, T. LIPSCOMB, T. ROWLES, C. SCHOLIN, T. SPRAKER, V.
	TRAINER, F. VAN DOLAH
10:30-10:45	(71) AFLATOXICOSIS IN SNOW GEESE FEEDING IN LOUISIANA
	CORN FIELDS.
	K. A. CONVERSE, L. SILEO, T. CORNISH, D. FINLEY

- 10:45-11:00 (72) WINTER MORTALITY IN WAXWINGS (BOMBYCILLA GARRULUS) CAUSED BY ETHANOL INTOXICATION.

  TORSTEN MÖRNER, ANNA-LENA BARMARK, ERIK NORDKVIST, DESIRÉE S. JANSSON, CARL HÅRD AF SEGERSTAD
- 11:00-11:15 **(73) RECENT POISONINGS OF WILDLIFE IN NEW YORK WITH ANTICOAGULANT RODENTICIDES.**WARD B. STONE, KEVIN P. HYNES, <u>JOSEPH C. OKONIEWSKI</u>, JAMES R. STEDELIN
- 11:15-11:30 (74) LEAD EXPOSURE IN BLACK DUCKS AND WATERFOWL MORTALITY FOLLOWING IMPLEMENTATION OF NONTOXIC SHOT.

  MICHAEL D. SAMUEL, E. FRANK BOWERS, LYNN H. CREEKMORE
- 11:30-11:45 **(75) METHYLMERCURY EXPOSURE IN WADING BIRDS: A COMPARISON OF CAPTIVE DOSING AND NATURAL EXPOSURE.**MARILYN G. SPALDING, PETER C. FREDERICK
- 11:45-1:05 **LUNCH**

#### **AVIAN INFECTIOUS DISEASE (1:05 – 2:35)**

Moderator: Page Luttrell

1:05-1:20 (76) THE OCCURRENCE OF DUCK PLAGUE VIRUS IN NON-MIGRATORY WATERFOWL IN THE CHESAPEAKE BAY AREA OF MARYLAND.

WALLACE R. HANSEN, DOUGLAS E. DOCHERTY, KATHRYN A. CONVERSE, SEAN W. NASHOLD

1:20-1:35	(77) ATOXOPLASMOSIS IN THE BALI MYNAH IN THE UK & THE TAXONOMIC STATUS OF ATOXOPLASMA.  PETER DASZAK, STANLEY JOHN BALL, DAVID JEGGO, ANDREW G. GREENWOOD
1:35-1:50	(78) SPIROCHETOSIS DUE TO AN UNUSUAL BORRELIA SP. IN A NORTHERN SPOTTED OWL.  NANCY J. THOMAS, ALAN G. BARBOUR, JONAS BUNIKIS, MARK J. WOLCOTT
1:50-2:05	(79) A SEROTYPE-SPECIFIC PCR ASSAY FOR AVIAN CHOLERA (PASTEURELLA MULTOCIDA SEROTYPE 1). TONIE E. ROCKE, SUSAN R. SMITH
2:05-2:20	(80) ENVIRONMENTAL CHARACTERISTICS ASSOCIATED WITH AVIAN CHOLERA OUTBREAKS IN WETLANDS.  MICHAEL D. SAMUEL, DIANA R. GOLDBERG, DANIEL J. SHADDUCK
2:20-2:35	(81) EFFECTS OF HEAVY METALS ON SURVIVAL OF PASTEURELLA MULTOCIDA (AVIAN CHOLERA) ORGANISMS.  JESSIE PRICE, MELODY MOORE, SEAN NASHOLD, DAN SHADDUCK
2:35-2:55	BREAK
	MAMMALS (2:55 – 4:25) Moderator: Joe Gaydos
2:55-3:10	(82) PATHOLOGY ASSOCIATED WITH AN EPIZOOTIC OF FIBROMATOSIS IN GRAY SQUIRRELS (SCIURUS CAROLINENSIS). SCOTT P. TERRELL, PAMELA E. GINN, DONALD J. FORRESTER
3:10-3:25	(83) KIDNEY AGENESIS IN TWO FLORIDA BLACK BEARS (URSUS AMERICANUS FLORIDANUS) FROM A NORTH CENTRAL FLORIDA POPULATION.  MARK W. CUNNINGHAM, SCOTT P. TERRELL, GARRY W. FOSTER, MIKE R. DUNBAR, DONALD J. FORRESTER
3:25-3:40	(84) SEROLOGIC SURVEY FOR CANINE CORONA VIRUS IN WOLVES FROM INTERIOR ALASKA. RANDALL L. ZARNKE, MARK McNAY, JAY VER HOEF

3:40-3:55	(85) SERUM NEUTRALIZING ANTIBODY TITERS IN RACCOONS (PROCYON LOTOR) SUSPECTED OF BEING INFECTED WITH THE CANINE DISTEMPER VIRUS.  CATHERINE M. BROWN
3:55-4:10	(86) HEALTH ASPECTS OF A REINTRODUCTION OF CANADA LYNX INTO COLORADO.  MARGARET A. WILD, HERMAN DIETERICH, SUSAN DIETERICH, TERRY R. SPRAKER, TANYA SHENK
4:10-4:25	(87) AN UNUSUAL OCCURENCE OF HARBOR PORPOISE STRANDINGS IN MARYLAND - MARCH - MAY 1999.  CINDY P. DRISCOLL, SUSAN KNOWLES, BRENDA KIBLER, BRETT COAKLEY
4:25	FINAL ANNOUNCEMENTS AND ADJOURN

# (1) THE RABIES PARADOX: WILL CHANCE STILL FAVOR THE PREPARED MIND?

CHARLES E. RUPPRECHT, Centers for Disease Control & Prevention, Atlanta, GA.

Rabies should continue to present a unique challenge to the wildlife disease specialist, on an international basis. It remains an acute, progressive, viral encephalitis transmitted by the bite of infected animals, possessing the highest case: fatality ratio of any agent once clinical signs develop, yet it is almost always preventable if exposure is obvious and prompt and proper prophylaxis is initiated. The disease beckons from antiquity, but novel aspects concerning its basic etiology and epizootiology readily appear. These negative-stranded RNA viruses, assigned to the Family Rhabdoviridae, Genus Lyssavirus, reside on all inhabited continents and contain at least seven putative genotypes, only one of which, rabies virus, occurs in the New World. Birds are susceptible under experimental conditions, while mammals are the only defined natural hosts. Although species span the gamut from armadillo to zebra, nevertheless reservoirs appear restricted primarily to the Orders Carnivora and Chiroptera. Initially believed to be a single entity, both antigenic and genetic data clearly demonstrate that rabies is caused by highly compartmentalized, dynamic virus variants persistent among several specific hosts. Dogs are prominent global reservoirs, particularly in developing tropical and sub-tropical countries, while in North America taxa include Arctic, red, and gray foxes, covotes, skunks, raccoons, mongoose, and bats. Neither current experimental nor epidemiologic data support the concept of "latency" among wildlife, but incubation periods can be extremely lengthy, figuratively ranging from days to months. Lyssaviruses do attain the title of penultimate neurotropic, neuroinvasive, and neurovirulent agents, while defying sensitive detection until reaching the CNS by as of yet inexplicable processes and retaining the capacity for non-neuronal infection. Swept into the collective consciousness by visions of stereotypic maniacal presentation and prominent Negri bodies, these obligate intracellular parasites still outwit field biologists and pathologists alike because associated signs of illness (clinically, grossly, and microscopically) may be quite subtle or lacking. Often subjectively labeled as defective in strategy due to catastrophic terminal outcome in vivo, from another perspective the host is merely excess baggage as the virus provides its own guaranteed transmission to a new site by surreptitious behavioral alteration and excretion well before death. As rabies is thought passe and controlled in one area, it then emerges, re-emerges and becomes entrenched in another. Numerous recent examples highlight that it may easily persist for centuries in some arenas, then complicated by the purposeful or accidental translocation of infected wildlife and invasion of unoccupied niches. Due to the ample public health, agricultural, and conservation threats posed by the ready local, regional, and international movement of potentially infected animals, many disciplines must share a primary responsibility in disease prevention and control to minimize the opportunity for an unrecognized biologic oddity to regale as tomorrow's outbreak. What will the next millennium have in store for wildlife disease professionals as regards rabies? Improved surveillance, diagnostics, and virus typing - or loss of basic infrastructure, control services, and research expertise? Significant strives towards regional wildlife rabies elimination - or the emergence of ever more vagile hosts assisted by human intervention? Development of more efficacious, safer, less expensive, oral vaccines, able to be extrapolated to a broader array of species (including bats), via more effective delivery systems - or failure of the basic concept based on complacency and planned obsolescence?

#### (2) RABIES VIRUS: REFLECTIONS ON ITS EVOLUTION.

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Although a large number of mammalian species are susceptible to infection with rabies viruses, only a few are recognized as important for the persistence of the disease in nature. In these principal host species, a prolonged enzootic existence is possible because of sets of coadapted traits of susceptibility, viral evasion of immune surveillance, long incubation, excretion in saliva, neurological disorders that promote transmission, host life history traits, social behavior, and population biology. *Chiroptera* (bats) are identified as hosts of lyssaviruses in Australia, Africa, Europe, and in the Americas. Different *Carnivora*, including domestic dogs, are the principal hosts for classical rabies (serotype 1) in Asia, Africa, Europe, and in the Americas. Despite the obvious potential for random viral mutation, overall high levels of conservation are found in wild rabies isolates. If "self-guided tours" in the fitness landscape were a possibility for rabies virus, one would expect that it should switch principal hosts opportunistically. This is obviously not the case; rabies genomes appear to be trapped at local fitness optima. Adaptations to new hosts or the adoption of other transmission strategies may both be difficult due to structural and functional constraints or may need too many simultaneous coadapted changes. Though intricate they may be, future invasions of new hosts are possible.

# (3) AUSTRALIAN BAT LYSSAVIRUS – CURRENT SITUATION AND SOME EXPERIMENTAL STUDIES.

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In 1996, surveillance for Hendra virus in fruit bats revealed the presence of Australian bat lyssavirus (ABL) in a fruit bat tested for rabies as a differential diagnosis. Since then, infections have been found to be widespread over the country, in 4 species of fruit bat (flying fox, *Pteropus* spp.), in insectivorous bats, the yellow-bellied sheathtail-bat (Saccolaimus flaviventris), and in 2 humans. The virus is most closely related to classical rabies virus. Antigenically, it has been included with rabies viruses in serotype 1, and genetically, although very close to rabies viruses, it has been classified as a new clade, genotype 7. Samples of field sera have shown antibody in fruit bats, suggesting non-lethal infections. Experimental animal studies have also produced some remarkable results. In each of these studies, concurrent intracerebral inoculations in mice with the same viruses were conducted successfully to validate the infectivity of the test viruses. In the first experiment, 7 grey-headed flying foxes (*Pteropus poliocephalus*) were challenged by intramuscular inoculation in the forelimb with 103.7 TCID50 of Australian bat lyssavirus (ABL), and another 7 by the same procedure with 103.3 TCID50 of a bat-derived rabies virus, Eptesicus 1 virus. One animal was held as an uninfected control. Only one bat succumbed, an ABLchallenged bat, which became paretic at 27 days post-inoculation (dpi). A second experiment with bats was conducted, this time with 10 bats receiving 105 TCID50 ABL and 4 with the same dose of Eptesicus 1 virus. One further bat was held as an uninfected control. Three of the 10 bats inoculated with ABL and 2 of 4 inoculated with the Eptesicus 1 virus developed clinical signs consistent with a lyssavirus infection, at 15, 23 and 24 dpi, and 16 and 17 dpi, respectively. All 5 affected animals had histological lesions in the CNS, 5 were positive for viral antigen by the fluorescent antibody test (FAT), but only 4 by indirect immunoperoxidase (IPX), and virus was isolated in cell culture from 3 of the 5 cases. The affected bat that was negative for viral antigen by IPX was only very weakly positive by FAT, and it was 1 of the 2 from which virus could not be isolated. Three mature male cats were inoculated intramuscularly into the forelimb with 105 TCID50 of ABL, one was inoculated with a similar dose of Eptesicus I, and one was an uninfected control. At the end of the 3-month-long trial, no cats had succumbed. However, all 3 ABL-infected cats had seroconverted, # 2 at 29 dpi, # 4 at 42 dpi, and # 3 at 95 dpi (euthanasia). In cat # 2, the anti-lyssaviral titre continued to rise until approximately 60 dpi, at which point the titre remained steady until euthanasia at 91 dpi. By contrast, in cat # 4 the titre continued to rise rapidly from 42 dpi until euthanasia at 95 dpi. Anti-lyssavirus antibody was found in the cerebrospinal fluid of cat # 3. All 3 ABL-infected cats showed mild, and transient, behavioural abnormalities throughout the trial (from as early as 12 dpi, and no later than 39 dpi). The single cat inoculated with Eptesicus I virus failed to seroconvert, although it too showed vague behavioural changes intermittently between 11 and 42 dpi. Histopathological examination of the spinal cord, and of 3 standard sites in the brain, failed to reveal lesions in any of the cats. Immunoperoxidase examinations of the CNS also failed to reveal viral antigen. At the time of writing this abstract (19th April, 1999), additional sites in the brains are being examined. Virus isolation on clinical and necropsy samples, FATs and histopathology on tissues outside the CNS are yet to be undertaken.

# (4) ECOLOGY & POPULATION BIOLOGY OF BAT RABIES IN THE UNITED STATES.

SHARON L. MESSENGER, Viral and Rickettsial Zoonoses Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333.

Insectivorous bats were not recognized as reservoirs for rabies viruses in the United States until 1953, but since that time, they have become associated with nearly all indigenously-acquired human rabies cases in the U.S. Despite the public health importance of bat rabies, little is known about the ecology and population biology of rabies in bats.

What impact does rabies have on a population of bats? It was initially speculated that large dieoffs of Mexican free-tailed bats (*Tadarida brasiliensis*) documented in the 1950's and 1960's
were the result of epizootics of bat rabies, but this hypothesis has not been upheld by the data.
Bat rabies is known to be widespread having been documented in 30 of the 38 known U.S. bat
species and from all U.S. states except Hawaii, but mortality and morbidity rates for any given
species of bat are unknown. Attempts to assess the impact of rabies on a single population (i.e., a
bridge community) of Mexican free-tailed bats in central Texas suggest that although rabies
contributes significantly to the overall mortality of bats in the population, the absolute number of
bats dying from rabies is relatively small. Whether these data can be generalized to other bat
species remains to be tested.

The low rabies-related mortality in such a high-density population begs the question as to whether or not bats have evolved mechanisms that enable them to survive exposure to rabies. Though there is no information to suggest recovery from clinical rabies in wild populations, there are some data that indicate that bats develop immunity to rabies upon exposure. Studies have documented a prevalence range of rabies neutralizing antibody in a wide variety of bat species depending in part upon the method of data collection, but at least one study following a single population of bats (*Tadarida brasiliensis*) long-term found a consistently high prevalence of rabies virus-neutralizing antibody throughout a season. The hypothesis that bats experience low mortality during rabies epizootics because of high levels of acquired immunity warrants further study.

Given that bat rabies is so widespread in the U.S., earlier researchers speculated that bats played an important role in initiating outbreaks of rabies among terrestrial mammal populations. Characterization of rabies virus variants using both monoclonal antibodies and DNA sequencing suggest that although spillover of rabies viruses from bats occurs infrequently, there are no data to suggest that terrestrial mammals can maintain sustained transmission of bat rabies variants. Phylogenetic analyses of rabies viruses that have spilled over from bats to terrestrial mammals indicate that only some bat species account for most of these events, and generally these are the most common bat species found in the geographic region where the infection presumably took place. The most notable exception to this pattern, however, is that in the northwestern and southeastern U.S., where most spillover events occur from silver-haired (*Lasionycteris noctivagans*) or eastern pipistrelle (*Pipistrellus subflavus*) bats despite the fact that they are not considered common bats in either region. It is striking that these two areas overlap the regions where most of the bat-related human rabies deaths have occurred.

#### (5) RABIES IN LATIN AMERICA: IT'S A JUNGLE OUT THERE!

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Latin America is a region of beauty, contrast and complexity. This land of wonders comprises scorching and frozen deserts, high mountains, enormous savannahs, the largest tropical forest of the world and a prodigious variety of mammalian species. Here, in ancient times humans admired, protected and worshiped their precious environment. Unlike their ancestors today, they are invading and modifying great expanses of their land.

Although the epidemiology of this disease is highly influenced by the dynamic forces of ecology and human activities, rabies in ever changing Latin America has been traditionally associated with only 3 major sources: domestic dogs in urban centers, and vampire bats (*Desmodus rotundus*) and imported mongooses (*Herpestes auropunctatus*) representing sylvatic fauna. The improvement of rabies surveillance programs and the molecular characterization of rabies virus isolates from different countries have revealed a very different epidemiologic situation, characterized by the presence of multiple antigenic and genetic virus variants circulating in the same or different mammalian species following complex intra- and inter-specific transmission pathways.

In some cases, dog-to-dog rabies transmission has been eliminated, allowing concentration upon other potential vectors. For example, in Chile, insectivorous bats have become the main rabies reservoir in urban centers, and are the source of infection for sporadic cases reported in domestic animals. Both *Tadarida brasiliensis* and *Lasiurus* species have been demonstrated as responsible for the maintenance of two of 5 different genetic variants identified to date in this country. Evidence of an active viral transmission between these two species has been discovered.

This biodiversity is also represented in the rabies virus population circulating among vampire bats throughout Latin America. Human invasions of virgin environments as a consequence of military exercises, farming, mining, logging or other industrial activities increases the frequency of contacts between humans and vampire bats, with the consequent increase of the risk of rabies infection. Despite the small number of primary vampire isolates studied thus far, 3 antigenic variants (AgV3, 5 and 11) and at least 3 genetic variants have been recognized. The distribution of AgV3 coincides with the geographical range of *D. rotundus* throughout Latin America. In contrast, AgV 5 and AgV 11 appear to have a more restricted distribution, having been detected only in Venezuela and Mexico, respectively. Similarly, the molecular epidemiology of vampire bat rabies in Brazil has revealed an intricate interspecific transmission cycle in which vampires

are the primary source of infection for several different species of insectivorous and frugivorous bats.

Although rabies virus has been sporadically isolated from numerous mammals throughout Latin America, there is little information about the existence of rabies endemic cycles in the indigenous wildlife of the region. Skunks in Mexico have been proposed as the reservoirs for 2 independent rabies endemic cycles in the country. In some portions of South America, important outbreaks in other species such as foxes have also been reported. Commercialization of exotic species and local customs of keeping wildlife as pets may create unexpected epidemiological situations in which animals not previously considered to play an important role in rabies transmission may become a risk for humans and their domestic animals. For example, in the northeastern part of Brazil, people and marmosets are in close contact, and these animals are kept as pets. Rabid marmosets have transmitted the virus to humans with fatal consequences.

In summary, the historical conventional view of simple canine rabies persistence in Latin America is gradually being replaced by one of a much more complex epidemiology involving multiple wild taxa, burgeoning and transient human and domestic animal populations, and a highly diverse array of rabies viruses that may come to that of Canada and the United States.

#### (6) A PROPOSED MIDDLE EAST REGIONAL RABIES CONTROL PROGRAM.

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Rabies in domestic and wild animals has been a problem in the Middle East for many years. Domestic dogs, red foxes, and golden jackals are the principal vectors. Annual human post-exposure treatments within the region are estimated at about 87,000. A four year European Union-funded Regional Rabies Control Program has been proposed by the veterinary and public health officials in Egypt, Israel, Jordan, and the Palestinian Authority. Preliminary investigations suggest that oral rabies vaccination (ORV) could be a useful control method, along with increased regional information transfer, enhanced surveillance and diagnosis, upgraded facilities and equipment, technical training, including extensive use of expert consultants, vector ecology studies, and initiation of ORV trials and a cooperative regional ORV program along the Jordan River Valley.

# (7) SUCCESS AND SETBACKS OF THE ORAL VACCINATION OF RED FOXES IN EUROPE.

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From 1989 to the end of 1998, rabies cases in the countries of the European Union (EU) have decreased from 14023 to 112 (- 99 %), thanks to the oral vaccination of foxes. In large parts of Austria, France and Germany rabies is no longer endemic. However, setbacks have occurred in many places that have led to contamination between neighbouring countries. Numerous examples can be evoked. Despite a very early start of vaccination programs in several Länder of the former West Germany, foci of rabies are still active in several of them. They may re-infect rabies free countries. In these examples the main cause of the problem remains with the lack of strategic analysis and international co-ordination.

Other causes could be attributed to a lack of efficiency of the vaccine-baits and/or the specific methods. When evaluating the efficiency of the vaccination programs on the basis of the number of vaccine baits used per square km of contaminated area, large discrepancies are apparent: for example, approximately 7500 and 2000 baits were necessary in Austria and Switzerland, respectively, to achieve the same reduction in the number of rabies cases, whereas there seem to be no obvious ecological differences between both countries. As a result, despite tremendous financial outlays, rabies is still present in the EU and may be on the increase in neighbouring countries.

In addition, a dramatic increase in fox population densities renders a complete elimination of the disease more difficult. When vaccination programs are insufficient to obtain rabies elimination, they produce a transient reduction of rabies incidence and an increase in the density of fox populations. Ironically, the subsequent recovery of the fox population favours the duration of epizootic episodes. Where rabies has been eliminated, disease-free populations are highly dense and therefore more susceptible to further rabies outbreaks.

To tackle these problems, several improvement should be explored. For addressing the problem of cross-border contamination, common plans of action must be organised on both sides of national borders. The plans must solve questions as simple as where oral vaccination of foxes has to be initiated or pursued. Several international consultations and vaccination plans supported by the European Commission lead neighbouring countries to adopt vaccination strategies more in accordance with the epidemiological constraints. However, there are few examples of closer collaboration between national teams at the field level.

Vaccination methods need to be improved, verified and validated. A very simple step would be to assess the potency and the stability of the vaccine baits. Strangely enough, in many European countries, no control of the vaccine titre is done neither before nor after distribution whereas most of the vaccines are attenuated strains of the rabies virus whose temperature sensitivity is well known. The stability of the bait casing should also be tested in the environment. Such controls have not been reported even when additional distributions of baits were organised during summer. Simple quality control and field protocols would probably give clues to the origin of some failures.

In addition to spring and autumn distributions of vaccine baits in the fields via hunters or aircraft, the following measures have been tried by several European teams:

- to increase the density of bait distributions to compensate for increases in fox density;
- the application of a second distribution of vaccine two weeks later, in hope that foxes will be boostered and will develop better protection, and that baits will be more evenly distributed in the environment and therefore will be offered to a larger proportion of foxes;
- vaccination of fox cubs by distributing vaccine baits at the entrance of dens inhabited by fox families.

All these measures are only based on hypotheses. However rational they may be, their real impact has never been measured in the field, or even confirmed in captive foxes. Because such measures require higher quantities of vaccines and more man-power, they are more expensive than usual campaigns. These problems identify the need for a common evaluation of a relative index of density: when and how should one adjust the density of baits to the density of foxes? Experimental studies are still needed on captive foxes to validate – in adults, the hypothesised increased protection conferred by a short delayed repeated vaccination – and in cubs, the protection given by passively acquired immunity and their ability to develop an active immunity despite antibodies of maternal origin. In addition, it will be necessary to verify in the field the cost-effectiveness of these proposed measures, if rabies elimination is to be a reality.

# (8) RABIES VIRUS (GENOTYPE 1): GENETIC DIVERSITY AND INSIGHT INTO THE EPIDEMIOLOGY OF VIVERRID RABIES IN SOUTHERN AFRICA.

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Rabies is an endemic viral disease affecting wildlife in many parts of the world. Previous studies have demonstrated the antigenic and molecular diversity of classical (genotype 1) rabies virus isolates. Four hundred nucleotides from the nucleocapsid gene (N-gene) from 396 classical rabies virus isolates were sequenced and compared with the published data from a further 189 viruses. Phylogenetic analysis of this data set confirmed the work of others and permitted previous findings to be directly compared. Detailed analysis of 54 closely related isolates from southern Africa confirmed the occurrence of a highly diverse viverrid virus group. Although most isolates were obtained from viverrids, mainly yellow mongoose (*Cynictis pencillata*), some spillover into other species is apparent. Clear geographically defined subgroups were evident within the group suggesting that outbreaks remain relatively isolated. Our analysis supports the view that viverrid rabies in southern Africa has a long evolutionary history.

# (9) AN OUTBREAK OF RABIES IN CENTRAL SAUDI ARABIA: HAS IT AFFECTED HOST POPULATION DEMOGRAPHY?

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Rabies is endemic in Saudi Arabia. In 1989 the 2244 km2 Mahazat as-Sayd protected area, in central Saudi Arabia, was fenced to exclude grazing and hunting, and to allow recovery of the native vegetation. Prior to 1997, rabies was reported only sporadically in the region, mainly affecting Red foxes (Vulpes vulpes arabica). In the final two months of 1997 and first four months of 1998, however, Mahazat as-Sayd, experienced an outbreak of fox rabies, with a total of 6 laboratory-confirmed cases of rabies in Red foxes, one in a Rüeppell's fox (Vulpes rueppelli sabaea), one in a Sand cat (Felis margarita), and one in a Ratel (Mellivora capensis). Additionally, 16 carcasses of Red foxes, two of Rüeppell's foxes, and one of a feral cat were found during this period, but no laboratory investigations were carried out due to their advanced state of decomposition. The first recorded cases occurred in Red foxes, whereas the first observed case in a Rüeppell's fox occurred three months later. Hypotheses concerning the apparent variation in the susceptibility of these two fox species are discussed. Populations of mammalian carnivores naturally occurring in the protected area have been studied during 1992-1994 and 1996-1998, and detailed estimates of densities have been made both inside and outside the reserve. Fox abundance was found to be markedly lower outside Mahazat as-Sayd. In a demographically unbalanced situation, theory suggests that rabies can result in the regulation of vector populations. Mathematical models have shown that the virus may spread across an area, at a rate and to an extent dependent on the density of fox populations, and on the presence of certain topographical features. However, fox demographic variations during the Mahazat as-Sayd outbreak proved to be difficult to interpret, and it is not clear whether the mortality of individuals attributed to rabies had significant population dynamic consequences. In particular it was difficult to distinguish between the effects of the disease and some other natural factors acting on fox populations.

# (10) ORAL WILDLIFE RABIES VACCINATION: BIOMARKER (TETRACYCLINE) ASSESSMENT.

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There are a number of ongoing projects in the United States evaluating rabies control through the distribution of vaccine-laden baits for free-ranging carnivores. Assessment based upon disease detection, or lack thereof, is constrained by passive case surveillance for public health needs. Serological monitoring requires intensive live-trapping within a limited time period after baiting (e.g., 3-12 weeks) for optimal detection of rabies virus neutralizing antibodies. A common additional tool to assess the proportion of sampled animals which consumed a bait is by analysis for a biomarker, such as tetracycline, a long-term, hard tissue (calciphilic) marker. This assumes routine quality assurance of biomarker incorporation in the bait during manufacture, as well as adequate field collection protocols for tissue selection and temporal collection. Previous studies have utilized teeth (e.g., premolars, canines, etc.) or jaws from live-trapped, euthanized or roadkilled animals as samples. The objective of this study was to examine the differential marking of tetracycline in mandibular bone, premolar and canine teeth from animals which consumed commercial rabies baits with the biomarker incorporated in the bait matrix. Approximately 8-10 micron sections of bone and teeth were cut, mounted, and observed by fluorescent microscopy for tetracycline marking. Among positive raccoon samples, a 37% (10/27) rate of discordant pairs was observed between canine teeth and mandibular bone samples. Discordant pairs consisted of 50% (5/10) of bone positive, tooth negative, and 50% of tooth positive and bone negative (5/10). Premolar samples were only positive (22%) when both bone and canine samples were also marked. If only mandibular bone or a canine tooth is examined, the sensitivity of tetracycline detection approximates 80%. When both samples were available, virtually 100% detection may be achieved. Examination of a premolar tooth alone yielded a sensitivity of 22% (78% false negative). Thus, post-mortem mandibular bone samples containing embedded canine teeth appear optimal for tetracycline analysis. Field collection of canine teeth from live-trapped animals for release is neither practical nor desirable and may underestimate tetracycline marking (e.g., up to 20% false negative). Similarly, field collection of a premolar tooth, while not as invasive as removal of a canine tooth, may considerably underestimate tetracycline (e.g., up to 78% false negative). To elucidate the value of tetracycline as a biomarker, examination of additional laboratory and field samples is warranted, to clarify the effects of age, reproductive status, dose and type of tetracycline, species differences, and related physiological variables.

# (11) RABIES VACCINE, LIVE VACCINIA VECTOR: BIOSIMILARITY COMPARISON BETWEEN THREE V-RG VACCINE DELIVERY SYSTEMS BY SEROLOGIC EVALUATION IN RACCOONS, *PROCYON LOTOR*.

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A new vaccine delivery system has been developed by Merial and the Southeastern Cooperative Wildlife Disease Study of the University of Georgia to deliver V-RG vaccine to wild raccoons. A biosimilarity study was initiated to test the new delivery system, identified as the Coated Sachet, in comparison with rectangular and square fishmeal polymer baits. Seventy-two rabies seronegative pen-raised raccoons were assigned to three vaccinate groups of 20, and one non-vaccinated group of 12 animals. Vaccinate groups were offered dog biscuits on days -4, -3, -2, and placebo contained in one of the three delivery systems on day -1 to accustom the raccoons to hand feeding. On the day of vaccination, raccoons were offered one of three different delivery systems containing Raboral V-RG®. Ninety-five percent of raccoons given the Coated Sachet ruptured the sachet containing vaccine. The rectangular and square fishmeal polymer baits were accepted by 90% and 75% respectively. Statistical analysis indicates no significant difference in seroconversion (RFFIT data to be presented) of captive raccoons between the currently licensed Raboral V-RG® fishmeal polymer baits and the smaller, lighter and less expensive Coated Sachet.

# (12) RABIES VACCINE, LIVE VACCINIA VECTOR ORAL VACCINATION OF COYOTES, CANIS LATRANS, IN SOUTH TEXAS USING AN EXPERIMENTAL COATED SACHET VACCINE DELIVERY SYSTEM.

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In 1995, the state of Texas began an intensive oral rabies vaccination campaign designed to contain the spread of the urban dog strain of rabies in coyotes. To date, 6.7 million units of Vaccinia Recombinant Glycoprotein (V-RG) vaccine inserted into a fishmeal-polymer bait have been distributed over South Texas. In 1997-1998, the Southeastern Cooperative Wildlife Disease Study and Merial Limited investigated the use of a novel vaccine delivery system targeting coyotes. Three configurations of the coated sachet, one, two, and three chamber were presented to captive coyotes. Through direct observation and fecal examination, it was found that the coyotes accepted and ruptured the different sized coated sachets at the same rate. In another study, six varieties of the coated sachet (no vaccine), as well as fishmeal-polymer baits were placed in track-stations in South Texas. Visitation and uptake of the coated sachets were similar to the fishmeal-polymer bait. Coated sachets containing V-RG, with no biomarker, were distributed in a ranch in South Texas over a 950 square mile area. Ninety-four percent of the juvenile coyotes, negative to the biomarker, were found to be positive for rabies neutralizing antibodies. These data show the feasibility of using this smaller, lighter and less expensive coated sachet vaccine delivery system instead of fishmeal polymer baits for oral rabies vaccination campaigns targeting coyotes.

# (13) DEATH IN THE PLEISTOCENE: DID EMERGING INFECTIOUS DISEASES CAUSE QUATERNARY MEGAFAUNAL EXTINCTIONS?

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During the late Quaternary, mammalian faunas were affected almost worldwide by an unusual series of extinctions that were highly regionalized and temporally disjunct. Thus, about 11,000 yrbp in the Americas 130+ species (including mammoths, mastodonts, "ground" sloths, sabertoothed cats) disappeared very suddenly, perhaps in the space of 400 yr. Similar but smaller losses occurred in Australia/New Guinea ~ 50,000 yrbp, in the West Indies ~ 5000 yrbp, and in Madagascar ~ 1000 yrbp. These extinctions are widely believed to have been anthropogenic in nature, and thus represent the "first" biodiversity crisis forced by our species. But what is the evidence for this conclusion? Overhunting is frequently mentioned as the leading cause, but for several reasons it is highly improbable that primitive peoples could have induced so many species losses in this way. Climate change is even more unlikely. As an alternative, I propose that most features of late Quaternary extinctions can be explained by inferring that the principal agency of loss was infectious diseases introduced into immunologically naive populations by colonizing humans or their commensals/synanthropics. The hyperdisease theory potentially (1) explains differential losses in K-selected vs. r-selected taxa (both young and old animals highly susceptible to disease, causing catastrophic depression in rate of natural increase in larger taxa); (2) requires no ad hoc explanations for the absence of mass kill sites in affected areas (mass kill sites absent because losses attributable to human hunting were usually negligible); and (3) accounts for pattern change in the character of anthropogenic extinction after first-contact losses. A methodology for testing this hypothesis, using "ancient DNA" techniques, is proposed.

# (14) NUCLEAR DNA FROM THE WOOLLY MAMMOTH (MAMMUTHUS PRIMIGENIUS): THE SEARCH FOR PLEISTOCENE VIRUSES.

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Proboscideans, particularly elephants, were a diverse and broadly distributed group during the Pleistocene, but were severely reduced by extinctions at the end of that epoch 10,000 years ago. The only survivors were the living Asian and African elephants and, surprisingly, the woolly mammoth, the last population of which persisted on Wrangel Island (Siberia) until approximately 4000 years ago. Fossil remains of the Wrangel mammoth population provides an abundant resource for testing the "hyperdisease theory", that infectious diseases were a leading cause of Quaternary extinctions among elephants and many other mammalian groups. To this end the preservation of nucleic acids in mammoth tissues is being systematically evaluated from samples collected from Wrangel Island. Samples from Alaska and Siberia are also being analyzed for control purposes. Molecular biological techniques have been applied by our group to the analysis of mitochondrial, high-copy nuclear, and single-copy nuclear DNA from mammoths. The DNA sequence data obtained is used to evaluate the presence of contaminating nucleic acids, the preservation state of the DNA, the phylogenetic relationships among the elephants, the ability to distinguish individual mammoths from one another, and the suitability of the extracted DNA's for analysis of viral nucleic acids. The work completed thus far establishes the framework from which to pursue reservoirs of benign and pathogenic microorganisms using samples that are extremely old and for which the DNA is both degraded and modified. We have confirmed that multiple samples from Wrangel Island and other locations are suitable for retrieval and analysis of nucleic acids. Additional work in progress includes the screening of the samples for viruses such as elephant herpesvirus and endogenous retroviruses.

# (15) WILDLIFE DISEASES & GLOBAL EXTINCTION: LESSONS FROM THE *PARTULA* SNAIL CONSERVATION PROGRAM

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A causal role for infectious disease in the global extinction of animal species has been hypothesized in a number of cases. Recently, the first definitive case of an infectious agent causing the extinction of its host has been reported. Snails of the genus *Partula* have been extirpated from much of their South Pacific island range following the introduction of alien predatory snails. Many species of *Partula* are now found only in captivity, and a number of these have become extinct during the past decade. *Partula turgida* became extinct on January 1st, 1996 and pathological investigations revealed a disseminated protozoal infection, with associated destruction of the digestive gland, within each of the last group of individuals to die. The impact of this and other protozoa in the success of *ex situ* captive breeding programs for *Partula* is discussed. Evidence for other infectious agents causing historical extinctions of wildlife species is reviewed and the concept of parasite conservation discussed.

# (16) PREDATORS, PATHOGENS, AND `ALALA: THE TRAVAILS OF ENDANGERED SPECIES REPATRIATION IN HAWAII.

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The alala (*Corvus hawaiiensis*) is one of the world's most endangered corvids with 4 adult birds remaining in the wild. Since 1993, the USFWS has been carrying out re-introduction of captive reared alala into their former native range on the Kona coast of Hawaii. These releases have met with mixed success and the cause of release failures (mortality) were not known because of unavailability of suitable diagnostic specimens. Since 1996, the HFS has received 13 carcasses of immature alala that have died within a year post release. Based on the number of carcasses scavenged by raptors, there appears to be significant interactions between endangered Hawaiian hawks (io) (*Buteo solitarus*) and alala. Of six alala carcasses suitable for laboratory assays, one bird died from erysipelas, one from mycotic arteritis, and 3 from toxoplasmosis. *Toxoplasma gondii* was cultured from the brain of a fourth partially scavenged alala, however, no lesions were seen in the brain. A fifth crow was found sick in the wild with high antibody titers to *T. gondii*; this animal was successfully treated with diclazuril thus providing a potential management option for the disease. Toxoplasmosis is a significant cause of morbidity and mortality in re-introduced alala. Io scavenge (and perhaps kill) alala and pose the management quandary of an endangered raptor killing an endangered scavenger.

(17) DETECTION OF THE CAUSATIVE AGENT OF DISSEMINATED VISCERAL COCCIDIOSIS (EIMERIA SP.) IN SANDHILL CRANES (GRUS CANADENSIS) AND WHOOPING CRANES (GRUS AMERICANA) BY POLYMERASE CHAIN REACTION AMPLIFICATION OF 18S rDNA.

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Disseminated visceral coccidiosis (DVC) is a disease characterized by the presence of disseminated lymphohistiocytic inflammatory lesions in sandhill cranes (*Grus canadensis*) and whooping cranes (*Grus americana*). The etiologic agent of DVC is a coccidian parasite of the genus *Eimeria*. Currently, diagnosis of this disease requires microscopic identification of the *Eimeria* parasite in tissue samples. However, microscopic identification of this parasite is often difficult due to the small numbers of organisms present or severe autolysis of field-collected specimens. A polymerase chain reaction (PCR) based assay was developed to detect *Eimeria* spp. DNA in frozen tissue samples from cranes known or suspected to have DVC. The PCR assay successfully detected *Eimeria* spp. DNA in tissue lesions known to contain coccidial organisms and also detected DNA in highly suspicious tissue lesions in which organisms were not microscopically visible. Tissue samples that did not contain lesions consistent with DVC and tissue samples from uninfected control birds did not produce a positive result with the PCR assay. This work provides a useful diagnostic tool, the PCR assay, to confirm the presence of coccidian DNA in tissue lesions suspected to be the result of DVC.

# (18) FLUOROQUINOLONE RESISTANT MYCOPLASMA GALLISEPTICUM FROM ENROFLOXACIN-TREATED HOUSE FINCHES (CARPODACUS MEXICANUS)

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There is an ongoing outbreak of *Mycoplasma gallisepticum* - associated conjunctivitis in house finches (*Carpodacus mexicanus*). House finches (N=57) presenting with *M. gallisepticum* - associated conjunctivitis were admitted to the Wildlife Rehabilitation Center of Minnesota during 1996 and 1997. Finches too debilitated for treatment were euthanized. Twelve birds were treated with oral enrofloxacin (Baytril, 15 mg/kg, BID x 21 days) and opthalmic gentamicin (BID x 21 days). A nested PCR was designed for sensitive and specific diagnosis of the presence of the organism. Following treatment, finches were held for up to six months and tested for the presence of *M. gallisepticum*. Seven out of twelve finches were positive. All finches were euthanized to prevent release of the organism. An *M. gallisepticum* isolate obtained from a post-treatment finch showed increased resistance to enrofloxacin as compared to pretreatment isolates. The results suggest that oral enrofloxacin and opthalmic gentamicin are not an effective treatment for the eradication of *M. gallisepticum* in house finches and show that nested PCR is a suitable method for detection of *M. gallisepticum* in house finches. Further, our results suggest the development of fluoroquinolone resistance in *M. gallisepticum* in a wildlife rehabilitation clinic

# (19) THE PREVALENCE OF *LEPTOSPIRA POMONA* IN CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*) ALONG THE CALIFORNIA COAST, 1996.

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Several severe outbreaks of renal disease in California sea lions (*Zalophus californianus*) stranded along the northern California coast have been attributed to leptospirosis. Although the microscopic agglutination test (MAT) has been used to diagnosis leptospirosis during such outbreaks, the usefulness of this test in California sea lions has never been examined. Evaluation of the sensitivity and specificity of the MAT as a method for detection of exposure to *L. pomona* in California sea lions was performed using sera from individuals with visible leptospires in kidney sections as positive controls and negative control captive individuals. Our results indicate that the MAT has 100% sensitivity and specificity for detection of *L. pomona* antibodies in California sea lions. Therefore, the MAT previously developed for cattle is a useful test for determining exposure to *L. pomona* in California sea lions.

Subsequently, sera from 225 California sea lions that presented to one of three participating California coast marine mammal rehabilitation centers in 1996 were evaluated for antibodies to *Leptospira pomona* using a microscopic agglutination test (MAT). Any animal with a titer of 1:100 or greater was considered positive. The overall prevalence was 38.22% (86/225), although the prevalence at each center varied from 100% (38/38) at the Marine Mammal Care Center (MMCC) at Fort MacArthur to 27.75% (48/173) at The Marine Mammal Center (TMMC) to 0% (0/14) at Sea World, San Diego. At TMMC, the majority of seropositive animals were subadults and adults; males were 4.67 times more likely to be seropositive to *L. pomona* than females; and the highest proportion of seropositive animals presented during the winter and autumn months. Our findings demonstrate that exposure of California sea lions to *L. pomona* may be more common in all regions of California than previously expected.

# (20) FIELD INVESTIGATION OF CONJUNCTIVITIS AND MYCOPLASMA GALLISEPTICUM INFECTIONS IN SONGBIRDS FROM NEW YORK.

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A field study was conducted to determine the prevalence of conjunctivitis and Mycoplasma gallisepticum (MG) infections in house finches (Carpodacus mexicanus) and other songbirds common to bird feeders in Tompkins County, New York. A total of 802 individuals of 23 species and nine avian families were captured and given physical examinations during the 14month study beginning in February 1998. Clinical conjunctivitis (eyelid or conjunctival swelling, redness, discharge) was observed in 9.7% (19/196) of house finches examined, and only in the winter months from November to March. Unilateral conjunctivitis was observed in 79% (15/19) of affected house finches; one case developed bilateral disease between 8 and 18 days following initial examination. Conjunctivitis was observed in a similar proportion of males and females sampled, and body condition scores and wing chord lengths were not significantly different between diseased and non-diseased house finches. M. gallisepticum was isolated from 76% (13/17) of finches with conjunctivitis and 2% (3/168) of clinically normal house finches during the study. DNA fingerprints of 11 MG isolates using random amplification of polymorphic DNA (RAPD) techniques showed no apparent differences in banding patterns over the course of the study, suggesting persistence of a single MG strain in the study population. The prevalence of conjunctivitis and MG infections declined in house finches between February/March 1998 and February/March 1999 (22.7% to 6.2%, and 20% to 4.6%, respectively), but only the former was significant (P < 0.05). Conjunctivitis was also observed in four American goldfinches (Carduelis tristis) and one purple finch (Carpodacus purpureus). M. gallisepticum infection was confirmed in the purple finch, the first documented case of MGassociated conjunctivitis in this species. The purple finch isolate was similar to house finch isolates from the study site by RAPD analysis. Positive plate agglutination (PA) tests were recorded in one other goldfinch and two purple finches, suggesting exposure of these individuals to MG. Positive PA tests were also obtained from two brown-headed cowbirds (*Molothrus ater*) and four tufted titmice (Parus bicolor), but MG infection could not be confirmed in these cases due to lack of samples. Based on these findings, the prevalence of MG infections in hosts other than house finches appear to be low in the population sampled. However, there is also growing evidence that songbird species other than house finches are susceptible to MG infection and disease.

# (21) WILDLIFE IN SOUTH CAROLINA AS RESERVOIR HOSTS FOR TRYPANOSOMA CRUZI.

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Trypanosoma cruzi, the etiological agent of American trypanosomiasis in humans, has been reported from a variety of reservoir hosts (e.g., raccoons and opossums). For sites representing the five different physiographic regions in South Carolina, serum was collected from a total of 167 raccoons and 135 opossums during the time period of April 1997 to May 1999. Serum was tested for anti-T. cruzi antibodies using the indirect immunofluorescent test (IFAT) with formalized Brazil strain epimastigotes as antigen. Fifty-one (50.9%) percent of raccoons tested were positive for T. cruzi, with a significantly higher prevalence in females (73.3%) than in males (47.6%). Trypomastigotes of T. cruzi isolated from the blood of two raccoons (T. cruzi-R) in Pickens Co. have been maintained in LIT culture as epimastigotes. When exposed to murine fibroblasts (L-cells), the culture forms of T. cruzi-R infected and multiplied in the L-cells, with trypomastigotes being released to invade adjacent cells. Preliminary data on virulence and pathogenicity were obtained by injection of four BALB/c mice with 10<sup>6</sup> culture forms of T. cruzi-R. Following recovery from the acute phase of infection, mice were challenged with a known virulent Brazil strain of T. cruzi to test for cross immunity between different strains.

## (22) ASSOCIATIONS BETWEEN WATER CHARACTERISTICS AND AVIAN CHOLERA AT THE SACRAMENTO NATIONAL WILDLIFE REFUGE, CALIFORNIA.

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Avian cholera, caused by infection with *Pasteurella multocida*, is an important cause of nonhunting mortality among wildfowl throughout North America. The respective roles of carrier birds versus wetlands as the source of *P. multocida* for epizootics remain unclear. During the winters of 1996-97 and 1997-98, we evaluated ten wetlands at the Sacramento National Wildlife Refuge complex, California, for their water characteristics and the presence of *P. multocida*; six wetlands had a history of mortality during the previous six years (epizootic sites) and four sites had little or no mortality during the previous six years (reference sites). We sampled for P. multocida in water and sediment of these wetlands before, during, and after epizootics. Pasteurella multocida was not recovered more frequently from epizootic sites than from reference sites and was not found consistently in water or sediment samples taken before, during, or two weeks after epizootics in 1996-97 and 1997-98. Water characteristics did not differ consistently between historical epizootic and reference wetlands, between wetlands experiencing epizootics during the study and wetlands with no mortality, or between wetlands from which P. multocida was isolated and those without the bacterium. Absence of P. multocida prior to epizootics and lack of consistent recovery of the bacterium during and after epizootics do not lend support to the hypothesis that *P. multocida* remain in natural wetlands for extended periods. Contrary to previous studies we found no consistent differences in water characteristics of sites with and without avian cholera.

## (23) ORAL TRANSMISSION AND EARLY LYMPHOID TROPISM OF CHRONIC WASTING DISEASE PrP<sup>res</sup> IN MULE DEER FAWNS.

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Chronic wasting disease (CWD), a transmissible spongiform encephalopathy, is a fatal disease of free-ranging mule deer (Odocoileus hemionus), white-tailed deer (Odocoileus virginianus), and Rocky Mountain elk (Cervus elaphus nelsoni) in northeastern Colorado and southeastern Wyoming, with recent emergence in game ranched elk in several states outside of Colorado. The natural route of transmission is currently unknown. We transmitted the disease experimentally via oral exposure of mule deer fawns to a brain homogenate prepared from deer with naturally occurring CWD. Fawns were necropsied and examined for PrPres, the abnormal prion protein isoform, between 10 and 80 days post-inoculation (pi) using an antibody against the prion protein and an immunostain assay. PrPres was detected in alimentary-associated lymphoid tissues (one or more of the following: retropharyngeal lymph node, tonsil, Peyer's patches, and ileocecal lymph node) as early as 42 days post inoculation (pi) and in all fawns examined thereafter (53 to 80 days pi). No PrP<sup>res</sup> staining was detected in lymphoid tissue of three control fawns receiving a control brain inoculum, nor was PrPres detectable in neural tissue of any fawn. These results indicate that CWD PrP<sup>res</sup> can be detected in lymphoid tissues draining the alimentary tract within a few weeks after oral exposure to infectious prions, early in the incubation period--and may reflect the initial pathway of CWD infection in deer. The rapid infection of deer fawns following exposure by the most plausible natural route is consistent with the efficient horizontal transmission of CWD in nature and enables accelerated studies of transmission and pathogenesis in the native species.

## (24) PRELIMINARY DISCUSSIONS ON THE EPIDEMIOLOGY OF BRUCELLOSIS IN WOOD BUFFALO NATIONAL PARK, CANADA.

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We present data on the epidemiology of brucellosis (*Brucella abortus*) in bison in Wood Buffalo National Park, Canada. Two hundred and seventy-six bison were captured and tested for brucellosis in the winter of 1997 (n = 136) and 1998 (n = 140). Sera were tested with the complement fixation test and buffered plate antigen test. Twenty-three percent (62/266) were positive, 70% (186/276) were negative and 7% (18/266) were suspicious reactors. Age significantly affected seroprevalence (Multiple logistic regression, Wald statistic = 16.5, d.f. = 5, p = 0.005, n = 266). Other factors did not contribute significantly to the logistic regression model (sex, region, body condition; p > 0.1). Using a weighted least-squares non-linear regression technique, we fit a sigmoidal curve to the age-stratified serological profile. Using this function we estimate a function for the age-specific force of infection, the instantaneous per capita rate at which susceptible individuals acquire infection. The resulting function is age-dependent in a non-linear fashion where young bison experience the greatest risk of infection. Above the age of five years, the force of infection drops to negligible levels. We discuss possible reasons for this pattern of infection.

### (25) GENETIC VARIATION OF EPIZOOTIC HEMORRHAGIC DISEASE VIRUS ISOLATES: SPATIAL AND TEMPORAL INFLUENCES.

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Epizootic hemorrhagic disease virus (EHDV) is a member of the genus *Orbivirus* which affects both wild and domestic ruminants, with periodic outbreaks involving high mortality. As a segmented RNA virus, EHDV is subject to a high level of mutation, and is capable of segment reassortment, both of which contribute to viral diversity. As is commonly seen in population biology, temporal and spatial factors contribute to the evolution of populations. In order to delineate the role of these factors in the genetic variation of populations of EHDV-2, we have isolated virus collected from white-tailed deer in seventeen states over a twenty- year period. Both conserved and variable genes have been sequenced from these isolates, and phylogenetic analysis performed. This data, in concert with sequence data from additional genes (including a gene encoding a highly variable surface protein) will provide a better understanding of the evolution of EHDV, and the generation of genetic diversity.

## (26) THYROID LESIONS IN BELUGA WHALES (DELPHINAPTERUS LEUCAS) FROM THE ST. LAWRENCE ESTUARY AND HUDSON BAY, QUEBEC, CANADA

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Thyroid follicular neoplasms have not been reported in marine mammals other than beluga whales (*Delphinaterus leucas*) from the St. Lawrence Estuary. In order to better characterize thyroid lesions in beluga whales, we compared the lesions of thyroid glands of animals from the St. Lawrence Estuary (n = 16) and from Hudson Bay (n = 15). Follicular adenomas, nodular follicular hyperplasia and cysts were found in eight, nine and nine adults from the St. Lawrence estuary (n = 10), respectively, and in four, six and four adults from Hudson Bay (n = 14), respectively. The size and the number of these lesions increased with age in both populations. Beluga whales from the St. Lawrence Estuary had larger and more numerous follicular adenomas, nodular follicular hyperplasia and cysts than animals from Hudson Bay. However, the beluga whales from the St. Lawrence Estuary sampled for this study were older than those from Hudson Bay. Beluga whales from both populations have unique thyroid lesions among marine mammals. Further studies using age-matched groups are needed to compare thyroid lesion of St. Lawrence Estuary beluga whales to those of other beluga whale populations.

## (27) SEROLOGIC AND PCR SURVEY FOR EHRLICHIAL ORGANISMS IN RACCOONS AND OPOSSUMS FROM AN *EHRLICHIA CHAFFEENSIS* ENDEMIC AREA.

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Ehrlichia chaffeensis, the causative agent of human monocytotropic ehrlichiosis, is maintained in nature in a cycle involving white-tailed deer ( $Odocoileus\ virginianus$ ) as reservoir host and lone star tick ( $Amblyomma\ americanum$ ) as vector. Antibodies reactive to  $E.\ chaffeensis$  also have been detected in raccoons ( $Procyon\ lotor$ ) and opossums ( $Didelphis\ virginianus$ ) in the Piedmont physiographic province of Georgia. To better understand if these antibodies resulted from previous infection with  $E.\ chaffeensis$  or represent a nonspecific cross reaction, blood samples were collected from 26 raccoons and 19 opossums trapped during February and March 1999 from a confirmed  $E.\ chaffeensis$ -endemic area. Samples were tested by indirect immunofluorescent antibody (IFA) assay for  $E.\ chaffeensis$  and  $E.\ canis$ . Eight (30.8%) raccoons had  $E.\ chaffeensis$ -reactive antibodies and six (23.1%) had  $E.\ canis$ -reactive antibodies at a titer of  $E.\ chaffeensis$ -reactive antibodies were not found in any opossums tested (0%). Testing of raccoons seropositive to  $E.\ chaffeensis$  and/or  $E.\ canis$  by bacterial wide nested polymerase chain reaction (PCR), and with primers specific to  $E.\ chaffeensis$  and  $E.\ canis$  is underway.

#### (28) SAFETY OF BRUCELLA ABORTUS STRAIN RB51 IN COLLARED LEMMINGS.

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Alaskan reindeer herds are known to be infected with *Brucella suis* biovar 4. One potential vaccine candidate to aid in the control of reindeer brucellosis is the live-attenuated, rough vaccine *B. abortus* strain RB51. However, vaccination with RB51 may inadvertently expose non-target species to this vaccine. The Alaskan collared lemming (*Dicrostonyx* spp.), a non-target host, has been reported to have a high degree of susceptibility for virulent *Brucella* spp. To evaluate the virulence of this vaccine in the collared lemming, 60 lemmings were divided into three groups. The groups consisted of 20 animals each, inoculated intraperitoneally with 1 x 10<sup>3</sup> colony forming units (CFU) of either virulent *B. abortus* strain 2308, rough *B. abortus* strain RB51 or saline. The animals were monitored daily and moribund lemmings were euthanized. At 7, 14, 17, and 32 days post-infection, 5 animals from each group were necropsied and samples obtained from the spleen and liver for bacteriologic and histologic analysis. By day 17 post-infection, all of the strain 2308 infected animals had succumbed to necrotizing hepatitis, whereas none of the strain RB51 lemmings when necropsied exhibited these lesions. Therefore, *B. abortus* strain RB51 did not cause mortality or severe pathology in collared lemmings when given intraperitoneally at this dose.

# (29) LAKE WHITEFISH, COREGONUS CLUPEAFORMIS, FROM THE ST. LAWRENCE RIVER, QUEBEC, CANADA; A BIOINDICATOR OF ENVIRONMENTAL CONTAMINATION.

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

## (30) HEALTH EVALUATION OF FREE-RANGING ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*) REQUIRING TRANSLOCATION IN SABAH, MALAYSIA.

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To minimize the risks involved in the translocation of wildlife including the introduction of new species of either competitors or pathogens, multiple measures ought to be taken. These include baseline information on the health status of free-ranging animals requiring relocation and an evaluation of the receiving habitat. The collection of blood, hair and fecal samples from 18 elephants requiring translocation was therefore incorporated into the capture and relocation of elephant protocol in Sabah, Malaysia. For the first time in Sabah, the combination of chemical immobilization and heavy machinery was successfully used for the health evaluation and translocation of wild Asian elephants (*Elephas maximus*). Blood samples were collected from thirteen sedated animals during the capture procedures. Fecal and fur samples were also collected from these elephants. The elephant's body condition, physiological data and effects of immobilization drugs were recorded. Elephants were marked for long and short-term identification. Two individuals were collared with radio tracking devices for follow-up studies. Sample analysis included basic hematology, serum chemistry, serum soluble elements, virology, bacteriology and parasitology. Blood samples analyzed were negative for brucellosis, bluetongue, bovine herpes 1 & 2 and parainfluenza-3. One elephant showed a weak positive response for Johne's disease. All but 2 samples analyzed were positive for one or more *Leptospira* serovars. Fecal samples were positive for nematodes including *Murshidia* and *Equinurbia*. Analyses for hemo-parasites were negative on peripheral blood films. Ecto-parasites in the louse family were seen on several individuals. Baseline data is now available on hematology, serum chemistry, serum soluble elements and circulating Vitamin A and E levels. Analysis of samples collected is providing objective information for the future management plans of these wild animals.

## (31) PRELIMINARY REPORT ON ADULT AND KITTEN SURVIVAL TIME OF FERAL CATS IN MANAGED COLONIES IN RANDOLPH COUNTY, NORTH CAROLINA.

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The appropriate management of feral cats is a controversial topic. Traditional control methods involve trapping and eradication, but programs to trap, neuter, and return feral cats to their habitats are being employed as alternatives to lethal measures. Since May 1998 we have been studying the population dynamics of nine established feral cat colonies managed under three different conditions: (1) control colonies, with reproductively intact males and females, (2) castration colonies, with males castrated and females spayed, and (3) vasectomy colonies, with males vasectomized and females spayed. Cats in all colonies are vaccinated (rabies, FVRCP), administered ivermectin annually, and provided food, water and some degree of shelter. Specific hypotheses are that (1) neutering feral cats stabilizes colony size by preventing reproduction within the colony and immigration from outside the colony, and (2) vasectomized male cats contribute more to stable colony size than do castrated male cats, being more likely to defend territories against intact male interlopers. Colony size at study inception averaged 11.67 + 6.24 adult cats, with at least 3 adult males per colony. Through April 1999, 130 cats (64 female, 66 male) have been enrolled. Median (+ SD) colony residence time for adult cats through April 1999 is 280 + 86 days, with no significant differences between sexes or among the different conditions. The proportion of kittens surviving to six months of age was 36% + 21% (mean + SD), with no significant differences between sexes or among the different conditions. Intact immigrant male cats have been observed in control and vasectomy colonies, while only previously neutered immigrant male cats have been observed in castration colonies. One immigrant female cat was briefly observed in a control colony. Kittens have been born in control colonies, but not in castration or vasectomy colonies, indicating that there have been no reproductively successful intact immigrants. Overall, control colony populations have continued to increase to (mean + SD) 128% + 75% of initial size, while castration and vasectomy colonies have decreased in size (89% + 8% and 81% + 10%, respectively). As the first full breeding season has progressed, conflict between males in control and vasectomy colonies has heightened and these aggressive interactions may further alter the stability of the colonies.

## (32) HELICOBACTER-LIKE ORGANISMS IN FREE-RANGING BOBCATS (FELIS RUFUS) IN THE SOUTHEASTERN UNITED STATES.

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Gastric spiral bacteria of the genus *Helicobacter* and *Helicobacter*-like organisms are gram negative, curved bacteria that have been described in many host species, including domestic and captive felids, but African lions are the only free-ranging species from which these bacteria have been described. Our objective was to determine if other free-ranging felids, such as the bobcat, are also infected with these organisms. To investigate this possibility, a retrospective study was conducted using banked formalin-fixed and paraffin-embedded gastric tissue of free-ranging bobcats from the Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, GA. Microscopic sections were stained with hematoxylin and eosin and Warthin Starry stains. Paraffin sections of stomach were examined by a multiplex PCR using primers specific for genus *Helicobacter* and for *H. pylori*; resulting products were sequenced. Formalin-fixed tissues were processed for and examined by electron microscopy. Stomach was available from 5 of 15 bobcats examined at the SCWDS between 1975 and 1998. All 5 had Helicobacter-like organisms in the gastric mucosa. The organisms were morphologically most similar to H. heilmannii. PCR amplification products had homology to other known Helicobacter spp. These results suggest that Helicobacter-like organisms are frequent in the gastric mucosa of free ranging bobcats, but their significance remains unclear. Further investigations are warranted to better characterize the distribution and prevalence of gastric spiral bacteria in other free-ranging wildlife.

#### (33) AUSTRALIAN BAT LYSSAVIRUS IN QUEENSLAND.

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Australia was considered free of the viruses in the genus *Lyssavirus* until the recognition in 1996 of Australian bat lyssavirus (ABL) as the cause of a rabies-like disease in a Black Flying-fox (*Pteropus alecto*) and a wildlife carer. ABL infection has been diagnosed in four species of Australian flying-fox (Black, Grey-headed, Little Red and Spectacled Flying-Fox) and an insectivorous bat, the Yellow-bellied Sheathtail-bat (*Saccolaimus flaviventris*). There have also been two fatal human cases of Australia bat lyssavirus infection.

The clinical signs of ABL infection in Australian bats are dominated by weakness and paralysis, particularly of the hind legs. Most infected bats are found on the ground or low in a tree or fence, and are quiet, depressed or moribund. There are few reports of infected bats being aggressive. Bats that have been in care while ill have shown progressive depression and paralysis and died within 1-2 weeks.

Between 27 October 1997 and 19 May 1999, 23 cases of Australian bat lyssavirus infection were diagnosed by fluorescent antibody test from 522 flying-foxes tested. Of these, none of the 231 clinically normal flying-foxes, most of which had been trapped for epidemiological investigations, were positive for ABL. The 23 cases constitute 8% of the remaining sick or injured flying-foxes, however ABL-infection was demonstrated in 39% of the 43 sick or injured flying-foxes that showed clinical nervous signs. There are species variations in the proportion of sick or injured flying-foxes diagnosed with Australian bat lyssavirus infection, it being most common in the Little Red Flying-fox. While only 5 Yellowbellied Sheathtail bats have been submitted, 4 (80%) have been ABL-positive.

## (34) IMMUNOHISTOCHEMICAL DIAGNOSIS OF CHRONIC WASTING DISEASE IN PRECLINICALLY AFFECTED ELK FROM A CAPTIVE HERD.

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Chronic Wasting Disease (CWD) is a condition seen in captive and free-ranging cervids, including mule deer, white-tailed deer, and Rocky Mountain elk. The disease is considered one of the Transmissible Spongiform Encephalopathies (TSE), a group of fatal diseases that affect the central nervous system and occur in several animal species, including human beings.

The diagnosis of CWD has traditionally relied on observation of characteristic clinical signs or histopathologic changes in brain tissue. This report presents evidence that neither of these diagnostic strategies is sufficient for identification of some infected animals.

An immunohistochemical (IHC) method was used to test brain tissues from 17 elk in a captive herd where CWD had previously occurred. The IHC technique detects a protein, the prion protein (PrP), that is considered a disease specific marker for TSEs, regardless of species affected. Of the 17 elk tested, 10 were found to be positive with the IHC test, whereas only 2 of the animals had shown clinical signs of CWD. Furthermore, only 3 animals, including both of the clinical cases, were definitely diagnosed as having the disease by histopathologic examination.

Application of the IHC method to different areas of the brain showed that the most consistently positive tissue was medulla oblongata, especially a region known as the obex. These results clearly show that an IHC test on the brain tissue, specifically medulla oblongata at the obex, is a more sensitive method than either clinical or histopathologic examination for diagnosing CWD. Therefore, the IHC test should be considered an essential component of any surveillance study intended to determine the incidence of CWD in captive or free-ranging cervids.

## (35) THE DEER MOUSE (*PEROMYSCUS MANICULATUS*) AS A POTENTIAL AMPLIFYING HOST FOR VESICULAR STOMATITIS VIRUS NEW JERSEY SEROTYPE.

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Vesicular stomatitis is a disease of horses, cattle, and swine, caused by two distinct but related viruses in the genus Vesiculovirus of the family Rhabdoviridae. Both viruses, vesicular stomatitis virus New Jersey (VSV-NJ) and vesicular stomatitis virus Indiana (VSV-I), exist in North, Central, and South America. Disease caused by both viral serotypes occurs in livestock in the western United States as periodic outbreaks, and at one site (Ossabaw Island, Georgia) in enzootic form (VSV-NJ). Despite having significant economic and regulatory impacts, the epizootiology of vesicular stomatitis remains largely undefined. Rodents have been suggested as potential reservoir and/or amplifying hosts for these viruses, and the goal of this research was to investigate whether the deer mouse (Peromyscus maniculatus) could serve as an amplifying host for VSV-NJ. Deer mice occur throughout all of the western United States, including sites where VSV-NJ (and VSV-I) outbreaks occur in livestock. Mice of three age classes (nestlings, juveniles, and adults) were inoculated intradermally with VSV-NJ, to simulate infection by a biological insect vector, and clinical outcome of infection and extent and duration of viremia were examined. Adult mice were generally refractory to infection, and failed to develop either viremia or clinical signs of disease. Nestling and juvenile mice were susceptible to infection via the intradermal route, and developed a detectable viremia that lasted from days 1-3 postinoculation (PI). Viremia in nestling and juvenile mice was followed by clinical signs of disease and death, usually by day 4 (nestlings) or 5-6 (juveniles) PI. These results suggest that nestling and juvenile deer mice are capable of serving as amplifying hosts for VSV-NJ, and also provide a good model for further virus/host/vector research.

## (36) DEVELOPMENT AND USE OF A RECOMBINANT CELL CULTURE BIOASSAY SYSTEM FOR THE DETECTION OF PETROLEUM EXPOSURE IN SEA OTTERS (ENHYDRA LUTRIS SPP.).

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Exposure to petroleum products has been well-documented to cause acute morbidity and mortality in marine vertebrate species due to physical coating, alterations in thermoregulatory mechanisms and organ toxicity. While the determination of extensive external exposure is oftentimes easy to accomplish by simple observation, light external oiling and the antemortem assessment of internal exposure is difficult to achieve in a time-, cost- and sample-effective manner. To address this limitation, a recombinant cell line, which responds to pollutant exposure with the induction of firefly luciferase, has recently been developed as a bioassay for the direct detection of such compounds in small amounts of serum. This method was optimized for use in sea otters (Enhydra lutris spp.) through a three-phased approach. First, the methodology was designed and optimized such that petroleum compounds (and polycyclic aromatic hydrocarbon, or PAH, components of oil) were able to be detected in "spiked" serum samples down to the picomolar (or parts per trillion) levels. Secondly, as part of a multifaceted study examining the physiological effects of oil on a model for otter species, serum from American mink (*Mustela vison*) externally or internally exposed to Alaska North Slope crude oil or Bunker C fuel oil was evaluated. Lastly, sera collected from oil-exposed sea otters (as part of the rehabilitation effort associated with the Exxon Valdez oil spill), otters being rehabilitated due to non-oil associated causes, and free-ranging Alaskan and Californian animals captured for other evaluative projects were analyzed using this system. The results of these analyses prove the validity of this testing method in the assessment and semi-quantitation of petroleum exposure in otter species.

### (37) VACUOLAR MYELINOPATHY IN WILD BIRDS FROM AN IMPOUNDMENT IN THE NORTH CAROLINA SANDHILLS.

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The history, pathology and epizootiology of a primary demyelinating disease in wild birds is summarized with an emphasis on the occurrence of the disease in a North Carolina impoundment. At this site, we have documented vacuolar myelinopathy in American coots (*Fulica americana*), mallard ducks (*Anas platyrhynchos*), ring-necked ducks (*Aythya collaris*) and one bald eagle (*Haliaeetus leucocephalus*). Field signs of affected birds include those consistent with central nervous system impairment of motor function (incoordination, abnormal movement and posture, weakness, paralysis); mortality of affected birds has also been documented. Lesions in the brain and spinal cord are characterized by splitting of myelin layers; their resemblance to lesions naturally- and experimentally-induced via certain chemicals support the current theory that the disease is caused by a neurotoxicant. Efforts to determine the cause of disease include epidemiological assessments (geographic extent, disease onset and duration, affected species, etc.), histopathology (disease progression, severity, and extent), and environmental chemistry / toxicology (analyses of environmental media, analyses of tissues of affected birds, and attempts to reproduce the lesion via field and laboratory exposures).

## (38) LEUCOCYTOZOONOSIS IN NESTLING BALD EAGLES, HALIAEETUS LEUCOCEPHALUS, IN MICHIGAN AND MINNESOTA.

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The bald eagle, *Haliaeetus leucocephalus*, has been listed as a threatened species in Michigan and Minnesota under the United States Endangered Species Act. Its status as a threatened species makes it somewhat difficult to study and there are still aspects of its life history that are poorly understood. Morbidity and mortality factors effecting populations are one of the areas that need further study. The purpose of this investigation was to look for occurrences of blood parasites in nestling bald eagles and to begin to assess the impact of blood parasites on bald eagle populations. Thirteen of 21 nestling bald eagles examined for blood parasites in Michigan and Minnesota during June and July 1997 had patent infections of *Leucocytozoon toddi*. No other parasites were seen. The degree of parasitemia was light and varied from 1 to 2 on the Ashford Scale. Several of the infected nestlings appeared to have elevated levels of heterophils in their peripheral circulating blood. One of the infected nestlings also showed signs of severe anemia. We believe this is the first report of *L. toddi* in the bald eagle. Ongoing studies will assist us in determining if spatial and temporal trends of prevalence exist.

### (39) AN INVESTIGATION OF SHELL LESIONS IN A POPULATION OF TURTLES IN A MARYLAND LAKE.

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In April 1997 a private citizen reported moribund and dead turtles in Tony Tank Lake, Salisbury, MD. Upon investigation by the Maryland Department of Natural Resources (MDDNR) painted turtles (Chrysemys picta) and red-bellied turtles (Chrysemys rubriventris) were found along the banks of the lake with disfiguring plastron and carapace lesions. A small subsample of turtles was sent to a local pathology lab for analyses and upon microscopic examination lesions were described, but the cause of the lesions was not determined. A pilot study was developed to collect and examine turtles throughout the summer and fall. Live turtles were sent to a local aquarium and a clinical evaluation was performed; additional turtles were sent for pathological analysis. Bacterial and fungal infections were reported, though the inciting cause of the lesions was not determined. In 1998, MDDNR began a mark and recapture study to determine the species affected and the extent of the lesion occurrence. During this investigation 234 individuals were captured and examined, including painted, red-bellied, stinkpot (Sternotherus odoratus), red-eared sliders (Chrysemys scripta elegans) and snapping turtle s (Chelydra serpentina),. Painted, red-bellied, and red-eared slider turtles were found affected. These turtles had either raised nodular lesions or excavated, penetrating lesions of plastron or carapace. Results from soil and water samples have been unremarkable thus far. In 1999 MDDNR will continue to document lesion occurrence, conduct water/sediment and fish sampling projects in the lake, and submit additional turtles for pathologic examination.

## (40) IMMOBILIZATION OF FREE-ROAMING RACCOONS (*PROCYON LOTOR*) AND OPOSSUMS (*DIDELPHIS VIRGINIANA*) WITH MEDETOMIDINE-KETAMINE AND REVERSAL WITH ATIPAMEZOLE.

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As part of a pilot study for an urban wildlife health assessment, six free-roaming adult raccoons (Procyon lotor) and nine free-roaming adult opossums (Didelphis virginiana) were live-trapped (Havahart; Lititiz, PA, USA) and immobilized with a combination of medetomidine hydrochloride (MED) and ketamine hydrochloride (KET) and reversed with atipamezolehydrochloride (ATI). Approximate weights were determined for the captured raccoons and they were given an injection IM of 0.075 mg/kg MED and 2.5 mg/kg KET; approximate weights were also determined for the opossums and they were given an injection IM of 0.100 mg/kg MED and 5.0 mg/kg KET. The induction times for raccoons and opossums were  $7 \pm 3$  (5-11) min and  $6 \pm$ 3 (3-10) min, respectively. Heart rate, respiratory rate, body temperature, presence of pedal withdrawal reflex and corneal reflex, and response to handling were recorded at 5, 10, 20, and 30 min. This combination provided at least 30 minutes of effective immobilization/restraint allowing for venipuncture, external parasite collection and physical examinations. After 30 min raccoons and opossums were given an injection IM of 0.375 mg/kg ATI and 0.500 mg/kg ATI, respectively. Raccoons and opossums given ATI were ambulatory in a mean of  $12 \pm 6$  min and  $6 \pm 3$  min, respectively. This combination of drugs provided a good level of immobilization and allowed for rapid recovery in comparison to other drug combinations.

### (41) DESCRIPTION AND CLASSIFICATION OF MALFORMATIONS IN THE NORTHERN LEOPARD FROG.

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Frog malformations have occurred in 43 states, in 38 species of frogs and 19 species of toads. Rear limb malformations are the primary malformations reported in free-living frogs, and were found in 86% (157/182) malformed Northern leopard frogs (*Rana pipiens*) submitted to the National Wildlife Health Center (NWHC) during 1997 and 1998 from studies conducted in Minnesota (1997, 1998), Vermont (1997), and Maine (1998). We did not attempt to infer incidence or frequency of malformations from the biased samples submitted to the NWHC. Instead, we considered all malformations to be important to the developmental story as even the unique malformations may represent a larger set of malformed frogs that were either not submitted, not observed, or did not survive. Because of this, detailed characterization of rear limb malformations were completed in all 157 newly metamorphosed leopard frogs based on external examination of frogs at necropsy, radiographic evaluation of the skeleton and microscopic examination of bones from selected frogs. The characterized malformations (represented by the 36 radiographs in this poster) were then organized into 6 major categories with the hope that complete and thoughtful classification of malformations seen in the field might provide clues to the dysfunctional developmental mechanisms occurring in nature.

#### (42) ORAL DELIVERY OF SEDATIVE AGENTS TO CAPTIVE WILDLIFE.

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Parenteral administration of immobilizing agents is the norm in veterinary anesthesia but oral delivery of agents may, in some circumstances, be a useful alternative. In captive animals, oral administration avoids the stress associated with darting and avoids darting-related morbidity. Orally delivered, transmucosally absorbed (ODTA) carfentanil citrate mixed with honey has been used successfully to immobilize adult black bears (6.8-18.8 µg/kg), polar bears (1.0-2.0 mg total dose), and a spectacle bear (1.5 mg total dose). A lower dosage of ODTA carfentanil citrate (2.0 µg/kg) has been used to sedate a wide variety of primates. In both bears and primates carfentanil produces profound bradypnea, resulting in lower than desired hemoglobin (oxygen) saturation. When we elect to use ODTA carfentanil, it is as an initial sedative, to make the animal indifferent to darting. Naltrexone, used to reverse the carfentanil effects, is administered by dart approximately 20 minutes after carfentanil administration. Dissociative and alpha-2agonist agents, used to maintain immobilization, are mixed with the naltrexone and administered in the same dart. Insufflation of the animals with oxygen, via a nasal tube, is routinely performed after the animals are safe to handle. Detomidine, an alpha-2-agonist marketed for use in horses, is also an effective sedative via ODTA route. Detomidine (0.5 mg/kg ODTA) has been used alone or in combination with ODTA ketamine HCl (10 mg/kg) to sedate servals, lions, a tiger, and African hunting dogs. Depth of sedation is, in part, determined by the animal's level of excitement prior to drug administration and success of drug delivery and contact time on the oral mucosa. Oral delivery of sedative agents offers a less noxious alternative to darting animals and may be useful to wildlife veterinarians and managers in certain situations.

#### (43) BEETLES, BIRDS AND BATS: CHLORDANE POISONING IN NEW JERSEY.

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Mass (425) chlordane poisonings of common grackles (Quiscalus quiscula), European starlings (Sturnus vulgaris) and American robins (Turdus migratorius) were recurring in July at a roost in suburban Scotch Plains, New Jersey. This paper reports on an investigation into the ecological aspects which account for the periodicity of these poisonings. Invertebrates with at least a portion of their life stages spent in the soil were collected by pit traps, light traps and pheromone traps in the vicinity of the suburban roost and a rural reference site during July 1998. Chlordanerelated compounds were present at concentrations above 1 ug/g in stag beetles (Lucanus sp.), Asiatic garden beetles (Maladera castanea), Japanese beetles (Popilla japonica), and oriental beetles (Anomala orientalis). Japanese beetles and oriental beetles had the highest mean tissue concentrations of chlordane-related compounds at 5.9 and 15.1 ug/g, respectively. Japanese beetles and oriental beetles collected from a rural reference site during the same period had chlordane-related compounds in low concentrations of <0.02 and 0.03 ug/g, respectively. Twenty-four fecal pellets collected at the roost contained the remains of 1-5 beetles each. Scarab beetles, including the oriental and Japanese beetles, accounted for 43% of the total number of insect parts from the stomach contents of 8 common grackles mist-netted at the roost. The peak month for the emergence of toxic beetles from the contaminated lawn soils (0.02 - 20.3 mg chlordane-related compounds/kg soil) at the roost corresponds to the July peak for chlordane poisonings of both passerine birds and Cooper's hawks (Accipiter cooperii). This suggests the hawks may be feeding on passerines debilitated with chlordane. However, scarab beetle parts in the stomach contents of an American Kestrel (Falco sparverius) diagnosed with chlordane poisoning suggests that the small falcons and hawks may derive their toxic dose directly by consuming contaminated beetles. Brains of 8 rabies negative big brown bats (*Eptesicus fuscus*) and 2 rabies negative little brown bats (*Myotis lucifugus*) submitted to the NJ Rabies Laboratory from northeastern New Jersey during July 1998 were analyzed for chlordane-related compounds. Half of the bats had chlordane-related compounds at concentrations greater than the mean background concentration (0.7 ug/g) in mist-netted grackles from the Scotch Plains roost. One big brown bat had brain concentrations of heptachlor epoxide (4.6 ug/g) and oxychlordane (4.8 ug/g) exceeding lethal levels for birds. This study demonstrates that bats have been impacted by chlordane probably through ingestion of nocturnal beetles (i.e. June beetles, masked chaffers). It also suggests the Japanese beetle has excellent potential as an indicator of local soil contamination and risks posed to insectivorous wildlife. These widely distributed beetles are easily collected in sufficient quantities for chemical analysis using commercially available pheromone traps. A survey of critical Cooper's hawk and other wildlife habitats using contaminated beetle indicators is currently being conducted in New Jersey.

## (44) CAPTURE, HEALTH, AND MORPHOLOGICAL ASSESSMENT OF FREE-RANGING MANTLED HOWLER MONKEYS (*ALOUTTA PALLIATA*) IN NICARAGUA.

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This paper describes the results of an inter-disciplinary project on free-ranging mantled howler monkeys (*Aloutta palliata*) on Ometepe Island in Lake Nicaragua. This project is unique in being the first in Nicaragua to assess the health of these biogeographically isolated monkeys. Eleven mantled howler monkeys (5 adult female, 5 adult male and 1 subadult female) in a group composed of 13 adults were captured, marked and released over a 4 day period in late July, 1998. Capture was affected using a CO2 propelled dart gun system delivering 120 mg of a combination of tiletamine and zolazepam (approximately 20 mg/kg). Unconsciousness occurred rapidly (1 - 4 minutes) with a mean recovery time of 2.53 +/- 0.56 hours. During the period of restraint, each animal was given a thorough physical examination; blood, feces and hair samples collected; and information gathered on temperature, heart and respiration rates, body weight, morphology (sitting height, tail length, upper and lower limb components), dental morphology, and estimates of age and reproductive status.

Overall the health of the animals was excellent. Mean body weights were 4.86 +/- 0.34 kg for females and 6.23 +/- 0.10 kg for males. Palpation indicated that 3 females were pregnant. Hematology performed in the field revealed median values for WBC (6,660/ml), RBC (4.09 x  $10^6$ /ml), and PCV (40%). Plasma, serum, feces, blood smears and clots were transported to a laboratory for analysis (DNA isolation, serum chemistry, differential blood counts, serology, blood parasite and fecal parasite examination). ELISA for measles and IFA for EEE, VEE, yellow fever, dengue, herpes plattyrhinus, and leptospirosis indicated no exposures. No blood parasites were observed in blood smears. Fecal samples were negative for giardia antigen by ELISA analysis. All fecal samples were negative for louse eggs, but all were positive for an unspeciated pinworm.

The lack of evidence of previous exposure to common diseases may indicate that the island howling monkey population is an at-risk population in terms of susceptibility to disease. The island is experiencing increased human activity through population growth and tourism. Howling monkey habitat is decreasing and becoming more fragmented resulting in increasing interaction of monkeys with people and domestic animals (horses, cattle, swine, fowl, and dogs).

## (45) MORBIDITY AND MORTALITY OF WEDDELL SEALS, *LEPTONYCHOTES* WEDDELLII, IN McMURDO SOUND, ANTARCTICA.

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The population of Weddell seals in McMurdo Sound, Antarctica, has been studied extensively by wildlife ecologists and physiologists. A demographic database, compiled over several decades, provides detailed histories on individual animals, a rarity in studies of wildlife health. The population's proximity to the largest human settlement in Antarctica makes it a good candidate for assessing anthropogenic impacts on Antarctic wildlife. Development of biomedical reference ranges is an important first step in assessing wildlife population health and is needed for evaluations of the relative impacts of environmental factors and human activities. We conducted physical exams and collected blood samples from 39 clinically healthy Weddell seals (17 adults, 22 pups) and from 14 seals (11 adults, 3 pups) with significant abnormalities on physical exam (e.g., suppurative wounds) during the 1996/97 and 1997/98 breeding seasons. Standard veterinary hematological and serum biochemical profiles were developed by age and sex class. We conducted post-mortem exams on four freshly dead seals encountered during the course of other activities. We also surveyed all seals at two sites for lesions (cf. McFarlane 1996; Aquatic Mammals 22:27-33). Our goals were to compile a baseline biomedical database for Weddell seals, including the prevalence of infectious and non-infectious disease. Most blood parameters were within reference ranges published for other phocid seals, though several varied with age and condition (p<0.05). Total white blood cell counts, monocyte counts, serum urea nitrogen and creatinine levels were higher in adults; serum glucose, cholesterol, triglycerides and iron were higher in pups. Clinically healthy adults had higher red blood cell counts, lower white blood cell counts, and fewer bands and neutrophils than seals with abnormal physical exam findings. Clinically healthy pups were larger (length and girth), had higher hematocrits and serum glucose levels, had fewer bands and lower serum creatinine kinase and lactate dehydrogenase levels than pups that were abnormal on physical exam. Physical injury (e.g., conspecific trauma) was the most common non-infectious lesion observed. No previously unreported parasites were detected during examinations of carcasses, feces, or blood smears. Finally, our preliminary pathogen seroprevalence data suggest that this is a relatively naïve population, which has important implications regarding its susceptibility to epizootics (cf. Duignan et al. 1995; Journal of Wildlife Diseases 31:491-501).

## (46) THE HOOK LAKE WOOD BISON RECOVERY PROJECT: AN ATTEMPT TO ERADICATE BOVINE TUBERCULOSIS AND BRUCELLOSIS FROM A WOOD BISON HERD IN NORTHERN CANADA.

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Free-ranging bison herds in the greater Wood Buffalo National Park (WBNP) area are infected with bovine brucellosis and tuberculosis. These two diseases pose a threat to long term conservation and recovery of healthy free-ranging bison populations, the ecological, cultural and economic value of bison to local aboriginal communities, and the disease-free status and international reputation of commercial bison and cattle industries in Canada. In 1990, a federally appointed Northern Diseased Bison Environmental Assessment Panel recommended depopulation of infected herds and their replacement with disease-free wood bison. That recommendation was rejected by the Canadian public. The public's major concerns were possible impairment of ecosystem integrity and loss of genetic diversity represented in the infected herds. An alternative option to depopulation is being developed as a pilot project by the Gov't of the NWTs and the community of Fort Resolution, NT. The Hook Lake Wood Bison Recovery Project involves a phased approach to disease eradication, genetic conservation, and recovery of an infected wood bison herd in an area northeast of WBNP. The program included capturing newborn calves, screening for brucellosis, strict isolation of orphaned calves in pairs, prophylactic antibiotic treatment, and intensive testing for both diseases. We captured newborn calves in May 1996, 1997 and 1998. In 1997 and 1998, we also screened calves for brucellosis and detected maternal antibodies to Brucella abortus in 10 of 52 calves (19%). In those years, only test negative calves were accepted into the recovery project and all three cohorts were handreared in an isolation facility at Fort Resolution, NT. During their first 14 days of captivity, we injected oxytetracycline and dihydrostreptomycin every other day; in 1998, oxytetracycline was givin orally. Enrofloxacin and isoniazid were administered for up to three months. In 1997 and 1998, rifampin was also administered through the milk ration. Preliminary analysis of peak serum levels and 24-hour pharmokinetic curves indicated satisfactory absorption and maintenance of blood levels for all three antibiotics. We have conducted whole-herd disease tests biannually using an array of serological tests for Brucella abortus and the caudal fold test and Blood Tuberculosis Test for *Mycobacterium bovis*. With the final capture of calves in May 1998, the captive bison herd consists of 58 founders in three separate cohorts. To date, repeated negative whole herd tests have been completed for the 1996 (six tests), 1997 (four tests) and 1998 (two tests) cohorts. The spring of 1999 represents the first of several additional tests for brucellosis in first-calf heifers and their calves. This spring, we will sample serum from nine pregnant females and their calves at 24 hours and 14 days post-calving to detect possible conversions of latent B. abortus infections.

#### (47) BOVINE TUBERCULOSIS IN MICHIGAN.

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Since 1994, the State of Michigan has recognized a problem with bovine tuberculosis (TB), caused by *Mycobacterium bovis*, in the wild white-tailed deer from a six-county area in northeastern lower Michigan. The disease has been found in other wildlife species and, in 1998, in domestic cattle. Recognizing the potential economic and public health consequences of *M. bovis* to the state, the governor has issued orders to eradicate *M. bovis* from the state's deer population. Unfortunately, the situation is unique in that there have never been reports of self-sustaining bovine TB in a wild, free-ranging cervid population in North America. There are no existing control programs for TB in wild deer, and there is much about TB in deer that is currently unknown. Scientists, biologists, epidemiologists, and veterinarians that have studied this situation have concluded that the most logical theory is that the supplemental feeding of wild deer serves to congregate deer, therefore contributing to the spread of TB. Supplemental feeding has been banned and baiting (the practice of hunting deer over feed) has been limited with the intention of reducing the spread of TB between deer and eventually eliminating this disease from the wildlife, therefore completing the eradication. In addition, deer densities are being reduced through hunting.

#### (48) NATURALLY OCCURRING TUBERCULOSIS IN WHITE-TAILED DEER.

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In 1997 tuberculosis was diagnosed in a captive herd of white-tailed deer (*Odocoileus virginianus*) in northeast Michigan. The herd was composed of approximately 350 head of white-tailed deer on 1500 acres, and was operated as a private hunting establishment. The decision to depopulate the herd was made by the owner, the Michigan Department of Agriculture, and the USDA. During the depopulation effort, postmortem examinations were done on 116 deer. Various tissues, feces, and swabs of the nasal, oral, and tonsilar crypt regions were collected for bacteriologic culture from all 116 deer. Tissues were collected for microscopic examination from deer with gross lesions suggestive of tuberculosis. Tuberculous lesions were seen in 9 of 116 deer. *Mycobacterium bovis* was isolated from 1 deer that had no gross lesions of tuberculosis. The most common sites to contain gross lesions were the medial retropharyngeal lymph nodes (6 of 9), and the lung (4 of 9). Three of ten tuberculous deer would have been missed had the examination been limited to the head and associated cranial lymph nodes; however only 1 of 10 would have been missed if the thoracic organs would have been examined in addition to the head.

Tuberculous deer ranged in age from < 1 yr to > 4 yrs of age, with deer 2-4 yrs of age most commonly affected. Swabs of the tonsilar crypt region from 1 of 116 deer contained *M. bovis*. All other swabs and fecal samples were negative for the presence of *M. bovis*. Pelleted feed, corn, hay, and soil were collected for bacteriologic culture from feeding sites on the 1500-acre premise in March, 1998 and again in November, 1998. Water was collected for bacteriologic culture from ponds used as watering sites by deer. *Mycobacterium bovis* was not isolated from any feed, hay, soil, or water samples.

Some surveys for tuberculosis in white-tailed deer involve examination of the head and cranial lymph nodes only. These results suggest that examination of the head alone, will underestimate the prevalence of disease in a naturally infected population; however, examination of the medial retropharyngeal lymph node is critical in cases of suspected tuberculosis in white-tailed deer.

### (49) IDENTIFICATION OF A PROTEIN IMPORTANT IN IMMUNODIAGNOSIS OF *PARELAPHOSTRONGYLUS TENUIS* INFECTIONS IN RED DEER.

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Experimental infections of *Parelaphostrongylus tenuis* were attempted in 12 of 15 red deer. We utilized three groups of four animals which were fed 10, 25 and 100 third-stage larvae ( $L_3$ ) of P. tenuis in saline, respectively, with a group of 3 additional red deer serving as non-exposed controls which received saline only. The four deer in each of the three groups of animals exposed to P. tenuis received a challenge exposure, equivalent to the initial exposure, at either 6, 12, 18, or 24 months post-initial exposure. Infections were monitored through bi-weekly blood and faecal sampling for up to 2.5 years in the red deer. Adult *P. tenuis* were not recovered from the control animals, but were recovered from each of the 12 experimentally exposed animals at necropsy. Adult *P. tenuis* were collected from road-killed white-tailed deer and protein samples derived from the adult stage of *P. tenuis* were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The membrane-transferred proteins were exposed to up to 11 serum samples obtained from each of the 15 red deer over the course of the experiment. Sera from each animal included: two samples taken pre-exposure to P. tenuis; and, up to nine samples at defined intervals post-exposure to P. tenuis (1, 2, 3, 5, 11, 17, and 23 months post-exposure as well as the serum samples taken one week post-challenge and those collected at euthanasia). Sera from control animals were taken at the same intervals and were recorded as post-saline. The antigen recognition profile of the sera was found to vary substantially between individual animals and, more importantly, with the duration of the infections. Although several adult worm proteins were identified only by sera from infected animals, the response was either inconsistent among all animals or the antibody response disappeared over time. A single protein band was recognized consistently by all infected animals throughout the course of the infections beginning at 1 month post-exposure to *P. tenuis*. Evaluation of the Western Blot assay, based on recognition of this single protein, resulted in a sensitivity of 99 % (89 of 90 positive serum samples recognized this band) and the specificity was calculated at 86 % (49 of 57 negative serum samples did not recognize this band). This research represents the first conclusive identification of a protein from *P. tenuis* with real potential for development of a reliable antemortem diagnostic procedure.

#### (50) XYLAZINE-INDUCED ASPIRATION PNEUMONIA IN SHIRA'S MOOSE.

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Large North American ungulates are often chemically immobilized with a combination of carfentanil and xylazine. This combination is thought to decrease drug volumes, decrease induction times, reduce undesirable side effects, and improve recovery. Eleven Shira's moose (Alces alces shirasi) were captured in northern Colorado via net gun and transported to Wyoming for captive research. Three weeks after this initial capture, these moose were anesthetized with 3 mg carfentanil and 30 mg xylazine. Nine of 11 moose subsequently developed pneumonia and died or were euthanized. This observation led to the hypothesis that the xylazine component of the drug mixture was responsible for abnormal postures that exacerbated aspiration pneumonia. Six female adult moose were then captured in western Wyoming and brought into captivity. Moose were allowed to acclimate for several months. Moose were immobilized three times each with 3 mg carfentanil only. Then moose were immobilized with 3 mg carfentanil and 30 mg xylazine and observed for 7 days at which time they were euthanized and necropsied. Moose were euthanized before 7 days if clinical pneumonia developed. As of May 10, 1999, no moose immobilized with carfentanil alone developed pneumonia whereas 3 of 4 moose immobilized with carfentanil/xylazine developed pneumonia (P = 0.003). Moose given carfentanil only remained in sternal recumbency whereas 4 of 5 moose given carfentanil/xylazine were unable to remain sternal (P = 0.02). There was no difference in induction times between moose given carfentanil and moose given carfentanil/xylazine (P = 0.13). These data suggest, but do not prove conclusively, that moose that are unable to maintain sternal recumbency develop aspiration pneumonia and that xylazine contributes to this loss of posture. Based on these data we suggest that Shira's moose be immobilized with 0.01 mg/kg carfentanil only.

### (51) REDUCING THE RISK OF TRANSMISSION OF BRUCELLOSIS FROM ELK TO CATTLE.

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Some elk ( $\underbrace{Cervus\ elaphus\ nelsoni}$ ) being fed in the winter on feedgrounds in western Wyoming are infected with  $\underbrace{Brucella\ abortus}$ , and it is feared that they may transmit the disease to cattle of the area. A formula examining the risk of brucellosis transmission from elk to cattle was developed. The probability of transmission occurring at least once in a given year is:  $P_{(trans.)} = 1 - (1 - (1 - 1/N_{cows})^{Ielkbelk@cow}))^{Nsuscows}$ , where  $P_{(trans.)}$  is the probability of transmission,  $N_{cows}$  is the total number of cattle grazing in the area inhabited by brucellosis infected elk,  $I_{elk}$  is the number of infectious elk,  $b_{elk@cow}$  is the number of infectious contacts made between cattle and one infectious elk (including its fetus), and  $N_{suscows}$  is the total number of susceptible cattle in the area. While the formula can't determine the actual risk of transmission, it indicates that only a few variables determine this risk. The risk can be most effectively reduced by vaccinating elk and cattle, reducing the feedground elk population, improving elk habitat, keeping elk and cattle separate from January through the end of June, and/or restrictions on cattle grazing.

#### (52) DIAGNOSIS OF CHRONIC WASTING DISEASE OF DEER AND ELK.

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Chronic wasting disease (CWD) is a spongiform encephalopathy of deer (*Odocoileus hemionus*, O. virginianus) and elk (Cervus elaphus nelsoni) in limited areas of Wyoming and Colorado and in privately owned elk in several western states and provinces. Diagnosis of CWD is by histopathology for spongiform encephalopathy and immunohistochemistry (IHC) for evidence of accumulation of PrP<sup>res</sup>, a marker of prion diseases. Western blots for PrP<sup>res</sup> and negative stain electron microscopy for scrapie associated fibrils are used as ancillary tests. Currently, diagnosis of CWD is based on examination of the brain, at a minimum to include the medulla oblongata at the obex. Sections of brain are treated with formic acid before embedding for inactivation of the CWD agent. Formic acid treatment and hydrated autoclaving are used for antigen retrieval prior to IHC. Both polyclonal and monoclonal antibodies against PrPres have been used as primary antibodies. Surveillance of hunter harvested free-ranging cervids (n=4,287) in the CWD endemic area relies on IHC for estimation of prevalence. Of deer diagnosed with preclinical or subclinical CWD (n=132), 57% had both spongiform encephalopathy and accumulation of PrP<sup>res</sup> in the brain detected by IHC; the remainder of deer were only positive by IHC. The earliest site of accumulation of PrP<sup>res</sup> was the lateral aspect of the parasympathetic vagal nucleus in the medulla oblongata. Immunohistochemistry for PrP<sup>res</sup> is essential for confirmation of CWD in clinically affected animals and for estimation of CWD prevalence in the endemic area.

### (53) CHRONIC WASTING DISEASE SURVEILLANCE IN ARIZONA USING HUNTER-KILLED DEER AND ELK

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Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy that affects native cervids, and was first diagnosed in the 1960s in captive mule deer (*Odocoilious hemionis*) and elk (*Cervus elaphus*) in north-central Colorado and Wyoming. CWD has been diagnosed from these species in the wild in the last decade from these two focal areas. While not diagnosed in free-ranging animals outside these areas, CWD has been found in captive cervids in several locations. Although the association is biologically inappropriate, the media has made ties between CWD and "mad cow disease," making it essential that wildlife agencies have information on the status of CWD in free-ranging populations. Because of the biopolitical nature of CWD, the Western Wildlife Health Cooperative has urged states/provinces in the West to begin surveillance for the disease. During the fall hunting season, Arizona initiated a sampling protocol using hunter-killed heads collected from taxidermists and meat processing plants. We were successful in collecting and assessing the CWD status for 199 deer and 80 elk. All tests were negative for CWD. The cost for collecting and submitting heads using this method was >\$10.00/sample. These data have been used to address public-media requests for information on the potential for having CWD in hunter-harvested wildlife.

### (54) SURVEY FOR SELECTED DISEASES IN HUNTER-KILLED ELK FROM ARKANSAS.

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In 1981 the Arkansas Game and Fish Commission, in cooperation with private citizens, initiated a plan to restore elk to the Ozark Mountains in northwest Arkansas. Between 1981 and 1985, 112 Rocky Mountain elk (Cervus elaphus nelsoni) from Colorado and Nebraska were released at five sites in Newton County near the Buffalo National River. Since 1985, the elk herd has increased to an estimated 450 animals, and in 1998 the first elk hunts were offered as part of the Arkansas Game and Fish Commission's long range elk management program. At the request of the Commission, personnel from the Southeastern Cooperative Wildlife Disease Study (SCWDS) attended the hunts and collected samples from hunter-killed animals for disease surveillance. Seventeen elk were examined and samples were collected for serologic testing, bacteriologic culture, and histologic examination. Ectoparasites and endoparasites were collected and identified, and nutritional condition was evaluated for each elk. There was no evidence of significant clinical disease in any of the elk examined, and all were in good to excellent nutritional condition. Serologic testing for a variety of viral and bacterial diseases demonstrated a very low level of exposure to a few pathogens, elk parasite burdens were minimal, and there was no evidence of infection with several diseases of special importance including chronic wasting disease, tuberculosis, brucellosis, and Johne's disease. One elk harbored a subclinical meningeal worm (Parelaphostrongylus tenuis) infection, and four additional elk from this herd have been diagnosed with clinical parelaphostrongylosis over the past two years by SCWDS. These results suggest that meningeal worm infection is the most significant disease present in the elk herd at this time. Plans have been made to continue this disease surveillance program at future elk hunts in Arkansas, to develop a more complete health database for this herd.

## (55) EVALUATION OF THE USE OF A SURROGATE SPECIES (NOTROPIS SCEPTICUS) FOR CAPE FEAR SHINER (NOTROPIS MEKISTOCHOLAS) RECOVERY PROGRAM HABITAT HEALTH RISK ASSESSMENT.

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First described in 1971, the Cape Fear shiner, *Notropis mekistochola* is, is now considered at serious risk of extinction and is found in only four small populations. While recovery efforts require an understanding of the health risks faced by the Cape Fear shiner in its current and potential future habitats, the limited population sizes severely hamper direct assessment of patho gen prevalences, particularly when lethal sampling methods are required. These studies examine the potential of using a closely related sympatric species, the sandbar shiner, *Notropis scepticus*, as a surrogate species for health risk assessment of potential Cape Fear shiner habitats. Five North Carolina sites representing current or historical habitat of the Cape Fear shiner or a potential future reintroduction site were sampled by seining in the fall, after the reproductive season. Ten *N. scepticus* were collected from each of the 5 sites. Based on current population estimates it was deemed appropriate to collect fifteen *N. mekistocholas* at sites 1 and 4, and 10 animals from site 5. Shiners were euthanized with 500 ppm tricaine methanosulfonate (MS 222, Argent) within 2 hours of collection.

Gender distribution across the five sampling sites and between species by site was roughly equivalent (P > 0.15). Lengths and weights were fairly consistent across sites for both species, however, *N. mekistocholas* at site 1 weighed less on average than those collected at sites 4 and 5 (P < 0.05). No gross external abnormalities were noted on any of the fish. The only internal gross lesions were diffusely pale, yellow livers and a pale, yellow caudal kidney. Findings on gill biopsies, fin clips, and skin scrapes were limited to mild parasitism and mild focal erythema and ballooning of gill lamellae. Gill protozoa (*Trichodina spp.*, and a *Chilodonella*-like species) were only identified from site 3 where only *N. scepticus* were collected. Cysts in the gill lamellae ranging from twenty to thirty microns were rare findings, identified in 5 *N. mekistocholas*, from sites 4 of the 5 where no similar lesions were found in *N. scepticus*. The two affected *N. scepticus* were from a site where no *N. mekistocholas* were collected.

Only one pooled sample of spleen, liver, caudal kidney, and intestinal tract in Hank's Balanced Salt Solution from 5 *N. scepticus* from site 5 resulted in positive viral isolation. This was a picornavirus of unknown pathogenicity. There were no statistically significant differences in the bacterial flora of the Cape Fear shiner and the surrogate species across the 3 sites where both species were sampled. Eighty-three aerobic bacterial isolates representing 13 species were cultured from shiner intestinal tracts. Although some bacterial species were identified in one shiner species and not the other, the prevalences of the most common bacterial isolates were similar.

Histological assessments of *N. scepticus* obtained in this study would tend to over represent the prevalence of most histological lesions expected in *N. mekistocholas*. Thirty-nine of the 90

shiners sampled (43.3%) had granulomas in coelomic organs with prevalences being essentially equal between *N. scepticus* and *N. mekistocholas* by site. The prevalence of encysted trematodes was equal between the two shiner species at two of the three sites where both species were collected, but no trematode cysts were detected in *N. mekistocholas* at site 1. Elliptical aggregates of bipolar, operculated, slightly refractile bodies with 1 or 2 nuclei were identified in the skeletal muscle or connective tissue of 14 (15.6%) shiners in the study, primarily *N. scepticus*. The only lesions of this type identified in *N. mekistocholas* were in 2 animals from site 1. Protozoal infections were detected histologically in from only 2 *N. mekistocholas*, from site 1, where the prevalence of these organisms in *N. scepticus* was dramatically higher than at other sites. Varying degrees of hepatocellular vacuolization were noted in 23 (25.6%) shiners, 22 *N. mekistocholas* and 1 *N. scepticus*, representing 55% of the 40 *N. mekistocholas* sampled. The prevalence of this finding in *N. mekistocholas* was similar for two of the three sites sampled but dramatically lower at site 1.

Distinct differences in the prevalence and patterns of pathogen detection and histological lesions were identified between sites in this study. While the value of the clinical assessment (gill biopsy, skin scrape, fin biopsy) of *N. scepticus* as a surrogate for *N. mekistocholas* remains equivocal due to the low prevalence of pathogens observed in this study, histological assessment of *N. scepticus* was an excellent conservative indicator of the presence of health risks that can be expected to affect *N. mekistocholas*.

# (56) A DIAGNOSTIC EVALUATION OF THREE SALAMANDER MORTALITY EVENTS ASSOCIATED WITH AN IRIDOVIRUS AND SUBSEQUENT GENOMIC COMPARISON OF THE VIRUS ISOLATES OBTAINED.

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In 1998, individuals from three separate salamander mortality events were submitted to the National Wildlife Health Center (NWHC) for determination of cause of death. These events occurred in North Dakota tiger salamander (Ambystoma tigrinum) larvae in May and June, Maine spotted salamander (*Ambystoma maculatum*) larvae in July, and Utah tiger salamander larvae in September. Intracytoplasmic inclusions suggestive of viral infection were seen in the tissues of representative salamanders submitted. Transmission electron microscopy was performed on thin sections of suitable infected salamander tissue and arrays of viral particles resembling iridoviruses were seen in the cytoplasm of infected cells. Tissue suspensions prepared from individuals from each event were inoculated into fathead minnow, bullfrog tongue, and rainbow trout gonad cell culture for virus isolation. Viral cytopathic effect was noted and transmission electron microscopy of the cell culture material revealed virus particles that were morphologically similar to an iridovirus. Representative isolates from Maine and Utah were identified, at the NWHC, as an iridovirus by comparison of restriction fragment length polymorphisms of the novel isolate with those of frog virus 3 (RV3), the type species of the genus ranavirus. Subsequently, representative iridovirus isolates obtained from each of these mortality events were molecularly characterized, at UMMC, by analyzing viral protein synthesis patterns, restriction enzyme digestion profiles, and the sequence of the major capsid protein gene. By all three criteria, the isolate from the spotted salamander (Maine) was similar to, but distinct from, FV3. Furthermore, the Maine isolate was markedly different from iridoviruses previously identified in tiger salamanders from Arizona and Saskatchewan. Work on the Utah and North Dakota isolates is in progress. Preliminary data suggests that these isolates are similar to other iridoviruses detected in tiger salamanders. Furthermore, these studies indicate that members of different salamander genera can be infected by distinct iridovirus species.

#### (57) CHYTRIDIOMYCOSIS OF AMPHIBIANS: INVESTIGATING AN EMERGING INFECTIOUS DISEASE OF WILDLIFE.

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Chytridiomycosis is an emerging fungal disease associated with mass mortalities and implicated in population declines of amphibians in the Australian and Central American Wet Tropics and at a single USA site. The causative agent is a new genus of chytrid fungus, *Batrachochytrium*, which represents the first example of a chytrid parasite of vertebrates. We review the latest available data on the biology, host-parasite ecology, phylogeny, origins and impact of this disease. We demonstrate that an ability of the pathogen to infect amphibian larvae without significant mortality, and to survive and reproduce outside the amphibian host allows it to persist at low host population densities and cause local host extinction. Epizootiological data suggests that the most parsimonious hypothesis for the origin of this disease is the recent introduction of the pathogen into naive populations of amphibians. A comparison of host ecology demonstrates that although many species are susceptible to this virulent pathogen, longterm declines occur in a specific group of species defined by common ecological characteristics. Finally future plans for research on this emerging pathogen are discussed.

#### (58) IMMUNOLOGY OF GREEN TURTLE FIBROPAPILLOMATOSIS IN HAWAII.

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Fibropapillomatosis (FP) is a neoplastic disease with global distribution that poses significant threat to marine turtles like greens, loggerheads and Olive Ridleys. In endemic areas, prevalence of FP in green turtles in Hawaii can reach up to 80%. Previous work in Hawaii revealed hematologic changes in green turtles afflicted with FP that were suggestive of immunosuppression. A trial with captive green turtles in Hawaii evaluated tools to assess immune response in this species and found that both cell mediated and humoral immune response can be measured. Cell mediated immune response of free-ranging green turtles with and without FP was measured on Oahu and Hawaii. Cell proliferation assays (CPA) indicate that turtles moderately to severely afflicted with FP are immunosuppressed; however, immunosuppression does not appear to be a pre-requisite for manifestation of FP in green turtles.

#### (59) CAPTIVE REARING BLANDING'S TURTLES (EMYDOIDEA BLANDINGII) AS PART OF A MANAGEMENT PROGRAM OF A THREATENED POPULATION.

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Prior to settlement, approximately 60% of DuPage County, Illinois (130,000 acres) was wetlands. At this time, they represent only two percent of the entire county and the majority of existing wetlands are in a poor natural condition due to siltation from farms, development of adjacent lands, historical drainage attempts, constant and excessive flooding, invasion by alien flora, chemical pollutants and lack of fire. Previously a common species in the County, Blanding's turtles (*Emydoidea blandingii*) are currently classified as state threatened. Recent amphibian and reptile surveys indicate that this turtle is present only as small groups of old individuals isolated from each other by development and land use. They persist only in preserves containing the highest quality wetland remaining. The Forest Preserve District of DuPage County has adopted a two-pronged approach designed to enhance the population: 1) habitat and ecosystem management; and 2) annual head starting of hatchlings.

During the summers of 1995-1998, visual surveys and live trapping of Blanding's turtles were conducted in the two forest preserves with significant populations. Individuals were marked for identification and fitted with radio transmitters. Females were radiographed during mid-June to determine if they were gravid. Those carrying eggs were held for induction of egg-laying on July 1. Oxytocin (10 mg/kg) was injected intramuscularly to initiate ovipositioning. Eggs were removed as they were layed and separated into incubators so as to produce both male and female hatchlings from each adult female. After hatching, young turtles were numbered to identify them with their parent and preserve of origin. Subsequent to absorption of their yolk sacs, hatchlings were housed in groups of four to seven in plastic tubs in three to four inches of water. Submerged filters were utilized to maintain water quality and sphagnum moss provided hiding places. Slate rocks were stacked to allow natural basking behavior and incandescent lights were set to a 12/12 hour light/dark cycle. They were fed two different types of commercially available aquatic turtle diets. Mass and carapace lengths were recorded weekly and individuals were sorted monthly to keep animals of similar sizes together to avoid out-competition of smaller hatchlings. Juveniles were moved to an outdoor facility for acclimatization between May and June. Prior to release in late June and July, approximately one-half of the individuals were fitted with transmitters for post-release studies.

To date, 28 captive reared individuals have been released with 32 additional turtles slated for release in June of 1999. These juveniles have attained in 10 months, a size comparable to that of five to six year-old wild Blanding's turtles. Due to some concerns over the long term health effects of such accelerated growth, a small study using hatchling Snapping turtles (*Chelydria serpentina*) is being planned.

# (60) EXPERIMENTAL TRANSMISSION OF A DOG-ORIGIN ISOLATE OF HEPATOZOON AMERICANUM TO COYOTES BY THE GULF COAST TICK, AMBLYOMMA MACULATUM.

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Two captive coyote pups were exposed to *Hepatozoon americanum* oocysts recovered from experimentally infected ticks and the resulting infection was followed. Both coyotes developed hepatozoonosis detectable by specific muscle and bone lesions beginning at four weeks after exposure. Bone lesions were detected grossly and histologically. Nymphal *Amblyomma americanum* that experimentally fed on one of the coyotes became infected and mature *H. americanum* oocysts were recovered from the hemocoel of the newly molted adult ticks. These results demonstrate that coyotes experimentally infected with *H. americanum* (dog origin) can develop clinical disease including bone lesions and that *A. maculatum* nymphs can acquire infection by feeding on them.

#### (61) ADAPTATIONS OF WINTER TICKS (DERMACENTOR ALBIPICTUS) TO INVADE MOOSE AND MOOSE TO EVADE TICKS

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Most, if not all North American moose south of 60<sup>0</sup> N, are infested with winter ticks, *Dermacentor albipictus*, annually. Moose commonly host many thousands of winter ticks and tick-associated die-offs of moose (such as occurred in many regions of Canada in 1998 – 1999) are reported often. Winter tick larvae display several behaviors, such as aggregating in clumps on the leeward side of vegetation and at heights of preferred ungulate hosts, which apparently help them survive and attach to vertebrate hosts. Moose, in turn, use several behavioral strategies to avoid or reduce infestation by ticks including evasion of tick larvae on vegetation, tick-removal grooming, such as oral grooming, scratching with hind foot and rubbing, and tolerance of tick-foraging by magpies. Recent findings that grooming by African antelope and North American bison and elk is centrally controlled (*i.e.*, driven by endogenous timing mechanism) and evolved to remove ticks before they attach and feed have not been supported for moose. This paper reviews host-finding adaptations of winter ticks, pathogenic characteristics of winter ticks for moose, the biological basis of grooming in moose, evasion of ticks by moose and the relationship between magpies and moose.

### (62) WILDLIFE SURVEILLANCE FOR THE SOUTH AFRICAN TORTOISE TICK (AMBLYOMMA MARMOREUM) IN FLORIDA.

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Surveillance is being conducted at selected sites in Florida to determine if wildlife or feral animals have become infested by Amblyomma marmoreum, the South African tortoise tick. Numerous species of exotic ticks are being brought into the United States on exotic reptiles imported for the pet trade, and are inadvertently being moved to areas throughout the state and to other areas of the United States as these animals are sold and relocated. Examinations of imported reptiles during an ongoing statewide survey begun in Florida in July 1997 have resulted in the recovery of several species of exotic ticks including A. marmoreum, a vector of Cowdria ruminantium, the etiologic agent of heartwater. Of the infested premises, most keep the imported reptiles indoors and there appears to be little opportunity for establishment in the environment. However, A. marmoreum was found on reptiles at five premises where the imported reptiles were kept outdoors. Infested premises in Florida that house imported reptiles outdoors afford an opportunity for these exotic ticks to infest native wildlife and feral animals and to become established in Florida. Of the premises where A. marmoreum- infested reptiles were held outdoors, three have so far been evaluated for potential exposure to wildlife, and wildlife surveillance was begun at all of the three sites. Wildlife surveillance has resulted in the recovery of specimens of Amblyomma dissimile, Amblyomma auricularium, and Ixodes scapularis from feral iguanas and opossums. Individual specimens of A. marmoreum were recovered from tick drags conducted during wildlife surveillance at two of the sites. Evaluations of additional premises are ongoing, and wildlife surveillance at infested sites will continue.

#### (63) TROMBIDIOSIS IN THE FLORIDA BLACK BEAR (URSUS AMERICANUS FLORIDANUS).

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We found infestation with trombiculid mites (Trombiculidae) in 3 of 12 (25%) free-ranging Florida black bears (*Ursus americanus floridanus*) from a small isolated coastal population (Chassahowitzka) live-captured between October 1997 and May 1999. An additional case involved an adult female black bear live-captured in a large north Florida/south Georgia population (Okeefenokee) in July 1994. Mites from this bear were identified as larval *Eutrombicula splendens*. In all Chassahowitzka cases trombiculid mites were distributed over the ventral abdomen and thorax including inguinal and axillary regions. A moderate to severe multifocal necrotizing pyogranulomatous and eosinophilic dermatitis was present on histologic examination. Intact attached mites and stylostomes were also identified. In one Chassahowitzka bear *Staphylococcus intermedius* was cultured from a secondary superficial pyoderma.

#### (64) THE CENTER FOR CONSERVATION MEDICINE: LINKING ECOSYSTEM HEALTH TO HUMAN AND ANIMAL WELL-BEING.

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The Center for Conservation Medicine (CCM) is a newly established environmental health collaborative with Tufts University School of Veterinary Medicine, Wildlife Preservation Trust International (WPTI), and Harvard Medical School's Center for Health and the Global Environment. The mission of CCM is to advance biological diversity conservation and ecosystem health through interdisciplinary biomedical research and education; and to foster human and animal well being by understanding the ecological context of health. Conservation medicine is an emerging discipline that links human and animal health with ecosystem health and global environmental changes. The conservation medicine approach engages the veterinary and medical health professions in the protection of biodiversity by studying the implications of environmental changes for human and animal health. Effective conservation efforts require an interdisciplinary team to address the complex environmental, social, medical and political issues faced by the world today. By bringing together veterinarians, physicians, ecologists and other conservation professionals, we will attempt to provide an ecological context to health management. The CCM has developed several programmatic and research initiatives based on critical species and ecosystem conservation concerns. These initiatives are focused on the intersection of ecosystem health, human health, and animal health. As an integral part of the Pacific Marine Ecosystem Health Initiative, we are developing a monitoring program of key sentinel species as indicators of marine health. Sea turtles and marine mammals are being targeted to examine the systemic health threats to these species. We proposed to systematically address emerging diseases in one of the richest ecosystems of the world. The CCM is also examining issues related to the use of science and public policy in the Yellowstone to Yukon (Y2Y). This Y2Y Conservation Initiative is addressing related to the linkage between wildlife, disease and the establishment of ecological connectivity in this increasingly fragmented landscape. Disease ecology and interspecies transmission have created a major public policy impasse in fostering free-ranging wildlife populations in the area. CCM is working through community-based conservation in the severely fragmented Atlantic Coastal Rain Forest of Brazil. We also are examining ecosystem health issues related to buffer zones and wildlife corridors. The Ecosystem Fragmentation and Health Implications Initiative is a collaboration with the Instituto de Pesquisas Ecologicas and the Center for Environmental Research and Conservation based at Columbia University. Education and training are critical components of the CCM providing an opportunity to engage talented veterinary and medical students and practicing health professionals through experiential learning approaches. These efforts complement WPTI distinguished record of training host country scientists and building the local capacity of wildlife veterinarians in tropical countries. By integrating animal and public health into biodiversity conservation, CCM will broaden the role of veterinarians and physicians in promoting ecological health and the public understanding of the interconnection of environmental issues.

# (65) HEALTH ASSESSMENT OF A MONITORED POPULATION OF FREE-RANGING BAIRD'S TAPIRS (*TAPIRUS BAIRDII*) IN CORCOVADO NATIONAL PARK, COSTA RICA.

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Despite the fact that tapirs are the largest land mammals in Central America, few studies have been done to establish their health status and susceptibility to disease. Currently, a small population of tapirs is being monitored for a related ecological study in Corcovado National Park, Costa Rica. During the course of immobilizing these animals, blood has been collected (n=17) for complete blood cell counts and serum chemistry. A comparison of these values from the free-ranging population and MedArks reference values is currently underway and results will be presented. Although few infectious diseases are reported to affect captive tapirs, several institutions recommend the vaccination of tapirs for EEE, VEE and WEE and Leptospirosis. This free-ranging population in Costa Rica was tested for the following serology: Eastern equine encephalitis, Venezuelan equine encephalitis, Western Equine Encephalitis, Brucella, Equine Infectious Anemia and Leptospirosis. Findings from this survery will be presented. Fecal material has been collected and analyzed for parasites. Ticks have been collected and have been identified as either *Amblyomma oblongoguttatum* or *A. coelebs*. Rectal bacterial cultures isolated have shown *Streptococcus* sp., *Rhodococcus* sp. and diptheroids. Information gained from this study will greatly contribute to concurrent tapir field studies as well as captive populations.

#### (66) HEALTH EVALUATION OF PAMPAS DEER (OZOTOCERUS BEZOARCTICUS CELER) IN ARGENTINA.

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The southernmost subspecies of pampas deer, (*Ozotocerus bezoarticus celer*), is seriously endangered with only two isolated populations remaining in the wild. Their dramatic decline has been attributed to over-exploitation, pressure of agriculture and livestock production and diseases introduced by cattle. However, no previous studies have been conducted to evaluate diseases affecting this species. As part of a long-term field study to evaluate the factors limiting pampas deer recovery, we conducted health evaluations in 1995 and 1998. Specific objectives were to: 1) establish baseline values for hematology, serum chemistries, electrolytes, vitamin, mineral and metal levels, 2) evaluate the serological evidence of infectious agents, 3) identify the presence of parasites, 4) and compare pampas deer findings with those of domestic cattle sharing their habitat. Fourteen pampas deer (seven females and seven males) were immobilized at Campos del Tuyu Wildlife Reserve, and neighboring ranches and 27 blood samples were collected from cattle. Tests were run at laboratories in Argentina and the US. The results from this study have provided the first data on the health status of free-ranging pampas deer and showed the potential risk of disease transmission from cattle.

## (67) CALF RECRUITMENT AND SURVIVAL IN A POPULATION OF BLACK RHINOCEROS, *DICEROS BICORNIS*, IN ZIMBABWE FOLLOWING IMMOBILIZATION AND DEHORNING.

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The long-term effects of chemical immobilization and horn removal in the black rhinoceros, *Diceros* bicornis, have been debated for several years. Recent publications suggest that dehorned black rhinoceros females in Namibia are unable to defend their calves against predators and that calf survival is so low that dehorning should not be considered. During the period 1991 – 1994, 586 immobilizations of both white, Ceratotherium simum (n=179) and black (n=407) rhinos were carried out in Zimbabwe, of which more than 400 animals were dehorned. A dehorning operation in the Sinamatella/Deka Safari Area in northwestern Zimbabwe in 1992 was conducted on a discrete population of black rhinos in the Sinamatella Intensive Protection Zone (IPZ). The area has healthy populations of lion, Panthera leo and spotted hyena, Crocuta crocuta. In 1994, a follow-up operation conducted to evaluate the effects of dehorning this population also allowed the evaluation of calf survival and recruitment. Of 59 black rhinoceros immobilized in 1992, 15 cow/calf pairs were recorded. Of these, 7 calves were estimated to be less than 6 months old and 8 were estimated to be older than 6 months when their mothers were dehorned. Of the calves less than 6 months old, 5 were re-located in 1994, representing a 71% survival rate. One cow/calf combination remained unaccounted for in 1994 and several poaching incidents in the area had been recorded between 1992 and 1994. At least one calf was poached in the same area. Excluding this animal the calf survival rate is at least 85%. If both calves had been poached, calf survival related to depredation would be 100%. Of the 8 cow/calf pairs with calves older than 6 months found during the initial operation, 5 were re-located in 1994. This represents at least 62% calf survival but as several of the older calves were estimated to be 2-3 years of age at the time of initial dehorning it is likely that the animals had dispersed and were simply not re-located in 1994. The Sinamatella IPZ is approximately 1200 km<sup>2</sup> and contains rugged terrain making aerial spotting and ground tracking difficult. Mortality as a result of depredation in older calves is unlikely, being more commonly due to poaching, intra-specific conflicts or malnutrition. During the 1994 operation, four new calves were located, all born to females previously dehorned in 1992. Several of these females had older calves at the time of initial dehorning that had entered the general population by 1994. One of the newly located calves did not survive and was assumed to have been killed by predators but the other three animals have recently been observed, representing a 75% survival rate. Dehorning of rhinos represents an intensive management strategy chosen to enhance the survival of wild rhinos in the face of an unsustainable loss from illegal hunting. In conservation terms, there is little point in persisting with dehorning if effects are negative, including continued illegal hunting pressure, compromised health from repeated chemical immobilization and dehorning complications, poor calf recruitment and failure of hornless mothers to defend calves from dangerous predators. However, despite initial failures with white rhinos, evidence from data collected in Zimbabwe suggests that dehorning has played a major part in reducing the risk of a rhino being hunted illegally especially with the establishment of IPZs and improved law enforcement. Many premature conclusions concerning the effect of dehorning on calf recruitment and survival have been reached and evidence from the Sinamatella operation and other IPZs in Zimbabwe suggests that the impacts of dehorning on adult black rhino are minimal, overall calf losses are sustainable and calf depredation by hyena and lion has little impact on the overall health of a population of recently dehorned black rhinoceros.

#### (68) SERO-SURVEILLANCE OF MALAYSIAN BATS FOR EVIDENCE OF NIPAH VIRUS INFECTION.

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A previously undescribed virus of the family Paramyxoviridae was responsible for a major outbreak of disease in pigs and humans in several areas of peninsular Malaysia between October 1998 and April 1999. A team of Malaysian, Australian and American scientists employed a multi-disciplinary approach to the investigation and control of the outbreak, pursuing parallel investigations of infection and disease in humans, domestic animals and wildlife species. The apparent close ultrastructural, molecular, and serologic relationship of the new virus (subsequently named Nipah virus) with Hendra virus (equine morbillivirus), focused the initial wildlife surveillance on bat species (Order Chiroptera), and particularly on pteropid bats, previously identified as a natural host of Hendra virus in Australia. A total of 315 individuals from 14 species of bat were sampled by shooting and netting. A sample size of 25 or greater was obtained in five species of frugivorous bats (*Pteropus vampyrus*, n= 57; *Pteropus hypomelanus*, n=42; *Cynopterus brachyotis*, n=74; *Eonycteris spelaea*, n=44; *Cynopterus horsfieldi*, n=25) and one species of insectivorous bat (*Scotophilus kuhlii*, n=49). While serology is incomplete at the time of writing, evidence of infection with Nipah virus or with a cross-neutralising virus has been demonstrated in both *P. vampyrus* and *P. hypomelanus*.

#### (69) AN EPIDEMIC OF BLINDNESS IN KANGAROOS CAUSED BY A VIRUS.

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In 1994 to 1996, there was a massive outbreak of blindness in kangaroos in Australia. Blind animals stumbled into bushes and other objects but otherwise were able to hear, move, feed, and maintain body condition. The following is a brief summary of cooperative investigations conducted by many field investigators and laboratories that indicated the cause and much of the epidemiology of the disease.

Orbiviruses of the Wallal and Warrego serogroups were isolated from tissues taken from various affected kangaroos, particularly from eyes, brains and blood. Histologically, blindness was confirmed by the appearance of very severe necrotising retinitis, well-developed choroiditis, occasional atrophy of optic nerves, and mild non-suppurative encephalitis. Polymerase chain reaction confirmed the principal presence in the eyes and brains of a Wallal serogroup virus and showed that it was dissimilar to a standard Wallal isolated from clinically-normal macropods in 1974. An indirect immunofluorescence test on formalin-fixed paraffin-embedded tissues showed reactions to Wallal serogroup specifically in the affected retinas of a number of cases.

Polymerase chain reactions showed that the sandflies, *Culicoides dycei* and *C. austropalpalis*, were carrying the viruses during the main part of this epidemic. This was probably not the first outbreak of this disease as a paraffin-embedded eye taken from a blind kangaroo in 1976 yielded a product equivalent to the specific Wallal orbivirus suspected in the 1994-1996 epidemic.

It now remained to finalise the case against the virus of the Wallal serogroup by animal transmission trials. Of 8 animals inoculated, 3 developed clinical and pathological evidence of the disease after incubation periods of about 4 to 5 weeks. The lesions in the retinas of their eyes were confirmed as specific to an orbivirus of the Wallal serogroup by polymerase chain reaction and by immunofluorescence.

\*There were numerous contributors to this work including R Lunt, A Gould, A Hyatt, G Russell, J Kattenbelt, S Blacksell, L Reddacliff, P Kirkland, R Davis, P Durham, A Bishop, J Waddington, A Philbey, L Vogelnest, F Hulst, A Deykin, and J Smith.

### (70) DOMOIC ACID TOXICITY IN CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS) STRANDED ALONG THE CENTRAL CALIFORNIA COAST.

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Between 15th May and 19th June 1998, 70 California sea lions (Zalophus californianus) and one northern fur seal (Callorhinus ursinus) in good body condition and displaying neurological signs stranded along the central California. Forty-eight of the 70 animals (67 %) died despite treatment. Of these 70 animals, 54 were adult females, 27 (50 %) of which were pregnant; five were immature females, one was a yearling female, six were subadult males and four were juvenile males. The predominant histologic lesion in affected animals was acute neuronal necrosis, that was most severe in zones CA3 and CA4 of the hippocampus and the dentate gyri. There were also intramyelinic and neuropil edema and occasional foci of gliosis. In some animals dying within 48 hours of stranding, there was mild multifocal myocardial edema and necrosis. Domoic acid was detected in serum from three and urine from a further three affected animals by both a microplate receptor assay method and High Performance Liquid Chromatography (HPLC). It was also detected by HPLC in feces of three of eleven animals tested. Two of these positive fecal samples were examined by scanning electron microscopy, and frustules of Pseudo-nizchtia australis observed. A bloom of Pseudo-nitzschia australis occurred in Monterey Bay during the later half of May 1998, reaching its peak on May 22. Plankton samples were also analyzed for domoic acid using a receptor binding assay. The rise and fall of domoic acid in these samples corresponded to the rise and fall of P. australis observed in the plankton. Anchovies collected from the bay on 22nd May 1998 had levels of domoic acid of 105.6 µg domoic acid/g tissue, while fish collected on 10th June 1998 had no detectable levels of domoic acid. Stomachs of anchovies collected during the peak of the *P. australis* bloom that contained high amounts of domoic acid were also filled with P. australis frustules. Feces of two affected California sea lions contained anchovy otoliths. The combination of clinical signs, histopathological, toxicological, epimemiological and oceanographic changes led to a diagnosis of domoic acid toxicity in the California sea lions. This event is the first documented case of domoic acid toxicity in marine mammals.

#### (71) AFLATOXICOSIS IN SNOW GEESE FEEDING IN LOUISIANA CORN FIELDS.

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Aflatoxins, produced by some strains of the fungi Aspergillus flavus and A. parasiticus, were first documented to cause hepatotoxicosis in domestic ducklings in 1961. Since that time, aflatoxicosis or other mycotoxicoses have been suspected in numerous epizootics involving sandhill cranes (Grus canadensis), snow geese (Anser caerulescens) and mallards (Anas platyrhynchos) that had ingested moldy waste peanuts or corn. Aflatoxicosis was rarely confirmed as the cause of the epizootics by both histopathological and toxicological data. Between December 1998 and March 1999, an estimated 10,500 snow geese and another 100 white-fronted and Ross' geese and mallards were found sick or dead in northeastern Louisiana. Waterfowl were observed feeding on unharvested waste corn that had aflatoxin concentrations that exceeded acceptable guidelines for domestic animal and human consumption. Necropsy and histopathological examinations confirmed the presence of severe diffuse hepatocellular necrosis with biliary hyperplasia and degenerative renal changes consistent with aflatoxicosis. Unfortunately ingesta was absent or scant in most geese. NWHC detected the presence of aflatoxin in the ingesta of one snow goose and pooled ingesta of three other snow geese by enzyme immunoassay; Aflatoxin B<sub>1</sub> and B<sub>2</sub> were quantified by thin layer chromatography in the unpooled sample. Queries about the hazards for waterfowl of aflatoxins in condemned crops are difficult to answer because the risks of exposure, extent of mortality, and efficacy of management efforts are unknown. Publicizing this Louisiana event may help stimulate development of programs to make condemned grain crops less accessible to wildlife species.

#### (72) WINTER MORTALITY IN WAXWINGS (BOMBYCILLA GARRULUS) CAUSED BY ETHANOL INTOXICATION.

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Bohemian waxwings (*Bombycilla garrulus*) breed in the Northern parts of Scandinavia and Russia. The birds migrate during the winter season southwards and are normally found in southern parts of Scandinavia. The birds feed in summertime on insects and in wintertime on fruits and berries, of which rowan berries (*Sorbus aucuparia*) are one of their favourite choices. During cold periods small groups of waxwings (2-10) can be found dead below their roosting trees in the morning. Post mortem examination of these dead birds normally show that they are in good body condition and that the primary cause of death is trauma with internal bleedings. The most prominent pathological finding is a severe fattening of the liver, with rupture of the capsule and internal bleedings. No other pathological findings are normally found in these birds. The pathological changes are similar to those seen in acute alcoholic liver disease in humans. Levels of ethanol in the liver were analysed by liquid chromatography using refractive index detection. In four investigated birds, levels of 0.02 - 0.05% ethanol were found in the liver. Liver levels of alcohol are lower than in the blood, demonstrating that these birds were affected by ethanol intoxication.

#### (73) RECENT POISONINGS OF WILDLIFE IN NEW YORK WITH ANTICOAGULANT RODENTICIDES.

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Anticoagulant poisons are currently the most commonly used rodenticides world-wide. In use since the development of warfarin in the 1940's, anticoagulants were not associated with notable wildlife mortality until so-called "second-generation" anticoagulants were developed in response to the appearance of warfarin-resistant rodent populations in some locations. Some of these newer compounds pose a much greater threat to wildlife largely due to a physiological persistence that allows single exposures to cause prolonged coagulopathy and, presumably, multiple sublethal exposures to have a cumulative effect. From January 1998 through April 1999 we documented 21 cases of wildlife mortality caused by these more persistent compounds. Thirteen of these cases were secondary poisonings of raptors: seven red-tailed hawks (*Buteo jamaicensis*), five great horned owls (*Bubo virginianus*), and one long-eared owl (*Asio otus*). Brodifacoum was the sole or contributing cause of hemorrhage or blood loss observed at necropsy in 18 cases. Bromadiolone was involved in four cases. We recommend that regulatory agencies world-wide consider further restrictions on the use of brodifacoum and similar products.

#### (74) LEAD EXPOSURE IN BLACK DUCKS AND WATERFOWL MORTALITY FOLLOWING IMPLEMENTATION OF NONTOXIC SHOT.

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Lead poisoning long has been recognized as an important disease of waterfowl with an estimated 1-4 million waterfowl dying annually prior to the implementation of nontoxic shot. Public concern over lead poisoning resulted in progressive prohibition of lead shot for waterfowl hunting until it was banned throughout the United States in 1991. However, few studies have been conducted to determine if replacing lead with nontoxic shot has reduced lead exposure in waterfowl. We compared lead exposure of American black ducks (*Anas rubripes*) banded in Tennessee during 1986-88 and 1997-99 (ten years after regional implementation of nontoxic shot). We also evaluated the long term trends in lead poisoning mortality for waterfowl submitted to the National Wildlife Health Center. Our data indicate that lead exposure and mortality events have significantly declined since lead shot was prohibited.

#### (75) METHYLMERCURY EXPOSURE IN WADING BIRDS: A COMPARISON OF CAPTIVE DOSING AND NATURAL EXPOSURE.

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We orally dosed captive great egret (Ardea albus) hatchlings with 0.5 mg/kg of methylmercury for 14 weeks, 6 weeks beyond the normal age for fledging, to approximate the current exposure of egrets in the Everglades. The uptake of mercury by storage organs, such as liver and especially feathers, delayed the increase of blood mercury concentrations until feathers ceased to grow, thus protecting young nestlings to some degree. Dosed birds experienced a decline in appetite, slowed growth, decreased PCV, a transient decline in circulating lymphocytes, histologic changes in immune organs, and a change in response to a skin test. Although these effects were not life threatening in well-fed captive birds, it is likely that their survival would have been compromised in a natural setting. By comparing responses in captive and freeranging birds, we suggest that thresholds differed due to detectability and synergism. Subtle, non-life-threatening effects of mercury were detected at lower observable adverse effect levels (LOAELs) in captive than in free-ranging birds due to the increased ability to detect a significant difference in a highly controlled laboratory study. Conversely, LOAELs for more severe changes occurred at lower concentrations in free-ranging birds than in captive birds due to the synergism of uncontrolled factors affecting health in the field. Thus we caution against equating LOAELs between captive and free-ranging situations.

#### (76) THE OCCURRENCE OF DUCK PLAGUE VIRUS IN NON-MIGRATORY WATERFOWL IN THE CHESAPEAKE BAY AREA OF MARYLAND.

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Duck plague, a herpesvirus disease of Anseriformes (ducks, geese and swans), causes annual mortality in the United States with the highest number of outbreaks occurring in Maryland since this disease was first identified in 1967. A study was conducted in Maryland to determine the presence of waterfowl that could be a reservoir source for duck plague outbreaks. Waterfowl were captured in three target populations including free-flying nonmigratory waterfowl (Freeflying), waterfowl raised and released for hunting (Released), and waterfowl maintained in private flocks (Captive) in three Maryland counties (Kent, Queen Annes, and Dorchester). A blood sample for serology and a cloacal swab for virus isolation and polymerase chain reaction (PCR) assay were collected from 527 birds and tested for evidence of duck plague virus infection. Duck plague virus was not isolated from any of cloacal swabs tested. Evidence for duck plague virus shedding (PCR) or exposure (serum antibody) were detected in 31.6% of 297 Released, 10.7% of 168 Free-flying and 1.6% of 62 Captive waterfowl sampled. The primary species infected were 16% of 37 Free-flying Canada geese (Branta canadensis) and 18% of 428 mallards (Anas platyrhynchos) collected; duck plague positive mallards included 32% of 297 Released and 9% of 131 Free-flying birds. One of 28 Captive dusky Canada geese (Branta canadensis occidentalis) was PCR positive and the remaining 34 birds from 15 waterfowl species were negative for duck plague. There was a progressive seasonal decline in the frequency of samples with duck plague virus (PCR) and antibody for the Free-flying population. A similar decline in PCR positive waterfowl occurred in the Released population, but antibody levels remained at a higher level. Duck plague appears to be enzootic in the waterfowl populations surveyed in three Maryland counties.

#### (77) ATOXOPLASMOSIS IN THE BALI MYNAH IN THE UK & THE TAXONOMIC STATUS OF *ATOXOPLASMA*.

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We present parasitologic and pathologic findings in the first report of fatal atoxoplasmosis in a captive Bali mynah (*Leucospar rothschildi*) in the UK. In a preliminary follow-up survey, bisporocystic oocysts were found in 18.25% of fecal smears taken from all UK birds and in 9/19 of captive collections. Detailed analysis of the prevalence data against sex, age, breeding status and other parameters are presented. These data suggest that atoxoplasmosis is as significant a threat to the UK collection of this endangered species as has been shown in the USA. Oocysts isolated from Bali mynahs were morphologically distinct from an *Isospora* recovered from feces of the European starling (*Sturnus vulgaris*). Studies on atoxoplasmosis have been hampered by problems in taxonomic classification of *Atoxoplasma* spp. These problems are reviewed in the light of recently published data which provides further evidence for a link between *Atoxoplasma* and *Isospora*.

#### 78) SPIROCHETOSIS DUE TO AN UNUSUAL *BORRELIA* SP. IN A NORTHERN SPOTTED OWL.

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Fatal systemic spirochetosis was diagnosed in an adult male Northern spotted owl (Strix occidentalis caurina) found dead in Kittitas County, Washington. The bird had been displaying normal behavior two weeks earlier. The gross necropsy findings included severe enlargement of the liver and spleen, and serofibrinous deposits on the serous membranes lining the thoracic and abdominal cavities, and in the pericardial and perihepatic sacs. Microscopically, macrophages and heterophils had infiltrated the periportal regions of the liver and some mild fatty degeneration and focal necrosis had occurred. The splenic enlargement was due to macrophage and reticular cell hyperplasia. Hemorrhage and acute inflammation were evident in the choroid plexus of the brain. No viruses or pathogenic bacteria were isolated from brain, liver, or spleen, and no parasites were found in blood smears or impression smears of the liver. Chlamydial culture attempts were unsuccessful and no chlamydial antibodies were detected in serum. By transmission electron microscopy and in silver-stained microscopic sections, numerous long, thin, spiral-shaped bacteria were seen in the liver, spleen, cerebral ventricles, and within blood vessels in many organs. The organism was identified as a member of the Borrelia genus using genus-specific primers for the 16s rRNA gene, the flaB gene and the P66 outer membrane protein gene. The most closely related species is B. hermsii, an agent of relapsing fever in humans. This is the first report of a relapsing fever-related *Borrelia* in a bird.

#### (79) A SEROTYPE-SPECIFIC PCR ASSAY FOR AVIAN CHOLERA (PASTEURELLA MULTOCIDA SEROTYPE 1).

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A serotype-specific polymerase chain reaction (PCR) assay was developed for the detection and identification of *Pasteurella multocida* serotype 1, the causative agent of avian cholera in wild waterfowl. Arbitrarily-primed PCR was used to detect DNA fragments that distinguish serotype 1 from the other 16 serotypes of *P. multocida* (with the exception of serotype 14). Oligonucleotide primers were constructed from these sequences, and a PCR assay was optimized and evaluated. PCR reactions consistently resulted in amplification products with reference strains 1 and 14 and all other serotype 1 strains tested, with cell numbers as low as 2.3 cells/ml. No amplification products were produced with other *P. multocida* serotypes or any other bacterial species tested. To compare the PCR with traditional culturing and serotyping techniques from diagnostic specimens, 64 Pekin ducks were inoculated with field strains of P. multocida serotype 1. PCR was as sensitive (50/51) as routine isolation (49/51) in detecting and identifying *P. multocida* serotype 1 from the livers of birds that became sick or died from avian cholera. PCR was also as sensitive (8/13) as routine isolation (3/13) in detecting the bacteria in birds that did not become sick and were euthanized 7-15 days post-inoculation (P<0.05). No product was amplified from tissues of 20 birds that received serotypes other than type 1 (serotype 3, 12 X 3, or 10) or 12 control birds. Also, PCR was much faster and less laborintensive than traditional culturing and serotyping procedures and could result in diagnosis of serotype 1 pasteurellosis within 24 hours of specimen submission.

#### (80) ENVIRONMENTAL CHARACTERISTICS ASSOCIATED WITH AVIAN CHOLERA OUTBREAKS IN WETLANDS.

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Avian cholera is an infectious bacterial disease that annually kills thousands of waterbirds in North America. Previous studies have linked a variety of environmental conditions including water temperature, chemical ion concentrations, and protein levels with the occurrence of avian cholera outbreaks in wetlands; however, many of these associations were based on laboratory studies or from field studies in local areas. During 1995-99, we investigated outbreaks of avian cholera throughout the United States. Our goal was to find common environmental conditions associated with outbreaks and identify potential management strategies to reduce the occurrence of outbreaks and subsequent cost of cleanup. We measured environmental conditions at outbreak wetlands and at nearby "control" wetlands, where avian cholera mortality was low or absent. We compared paired outbreak and control wetlands, but found only weak associations between environmental conditions and the risk of avian cholera outbreaks. We suspect that other factors related to bird use or weather conditions may be more important in determining the risk of outbreaks.

### (81) EFFECTS OF HEAVY METALS ON SURVIVAL OF *PASTEURELLA MULTOCIDA* (AVIAN CHOLERA) ORGANISMS.

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Contamination of wetlands by a wide variety of agricultural and industrial chemicals with the release of heavy metals is of growing concern regarding the health and well-being of migratory birds and other wildlife dependent upon wetlands for all or a portion of their needs. Sources of this contamination include agricultural drainwater, municipal and industrial discharges, and hazardous waste dumps. National Wildlife Refuges are often at the lower end of the terrestrial water supply systems and are the recipients of heavy metals and toxic chemicals leached from other areas and are concentrated in lakes and marshes on the refuges. Very little is known about the interactive effects of infectious disease agents and environmental metals in migratory birds and other wildlife despite the opportunities for such interactions to occur. One disease problem that warrants investigation is avian cholera caused by *Pasteurella multocida* (Pm).

This study was designed to determine the effect(s) of different concentrations of heavy metals in physiological saline on survival of Pm organisms *in vitro* and *in vivo*. Three concentrations (2ppm, 20ppm, and 200 ppm) of 4 heavy metals, cadmium, copper, iron, and magnesium were used as treatments. One ml of an 18 hr brain heart infusion broth culture of a virulent Pm waterfowl isolate containing 5.6 X 10<sup>10</sup> cfu /ml was inoculated into 99ml of each heavy metal concentration. The samples were incubated at 37 C and each culture suspension was sampled at seven intervals over time from 0h to and including 336h by doing plate counts of the viable organisms. Physiological saline and brain heart infusion broth control samples were tested at the same time. The Pm cells in each metal treatment were dead at the 124h sampling time except for the 2ppm and the 20ppm concentrations of magnesium. Both of these treatments survived at 37 C well beyond this study, with the 2ppm treatment surviving for over 7 years.

The same heavy metal concentrations were inoculated into embryonated chicken eggs with and without Pm cells. The treatments with Pm cells were incubated for 30h at 37 C before inoculating 0.2 ml of each concentration into chicken embryos via the allantoic route, 5 embryos/treatment. The magnesium mixed with the Pm cells was the only metal-bacterial mix that killed embryos. Each of the heavy metal concentrations without Pm cells killed 1 of 5 to 5 of 5 embryos with the exception of iron at 200ppm. These embryos survived. These results suggest the need for further investigation of *P. multocida*-heavy metal interactions that may have an effect on the virulence and survival of avian cholera disease organisms.

### (82) PATHOLOGY ASSOCIATED WITH AN EPIZOOTIC OF FIBROMATOSIS IN GRAY SQUIRRELS (SCIURUS CAROLINENSIS).

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Squirrel fibromatosis is an uncommon disease in the southeastern United States. Cases of fibromatosis are sporadic and mortality associated with fibromatosis is generally low. Beginning in the fall of 1998 and extending into the spring and early summer of 1999 there was a large epizootic of squirrel fibromatosis involving 6 counties in peninsular Florida. Hundreds of grey squirrels (Sciurus carolinensis) with multiple cutaneous tumors were submitted or reported to veterinary hospitals and private wildlife rehabilitators. Most squirrels died or were euthanized soon after submission. Ten randomly selected squirrels were submitted to the University of Florida College of Veterinary Medicine for necropsy. The majority of the squirrels examined were juvenile (6/10) and male (8/10). The number and location of tumors varied widely among the affected squirrels, however a consistent finding was involvement of the eyelids (10/10). Histopathology revealed a neoplastic population of mesenchymal cells within the dermis and marked ballooning degeneration of keratinocytes in the overlying epidermis. Intracytoplasmic viral inclusions were present in both the neoplastic cell population and the degenerating keratinocytes. Ulceration and necrosis of the surface of the tumors or associated tissues was present in 7 of the 10 squirrels. Metastasis of neoplastic cells to regional lymph nodes was rare (1/10) and mestastasis to major organ systems (liver, spleen, kidney, heart, lung) was not identified. Electron microscopy of tissue samples collected from a representative tumor demonstrated characteristic pox virus particles in the cytoplasm of degenerating keratinocytes. The death of the squirrels was attributed to emaciation, tissue damage and severe negative energy balance associated with pox virus infection and massive tumor growth.

### (83) KIDNEY AGENESIS IN TWO FLORIDA BLACK BEARS (URSUS AMERICANUS FLORIDANUS) FROM A NORTH CENTRAL FLORIDA POPULATION.

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Unilateral or bilateral kidney agenesis is a congenital condition defined as the complete absence of one or both kidneys. Bilateral kidney agenesis is incompatible with postnatal life while unilateral agenesis usually results in compensatory hypertrophy of the remaining kidney and normal homeostasis. The etiology is unknown but may be hereditary and the condition is often associated with other anomalies of the urogenital tract. Kidney agenesis has been reported in humans and domestic animals but has not been reported in black bears.

We found unilateral kidney agenesis in 2 of 108 (1.9%) Florida black bears (*Ursus americanus floridanus*) necropsied August 1993 to May 1999. Both cases were females dying from vehicular collision and were of 52 (3.8%) necropsied from a large central Florida population (Ocala). The first case was a 2.8 year-old black bear with left kidney agenesis and compensatory hypertrophy of the right kidney. No attempt was made to locate the left ureter and the reproductive tract was not examined. The second case was a 1 year-old with right kidney agenesis. Renal or ureteral tissue could not be identified following gross and histologic examination of right sublumbar retroperitoneal tissue. The left kidney was of normal size. Representative sections of the reproductive tract were normal grossly and histologically. Blood urea nitrogen and creatinine on postmortem serum chemistry were within normal limits.

Case #2 also had generalized demodicosis, a condition rare in other populations and subspecies but relatively common in the Ocala population. Although the cause of these conditions is unknown, the relatively high prevalence of kidney agenesis and demodicosis in the Ocala population may be the result of a common or related etiology.

#### (84) SEROLOGIC SURVEY FOR CANINE CORONA VIRUS IN WOLVES FROM INTERIOR ALASKA.

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Wolves were captured during spring (March-April) and autumn (October-November) each year from 1995 – 1998 (N=200). Sera were tested for evidence of exposure to canine corona virus by means of a serum neutralization method. Serum antibody prevalence averaged 75% during the spring period, and 25% during the autumn period. Prevalence was 0% in the pup cohort (age 5 months) during the autumn period. Prevalence was 72% in the yearling cohort (age 10 months). These results indicate that:

- (1)transmission occurs primarily during the winter, and
- (2)antibody levels decline rapidly.

## (85) SERUM NEUTRALIZING ANTIBODY TITERS IN RACCOONS (*PROCYON LOTOR*) SUSPECTED OF BEING INFECTED WITH THE CANINE DISTEMPER VIRUS.

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In 1996, animal control officers in DuPage County, Illinois started picking up raccoons (*Procyon lotor*) that were described as curious or friendly to humans, exhibited variable degrees of ataxia, were in fair to good body condition, and did not display significant respiratory signs. Their symptoms appeared to be consistent with the neurologic form of canine distemper, but represented a change from the clinical picture of distemper that had been common in this area. In an attempt to clarify the current clinical syndrome, serum samples were taken from 41 raccoons presented to the Willowbrook Wildlife Center and submitted for Canine Distemper Virus Serum Neutralization (CDV-SN) tests. Initial results indicate that most of these animals tested positive. Four individuals that were considered to have the more "classic" respiratory form of the disease were negative for serum neutralizing antibodies. Attempts to interpret these results on the basis of pathogenesis of the disease are made.

#### (86) HEALTH ASPECTS OF A REINTRODUCTION OF CANADA LYNX INTO COLORADO.

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Canada lynx (*Lynx canadensis*) are believed to have been extirpated from Colorado by the early 1970's. In an effort to re-establish the animal, 42 wild lynx were trapped in Canada and Alaska and transported to Colorado for reintroduction into the southern San Juan Mountains in February – May 1999. Prior to transport, lynx received physical examination and anti-parasitic treatment (0.3 mg/kg ivermectin, 5 mg/kg praziquantel, and dusting with carbaryl). In Colorado, lynx were housed individually or in pairs in holding pens (10 m<sup>2</sup>) with an attached nest box (1 m<sup>2</sup>). A variety of foods were provided and intake measured. All lynx were immobilized with 3-5 mg/kg telazol IM at least once for examination, sampling, and marking. Four lynx had lesions or fractures of 1-3 toes that required medical treatment or digit amputation. One female lynx required surgical repair of an abdominal hernia. One male lynx was euthanized due to illness. Otherwise, lynx remained healthy and markedly improved their body condition while in captivity. Age status of juvenile lynx was determined radiographically by lack of closure of the physis of the radius and ulna. Pregnancy was confirmed in six lynx using abdominal radiographs taken in early May. An initial release of four lynx was made in February within 1 week of their arrival in Colorado. Within 7 weeks, three of these lynx died and the fourth was recaptured in a state of severe emaciation. Post-mortem examination and histopathology revealed lesions compatible with starvation and an incidental finding of mild infection with *Trichinella spiralis*. Rehabilitation of the emaciated lynx was successful. Subsequently, lynx were held in captivity in Colorado >3 weeks for acclimation and fattening prior to release. Status of these lynx will be monitored over the next 2-4 years.

#### (87) AN UNUSUAL OCCURENCE OF HARBOR PORPOISE STRANDINGS IN MARYLAND - MARCH - MAY 1999.

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While marine mammal strandings are a common occurrence in coastal areas, Maryland experiences on average only ten strandings per year. Historically, harbor porpoise (*Phocoena phocoena*) strandings have been low, averaging four per year. In March 1999 a pulse of harbor porpoise strandings occurred along the East Coast from Maine to North Carolina. Approximately 170 porpoises stranded during this time more than tripling stranding numbers in the previous year. In Maryland from March to May twenty harbor porpoises stranded dead including one atypical stranding inside the Chesapeake Bay. Various stages of decomposition ranging from freshly dead animals to skeletal remains were evident in both male and female carcasses examined. Most animals were in good body condition. External examination of all carcasses documented net marks in eleven out of the twenty animals. Complete necropsies documented many porpoises with adequate fat reserves and full stomachs. Samples for microbiology and histopathology were taken from one freshly dead animal and results are pending. The value of conducting comprehensive examinations on marine mammals in all stages of decomposition is essential to document the cause of death of stranded animals.

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