8th CONFERENCE of the

ewda

EUROPEAN WILDLIFE DISEASE ASSOCIATION

ROVINJ - CROATIA

2-5 October, 2008

Focusing on Diseases of European Wildlife and Recent Changes in Disease Distribution

WDA
Wildlife Disease Association

EWDA
European
Wildlife Disease Association

Croatian Veterinary Institute
1933
Zagreb

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Croatian Veterinary Institute
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BOOK OF ABSTRACTS of the

8th CONFERENCE of the ewda
EUROPEAN WILDLIFE DISEASE ASSOCIATION

ROVINJ - CROATIA
2-5 October, 2008

EWDA
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SCIENTIFIC PROGRAMME
SUPPORTED BY:

OIE

Organisation Mondiale de la Santé Animale	World Organisation for Animal Health	Organización Mundial de Sanidad Animal

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CROATIA
The Mediterranean As It Once Was

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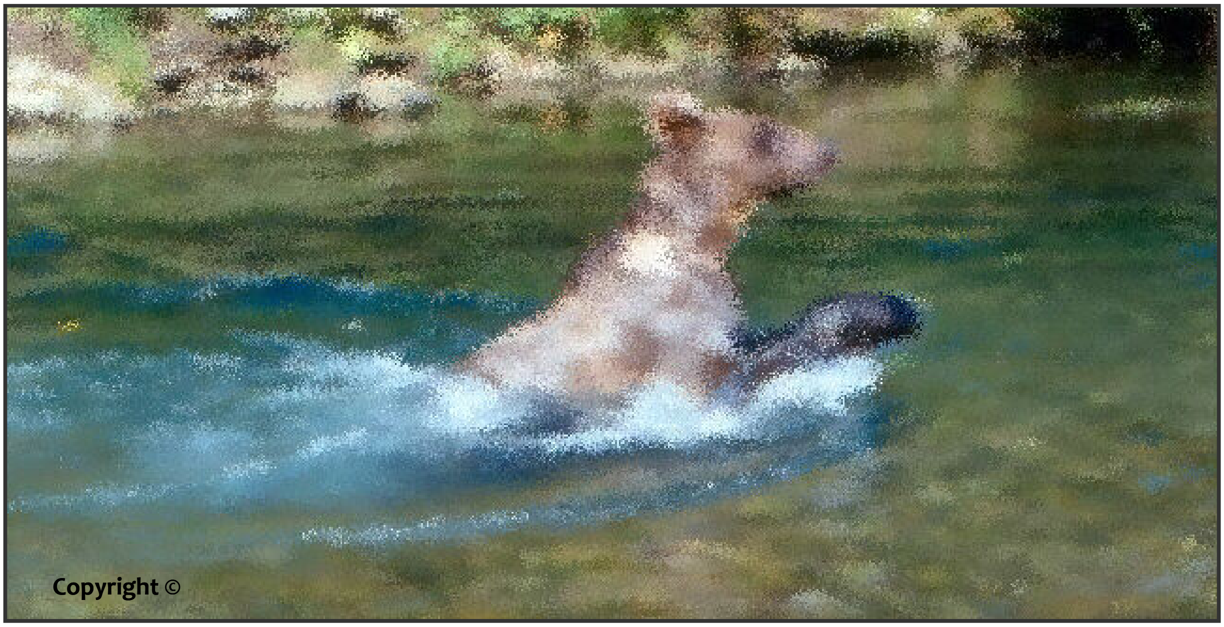
Welcome!



On behalf of the conference host and the European Wildlife Disease Association it is our great pleasure to welcome you to Croatia and to the 8th Biennial Conference of the European section of the Wildlife Disease Association.

Located on the west coast of the Istrian peninsula the town of Rovinj, also known by its Italian name Citta' di Rovigno, or locally known as the 'Blue Pearl of the Adriatic', is a small, pretty, picture postcard harbour town, and one of the most visited seaside resorts in Croatia. For those wanting to explore the delights of the local wildlife and especially the bird-watching of the Northern Adriatic, then Rovinj is an excellent starting point.

We are proud to welcome you to a conference with a wealth of wildlife health topics, that are linked to the ever present and global issues of species interface concerns. From new scientific breakthroughs to exciting case reports with many interesting presentations, and delegates from all continents, the conference covers a wide range of topics. We wish you an enjoyable, rewarding, professionally stimulating, and personally fulfilling meeting in a very special environment.

Through this year's participation of OIE identified experts in the fields of diseases of European wildlife and recent changes in the distribution of these diseases, with exactly 151 presentations, 49 oral addresses and 102 posters on exhibition, we are honoured to have the opportunity to host this conference. We are convinced that it will be an interesting week for all participants.

We hope you will enjoy the Conference and your stay in Rovinj and Croatia!

Mirko Lojkic	*Branko Sostaric*	*Ivan Vickovic*
Chair Organizing Committee Croatian Veterinary Institute	Chair Scientific Committee Croatian Veterinary Institute	Scientific Programme Planning Co-Chair Croatian Veterinary Institute

Programme Overview

Oral presentations schedule

Thursday, October 2, 2008

08:00	**Registration**	**Hotel Eden Convention Centre Reception area**
09:00 - 9:30	Welcome and opening ceremony	Chair: **Dolores Gavier-Widen** Speakers: **Branko Sostaric, Croatian Veterinary Institute** **Dolores Gavier-Widen**, EWDA Chair **Charles van Riper III,** WDA President
09:30 - 10:30	**Session: Wildlife disease reporting and surveillance**	Chairs: **Dolores Gavier-Widen** and **Ivan Vickovic**
09:30 - 10:45	**OIE European wildlife disease monitoring**	
09:30 - 10:30	Important diseases in European wildlife - the past, present and future (1)	Keynote Speaker: **Prof. Torsten Morner**
10:30 - 10:45	Global wildlife disease monitoring: an attempt by the OIE working group on wildlife diseases to collect annual data on relevant events across the world (results over 16 years) (2)	**Marc Artois**, Roy Bengis, Chris Bunn, John Fischer, Frederick Leighton, Torsten Morner, and Mike Woodford
10:45 - 11:15	Refreshment Break	
11:15 - 12:15	**Session continued:** **Wildlife disease reporting and surveillance**	Chairs: **Dolores Gavier-Widen** and **Ivan Vickovic**
11:15 - 11:30	Sharing and disseminating wildlife disease information (3)	**Joshua Dein**, Megan Hines, Cris Marsh, Steven Gustafson, Barabara Nash, Erica Schmitz, and Laura Wyndholds
11:30 - 11:45	Results of passive surveillance on the causes of wild ungulate mortality in Spain (4)	**Mariana Boadella**, Joaquin Vicente, Alvaro Oleaga, Isabel G. Fernandez-De-Mera, Maria Paz Martin-Hernando, and Christian Gortazar
11:45 - 12:00	The garden bird health initiative – a novel citizen science approach to wildlife disease surveillance (5)	**Becki Lawson,** Malcolm Bennett, Julian Chantrey, Andy Evans, Laura Hughes, James K. Kirkwood, Kirsi Peck, Tom Pennycott, Rob Robinson, Vic Simpson, Mike Toms, and Andrew A. Cunningham
12:00 - 12:15	Reviewing sample methodology of low pathogenic avian influenza (LPAI) surveillance studies in wild birds (6)	**Elisa Perez-Ramirez**, and Ursula Hofle
12:15 - 13:30	Lunch	

13:30 - 15:15	**Session: Emerging diseases and the role of wildlife**	Chairs: **Thijs Kuiken** and **Anthony Sainsbury**
	In focus: Bluetongue in Europe, a serious threat with critical questions	
13:30 - 14:30	Expansion of bluetongue in Europe and climate change (7)	Keynote Speaker: **Prof. Philip Mellor**
14:30 - 14:45	Bluetongue in wild red deer (*Cervus elaphus*) in southern Belgium (8)	**Annick Linden**, Benedicte Mousset, Fabien Gregoire, David Hanrez, Elise Vandemeulebroucke, Frank Vandenbussche, and Kris De Clercq
	Avian influenza	Chairs: **Julie Langenberg** and **Caroline Brojer**
14:45 - 15:00	Involvement of wildlife in the epidemic of highly pathogenic H5N1 avian influenza (9)	**Thijs Kuiken**
15:00 - 15:15	Red knots (*Calidris canutus*) with higher plasma concentration of corticosterone excrete more avian influenza H5N1 virus (10)	**Leslie Reperant**, Marco Van De Bildt, Geert Van Amerongen, Debbie Buehler, Albert Osterhaus, Andy Dobson, Theunis Piersma, and Thijs Kuiken
15:15 - 15:45	Refreshment Break	
15:45 – 16:55	**Session: West Nile fever and exotic diseases as issues of public health concern**	Chairs: **Ursula Hoefle** and **Vic Simpson**
15:45 - 16:25	West Nile fever: the European situation and the role of wildlife (11)	Keynote Speaker: **Dr. Rosella Lelli**
16:25 - 16:40	Surveillance of West Nile virus in France - seven years of monitoring in wild birds (12)	**Jean Hars**, Marion Mortamais, Jennifer Pradel, Philippe Auge, Elsa Jourdain, David Chavernac, Jerome Languille, and Herve Zeller
16:40 - 16:55	Disease and mortality in red footed falcon (*Falco vespertinus*) nestlings - challenges facing a long distance migrant species at its breeding habitat (13)	**Karoly Erdelyi**, Tamas Bakonyi, Adam Dan, Peter Fehervari, Miklos Gyuranecz, Tibor Juhasz, Laszlo Kotyman, Peter Palatitz, Szabolcs Solt
16:55 - 17:40	**Session: Epidemiology and environmental drivers of wildlife diseases**	Chairs: **Christian Gortazar** and **Jean Hars**
	Diseases caused by protozoa	
16:55 - 17:10	*Sarcocystis neurona*: exploring the fatal link between opossums (*Didelphis virginiana*) and sea otters (*Enhydra lutris nereis*) in coastal California (14)	**Daniel Rejmanek**, Elizabeth Vanwormer, Melissa A. Miller, Jonna A. Mazet, Michael E Grigg, and Patricia A. Conrad
17:10 - 17:25	Prevalence and molecular characterization of *giardia* isolates from wild mammals (15)	**Relja Beck**, Albert Marinculic, Snjezana Lucinger, Daniele Tonanzi, Edoardo Pozio and Simone M Caccio
17:25 - 17:40	Determining the genetic diversity of *Theileria equi* and *Babesia caballi* in common and mountain zebra (*Equus quagga* and *Equus zebra*) (16)	**Raksha Bhoora**, Barend L. Penzhorn, Alan J. Guthrie, and Nicola E. Collins
18:00	**Welcome party –** hotel Eden, Terrace in front of the Conference hall	

Friday, October 3, 2008

08:00	**Registration**	**Hotel Eden Convention Centre Reception area**
09:15 - 10:15	**Session: Wildlife handling and immobilization**	Chairs: **Ezio Ferroglio** and **Djuro Huber**
09:15 - 10:15	Wildlife chemical immobilization in the 21st century: the future, or back to the future? (17)	Keynote Speaker: **Dr. Terry J. Kreeger**
10:15 - 10:45	Refreshment Break and Poster Presentations	
10:45 - 12:00	**Session: Diseases of ungulates**	Chairs: **Torsten Morner** and **Are Berentsen**
	Ruminants	
10:45 - 11:00	Retrospective study of pestivirus infection in southern chamois (*Rupicapra pyrenaica*) in the Pyrenees (18)	**Ignasi Marco**, Rosa Rosell, Oscar Cabezon, and Santiago Lavin
11:00 - 11:15	An outbreak of foot necrobacillosis in a wild reindeer (*Rangifer tarandus tarandus*) population in Norway (19)	**Kjell Handeland**, Bjarne Bergsjo, Mette Boye, and Jorgen S. Agerholm
11:15 - 11:30	Investigations on the epizootiology of *Babesia capreoli* infections in free-ranging wild ungulates from Switzerland (20)	Stefan Hoby, Alexander Mathis, Nicole Schmid, Peter Deplazes, Nadia Robert, and **Marie-Pierre Ryser-Degiorgis**
	Suids	
11:30 - 11:45	Oral vaccination of wild boar (*Sus scrofa*) against classical swine fever: efficacy of the baiting process (21)	**Sophie Rossi**, Jean Hars, Marie-Frederique Le Potier, Nathalie Masse-Provin, and Anne Bronner
	General	
11:45 - 12:00	Brucellosis in wild ungulates in Spain (22)	**Pilar Mª Munoz Alvaro**, Maria Jesus De Miguel Lopez, Mari Cruz Arnal Barrera, David Martinez Duran, Miguel Revilla Calavia, Mariana Boadella, Joaquin Vicente, Clara Mª Marin Alcala, Montserrat Barberan Pelegrin, Jose Miguel Prieto Martin, Christian Gortazar, Daniel Fernandez De Luco Martinez, and Jose Maria Blasco Martinez
12:00 - 13:15	Lunch	
13:15 - 15:20	**Session: Diseases and preservation of carnivores**	Chairs: **Ignasi Marco** and **Annick Linden**
13:15 - 14:05	Biology and health of Croatian large carnivore populations (23)	Plenary Speaker: **Prof. Djuro Huber**
	Mustelids	
14:05 - 14:20	Tyzzer's disease in a Eurasian otter (*Lutra lutra*) in Scotland (24)	**Vic R. Simpson**, J. Hargreaves, R. J. Birtles, H. Marsden, and D. L. Williams
	Felids	
14:20 - 14:35	Does feline leukaemia virus modify parasitic fauna in wild-living cats? (25)	**Pauline Perrot**, Eve Afonso, Marc Artois, Estelle Germain, Marie-Pierre Callait-Cardinal, and Emmanuelle Gilot-Fromont
	Canine distemper in canids, mustelids, and procyonids	
14:35 - 14:50	Report on a canine distemper virus epizootic in foxes (*Vulpes vulpes*) and badgers (*Meles meles*) in the Italian eastern Alps (26)	**Carlo V. Citterio**, Mariapia Cova, Karin Trevisiol, Marco Bregoli, Gabriella Conedera, Krizia Cecchettin, Monica Lorenzetto, and Claudio Pasolli

14:50 - 15:05	Outbreak of canine distemper in raccoons (*Procyon lotor*) in Germany (27)	**Gudrun Wibbelt**, Stephanie Speck, Joerns Fickel, Berit Koehnemann, and Frank-Uwe Michler
15:05 - 15:20	**Session: Disease issues at wildlife-human-livestock interface** (session continues October 4)	
15:05 - 15:20	The influence of human visitor activity on spatial patterns of parasite infection (28)	Chris O'Brien, and **Charles van Riper III**
15:30	Departure to the medieval town of Motovun, and **EWDA Auction - PLEASE BRING AUCTION ITEMS WITH YOU** Buses depart in front of Hotel Eden - (see attached brochure)	

Saturday, October 4, 2008

08:00	**Registration**	**Hotel Eden Convention Centre Reception area**

DAY 3		
09:00 - 10:25	**Session: Marine mammals and health of marine ecosystems**	Chairs: **Branko Sostaric** and **Aleksija Neimane**
09:00 - 9:40	Cetacean mortality along the Croatian Adriatic coastline (29)	Plenary Talk: **Martina Duras Gomercic**, Ana Galov, Tomislav Gomercic, Hrvoje Lucic, Snjezana Vukovic, Darinka Skrtic, Snjezana Curkovic, and Hrvoje Gomercic
09:40 - 9:55	A review of marine mammal *Brucella* in british waters 1994 – 2007 (30)	**Emma Stubberfield**, Claire Dawson, Lorraine Perrett, Simon Brew, and Judy Stack
	In focus: Morbiliviridae of marine mammals – Phocine distemper virus	
09:55 - 10:10	The dynamics of Phocine distemper virus epizootics in European harbour seals (*Phoca vitulina*) (31)	**Petra Klepac**
10:10 - 10:25	Host specificity based on a three-dimensional structure model of marine mammal signalling lymphocyte activation molecule (SLAM), a receptor of morbillivirus (32)	**Kazue Ohishi**, Akiko Ando, Rintaro Suzuki, Kiyotaka Takishita, Masaru Kawato, Etsuko Katsumata, Dai Ohtsu, Kenji Okutsu, Koji Tokutake, Hirokazu Miyahara, Hirotaka Nakamura, Tsukasa Murayama, and Tadashi Maruyama
10:25 - 10:55	Refreshment Break and Poster Presentations	
10:55 - 12:25	**Session: Mycobacterial diseases**	Chairs: **Erik Agren** and **Richard Delahay**
10:55 - 12:10	**In focus: TB as re-emerging disease of the wildlife**	
10:55 - 11:10	The "no visible lesion" form of *Mycobacterium bovis* infection in a wide range of wildlife hosts (33)	**Dolores Gavier-Widen**, Michele Cooke, John Gallagher, Mark Chambers, and Christian Gortazar
11:10 - 11:25	Comparative diagnosis of bovine tuberculosis in wild ungulates from Spain (34)	**Maria Paz Martin-Hernando**, M. Jose Torres, Joaquin Vicente, Manuel Reglero, Jose De La Fuente, Javier Aznar-Martin, Juan Jose Negro, and Christian Gortazar
11:25 - 11:40	Experimental infection of European wild boar (*Sus scrofa*) with *Mycobacterium avium avium* and *Mycobacterium bovis* (35)	**Christian Gortazar**, Joseba Garrido, Joaquin Vicente, Ruth Galindo, Jose Manuel Perez De La Lastra, Esmeralda Minguijon, Alicia Aranaz, Ramon Juste, and Jose De La Fuente
11:40 - 11:55	Progress towards licensed tuberculosis vaccines for Eurasian badgers (*Meles meles*) (36)	**Mark A. Chambers**, Sandrine Lesellier, Allan Nadian, Deanna Dalley, Sonya Gowatage, Roland Ashford, Si Palmer, Pinar Court, Dipesh Dave, and R. Glyn Hewinson

11:55 - 12:10	Diagnosing bovine tuberculosis in live-sampled wild animals: just how accurate are the tests? (37)	**Julian Drewe,** and Gareth Pearce
	Other mycobacterial diseases	
12:10 - 12:25	Prevalence of *Mycobacterium avium paratuberculosis* in wild ruminants (*Cervus elaphus*, *Dama dama,* and *Sus scrofa*) from Donana national park (38)	**Tania Carta**, Christian Gortazar, Joaquin Vicente, Rafael Reyes-Garcia, Jose Manuel Perez-De-La-Lastra, Maria Jose Torres Sanchez, Juan J. Negro, and Javier Aznar Martin
12:25 - 13:40	Lunch	
13:40 - 15:25	**Session: Disease issues at wildlife-human-livestock interface** (continued from October 3)	Chairs: **Marc Artois** and **Petra Klepac**
	In focus: translocation, cohabitation, and human intervention	
13:40 - 13:55	Managing TB in badgers (*Meles meles*): a lesson in the role of ecological complexity in the transmission of disease between wildlife and domestic animals (39)	**Richard J. Delahay**
13:55 - 14:10	Veterinary risk assessment - likelihood and impacts of transmission of selected infectious diseases between free-ranging wild boar *(Sus scrofa)* , humans and domestic livestock in England (40)	**Matt Hartley**
14:10 - 14:25	Brucellosis in wapiti (*Cervus elaphus*) and bison (*Bison bison*) in the United States: a classic wildlife-human-livestock problem (41)	**Terry J. Kreeger**
14:25 - 14:40	Evaluating risks associated with the transmission of bovine tuberculosis from white-tailed deer (*Odocoileus virginianus*) to cattle in Michigan, USA: preliminary results from year one (42)	**Are R. Berentsen**, Ryan S. Miller, Mike R. Dunbar, and Regina Ebersole
14:40 - 14:55	Suspected spill-over infections with garden bird associated *salmonellae* to other species (43)	**Paul J. Duff**, J. Paul Holmes, and C. Papadopoulou
14:55 - 15:10	Developing the methodology for disease risk analysis for translocations undertaken for biodiversity conservation (44)	**Anthony W. Sainsbury**, Fieke M. Molenaar, and Katie Colvile
15:10 - 15:25	Ljungan virus (a possible cause of several human health conditions) found in bank voles (*Myodes glareolus*) and yellow-necked mice (*Apodemus flavicollis*) for the first time in northern Italy (45)	**Heidi C. Hauffe**, Bo Niklasson, Therese Olsson, Alessandro Bianchi, Annapaola Rizzoli, and William Klitz
15:25 - 15:45	Refreshment Break 20 min	
15:45 - 16:30	**Session: Birds and amphibians**	Chairs: **Karoly Erdelyi** and **Paul J. Duff**
	Diseases of birds	
15:45 - 16:00	Invasive species-associated emerging diseases in water birds in North America (botulism type E and trematodiasis) (46)	**Julie Langenberg,** David Blehert , and Rebecca Cole
16:00 - 16:15	Lead intoxication in white-tailed sea eagles (*Haliaeetus albicialla*) – a joint project on the causes and approaches to solutions in Germany (47)	**Oliver Krone**, Norbert Kenntner, Mirjam Nadjafzadeh, Friederike Scholz, Petra Schuck-Wersig, Justine Sulawa, Anna Trinogga, Katrin Totschek, and Roland Zieschank
16:15 - 16:30	Environmental toxins: a threat to reintroduced red kites (*Milvus milvus*) in England (1989-2007) (48)	**Fieke M. Molenaar**, Ian Carter, Mark Fletcher, Richard Shore, and Anthony W. Sainsbury

	Diseases of amphibians	
16:30 - 16:45	Treatment of chytrid (*Batrachochytrium dendrobatidis*) infection in frogs at Perth zoo - four case reports (49)	**Paul Eden**, Simone Vitali, Glen Gaikhorst, Anna Le Souef, Rebecca Vaughan, and Helen Roberston
16:45 - 17:00	**Closing remarks**	
17:00 - 17:45	**EWDA-activities discussion meeting** (EWDA Business Meeting) and **Best student presentations recognition awards**	All participants
19:30	**EWDA Banquet - the Island of St. Andrew** Boat departs from Rovinj Harbour at 19:40 (Street: Šetalište Vijeća europe / Lungomare del Consiglio d'Europa – (see attached brochure)	Optional event

Sunday, October 5, 2008

08:15 – 15:00	**Field trip - Brijuni National Park** Buses depart in front of Hotel Eden - (see attached brochure)	Optional event Lunch included. The EWDA Boat departs from the Island of Brijuni at 14:00 and reaches the mainland in Fazana after a 15-minute ride. For those of you catching flights after the field trip a shuttle will be waiting to take you to the Airport. Fazana is 16 km from the Pula Airport and it should not take you more than 30 minutes. Should you need to leave earlier there are also boats departing at 11:00, and 13:00 hours. Make sure to arrange for any special requests with the Conference Registration staff prior to the Field trip. The regular EWDA bus will be back in Rovinj at around 15:00. If you wish to stay longer on the Island, boats also depart at 15:00; 15:45; 17:30; 19:15; and 23:00 hours

Poster programme

Friday, October 3 & Saturday, October 4

1	Hypertrophic osteopathy in a roe deer (*Capreolus capreolus*) in Sweden	**Erik Agren**, and Desiree Jansson
2	Infection with *Taenia* spp. In rodents in Danish woodlands	**Mohammad Al-Sabi**, Per Jensen, Alexander Mathis, Peter Depazes, and Christian Kapel
3	Dynamics of an outbreak of infectious keratoconjunctivitis (*Mycoplasma conjunctivae*) in Pyrenean chamois (*Rupicapra pyrenaica*) in Aragon, Spain: preliminary data	**Maricruz Arnal**, Christian De La Fe, Miguel Revilla, Carlos Prada, Juan Carlos Corrales, David Martinez, Olatz Fernandez, Alicia Garcia-Serrano, Antonio Sanchez, Juan Herrero, Antonio Contreras, and Daniel Fernandez De Luco
4	Virus or parasite: what killed roe deer (*Capreolus capreolus*) in France?	**Marc Artois**, Flavien Gallo, Sandrine Flamand, Marie Eve Terrier, Alain Viry, and Olivier Mastain
5	Development of semi-quantitative RT-PCR cytokine assays for the Eurasian badger (*Meles meles*)	**Roland Ashford**, Brian Catchpole, Dipesh Dave, Deanna Dalley, Sandrine Lesellier, R. Glyn Hewinson, and Mark Chambers
6	Preparation and characterization of bait for the oral immunization of the European wild boar (*Sus scrofa*)	**Cristina Ballesteros**, Christian Gortazar, Joaquin Vicente, Ricardo Carrasco-Garcia, Jesus Carrasco, Rafael Mateo, Pablo Camarero, Mario Canales, Angelo Lasagna, and Jose De La Fuente
7	First report of ranavirus in the common midwife toad (*Alytes obstetricans*) in Spain	Ana Balseiro, Kevin P. Dalton, Ana Del Cerro, Isabel Marquez, Andrew A. Cunningham, Francisco Parra, R. Casais, and **Jose M. Prieto**
8	Seroprevalence and identification of *babesia* species infecting roe deer (*Capreolus capreolus*)	**Suzanne Bastian**, Nadine Brisseau, Maggy Jouglin, Geraldine Klegou, Laurence Malandrin, Monique L'hostis, and Alain Chauvin
9	Presence of the eastern cottontail (*Sylvilagus floridanus*) in the province of Perugia, Italy	**Lorenzo Battistacci**, Luca Convito, Michele Croce, Silva Costarelli, Piermario Mangili, Marco Sensi, and Livia Moscati
10	Health status of the eastern cottontail (*Sylvilagus floridanus)* in the province of Perugia: results of a serological survey	**Lorenzo Battistacci**, Luca Convito, Michele Croce, Silva Costarelli, Piermario Mangili, Caterina Mariotti, Marco Sensi, and Livia Moscati
11	Red deer (*Cervus elaphus*) are not a perfect host for *Fascioloides magna:* evidence from a histopathological study	**Ana Beck**, Relja Beck, Vanja Vrkic, Ivan Conrado Sostaric Zuckermann, Marko Hohsteter, Branka Artukovic, Zdravko Janicki, Dean Konjevic, Albert Marinculic, and Zeljko Grabarevic
12	Epizootiologic survey for zoonotic *babesia* isolates among mouse-like rodents and cervids in Croatia	**Relja Beck**, Lea Vojta, Snjezana Curkovic, Vladimir Mrljak, Josip Kusak, Nenad Turk, Josip Margaletic, and Albert Marinculic
13	Evaluating barriers and corridors to raccoon (*Procyon lotor*) strain rabies movement in Cleveland, Ohio, USA: research update	**Are R. Berentsen**, Mike Dunbar, and Chad Fitzpatrick
14	Phylogenetic characterization of canine distemper virus (CDV) from red foxes (*Vulpes vulpes*) in Greece	**Charalambos Billinis**

15	Detection and genetic characterization of the first encephalomyocarditis (EMC) isolates from wild boar (*Sus scrofa*) in Greece	**Charalambos Billinis**, Periklis Birtsas, and Vassiliki Spyrou
16	Border disease virus shedding and detection in organs of naturally-infected southern chamois (*Rupicapra pyrenacia*)	**Oscar Cabezon**, Rosa Rosell, Roser Velarde, Gregorio Mentaberre, Encarna Casas, Santiago Lavin, and Ignasi Marco
17	Rabies monitoring in Croatian wildlife	**Zeljko Cac**, Mirko Lojkic, Ivana Lojkic, and Tomislav Bedekovic
18	Scavenging on ungulate carcasses in Mediterranean habitats	**Ricardo Carrasco-Garcia**, Joaquin Vicente, Jesus Carrasco, Rafael Reyes, Manuel Reglero, Jose Antonio Gamarra, Carlos Rodriguez, Alfonso De Ancos, Angel Gomez, Juan Antonio Fernandez, Juan Jose Negro, and Christian Gortazar
19	The relationship between environmental contamination and pathology in stranded cetaceans (*Odontocetes*) in the canary inlands	**Matilde Carballo**, Fernando Esperon, Manolo Arbelo, Miguel Gonzalez, and Maria Jesus Munoz
20	West Nile virus: two years of ornithological and entomological surveillance in Padule di Fucecchio, Tuscany, Italy	**Erika Ciarrocca**, Micaela Cipriani, and Rossella Lelli
21	Clinical influenza type a in ferrets (*Mustela putorius furo*)	**Maja Conc**, Jozko Racnik, Brigita Slavec, Marko Zadravec, Cvetka Marhold, Petra Bandelj, Alenka Dovc, and Olga Zorman-Rojs
22	Salmonella infection in UK wild birds	**Aonghais Cook**
23	A survey of the health status of hooded crow (*Corvus cornix*) and magpie (*Pica pica*) populations in central Italy	Silva Costarelli, Livia Moscati, Silvia Crotti, Marcella Ciullo, Serenella Orsini, **Lorenzo Battistacci**, and Marco Sensi
24	Epidemiology of respiratory diseases in chamois (*Rupicapra rupicapra*) in Trentino, north-eastern Italy	Mariapia Cova, **Anna Roccato**, Michela Grisenti, Giovanni Farina, and Claudio Pasolli
25	Ovarian carcinoma in a great bustard (*Otis tarda*)	**Elena Crespo**, Tomas Castiella, and Ursula Hofle
26	*Trichinella nativa* muscle larvae from foxes (*Vulpes vulpes*) are able to withstand repeated freeze/thaw episodes with little effect on infectivity	Rebecca K. Davidson, **Kjell Handeland**, and Christian M.O. Kapel
27	Presence of *Babesia* spp. In the Croatian red fox (*Vulpes vulpes*) population	**Danko Dezdek**, Relja Beck, Zoran Lipej, Alen Slavica, Snjezana Curkovic, Lea Vojta, Damir Mihelic, Dinko Novosel, and Zeljko Mihaljevic
28	Risks of game farming: enterobacterias in farmed and restocked red legged partridges (*Alectoris rufa*) in Spain	**Sandra Diaz**, Ursula Hofle, Lorenzo Perez-Rodriguez, Francois Mougeot, and Elisa Perez-Ramirez
29	Histopathological findings from the sub-mandibular lymph-nodes of red deer (*Cervus elaphus*)	**Lorenzo Domenis**, Serena Robetto, Erica Pepe, and Riccardo Orusa
30	Surveillance for salmonellae in wild birds in England and Wales	Paul J. Duff, **Paul J. Holmes**, and C. Papadopoulou
31	Managing the biosecurity of an amphibian breeding and research programme, Perth zoo, Australia	**Paul Eden**, Simone Vitali, Helen Robertson, and Glen Gaikhorst
32	Herpes-simplex like sequences in stranded *Delphinidae* spp. in the canary islands	**Fernando Esperon**, Manuel Arbelo, Edwige Belliere, Matilde Carballo, Maria Jesus Munoz, Antonio Fernandez, and Jose Manuel Sanchez-Vizcaino
33	Adenovirus and mycoplasma infection in an ornate box turtle (*Terrapena ornata*)	**Szilvia L. Farkas**, Rachel E. Marschang, and Janos Gal

34	Hematology and serum chemistry in southern chamois (*Rupicapra pyrenaica*) naturally infected with pestivirus	**Laura Fernandez-Sirera**, Gregorio Mentaberre, Jorge Ramon Lopez-Olvera, Rafaela Cuenca, Santiago Lavin, and Ignasi Marco
35	*Leishmania infantum* in foxes (*Vulpes vulpes*) from the province of imperia, north-west Italy	**Ezio Ferroglio**, Walter Mignone, Angelo Romano, Piero Caroggio, Marco Poggi, and Anna Trisciuoglio
36	*Physaloptera sibirica* (nematoda) in foxes (*Vulpes vulpes*) and badgers (*Meles meles*) from the north western Alps, Italy	**Ezio Ferroglio**, Bruno Bassano, and Charlotte Ragagli
37	Detection of *Chlamydophila pecorum* in the lung of an alpine chamois (*Rupicapra rupicapra*) in northern Italy	**Alessandra Gaffuri**, Claudio Monaci, Nadia Vicari, Franco Paterlini, and Simone Magnino
38	Wildlife health monitoring in Bergamo province, northern Italy: a coordinated program between the official veterinary service, the public administration and the hunters	**Alessandra Gaffuri**, Claudio Monaci, Eugenio Testa, Giancarlo Bosio, Luigi Pisoni, Giacomo Moroni, and Franco Paterlini
39	Interspecies transmission of pathogens between domestic and wild ungulates changes their biological properties and thus epidemiological features	**Dominique Gauthier**, Yahia Chebloune, Francois Guiguen, Leila Mselli-Lakhal, Esadk Erhouma, Timothy Greenland, Theodore Alogninouwa, Angeli Kodjo, Laurence Villard, Celine Richomme, Paul Revelli, Bertrand Letallec, Yvette Game, and Emmanuelle Gilot-Fromont
40	Multilocular liver cysts in old-aged chamois *(Rupicapra rupicapra)* in Austria	**Walter Glawischnig**, and Zoltan Bago
41	The prevalence of congenital umbilical hernia in common bottlenose dolphins (*Tursiops truncatus*) in the Adriatic	Tomislav Gomercic, **Martina Duras Gomercic**, Hrvoje Lucic, Ana Galov, Darinka Skrtic, Snjezana Curkovic, Snjezana Vukovic, and Hrvoje Gomercic
42	Evidence of European brown hare syndrome in southern Belgium	**Fabien Gregoire**, David Hanrez, Benedicte Mousset, Adrien Nahayo, Pascale Pirot, and Annick Linden
43	Carbon source utilization of *Francisella tularensis* strains isolated in Hungary	**Miklos Gyuranecz**, Karoly Erdelyi, Laszlo Fodor, Katalin Janosi, Balint Szepe, Miklos Fuleki, Istvan Szoke, and Laszlo Makrai
44	Establishing zoo mortality patterns using animal record keeping software (ARKS) records	Rebecca Hartley, Lisa Yon, and **Rachael Tarlinton**
45	West Nile fever in eagles in Spain	**Ursula Hoefle**, Elena Sotelo, Juan Manuel Blanco, Julia Rodrigue Jovita Fernandez-Pinero, Francisco Llorente, and Miguel Angel Jimenez-Clavero
46	Using movements of aquatic wild birds to identify risks of H5N1 highly pathogenic avian influenza (HPAI) spread amongst Spanish wetlands	Irene Iglesias Martin, Marta Martinez Aviles, Mª Jesus Munoz Reoyo, **Belen Vazquez Ruiz**, and Ana De La Torre De La Reoyo
47	Hares as sentinels for *Yersinia pseudotuberculosis* in the ecosystem	**Marja Isomursu**, Varpu Hirvela-Koski, Leila Sihvonen, and Anja Siitonen
48	Skin test response of European wild boar (*Sus scrofa*) to mycobacterial and non-mycobacterial antigens	**Raquel Jaroso**, Joaquin Vicente, Isabel G. Fernandez-De-Mera, Maria Paz Martin-Hernando, Alicia Aranaz, and Christian Gortazar
49	Molecular analysis of *Mycobacterium bovis* isolated from buffaloes (*Syncerus caffer*) in Hluhluwe Imfolozi park, South Africa	**Akinbowale Jenkins**, Lizma Streicher, Estelle Venter, and Jacques Godfroid
50	Ljungan virus prevalence in German wild rodents	**Rene Kallies**, Juliane Paul, Rainer G. Ulrich, Mathias Schlegel, Thomas Buechner, and Matthias Niedrig
51	Retrospective analysis of the deaths of three Siberian tiger (*Panthera tigris altaica*) cubs in Zagreb zoo	**Dean Konjevic**, Ruza Sabocanec, Zeljko Grabarevic, Andreas Zurbriggen, and Ingeborg Bata

52	Pathology of post-weaning multi-systemic wasting syndrome in wild boar (*Sus scrofa*) in Croatia	**Zoran Lipej**, and Dinko Novosel
53	Serological survey of selected infectious diseases in mouflon (*Ovis aries musimon*) from south central Spain	**Jorge Ramon Lopez-Olvera**, Dolo Vidal, Joaquin Vicente, Marta Perez, Lluis Lujan, and Christian Gortazar
54	Cadmium contamination of the food chain in a forest ecosystem	**Ioan Macinic**, Alexandra Trif, and Florin Muselin
55	Haematological and biochemical investigations in fallow deer (*Dama dama*) from western Romania	**Ioan Macinic**, Teodor Mot, and Catalin Macinic
56	Importance of lynx (*Lynx lynx*) attacks to livestock as perceived by inhabitants of lynx areas in Slovenia and Croatia	**Aleksandra Majic Skrbinsek**, Magda Sindicic, Ivan Kos, and Djuro Huber
57	Red sheep tick (*Haemaphysalis punctata*) in Scandinavian moose (*Alces alces*)	**Jonas Malmsten**, and Jan Chirico
58	Characterization and antibiotic sensitivity of *Escherichia coli* strains isolated from cotton-tail rabbits (*Sylvilagus floridanus*) in Umbria, Italy	Piermario Mangili, Silvia Crotti, Nicoletta D'avino, Annalisa Dettori, Silva Costarelli, **Lorenzo Battistacci**, and Chiara Magistrali
59	Blood lipids and liver fatty acid composition in brown hares (*Lepus europaeus*) from two different Croatian habitats	Terezija Silvija Marenjak, **Krunoslav Pintur**, Alen Slavica, Nina Popovic, Nina Poljicak-Milas, Kresimir Krapinec, and Ivan Vickovic
60	Breeding distribution and population size of the grey heron (*Ardea cinerea*) in Slovenia	**Cvetka Marhold**, Primoz Kmecel, Vojko Havlicek, Jernej Polajnar, and Tomaz Mihelic
61	Serological survey for infectious abortive agents in free-ranging alpine ibex (*Capra ibex*) in Switzerland	**Nelson Marreros**, Sarah Albini, Daniela Huessy, Nathalie Holzwarth, Nicole Borel, Christian Willisch, and Marie-Pierre Ryser-Degiorgis
62	Considering the red deer (*Cervus elaphus*) as a potential definitive host and reservoir of liver flukes (*Fasciola hepatica*, and *Dicrocoelium dendriticum*) in northwest Spain	**Angelica Martinez**, Natividad Diez, Mª Jose Garcia, Claudia Perez, and Mª Rosario Hidalgo
63	Serological monitoring of mouflon (*Ovis orientalis musimon*) in the Arcipelago Toscano national park, Italy	Loretta Masoero, Chiara Guglielmetti, **Monica Pitti**, Luisa De Marco, Ezio Ferroglio, Francesca Giannini, and Silvia Gennero
64	Results of a survey for chronic wasting disease in Italian cervids	Daniela Meloni, Cristiana Maurella, Letizia Carnieri, Maria Cavarretta, **Riccardo Orusa**, Cinzia Cocco, Giuseppe Ru, and Elena Bozzetta
65	Leptospirosis in wild and domestic carnivores in Andalusia, Spain	**Javier Millan**, Monica G. Candela, Jose Vicente Lopez-Bao, Marian Pereira, Maria Angeles Jimenez, and Luis Leon-Vizcaino
66	Seroprevalence of feline viruses in free-living European wildcats (*Felis silvestris*) in Spain	**Javier Millan**, and Alejandro Rodriguez
67	Leptospirosis in endangered European mink (*Mustela lutreola*) and sympatric mustelidae and viverridae in south-western France	**Marie Moinet**, Christine Fournier-Chambrillon, Genevieve Andre-Fontaine, Alain Mesplede, Beatrice Blanchard, Veronique Descarsin, And Pascal Fournier
68	Indices of welfare and health in farmed wild boar (*Sus scrofa*)	**Livia Moscati**, Silva Costarelli, Marco Sensi, Fulvio Biancifiori, Michela Pela, Monica Timi, and Lorenzo Battistacci
69	Serum biochemistry values of fallow deer (*Dama dama*) in central Italy	**Livia Moscati**, Silva Costarelli, Marco Sensi, Francesca Urbani, Silvia Marchi, and Lorenzo Battistacci
70	Reasons for the presentation of badger (*Meles meles*) casualties to a veterinary hospital and outcomes following treatment	**Elizabeth Mullineaux**, Pauline Kidner, and Sarah Edwards
71	A survey for diseases in yellow-legged gulls (*Larus michahellis*) feeding at Majorca garbage dump, Spain	Nieves Negre, Ana Bistuer, and **Javier Millan**

72	Monitoring wild birds for avian influenza in north-western Italy (2005-2008)	Riccardo Orusa, Elisa Barcucci, Cristina Guidetti, F. Rizzo, Serena Robetto, and **Maria Lucia Mandola**
73	Tissue responses associated with abomasal parasites in roe deer (*Capreolus capreolus)*	Ernesto Pascotto, Paola Beraldo, and **Roberta Codolo**
74	Bluetongue virus in wild ruminants: a serological survey in piedmont, Italy	Monica Pitti, Chiara Guglielmetti, Luisa De Marco, **Silvia Gennero**, and Loretta Masoero
75	Polycyclic aromatic hydrocarbons (PAHs) in the black sea dolphins (*Phocaena phocaena* and *Delphinus delphis*) and in the food web (sediment-fish-dolphins)	**Oana Melania Popa**
76	Pathogen surveillance in wild birds in Zagreb	**Estella Prukner-Radovcic**, Zeljko Gottstein, Danijela Horvatek, Irena Ciglar Grozdanic, and Hrvoje Mazija
77	Avian paramyxovirus subtype 2 (APMV 2) in robins (*Erithacus rubecula*) in Slovenia	**Jozko Racnik**, Brigita Slavec, Tomi Trilar, Marko Zadravec, Alenka Dovc, Uros Krapez, and Olga Zorman Rojs
78	Evaluation of a mitochondrial genetic marker for terrestrial and marine wild mammal species identification	Maria Vittoria Riina, Silvia Colussi, Simone Peletto, Silvia Trisorio, Walter Mignone, Monica Dellepiane, Serena Robetto, **Lorenzo Domenis**, Riccardo Orusa, and Pier Luigi Acutis
79	Monitoring of *Salmonella* spp. in wild animals in Piedmont, Liguria and Valle d'Aosta in the period 2007/2008	Serena Robetto, Cristina Guidetti, Erika Pepe, Lorenzo Domenis, And **Riccardo Orusa**
80	Pathological findings in some predatory birds	**Serena Robetto**, Riccardo Orusa, Erica Pepe, Cristina Guidetti, and Lorenzo Domenis
81	Avian tuberculosis in wild birds in south central Spain	Julia Rodriguez-Ramos, Ursula Hoefle, Juan Manuel Blanco, Vanesa Arias, Vanesa Rodriguez, **Elena Crespo**, and Silvia Villaverde
82	Genetic characterization of *Trichomonas gallinae* indicates correlation with its pathogenicity	**Jose Sansano**, Mª Magdalena Garijo-Toledo, and Mª Teresa Gomez-Munoz
83	Morphological and molecular analysis of mammalian hair: a forensic approach	**Itaru Sato**, Shinichi Nakaki, Koichi Murata, and Keiko Shimizu
84	Offspring mortalities in captive-bred brown hares (*Lepus europaeus*) caused by *Bordetella bronchiseptica*	**Kresimir Severin**, Dean Konjevic, Kresimir Matanovic, Zdravko Janicki, Branka Seol, and Alen Slavica
85	Mortality of reintroduced Eurasian lynx (*Lynx lynx*) in Croatia	**Magda Sindicic**, Alojzije Frkovic, Djuro Huber, Tomislav Gomercic, and Josip Kusak
86	Molecular analysis of H5N1 highly pathogenic avian influenza virus (HPAIV) isolates from Slovenia in 2006	**Brigita Slavec**, Josko Racnik, Olga Zorman-Rojs, Uros Krapez, Alenka Dovc, Marko Zadravec, Renata Lindtner Knific, and Elke Starick
87	Wild boar (*Sus scrofa*) and domestic pig (*Sus scrofa scrofa*) inbreeding problem in Croatia	**Branko Sostaric**, Josip Toncic, and Ivan Vickovic
88	Toxic metal concentrations in tissues of wild boar (*Sus scrofa*) from lowland Croatia	**Emil Srebocan**, Andreja Prevendar Crnic, Jasna Jurasovic, and Maja Lazarus
89	Presence of *Trichinella* spp. in Swedish lynx (*Lynx lynx*)	**Anna Stromquist**, Dan Christensson, and Goran Hartman
90	Improvement of the organisation of wildlife disease surveillance in Belgium: the *WILDSURV* project	**Paul Tavernier**, Annick Linden, Pascale Pirot, Paul Heyman, Rik Dessers, Alexandre Dobly, Marc Dispas, and Stefan Roels
91	Development of an enzyme linked immunosorbent assay for the metastrongyloid nematode, *Otostrongylus circumlitus*, and its application to pinnipeds of unknown infection status	**Amanda Taylor**, Jocelyn Elson-Riggins, and Brian Aldridge

92	A survey for European brown hare syndrome in Friuli Venice Giulia region, north-eastern Italy	**Daniele Todone**, Marco Bregoli, Michela Favretti, Andrea Passera, Antonio Lavazza, Massimo Zanetti, and Elena Mazzolini
93	The health status of wild ungulates in the Friuli Venice Giulia region of the Italian-Slovenian border	**Daniele Todone**, Marco Bregoli, Michela Favretti, Umberto Fattori, Elena Mazzolini, and Gabriella Conedera
94	Interpretation of an apparent transient seropositive response to *Mycobacterium bovis* infection in Eurasian badgers (*Meles meles*)	**Alexandra Jane Tomlinson**, Mark Chambers, Neil Walker, and Richard Delahay
95	A survey for several zoonotic pathogens in free-living feral pigeons (*Columbia livia*) in Madrid	**Belen Vazquez**, Fernando Esperon, Juan Lopez, Carlos Ballesteros, Maria Yuste, Maria Jesus Munoz, and Jose Manuel Sanchez-Vizcaino
96	The transmission of the large American liver fluke (*Fascioloides magna*) from red deer (*Cervus elaphus*) to domestic animals in Croatia	Ivan Vickovic, **Branko Sostaric**, Radovan Fuchs, Josip Toncic, and Ivan Tarnaj
97	A survey for transmissible spongiform encephalopathies in European mouflon (*Ovis gmelini musimon*) in Germany	**Anke Wiethoelter**, Matthias Kramer, Kai Froelich, and Volker Stefanski
98	Development of a one-step, real time PCR assay for rapid detection of avian influenza virus subtype H7	**Maria Yuste**, Jovita Fernandez, Elena Sotelo, Ana Robles, M^{a} Concepcion Gomez, Montserrat Aguero, and Marisa Arias
99	Isolation and characterization of *salmonella* strains from wild boar (*Sus scrofa*) in northern Italy	Mariagrazia Zanoni, Mario D'incau, Matteo Spisani, **Alessandra Gaffuri**, Silvia Tagliabue, and Giovanni Loris Alborali
100	Parasites of *Knipowitschia croatica* – an endangered endemic Croatian fish	**Snjezana Zrncic**, Drazen Oraic, and Davor Zanella
101	A cognitive approach to risk assessment in wildlife welfare	**Paolo Zucca**
102	Impact of infectious diseases on the management and conservation of ursids	**Ezequiel Hidalgo**, and Angela M^{g} Martino

Abstracts

Oral Presentations

Focusing on Diseases of European Wildlife and Recent Changes in Disease Distribution

The 8th Conference of the EWDA focuses its scientific programme on the issues of diseases of European wildlife and recent changes in disease distribution. With particular interest on the issues of the role of wildlife and the influence of (re)emerging diseases of public concern on wild fauna, and on novel questions that have arisen in the cases of diseases such as tuberculosis, bluetongue, and West Nile fever. The Conference also includes relevant and authoritative information on the subject of chemical immobilization of wild animals, as well as the health of large carnivore populations on the European continent, and discuses issues in diseases of marine mammals. We are grateful that many of the keynote talks and designated experts are supported by the OIE.

INTRODUCING THE KEYNOTE SPEAKERS AND SPEAKERS FOR PLENARY SESSIONS:

Dr. Torsten Morner

KEYNOTE SPEAKER

Supported by

Important diseases in European wildlife - the past, present and future

Professor Morner from the Department of Pathology and Wildlife Diseases (formerly Department of Wildlife, Fish, and Environment) of the National Veterinary Institute (Uppsala, Sweden) and a former president of the WDA will be giving the keynote address on the topic of the current and most important wildlife diseases in Europe. Dr. Morner is an active member of the OIE group putting together the data of the disease situation in wildlife in Europe and thus has an excellent overview of diseases of the wildlife.

Dr. Philip S. Mellor

KEYNOTE SPEAKER

Supported by

Expansion of bluetongue in Europe and climate change

Professor Mellor from the School of Biological Sciences of the University of Aberdeen and the Department of Arbovirology of the Institute for Animal Health, Pirbright Laboratory (Surrey, UK) is an OIE international expert for bluetongue and African horse sickness. Professor Mellor will be giving an address on the epidemiology of bluetongue in Europe focusing on climate-change as the driving force behind the recent and unprecedented extensions in its distribution.

Dr. Rossella Lelli

KEYNOTE SPEAKER

Supported by

West Nile fever: the European situation and the role of wildlife

Dr. Lelli from the National Reference Centre for Exotic Diseases of the Institute for Animal Health Research (Istituto Zooprofilattico Sperimentale, Abruzzo and Molise Region, Teramo, Italy) is an OIE international expert for West Nile fever and has been involved with the disease since the Italian outbreak in 1998. Dr. Lelli will be giving an address on the epidemiology of West Nile fever focusing on the risks of the spread of the disease among European wildlife and its multiple profiles of the contemporary epidemic transmission throughout the world.

Dr. Terry J. Kreeger

KEYNOTE SPEAKER

Chemical immobilization in the 21st century: the future or back to the future?

Dr. Kreeger from the Veterinary Services Branch of the Wyoming Game and Fish Department (Wheatland, WY, USA) will be giving the keynote address on the topic of chemical immobilization and wildlife handling covering general topics on wildlife chemical immobilization and handling applicable to European wildlife species with suggested responses to animal and human emergencies in the field. Dr. Kreeger is also the author of the renowned Handbook of Wildlife Chemical Immobilization.

Dr. Djuro Huber

PLENARY SPEAKER

Biology and health of Croatian large carnivore populations

Professor Huber and his Large Carnivore Research Team of the Zagreb Faculty of Veterinary Medicine (Croatia) have been studying the biology and health status of brown bears, grey wolves and Eurasian lynx in Croatia ever since 1981, and the yielded results were all applied in conservation and management of those species. Along with the Scandinavian large carnivore populations, the Croatian populations are probably, the best studied and managed in Europe.

Dr. Hrvoje Gomercic's
Marine Mammal Research Group

PLENARY TALK

Cetacean mortality along the Croatian Adriatic coastline

Professor Gomercic's Group from the Faculty of Veterinary Medicine in Zagreb (Croatia) is amongst the most active sea-mammal fact-finding assemblies on the Mediterranean focusing on whales, and dolphins primarily. They will be offering an insight into causes of death, pathomorphologic findings, species abundance, and influence of anthropogenic activity in cetaceans through retrospective studies and 20 years of observations on the Croatian Adriatic coast.

Oral Presentations

Thursday, 2 October

Oral Presentation 1

Keynote Address

IMPORTANT DISEASES IN EUROPEAN WILDLIFE THE PAST, THE PRESENT, AND THE FUTURE

TORSTEN MORNER
National Veterinary Institute, Department of Pathology and Wildlife Diseases, SE 751 89 Uppsala, SWEDEN; (e-mail: torsten.morner@sva.se)

Surveillance of diseases in wildlife in Europe has been performed in several different institutions since the early 1940's. The major role of these monitoring programs has been to discover new diseases and pathogens, to document the impact of the different diseases on wildlife populations, and to investigate the role of diseases in wildlife as a source for diseases in domestic animals and humans.

In a long term historical perspective some diseases have been eradicated, while others for a long time have been, and still are, present in wildlife in Europe. There are diseases, like for example rinderpest that are since long eradicated in Europe. Other diseases have been present for a very long time, such as rabies, canine distemper, fox encephalitis, classical swine fever, Newcastle disease, pseudotuberculosis, bovine tuberculosis and Johne's disease. Some diseases, like tularemia and sarcoptic mange have been present in Europe for a long time, but have during the 20th century expanded in Europe and appear nowadays with a somewhat different epidemiology.

Many new diseases have been detected since the 1940's like myxomatosis, rabbit haemorrhagic disease (RHD), European brown hare syndrome (EBHS), phocine distemper, pestivirus infections in different ungulates, avian influenza and others. Some of these diseases have been introduced into Europe, while others could be a result of a mutation from an avirulent virus, rather than a completely new disease.

There are several different factors explaining why some diseases persist in wild animal populations, and why others are eradicated. Among such factors are; morbidity, incubation time, duration of disease, virulence, mortality, the appearance of asymptomatic carriers, reservoir hosts within the same species or other species serving as reservoir hosts, immune status of animals and environmental factors, are all important for the longevity of a disease.

The importance of different diseases, economically and ecologically, varies a lot. In a long-term perspective rabies, classical swine fever, bovine tuberculosis, tularemia and sarcoptic mange probably have been the most important diseases, found all over Europe and being of importance both as potential zoonotic diseases but also of large economical concern.

It is highly likely that we will continuously discover new diseases in wildlife in Europe. One reason for this is the more sophisticated diagnostic tools that will enable the finding of pathogens that have not been observed earlier. Another factor that will lead to introduction of new diseases is the increasing number of animals and humans that are moved all over the world and hereby potentially introducing new pathogens to new areas and new hosts. The ongoing climate change will also influence the disease pattern in wildlife in Europe and we will probably see more tropical diseases in Europe in the future.

Oral Presentation 2

GLOBAL WILDLIFE DISEASE MONITORING: AN ATTEMPT BY THE OIE WORKING GROUP ON WILDLIFE DISEASES TO COLLECT ANNUAL DATA ON RELEVANT EVENTS ACROSS THE WORLD (RESULTS OVER 16 YEARS)

MARC ARTOIS[1,2,9], ROY BENGIS[1,3], CHRIS BUNN[1,4], JOHN FISCHER[1,5], FREDERICK LEIGHTON[1,6], TORSTEN MORNER[1,7], and MIKE WOODFORD[1,8]
[1]OIE Working Group on Wildlife Diseases, World Organization for Animal Health, 12 rue de Prony, 75017 Paris France; [2]Universite J. Fourier, Laboratoire TIMC-IMAG, Grenoble & Ecole nationale veterinaire de Lyon, France; [3]Veterinary Investigation Centre P.O. Box 12, Skukuza 1350 South Africa, [4]Office of the Chief Veterinary Officer Department of Agriculture, Fisheries and Forestry, GPO Box 858 Canberra ACT 2601 Australia, [5]Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine University of Georgia, Athens - GA 30602 USA, [6]Canadian Cooperative Wildlife Health Centre, Western College of Veterinary Medicine, University of Saskatchewan, Canada, [7]Department of Wildlife, Fish & Environment National Veterinary Institute 751 89 Uppsala Sweden, [8]Apdo: 1084 8100 Loule, Algarve Portugal; [9]Corresponding author (e-mail: m.artois@vet-lyon.fr)

"Wildlife diseases" (or more precisely, diseases of livestock and humans carried or transmitted by wildlife), are perceived as important by national veterinary services in the context of the protection of the health of domestic animals and of animal product consumers. In many situations, exposure to wild animals is a health risk factor. Many of the "emerging diseases" of public health concern have arisen from infections and parasites which recently have spilled over to the people (influenza, rabies, SARS corona viruses); some of them have become infectious agents that now are transmitted directly from person to person (AIDSv). In addition, some pathogens propagating in wildlife can cause important diseases in domestic animals (AFV, BTb and CSF). Increasingly as well, public opinion is adopting the position that we have to protect wildlife as an important component of natural heritage and thus is concerned with the effects of diseases on wild animals themselves.

Recognition of the global significance of many diseases in wildlife caused the World Organisation for Animal Health (OIE, created in 1924) to establish a Working Group on Wildlife Diseases, which has met 16 times since 1993 and has provided the OIE with detailed annual reports on the worldwide disease situation amongst wildlife. Through a questionnaire sent to all OIE 172 member countries and through personal contacts, this Working Group has collected each year, information on occurrences of wildlife diseases and has provided the OIE with an overview of the most relevant events occurring across the world. This Working Group has received constant support from the OIE International Committee, which has encouraged it to extend its coverage of disease events by geographical regions, disease agents and host animal species. In Europe, members of the EWDA have been among the most constant and supportive providers of data for this annual review.

A recent analysis of the 16 reports* of the Working Group has resulted in comprehensive overview of the regions of the world which have the capacity to collect and report wildlife disease information, and identification of the main diseases in wildlife identified and reported. Foot and mouth disease (FMD), anthrax and bovine tuberculosis in wild animals were reported in each of the 16 annual reports as being of major significance in the global disease situation in wildlife. Twenty other diseases were each mentioned at least nine times in these reports; among these 20 diseases, only three were not on the official list of diseases with which the OIE is concerned. This emphasizes that many of the diseases of greatest concern in livestock and in public health are diseases that also affect wildlife. This analysis also highlights that the capacity to detect and report the occurrence of diseases of major importance in wild animals remains to be improved in many countries.

* The reports since 2001 are available in PDF format at http://www.oie.int/wildlife/eng/en reports.htm

Oral Presentation 3

SHARING AND DISSEMINATING WILDLIFE DISEASE INFORMATION

JOSHUA DEIN[1,2], MEGAN HINES[1], CRIS MARSH[1], STEVEN GUSTAFSON[1], BARABARA NASH[1], ERICA SCHMITZ[1], and LAURA WYNDHOLDS[1]
[1]NBII Wildlife Disease Information Node, US Geological Survey - National Wildlife Health Center and University of Wisconsin-Madison - Nelson Institute for Environmental Studies, Madison, Wisconsin, USA; [2]Corresponding author (e-mail: fjdein@usgs.gov)

Increasing international attention to emerging diseases in wildlife, and their potential impacts on populations, public health, and livestock has highlighted the importance of disease surveillance in wildlife, and the sharing of data and information across national boundaries. Efforts are underway through international agencies and through a Wildlife

Disease Informatics Working Group to identify and potentially link national wildlife disease data systems. This will require extended discussions on the selection of data to be shared and the consideration of international data standards to be employed.

The US National Biological Information Infrastructure (NBII; www.nbii.gov) Wildlife Disease Information Node (WDIN; http://wildlifedisease.nbii.gov), is a collaboration of the USGS National Wildlife Health Center and the UW-Madison Nelson Institute for Environmental Studies, along with other partners. WDIN is working on projects to aid this effort, and to assist and promote the collection and dissemination of wildlife disease data and information, as well as provide tools that link the international community. The Wildlife Disease News Digest (http://wdin.blogspot.com) is a collection of reports taken from a wide range of open source media, available on an almost daily basis. The Digest can be access through the website, subscriber email alerts, an RSS feed, or a Gadget that one can use on their personalized news page. Items selected for the Digest can also be viewed geospatially through the Global Wildlife Disease News Map (http://wildlifedisease.nbii.gov/wdinNewsDigestMap.jsp). Those interested in contributing news items or reports can send them to wdin@usgs.gov. WDIN Highlights is a monthly publication that provides updates on WDIN products and resources. The Wildlife Health List is a subscription email list server that provides information on academic and employment opportunities, notices of upcoming meetings, new resources available, and can also be used as a discussion forum. Other discussion forums available are the Implanters List, for those interested in the surgical implantation of radio transmitters to track wildlife, and the Wildlife Health Informatics List, for those participating in the Wildlife Disease Informatics Working Group. WDIN will also consider hosting other appropriate forums by request. Additional information on all these news sources is available at: (http://wildlifedisease.nbii.gov/wildlifediseasenews.jsp).

The Wildlife Health Monitoring Network (http://wildlifedisease.nbii.gov/cwddc.jsp) is a collection of wildlife disease surveillance tools, including the US National Highly Pathogenic Avian Influenza Data System (HEDDS), the Seabird Ecological Assessment Network (SEANET), and the Chronic Wasting Disease Data Clearinghouse. Additional efforts are being made to expand this network through the development of a web based collection of tools and applications that will available for anyone to use for wildlife disease surveillance, and offers the capability to share these data with others. This project is a collaborative effort with the Wildlife Conservation Society to create the Wildlife Information System for Disease Observation and Monitoring (WISDOM).

Oral Presentation 4

EWDA Best Student Presentation Award applicant

RESULTS OF PASSIVE SURVEILLANCE ON THE CAUSES 0F WILD UNGULATE MORTALITY IN SPAIN

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Knowledge of diseases circulating in wildlife populations can be important not only for conservation and livestock production but also for public health. Pathologic findings may provide valuable information about the contribution of different disease conditions to mortality and about environmental/management factors influencing wildlife health. We carried out a retrospective study in order to examine causes of mortality in wild ungulates from Spain from June 2000 to May 2008. The sample included 285 animals belonging to 258 case events. We report geographic origin, estate management category (open fenced game farm), season, age, sex and body condition, by disease condition. Species studied included Barbary sheep (*Ammotragus lervia*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*), European wild boar (*Sus scrofa*), Iberian ibex (*Capra pyrenaica*), mouflon (*Ovis aries musimon*) and Pyrenean chamois (*Rupicapra pyrenaica parva*). All animals of this study were found dead or sick and subsequently euthanized. Ethiologies were divided into major categories including bacterial, parasitic and viral disease, traumatic injuries, miscellaneous causes and unknown. Definitive diagnosis of specific conditions was not always possible due to diverse limiting factors such as carcass degeneration or availability of diagnostic tests.

Bacterial infections, mainly including bovine tuberculosis and pasteurellosis, were the most frequently diagnosed mortality causes (42%). Other important ethiologies included handling (trauma and stress, 8%), parasitic infections (8%), viral infections (7%) and traumatisms (7%). The cause of death could not be determined in 18% of the cases. Among red deer, differences between sexes were important (73% males), but sexes were equally represented in wild boar (46% males). Bacterial infections among red deer represented 40% of the cases and no viral infection were diagnosed, while in wild boar, viral infections were diagnosed in 17 animals (18%) and bacterial disease was detected in 50 cases (54%). Establishment of a passive disease surveillance network is fundamental so that clinical events in wild ungulates can be detected, submitted and studied. Further research and more detailed studies, including the assessment of diagnostic techniques and individual marking, should be carried out in order to better understand the contribution of diseases to host demography. However, data collected contribute to show that endemic disease such as bTB are among the frequent mortality causes of wild animals of recreational and economic value.

Oral Presentation 5

EWDA Best Student Presentation Award applicant

THE GARDEN BIRD HEALTH INITIATIVE A NOVEL CITIZEN SCIENCE APPROACH TO WILDLIFE DISEASE SURVEILLANCE

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Citizen science networks provide a useful and cost-effective approach for disease surveillance in free-ranging wildlife through reporting of mortality incidents. However, the *ad hoc* nature of submissions collected through such schemes may be influenced by temporal, spatial and other potential biases.

The Garden Bird Health initiative (GBHi) was launched in 2005 to investigate the causes and epidemiology of disease and mortality incidents in British garden birds. Risk factors for disease outbreaks, once identified, will be used to inform the development of science-based guidance on feeding garden birds with the aim of maximising welfare and conservation benefits in the future. The scheme benefits from collaboration between veterinarians, ornithologists, animal welfare organizations and the garden bird feed industry.

A co-ordinated national scheme utilizing two complementary but independent citizen science networks has been in operation for three years. Members of the public participate by opportunistically reporting garden bird mortality incidents to a national reporting line and the RSPB Wildlife Enquiries Unit. A questionnaire-based history is taken and disease investigation performed where appropriate.

Systematic monitoring for garden bird mortality, with consistent temporal and spatial effort, has been achieved through collaboration with the established British Trust for Ornithology Garden BirdWatch. A network of circa 1000 individuals was recruited from across Britain. Participants record on a weekly basis, 1. details of feeding practise (e.g. volume and type of food, feeder type and hygiene practises), 2. the species and numbers of birds visiting their garden and 3. evidence of sick or dead birds in their garden.

A network of disease investigation centres perform post mortem examinations on birds submitted through both the opportunistic and systematic networks following standardised protocols. A cause of death category is allocated for each post mortem examination, with the categories of "Infectious Disease", "Trauma", "Predation", "Other" and "Undetermined". The relative proportions of these cause of death categories has remained remarkably similar across each year of the GBHi.

In excess of 1500 post mortem examinations have been performed between April 2005 and March 2008 with good geographical coverage across Britain on a total of 49 species from 24 families. The greenfinch (*Carduelis chloris*) (n=539), chaffinch (*Fringilla coelebs*) (n=276) and house sparrow (*Passer domesticus*) (n=109) were the most frequently examined species in rank order.

Marked variation was found in the percentage of "infectious disease" cases between families, whilst comparison of this percentage between species within families was frequently similar. Infectious disease was the sole cause, or an important contributory factor to the cause, of death in 80% of Fringillidae (804/1004 individuals), 72% of Columbidae (64/89 individuals), 58% of Passeridae (71/122 individuals), and 12% of Turdidae (17/140 individuals). Trichomonosis, salmonellosis, colibacillosis and avian pox were the most frequently diagnosed infectious diseases of British garden birds.

Oral Presentation 6

EWDA Best Student Presentation Award applicant

REVIEWING SAMPLE METHODOLOGY OF LOW PATHOGENIC AVIAN INFLUENZA (LPAI) SURVEILLANCE STUDIES IN WILD BIRDS

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The ongoing HPAI H5N1 epidemic has caused great losses to the poultry industry and a serious public health problem. The situation has triggered worldwide surveillance programs on both domestic and wild birds. It is now well recognized that influenza virus surveillance in wild birds can provide "early warning" signals for the introduction of HPAI H5N1 into new regions. For proper risk assessment studies, better understanding of circulating low pathogenic subtypes, their ecology and virulence, can help to achieve preparedness for the protection of livestock and humans. Recently, experimental studies have shown important differences in quality of AIV RNA and thus in RTPCR results depending on the type of sample and handling during transportation and laboratory analyses. Also, sampling methods, i.e. capture or sampling of fresh faeces may select for the species or order sampled. Finally, different patterns of AIV virus prevalence have been reported throughout the year. This may mean that depending on when and how sampling for LPAI viruses is carried out results could vary greatly and lead to differing interpretations.

For this reason here we review 24 publications on studies of the prevalence of LPAI in wild birds that have been carried out in the five continents between 1976 and 2007. In our study we pretend to evaluate if type of habitat, timing of sampling in relation to migration, breeding or wintering, sampling/capture method and geographical location have an influence on the data obtained for overall prevalence, species prevalence and the subtypes detected..

Of the articles included in this study, 5 reported data from the North American Continent, 11 were studies from Europe, while only 2, 2, 3 and 1 article respectively reported data from South America, Asia, Oceania and Africa. The range of the overall prevalences of LPAI viruses in birds was greater in Europe (0.26-12.5%) and North America (6.5-25.5%), from where more studies were available, when compared to the range of prevalences obtained for Asia (0.8-1.8%), South America (1-1.2%), Oceania (1.87-5.78%) and Africa.

Most of the studies (60.8%, 14 out of 23) were based on cloacal swabs from captured birds, while only three studies (13%) used fresh faeces and the rest of the studies combined different methods. Most studies were carried out in one (9 out of 13, 39%) or two (10 out of 23, 43%) seasons (mostly during wintering and spring migrations), only 2 of the studies were carried out throughout the whole year. When using data from studies on LPAI prevalence for the analysis of risks for the introduction of HPAI viruses in certain regions, it may be of interest to take into account information related to sample collection to avoid bias associated to sampling methodology.

Oral Presentation 7

Keynote Address

EXPANSION OF BLUETONGUE IN EUROPE AND CLIMATE-CHANGE

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The presentation will begin with a brief discussion of those climatic variables that are likely to influence the distribution and incidence of vector-borne diseases such as bluetongue. An explanation of how these variables may induce their own particular effects will be included.

The talk will then move on to describe the disease caused by bluetongue virus (BTV) in its ruminant hosts, including certain wildlife species and, will then concentrate on recent changes in the world distribution of BTV and its vectors - focussing on Europe from 1998 until 2008. It will be argued that the recent changes, both in terms of virus distribution and the species of vectors transmitting the virus can be linked to climate-change.

Suggestions of what this might mean for the future, in a time of on-going climate-change will be set out.

Oral Presentation 8

BLUETONGUE IN WILD RED DEER (*CERVUS ELAPHUS*) IN SOUTHERN BELGIUM

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In August 2006, the bluetongue virus serotype 8 (BTV-8) was identified for the first time in North-Western Europe. The Netherlands, Belgium and Germany were the three initially-affected countries. The virus subsequently spread rapidly and new outbreaks of BTV-8 occurred in 2007 in neighbouring countries indicating that the virus had successfully overwintered. From August 2006 until December 2007, more than 7500 cases (domestic ruminants) were notified in Belgium (http//www.afsca.be). Measures enforcing restrictions of animal movements were taken, vector control measures were applied and vaccination campaigns of domestic ruminants started in May 2008. As an exhaustive knowledge of the virus biology is crucially needed in this context, the following serological and virological survey was carried out to address the question of the possible involvement of wild ruminants in the epidemiology of the disease. Through an active surveillance program, 1182 wild hunter-killed red deer (*Cervus elaphus*) were sampled (serum and/or spleen) during autumns of 2005 (n = 200), 2006 (n = 469) and 2007 (n = 513). Geographic distribution of the samples covered 25 of the 37 district forests known to shelter wild cervids in Southern Belgium. Serum samples were screened for anti-BTV antibodies using the “ID Screen Bluetongue Competition” assay (competition ELISA from ID VET, Montpellier, France) and spleen fragments were sought for viral RNA either by RT-PCR (2006) or by real-time RT-PCR (RT-qPCR, 2007). Each test was performed in parallel with an internal beta-actin control. All serum samples were negative in 2005 but seroconversion was detected after, the apparent seroprevalence being 0.9 % in 2006 and 40.4 % in 2007. In 2006, all spleen samples were viro-negative while 9.0 % were positive in 2007. Agreement between c-ELISA and RT-qPCR results will be discussed orally. Meanwhile, 118 (2005), 130 (2006) and 133 (2007) found-dead wild red deer were enrolled in the passive surveillance program. There was no indication of massive mortality in 2007 despite the dissemination of the virus over the entire region during this period. However, systematic necropsic examination and spleen testing revealed a few animals presenting both lesions suggestive of BTV and positive results by RT-qPCR. Our results suggest that the current BTV-8 is circulating in the wild red deer populations in Southern Belgium, which does not result in a significant excess mortality. The epidemiological modelling of the current BTV-8 spread, persistence and overwintering must therefore take this aspect into account.

Oral Presentation 9

INVOLVEMENT OF WILDLIFE IN THE EPIDEMIC OF HIGHLY PATHOGENIC H5N1 AVIAN INFLUENZA

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The currently ongoing outbreaks caused by highly pathogenic avian influenza A virus of the subtype H5N1 not only involves the poultry industry and public health, but also that of wild animals. In recent years we have studied several unusual aspects of H5N1 virus. First, H5N1 virus infection results in disease and mortality in multiple other species that were not previously known to become ill from influenza A virus infection. For example, during the 2003 to 2004 outbreak of H5N1 influenza in Asia there were reports of fatal H5N1 virus infection of domestic cats and zoo felids after feeding on virus-infected chickens. This is most unusual, because domestic cats are generally considered to be resistant to disease from experimental influenza A virus infection, and reports of natural disease are rare. To determine the pathogenicity of this virus for domestic cats, we experimentally infected cats with H5N1 virus by different routes and examined them by virologic and pathologic techniques. The results demonstrated that H5N1 virus can productively infect domestic cats, cause diffuse alveolar damage, and result in clinical disease or death. Second, H5N1 virus in humans and other mammals is not limited to the respiratory tract, as is usually the case with influenza A virus infection, but may also extend to other organs. To study this question, we experimentally infected domestic cats with H5N1 virus by different routes of inoculation. Our results showed that virus replicated not only in the respiratory tract but also in multiple extra-respiratory tissues, and was associated with severe necrosis and inflammation. An interesting observation in some cats was virus-associated ganglioneuritis in the submucosal and myenteric plexi of the small intestine, suggesting direct infection from the intestinal lumen. Third, free-living wild birds appear to act as a vector for H5N1 virus. Traditionally, spread of highly pathogenic avian influenza virus among poultry flocks is thought to occur by transport of infected poultry, contaminated equipment, and people associated with the poultry industry. However, in the current outbreaks wild birds are suspected to play a significant role as long-distance virus vectors. To test the hypothesis that wild waterbirds can excrete H5N1 virus in absence of debilitating disease and so act as potential long-distance virus vectors, we experimentally infected six species of wild ducks with a recent avian isolate of H5N1 virus from Europe. All six species are listed as presenting higher risk to avian influenza by the European Union. We found that some species of ducks excreted significantly more virus than others, while only two of six species became ill or died. These findings suggest that some wild duck species are potential long-distance vectors of H5N1 virus, while others are more likely to act as sentinels.

Oral Presentation 10

EWDA Best Student Presentation Award applicant

RED KNOTS (*CALIDRIS CANUTUS*) WITH HIGHER PLASMA CONCENTRATION OF CORTICOSTERONE EXCRETE MORE AVIAN INFLUENZA H5N1 VIRUS

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Migratory birds may become more susceptible to infection during migration, due to migratory restlessness and higher plasma concentration of corticosterone, which is known to interfere with the immune system. Increased susceptibility to infections may result in higher morbidity or mortality, which may hamper wild birds to undertake migratory flights, thus limiting their role in the long-distance spread of pathogens. It may also result in higher or longer excretion of pathogens, which may favour their long-distance spread by migratory birds. Reactivation of latent infections with *Borrelia burgdorferi* has been evidenced in songbirds as a result of migratory restlessness, without resulting in morbidity, suggesting that wild birds may contribute to the long-distance spread of Lyme disease in North America. It remains unknown whether higher plasma concentration of corticosterone during migration is associated with increased susceptibility of migratory birds to viral infection, and in particular to highly pathogenic avian influenza (HPAI) H5N1 virus infection. Because migratory changes, including the increase in plasma concentration of corticosterone, still occur in captive red knots, independently of the actual migratory journey, this species is well-suited for laboratory studies of migration physiology. Therefore, we used this species as a migratory bird model to determine whether birds were more susceptible to infection with HPAI H5N1 virus during spring migration in relation with the observed increase in plasma concentration of corticosterone. Groups of five to six red knots were inoculated before, during and after the period of spring migration intra-tracheally and intra-oesophageally with 10^6 $TCID_{50}$ of A/turkey/Turkey/1/2005. Blood samples were collected before inoculation to measure plasma concentration of corticosterone and to determine correlates of constitutive immunity prior to infection. The birds were observed for clinical signs, and pharyngeal and cloacal swabs collected daily until quantitative PCR demonstrated that they stopped excreting. Birds showing severe clinical signs or birds which stopped excreting for 2 consecutive days were euthanized and organs were collected for virological, histopathological and immunohistochemical examinations. Red knots excreted virus from the pharynx for up to 5 days post inoculation (dpi) and viral titers peaked at up to $10^{5.2}$ $TCID_{50}$/ml. No virus was recovered from cloacal swabs of any birds. Two birds infected before migration, and one bird infected at the time of migration developed neurological signs between 5 and 6 dpi, associated with encephalitis. Although plasma concentration of corticosterone did not differ significantly between the three groups of birds, pharyngeal excretion of HPAI H5N1 virus was positively correlated with plasma concentration of corticosterone. These results demonstrate that red knots with higher plasma concentration of corticosterone excrete more HPAI H5N1 virus. If this is a more general phenomenon, the physiological changes at the time of migration might favour the long-distance dispersal of viruses by migratory birds.

WEST NILE FEVER: THE EUROPEAN SITUATION AND THE ROLE OF WILDLIFE

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West Nile virus (WNV) belongs to the genus *Flavivirus* (fam. *Flaviviridae*) that contains approximately 70 members, most of which are transmitted by mosquitoes or ticks. West Nile Fever is a meningoencephalitis of birds, horses and humans. It was first isolated from a febrile woman in the West Nile district of Uganda in 1937 and subsequently was associated with sporadic cases of disease as well as major outbreaks in Africa, Europe and Middle East.

At the beginning of 1990s outbreaks began to occur more frequently, especially in Europe (Romania, France, Italy) and in the Mediterranean Basin (Algeria, Morocco, Tunisia, Israel) and were associated with increased numbers of cases with severe disease including viral encephalitis and neurological symptoms in humans and horses. In the summer of 1999 WNV appeared in New York City, and since 2002 USA has been declared endemic.

WNV is transmitted primarily by the bite of infected mosquitoes, predominantly *Culex* species, which acquire the virus by feeding on infected birds. The strain of WNV circulating within the ecosystem along with biological factors of the hosts and vectors and the environmental conditions affect the local ecology and transmission dynamics. For these reasons the ecology and epidemiology of WNV greatly differ between regions and within and across continents. Several hosts and virus related factors have been elucidated during the experimental infection studies: host-feeding preferences and vector competences combined with vertebrate ecology and ethology, determine the primary host species for the virus. Although reservoir hosts and vector species differ on a regional level, wild birds are recognized as the primary hosts of WNV throughout its geographical range.

Until year 1999, infection of wild birds with WNV has produced mostly unapparent and sub clinical responses with varying titres of circulating virus depending on bird species and virus strain tested. However, the emergence of a new, more virulent strain of WNV in Israel and in the USA has showed a different pattern of disease in wild birds.

Birds, in general, are also considered to be the most important reservoir of infection, some of them being able to develop high level and long viremia to sustain the transmission cycle. Moreover, species belonging to the orders Passeriformes, Charadriiformes, Anseriformes, Strigiformes and Falconiformes can develop viremia levels to infect most mosquitoes whereas species of Columbiformes, Piciformes, and Galliformes do not develop an adequate level of viremia for infecting vectors. Local movements of sedentary and long range migratory birds may both contribute to the spread of WNV.

Humans and horses, even when immunodepressed, are commonly described as dead-end hosts as their level of viremia is too low to permit virus transmission to uninfected vectors.
Introduction of WNV by migratory birds is worldwide recognized as most likely source of infection for free area and the main way of spread of infection.

WNF appeared in Italy for the first time in 1998, in the Padule di Fucecchio area of Tuscany region. During the outbreak 14 horses showed neurological symptoms and 6 animals died.
The Padule di Fucecchio area was subsequently selected for the EDEN project (Emerging Diseases in a changing European eNvironment) founded by EU, subproject West Nile, that aims to identify and catalogue those European ecosystems and environmental conditions which can influence the spatial and temporal distribution and dynamics of human pathogenic agents.

The knowledge of biology and the migratory behaviour of wild birds can be considered a milestone to understand the ecology of WNV in each ecosystem.
Since the Mediterranean Basin falls under the main trans-Saharan migratory routes, directly connecting free areas in Europe with endemic zones (sub-Saharan Africa), entire Mediterranean area could be considered as risk-area for WNV introduction and establishment.
The presence of early warning systems is the main instrument to fight against the spread of WNV through the Mediterranean, and to be effective it is necessary to include surveillance on wild birds both sedentary and migratory.

Oral Presentation 12

SURVEILLANCE OF WEST NILE VIRUS IN FRANCE - SEVEN YEARS OF MONITORING IN WILD BIRDS

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West Nile fever is a mosquito-borne viral disease including wild birds as amplifying hosts, and humans and horses as sensitive hosts (accidental victims). West Nile virus (WNV) was first recognized in southern France (Camargue) in 1962-1965. It reappeared in 2000 in the same region affecting only horses (76 clinical cases, 21 deaths). Shortly after the outbreak, the French game and wildlife agency (ONCFS) conducted a serological study in 5 bird species and found low seroprevalences in Mallards (*Anas platyrhynchos*, 8%) and European magpies (*Pica pica*, 22%). A long-term epidemiosurveillance program was established in 2001: it was based on the detection of encephalitis cases in horses and humans, the surveillance of abnormal mortality in wild birds (SAGIR network) and a serological monitoring of sentinel birds (mallards used as live decoys by hunters and domestic chicken) located in 30 sites distributed along the Mediterranean coast. Forty one equine cases (including 9 mortalities) were reported from different sites in 2003 (Var district), 2004 (Camargue area) and 2006 (Pyrénées Orientales district) and 7 clinical human cases (no fatal infections) were reported in the Var district in 2003. No abnormal mortality due to WNV was reported in the avifauna but passive surveillance allowed to isolate WNV in 2004 from two free-ranging passerines found moribund in the Camargue area: a European magpie and a House sparrow (*Passer domesticus*). Seroconversions were detected in sentinel birds from the Camargue area in 2001 and 2002, revealing an ongoing circulation of the virus despite the absence of detection of clinical cases in sensitive hosts. Additionally, in 2004, simultaneous seroconversions of several sentinel birds in southern Camargue allowed early detection of a high WNV transmission level that was followed by the emergence of 32 equine cases in late summer 2004. These results outline the importance of monitoring WNV circulation in birds as an early warning system. This system proves particularly relevant for areas such as southern France where WNV is responsible for minor outbreaks in horses and humans that appear unpredictably both in space and time.

Oral Presentation 13

DISEASE AND MORTALITY IN RED FOOTED FALCON (*FALCO VESPERTINUS*) NESTLINGS - CHALLENGES FACING A LONG DISTANCE MIGRANT SPECIES AT ITS BREEDING HABITAT

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All long distance migratory species are extremely vulnerable to a wide range of environmental risk factors, climatic to anthropogenic, which are capable of unpredictably increasing bird mortality on breeding and wintering grounds.
Red footed falcons (*Falco vespertinus*) are long distance, Sub-Saharan migrants, breeding in colonies in the area stretching from Central-Eastern Europe to the Baikal Lake region in Central Asia. They occupy nests built by other species, e.g. rook (*Corvus frugilegus*) colonies and solitary magpie nests as well as artificial nest boxes provided to them. Red footed falcon breeding colonies in the Koros-Maros National Park in South-Eastern Hungary were monitored during the 2007 breeding season. 183 nestlings were ringed and banded with individual colour band combinations. The clinical status and body condition of the nestlings was assessed. Samples were taken for bacteriology and virology. One kestrel and nine red-footed falcon nestling carcasses were recovered and presented for post mortem examination.

Macroscopic lesions compatible with various stages of cutaneous poxvirus infections were detected in 30 nestlings (16,4%), primarily on eyelids. An avian poxvirus, clustering with other Falconpox isolates, was identified in the lesions by PCR and sequence analysis.

Clinical disease comprising transient suppurative konjunctivitis, rhinitis and weak body condition was seen in 9 nestlings. In further 4 cases this clinical condition was associated or superseded by splay leg and perosis-type tarsometetarsal deformity. Mycoplasma spp. strains were isolated both from the ill nestlings and their clinically healthy siblings. The cases involving skeletal deformity proved invariably fatal.

West-Nile virus was detected in carcasses of 3 red-footed falcons and one kestrel nestling. Similarly to earlier findings in birds of prey, the most pronounced microscopic lesions were the focal, lymphocytic encephalitis and myocarditis, associated with large amounts of viral antigen. Additionally, a clinical WNV infection was diagnosed in a red-footed falcon fledgling found in the Borsodi mezoseg region presenting neurological symptoms.

Presence of ectoparasites was recorded on 6 nestlings and 3 red-footed falcons (adults and fledglings) succumbed to traumatic, car-collision injuries. Some nestlings could not be accounted for at subsequent nest controls and no clue could be established regarding the cause (e.g. predation or disease) of the presumed mortality.

The detection of WNV in the breeding population of a Sub-Saharan migrant species was not too surprising however the extent of associated mortality needs to be investigated further. The emergence of multiple cases of leg deformity was unexpected and its aetiology is so far unclear. The same disease has also been repeatedly detected in Saker falcon nestlings.

Our study identified several disease conditions which may potentially influence the breeding success of Red footed falcons. Further monitoring and analysis is needed to assess the real impact of these diseases and to identify the key factors contributing to their occurrence.

Oral Presentation 14

EWDA Best Student Presentation Award applicant

SARCOCYSTIS NEURONA: EXPLORING THE FATAL LINK BETWEEN OPOSSUMS (*DIDELPHIS VIRGINIANA*) AND SEA OTTERS (*ENHYDRA LUTRIS NEREIS*) IN COASTAL CALIFORNIA

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Despite intense recovery efforts and federal protection of the southern sea otter (*Enhydra lutris nereis*), the population of approximately 2700 individuals remains threatened. Spending their entire lives within 1 km of the shoreline sea otters inadvertently function as sentinels for both chemical and biological pollution. Protozoal encephalitis due to *Sarcocystis neurona* has emerged as one of the primary causes of morality for southern sea otters. The definitive host for *S. neurona* in North America is the Virginia opossum (*Didelphis virginiana*) which sheds sporulated oocysts and sporocysts in its feces. Despite their ubiquitous presence in California, opossums were introduced to the state barely 100 years ago. The focus of this study was to better understand the epidemiology of *S. neurona* and to assess whether *S. neurona* parasites shed by opossums are the same as those infecting sea otters in coastal California. The objectives of this study were: 1) determine the prevalence of *S. neurona* infection in opossums along the California coast; 2) identify associated risk factors for infection, and 3) molecularly characterize *S. neurona* isolates from sea otters and opossums to determine their relatedness. Live-trapped and traffic-killed opossums from central California were used in this study. *Sarcocystis* oocysts/sporocysts were visually and molecularly identified in the feces of live opossums and in the intestinal scrapings of dead opossums. *Sarcocystis neurona* parasites were also cultured from brain samples of stranded, freshly dead sea otters. To date, 275 opossums have been sampled and the observed *S. neurona* infection prevalence is 5.8%. Among risk factors tested, location and season were both significantly associated with *S. neurona* infection in opossums. Opossums sampled along the coast were significantly less likely to be infected with *S. neurona* than those sampled further inland. The reason for this difference is not clear although climate differences or intermediate host availability may play a role. Adult opossums at all locations were significantly more likely to be infected with *S. neurona* during the spring and summer months. Interestingly, this is also the time of year when sea otters are most likely to become acutely infected with *S. neurona*. Finally, preliminary DNA sequencing data using two polymorphic markers, ITS-1 and 25/396, from several sea otter and opossum *S. neurona* isolates show complete agreement over nearly 800 base pairs of sequence. This is a strong indication that the *S. neurona* parasites being shed by opossums are genotypically the same as those infecting sea otters. More detailed characterization using microsatellites and other polymorphic markers is currently underway. This is the first study to molecularly and seasonally link sea otter mortality to introduced opossums.

Oral Presentation 15

PREVALENCE AND MOLECULAR CHARACTERIZATION OF *GIARDIA* ISOLATES FROM WILD MAMMALS

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Wild mammals are commonly infected with *Giardia* spp., with prevalence rates often over 50%, but there is surprisingly little information on what *Giardia* species and/or G. *duodenalis* assemblages they carry. The limited information available indicates that rodents are commonly infected with the non-zoonotic species G. *muris* and G. *microti*, whereas other wild animals are infected with both, host-adapted (C, D, and E) and zoonotic assemblages (A and B) of G. *duodenalis*.
In this work, faecal samples were collected in Croatia from red deer (*Cervus elaphus*, n=285), roe deer (*Capreolus capreolus*, n=14), wild boars (*Sus scrofa*, n=144), foxes (*Vulpes vulpes*, n=65), bears (*Ursus arctos*, n=19), wolves (*Canis lupus*, n=122), jackals (*Canis aureus*, n=7), and hares (*Lepus europeus*, n=73) were examined for the presence of *Giardia* cysts using microscopy. The prevalence ranged from very low (0% in bears and hares, 1% in deer) to moderate (1.7% in wild boars, 4.5% in foxes, 10% in wolves, 12-5% in jackals) to relatively high (27% in roe deer).
DNA was extracted from positive samples and used for PCR experiments. Amplification and sequencing of a fragment of the small subunit ribosomal RNA (ssu-rRNA) gene identified assemblage A in wolves (5 isolates), foxes (1 isolate) and red deer (1 isolate), assemblage C in wolves (2 isolates), a mixed infection with assemblage A and C in a wolf isolate, and assemblage D in red deer (2 isolates) and roe deer (1 isolate). Finally, G. *microti* was found in a wolf isolate, possibly resulting from the mechanical passage of cyst from an infected prey.
This survey, therefore, suggests that the zoonotic risk is mainly linked to the shedding of G. *duodenalis* assemblage A cyst by carnivores and wild hoofed animals. However, the lack of intra-assemblage sequence variability in the ssu-rRNA gene limits the possibility to evaluate the actual zoonotic potential of G. *duodenalis* cysts typed as assemblage A. For this purpose, a comparative analysis of subtypes defined by sequencing more variable genes (such as the triose phosphate isomerase) is required. This work is in progress in our laboratory, and the data generated so far indicated the presence of subtype AI in isolates from a wolf and a red deer, and subtype AIII in a wild boar isolate. As these subtypes are rarely found (AI) or absent (AIII) in G. *duodenalis* isolates from humans, the role of wild mammals in the transmission of giardiasis to humans appears to be minimal.

Oral Presentation 16
Student Presentation

DETERMINING THE GENETIC DIVERSITY OF *THEILERIA EQUI* AND *BABESIA CABALLI* IN COMMON AND MOUNTAIN ZEBRA (*EQUUS QUAGGA* AND *EQUUS ZEBRA*)

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Extensive sequence variation in the V4 hypervariable region of the 18S rRNA gene of *Theileria equi* has led to the identification of new South African *T. equi* genotypes in horses. A study was thus undertaken to determine whether the same level of genetic variation exists in the 18S rRNA genes of zebras infected with *T. equi* and/or *Babesia caballi*. Seventy EDTA blood samples collected from Plains zebra (*Equus quagga*) and Mountain zebra (*Equus zebra*), were obtained from the Kruger National Park (n=20), the Wildlife Breeding Research Centre (n=14), and the Equine Research Centre (n= 36). Samples were screened for the presence of parasite DNA using the reverse line blot hybridization (RLB) assay, and a *B. caballi*-specific TaqMan real-time PCR test. RLB results indicated that 45 samples were negative for the presence of piroplasms. Seventeen samples were positive for *T. equi*, while no samples hybridized to the *B. caballi* probe. Eight samples hybridized only to the *Theileria/Babesia* genus-specific probe and not to any of the species-specific probes. Thirteen of the 17 *T. equi* positive zebra samples also hybridized to the *Babesia* genus-specific probe but not to the *B. caballi* species-specific probe. These results indicate either a possible mixed infection with *B. caballi* at a level below the detection limit of the *B. caballi* RLB probe, or the presence of a novel *Babesia* species or genotype. A TaqMan MBG real-time PCR assay, specific for the detection of *B. caballi* infections, was able to detect *B. caballi* parasite DNA in fourteen of the 25 RLB positive samples. The V4 hypervariable region of the 18S rRNA gene from the positive samples was amplified, cloned and sequenced. Nucleotide sequence data was obtained from 15 of these positive samples and BLASTN analysis revealed that the sequences were most similar to *T. equi* genotypes and not *B. caballi* genotypes. Although *Babesia* parasites were present in some of these samples, the parasitaemia may have been too low to allow detection by cloning of PCR products from a mixed infection. Based on these findings and our previous results obtained for parasites from horses, we conclude that there are three groups of *T. equi* genotypes in South Africa. One genotype has only been isolated from horses in South Africa; another genotype has thus far only been identified in zebra in South Africa, while the third genotype is present in both horses and zebra.

Friday, 3 October

Oral Presentation 17

Keynote Address

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The use of chemicals to capture wildlife dates back 50 years in which there have been significant improvements in both drug efficacy and safety. There are many reasons that a wildlife biologist or veterinarian needs to capture animals. For captive animals in zoos and research facilities, chemical capture is often required to handle and treat large and dangerous animals. Wildlife biologists need to capture free-ranging wildlife for research purposes or for translocations to augment existing populations or begin populations in new areas. An even more common use is to remove wild animals that have wandered into populated areas where they present a safety risk either to themselves or to humans, or get themselves trapped in dangerous situations from which they need to be extricated. Capture drugs should be viewed simply as tools to be used by wildlife biologists or veterinarians to conduct their jobs in a professional manner.

Some drugs used for animal capture are extremely potent, but that potency should not be used as an excuse to substitute less-than-optimal drugs that could endanger the animal or risk the safety of humans. Many individuals, and some entire wildlife agencies, are reluctant to use drugs, either through unfamiliarity or concerns about human safety. The decision to use, or not to use, chemicals to capture animals often have philosophical underpinnings which are anthropomorphic and have little basis in science. For instance, some veterinarians adhere strictly to the mantra that “above all, cause no harm.” Because anesthesia can be viewed as a near death experience, it is likely that some harm will occur to some animals some of the time. Others demand that the capture episode be as least stressful as possible. This criterion is probably impossible to achieve. Animals that are chased by helicopters, caught in traps, or surrounded by people are highly stressed. There is little that can be done to alleviate that stress, other than to capture, process, and release the animal as quickly as possible. A pragmatic philosophy, then, is to capture animals with one injection using the most efficient drug available. The use of inappropriate drugs, or underdosing with an appropriate drug, might be considered the most stressful or inhumane aspect of chemical capture.

To capture an animal with a single injection requires potent drugs that can be delivered in small volumes. Historically, the first capture drugs were paralytic drugs, such as nicotine sulfate and succinylcholine. Nicotine sulfate is no longer used because of its potential toxicity to both animals and humans. Succinylcholine, on the other hand, is widely used in humans, and on animals under selected circumstances. For decades, succinylcholine has been condemned by certain groups because the drug paralyzes the animal without any effect on its senses. That is, the animal can see, hear, and smell or feel pain. However, these same sensations would exist within the animal if it was physically restrained, such as in a trap or corral chute. One could argue that physical restraint, which is often considered perfectly acceptable, is just as stressful to the animal. Succinylcholine also has a very low therapeutic index: the animal can suffocate with just a slight overdose. Despite these shortcomings, succinylcholine has many desirable attributes. These include: 1) the most rapid induction of any capture drug (1-2 minutes); 2) a quick recovery without the need for an antagonist (15-20 minutes); 3) safe for humans should accidental injection occur; 4) safe for predators or scavengers, should the animal die in the field; and lastly 5) succinylcholine is the least expensive capture drug available (e.g., < 0.25 € to capture a red deer).

More currently, animals have been immobilized with a combination of a primary anesthetic and a tranquilizer or sedative. The advantages of combining drugs include: 1) potentiation of both drugs, 2) reduction of undesirable side effects, 3) decreased induction times, and 4) improved recoveries. Tranquilizers or sedatives include, but are not limited to: diazepam, acepromazine, azaperone, xylazine, and medetomidine. Of these, xylazine and medetomidine are probably the most desirable because of their potency and ability to be antagonized. Drugs considered anesthetics include ketamine, tiletamine (in Zoletil/Telazol), fentanyl, sufentanil, carfentanil, thiafentanil (A-3080), and etorphine (M99).

Ketamine, when combined with diazepam or acepromazine, is a useful combination for species as polecat, pine marten, beaver, fox, otter, mink, marmot, muskrat, wildcat, and other small mammals. Ketamine plus xylazine, or ketamine plus medetomidine, or Zoletil (with or without additional tranquilizers) can be used on medium to large mammals, such as badger, chamois, wild boar, wolf, roe deer, reindeer, mouflon, wolverine, and brown bear. Much pioneering work has been conducted in Europe in the development of ketamine-medetomidine combinations. The advantage of this combination is that the potency of medetomidine greatly reduces the amount of ketamine required for immobilization. This reduced amount of ketamine is more rapidly cleared and when the medetomidine is antagonized, the residual ketamine remaining in the animal is insufficient to maintain immobilization. Thus, reversals are rapid and fairly complete so that the animal returns to normalcy quickly. The downside to reducing the amount of ketamine is that the animal can awake abruptly, which can be disconcerting when dealing with large and dangerous carnivores.

Zoletil is a lyophilized combination of two drugs: tiletamine and zolazepam. An advantage of Zoletil is that by adding a small amount of solvent (e.g. 2 mL), one can obtain a potent and concentrated mixture sufficient for the immobilization of large carnivores such as bears. Additionally, instead of using sterile water for the solvent, one can add other drugs such as ketamine, xylazine, or medetomidine to provide an even more potent mixture in a small volume.

Although usually classified as anesthetics (agents that induce unconsciousness), there is some doubt that the opioids (fentanyl, sufentanil, carfentanil, thiafentanil, and etorphine) are true anesthetics and they should probably be considered to be somewhere between paralytics and anesthetics. The opioids are the most effective capture drugs for larger species such as red deer, ibex, mouflon, roe deer, European moose, muskox, bison (wisent), and large bears. The advantages of the opioids are that they are good analgesics that can be rapidly and completely antagonized. Disadvantages of opioids include respiratory depression, alteration of thermoregulation, and potential toxicity to humans should accidental exposure occur. There has been much concern about this latter aspect regarding the most potent opioids, such as carfentanil and thiafentanil. However, these drugs have high therapeutic indices and there have been no reported human deaths due to the use of these drugs, despite tens of thousands of doses being administered worldwide.

Equipment used to remotely deliver drugs has fundamentally remained unchanged for the last half-century. It has, however, become more sophisticated. Dart rifles, utilizing gun powder blanks to propel the dart, are still the most commonly used delivery devices worldwide. More recently, dart rifles utilizing compressed carbon dioxide or air have become the preferred delivery device because they can be finely adjusted to change dart velocity and thus distance. Darts can inject the drug into the animal either through compressed air, compressed gas, or explosive charge. Darts can be modified to take tissue samples, incorporate radio transmitters for location, or even euthanize animals.

When finally immobilized, the animal should immediately be observed for respiratory depression. Although pulse oximeters are good devices for monitoring respiratory efficiency, they are no substitute for vigilance. Manual resuscitation through chest compression is probably the easiest and most effective method to support respiration. The second most common problem encountered is hyperthermia. Constant monitoring of temperature is required when ambient temperatures are warm or the animal has been physically exerted. The single most rapid method to cool an animal is whole body immersion in water. Other medical concerns include hypothermia, bloat, aspiration, seizures, and capture myopathy. Prolonged physical exertion is probably the most significant threat to the health of the animal, particularly the ungulates.

Although virtually all the drugs used to capture animals can have a clinical effect in humans, the number of reported incidences is surprisingly rare. This is probably because most exposures are superficial, resulting in very small amounts of drug being absorbed. There have been only two reported deaths resulting from etorphine injection. There have been no deaths reported due to the accidental injection of other capture drugs, even though humans have been hit with darts.

What will the chemical immobilization of wildlife look like in the 21st century? It is unlikely that many new drugs will be developed, at least in the United States, because of the extremely high cost (> US$250 million) and years of development to bring a new drug to market. The market for veterinary drugs is probably too small to justify such an investment. Chemical immobilization advances will probably be dependent on new drugs being developed for humans that may be applicable to animals. Although the potent opioids are the preferred drug for the capture of most large animals, opioid development for human use is tending to emphasize ultra short-acting drugs that require constant infusion because of their short half-lives. Such short acting drugs would probably be of little use for animal capture because the animal could arouse even before approached and handled. Sufentanil is perhaps closer to the future than any of the other drugs. It has a high affinity for the mu receptor, and has an extremely high therapeutic index (25,000). Current formulations of sufentanil, however, are very dilute, making it unsuitable to inject small volumes to achieve immobilization. However, this drug can be reformulated to achieve higher concentrations.

One of the more intriguing developments in anesthesiology will be drugs that manipulate the endogenous central nervous system transmitters, which can produce profound analgesia and sleeplike states that mimic hibernation. Hibernation results in decreased body temperature, decreased oxygen use, reduced heart rate, and reduced respiratory rates. All of these can be immediately reversed with naloxone, or other opioid antagonists. During a state of hibernation, the animal's stress hormones are also dramatically reduced and the whole state of anesthesia can be considered less "stressful.' Anesthesia of the future may well mimic animal hibernation.

More disturbing in the field of wildlife immobilization is not the lack of new drug development, but rather the potential loss of existing drugs. It is not hard to imagine that a single terrorist incident in the use of potent opioids, such as carfentanil or thiafentanil, could result in the immediate banning on all uses of such drugs. Even today, it can be very difficult for some countries to import any of the immobilizing drugs, sometimes taking up to a year to receive the drugs after being ordered. Also in the United States, there is only a single supplier of carfentanil and thiafentanil. What will happen if this company goes out of business? The federal government regulatory hurdles facing a potential new supplier of these drugs are formidable and no one may wish to invest the time and money for such a limited market. Additionally, the cyclohexanes (ketamine, tiletamine) are increasingly being used by humans for illicit purposes. It is not hard to imagine future severe limitations on the availability and use of this class of drugs, if not outright removal from the market.

The future of wildlife chemical immobilization may be a trip "back to the future." It may well be that the only drugs remaining in the 21st-century will be the ones that were first used for wildlife chemical immobilization: the paralytics.

Oral Presentation 18

RETROSPECTIVE STUDY OF PESTIVIRUS INFECTION IN SOUTHERN CHAMOIS (*RUPICAPRA PYRENAICA*) IN THE PYRENEES

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In 2001 a new Pestivirus (Family Flaviviridae) was associated with an outbreak of a previously unreported disease in Southern chamois (*Rupicapra pyrenaica*) in the Pyrenees (NE Spain). Molecular characterization assigned this virus to the Border Disease Virus (BDV) cluster, BDV-4 genotype. Later studies showed that the disease has become endemic in the area and that it could have a significant impact on chamois population dynamics. Between 2005 and 2007 two further outbreaks were reported in two different areas and the same BDV was detected and characterized. A retrospective study was performed to detect Pestivirus and antibodies to these viruses in archived sera and frozen tissues (spleen) of 74 southern chamois from the Pyrenees between the years 1990 and 2000.
Thirty-six of 74 (48.6%) sera were positive by an ELISA antibody test. Comparative virus neutralization tests (VNT) performed on 26 seropositive samples with 6 pestivirus strains yielded higher titres to BDV Spain 97, followed by BDV chamois, BDV 137/4, Bovine Diarrhoea virus-1 (BVDV-1) NADL, BDV Moredum and BVDV-2 atypical.
An ELISA antigen test was performed on 37 seronegative chamois and yielded positive results in one animal and inconclusive result in two animals. RT-PCR and virus isolation performed on spleen samples from these animals gave positive results in the positive and one inconclusive animal. These two chamois were studied in 1996 at Freser-Setcases National Hunting Reserve, an area not affected to date by any outbreak of Pestivirus-associated disease. Sequence analysis in the 5' unstranslated region (5'-UTR) revealed that the two viruses were grouped into the BDV-4 genotype. Virological and serological data of the present study indicate that BDV infection has been present in the chamois population since at least 1990, 11 years before the first outbreak of disease. Therefore, the emergence of the disease in 2001 is apparently due to other factors rather than the introduction of a new virus in the chamois population.

Oral Presentation 19

AN OUTBREAK OF FOOT NECROBACILLOSIS IN A WILD REINDEER (*RANGIFER TARANDUS TARANDUS*) POPULATION IN NORWAY

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Norway harbours the last remnants of the original wild tundra reindeer (*Rangifer tarandus tarandus*) in Europe. The animals live in the alpine regions of southern Norway, and are divided into 23 sub-populations. An outbreak of foot necrobacillosis was diagnosed in one of these sub-populations located in the mountain area of Rondane. The population count is approximately 3,000 animals in winter and the disease was detected during the hunting season (August 20 September 30) in 2007. About 100 animals, mainly calves, were affected. They showed signs of severe lameness and swelling of the feet, mainly restricted to one limb. Most of the affected animals were shot by hunters, whereas a few were found dead. Materials from 12 animals were submitted for laboratory examination. Pathological examination revealed swelling of the foot, affecting an area from the coronary band up to the fetlock in one or both of the toes. The condition was further characterized by an eroded wound in the skin with necrotic-purulent surface, cellulitis, pus formation and proliferation of granulation tissue in deeper structures. The joints and synovial sheaths were involved to various degrees. One animal also had a severe osteomyelitis. Conventional bacteriological examination on calf blood agar plates incubated aerobically and anaerobically, revealed the growth of several bacteria, including *Arcanobacterium pyogenes*, *Streptococcus agalactiae*, *Staphylococcus aureus* and *Escherichia coli*. From two of the animals *Fusobacterium* sp. was isolated after anaerobe cultivation. PCR examination of the 16S r-RNA-gene of these isolates revealed sequences that differed by one in 671 nucleotides from those given for *Fusobacterium necrophorum* at the BLAST sequence data bank. Gram negative filamentous and pleomorph bacteria resembling *Fusobacterium* were abundant in tissue sections from all animals. Tissue sections were also examined by fluorescence in-situ hybridization with an oligonucleotide probe specifically targeting 16S r-RNA-gene in *F. necrophorum*. The examination identified an abundance of this bacterium in lesions from all animals. Foot necrobacillosis was previously a relatively common disease in domesticated reindeer herds in Norway, Sweden and Russia. However, the exact identification of the causative bacterium by modern molecular-biological methods has been lacking. The present study confirmed that foot necrobacillosis in reindeer is linked to infection with *F. necrophorum*. However, the bacterium seemed to belong to a genotype that has so far not been recognized as causing necrobacillosis in domestic ruminants.

Oral Presentation 20

INVESTIGATIONS ON THE EPIZOOTIOLOGY OF *BABESIA CAPREOLI* INFECTIONS IN FREE-RANGING WILD UNGULATES FROM SWITZERLAND

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Fatal babesiosis due to *Babesia capreoli* was recorded for the first time in free-ranging chamois (*Rupicapra rupicapra*) from two distinct regions of the Swiss Alps. This pilot study aimed to determine the origin of the disease and to assess the ecological factors potentially leading to the apparently emerging character of fatal *B. capreoli* infections in chamois. Ticks, blood and demographic data of 48 chamois, 46 roe deer (*Capreolus capreolus*), and nine red deer (*Cervus elaphus)* from both affected regions were collected in 2006-2007. None of the sampled animals showed clinical signs of babesiosis. Ticks were identified to species level. Blood was investigated microscopically and by PCR/sequencing using primers specific for *Babesia* spp.

All ticks were identified as *Ixodes ricinus*, and prevalence of tick infestation was significantly higher in roe deer than in chamois. No inclusions were observed on any of the blood smears. PCR/sequencing revealed a significantly higher prevalence of *B. capreoli* in roe deer than in chamois. Only one of the investigated red deer was infected.

Our results indicate that healthy roe deer may play an important role as reservoir hosts of *B. capreoli* in Switzerland. Factors that facilitated the spillover of *B. capreoli* to chamois need to be investigated. An expansion of *I. ricinus* to higher altitudes as a consequence of climatic changes might be a potential hypothesis.

Oral Presentation 21

ORAL VACCINATION OF WILD BOAR (*SUS SCROFA*) AGAINST CLASSICAL SWINE FEVER: EFFICACY OF THE BAITING PROCESS

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Oral vaccination has been performed in France and neighbour countries since the early 2000's with the aim to eradicate CSF in wild boar populations. The adopted process follows the recommendations by Kaden et *al.* (2004). Although the

vaccine is fully efficient at the individual level the efficacy of this oral vaccination is still debated because infection may persist several years in spite of a high level of herd immunity (>70%) (Von Ruden et *al.* in press). In this paper we analyse the efficacy of the baiting process and discuss its possible influence on the efficacy of oral vaccination.
The study area is located in the Vosges mountains, France that is infected since 2003 and were approximately 509 000 baits are released every year by hunters. The bait is a hard square composed by maize with almond aroma and including a plastic blister that contains the C-strain vaccine in a liquid form (Riemser Arznzimittel AG, Germany). We first analysed the bait uptake using questionnaires to hunters in order to test for seasonal and spatial variation of the bait uptake. We then analysed in deep the efficacy of baiting in young wild boar using two approaches: first, we observed the behaviour of animals on feeding grounds using camera-traps or direct observations. Then we studied the kinetics of antibodies in 1-6 months old captured piglets.
The proportion of effective feeding grounds was lower in September-October compared to February-March or June-July, especially in feeding grounds located near crops or oak trees that are likely to be attractive for wild boar. Wild boar was the species that ate baits in >90% of observation, anyway piglets 3-6 months old were not observed eating baits. The analysis of the kinetics of antibodies showed that neither the vaccination performed in June-July nor the vaccination performed in September-October generated an increase of seroprevalence among 3-6 months old piglets, so that the proportion of immune piglets observed in autumn was rather resulting of CSF infection than from effective vaccination. The baits are attractive for adults but the size and texture does not seem suitable for 3-6 young wild boar.
The heterogeneity of baiting delivery over space, time and the low efficacy of baiting in young may favour the survival of infection. With currently available baits the only strategy to increase the immunity in young is to delay the delivery of baits to November-December when piglets are older, but then the bait uptake would be dependent on the oak mast production. So that the delivery of baits adapted to piglets during early summer would be more suitable in the future.

Oral Presentation 22

BRUCELLOSIS IN WILD UNGULATES IN SPAIN

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Several *Brucella* species are zoonotic agents shared among domestic and wild animals but the role of wildlife as brucellosis reservoir is not well known. The availability of adequate indirect diagnostic tests is an essential requisite to determine properly the prevalence of brucellosis in wildlife. We have developed and standardised a multi-specific indirect enzyme immunoassay (i-ELISA) as a previous step to determine the prevalence of brucellosis in wild ungulates. Due to the lack of “Gold-Standard” sera from wild ungulates, sera used for the ELISA standardisation were taken from *Brucella* culture positive and *Brucella*-free phylogenetically related domestic species. Different antigens, conjugates and dilution factors were tested and those giving the largest differences in optical density (OD) between culture positive and *Brucella*-free controls were chosen as the optimum. This i-ELISA was performed with a phenol-water extract from *B.melitensis*16M as antigen and recombinant protein G-peroxidase as conjugate. A cut-off allowing the highest sensitivity and 100% specificity with the “Gold-standard” sera was selected to determine the seroprevalence in wild ungulates. Animal samples were obtained between 2003 and 2008 from several Spanish Regions affected by brucellosis in domestic species. Sera from 4616 red deer *(Cervus elaphus)*, 850 Spanish ibex (*Capra pyrenaica*), 3019 European wild boar (*Sus scrofa*), 1234 chamois (*Rupicapra pyrenaica)* and 532 from fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*), were tested using the i-ELISA described above. When feasible, necropsy samples (lymph nodes, spleen and sexual organs) of seropositive animals were submitted to bacteriological analysis.
With the exception of wild boar and red deer, the prevalence of brucellosis in the wild species tested was very low if any. The whole of roe and fallow deer tested were found free from infection. Only few positive reactions (less than 0,5 %) were detected in chamois and Spanish ibex. By contrast, around 2% of the red deer sera were identified as positive in Aragon, Asturias and Castile-Leon regions. Surprisingly, the prevalence of brucellosis in wild boar was very high. Hunting areas of Castile la Mancha showed seroprevalences near to 40%. High values were also obtained in Aragon (26%) and bordering areas of Navarre (19%). The lowest prevalence (11%) was found in Asturias. A total of 665 seropositive wild boar and 86 deer were submitted to bacteriological analysis. Up to 85 bacterial isolates were obtained

from wild boar, all them identified as *B. suis* biovar. 2. Whereas, only one red deer was found infected by *B. abortus* biovar. 1.
As conclusion, brucellosis in wild boar (caused exclusively by *B. suis* 2) is a generalised problem in Spain, representing a potential threat for the domestic extensive Iberian porcine population. By contrast, other wild ruminant species seem not to be an important reservoir of the disease.

Oral Presentation 23

Plenary Speaker

BIOLOGY AND HEALTH OF CROATIAN LARGE CARNIVORE POPULATIONS

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Croatia holds a population of 600-1000 brown bears (*Ursus arctos*), 200-220 gray wolves (*Canis lupus*) and 40-60 Eurasian lynx (*Lynx lynx*). Bears are game animals, while wolves and lynx are strictly protected. All populations belong to the Dinara mountain range and are shared with neighboring Slovenia and Bosnia and Herzegovina. Careful management based on detailed management plans is keeping the bear population increasing, wolf population stable, while lynx is still slightly decreasing and requires most attention.

The Large Carnivore Study Team of the Zagreb Faculty of Veterinary Medicine has been studding the biology and health status of brown bears, gray wolves and Eurasian lynx in Croatia since 1981. The approach included life capturing of wild animals and collection of dead specimens. So far we have captured and handled 56 wild brown bears (35 radio-tracked), 19 wolves and 7 lynx (all radio-tracked). In addition we performed 110 handlings of captive bears. Bodies of retrieved dead large carnivores included 110 bears, 104 wolves and 7 lynx, and have undergone various degrees of investigation depending on the state and completeness of remains. Human caused mortality dominated among all three investigated species. That included legal and illegal shooting and traffic kills. The other pathology determined at post mortems was rarely the cause of death. The health status has been additionally monitored through serum antibodies and biochemistry of living animals. In more recent years the genetic diagnostic approach has been used for parasitological investigations. Genetic studies of large carnivores themselves are used for insight into genetic diversity and population sizes.

The results yielded in over 120 publications which can be roughly grouped like following: health status and pathology (including parasitology, genetics and immobilization) 43, biology and ecology 21, status, management and human attitudes 57 papers. Most of results were applied in conservation and management. As usual, the more data collected, the more new questions open and ask for more research. We are aware of data gaps and are seeking the ways to fill them. The fully international approach in research and management is the way to secure the future of large carnivores in Europe.

Oral Presentation 24

TYZZER'S DISEASE IN A EURASIAN OTTER (*LUTRA LUTRA*) IN SCOTLAND

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Tyzzer's disease is an acutely fatal disease caused by infection with *Clostridium piliforme*. It affects a wide range of species, especially laboratory rodents, and has been recorded in both captive and free-living wildlife in North America and Australia. An otter cub aged approximately 12 weeks was found in weak condition on the Isle of Harris off the west coast of Scotland. It was taken to an otter rehabilitation centre on the Isle of Skye where, following initial improvement, it developed bilateral corneal opacity. It received antibiotic treatment but died on day 26. Post-mortem examination revealed corneal oedema and ulceration, excess clear fluid in the body cavities and a swollen liver with numerous pale focal lesions and petechial haemorrhages. The thymus gland was regressed and the adrenals were enlarged. Bacterial cultures of major organs were negative and no virions were seen on electron microscopy. Histopathological examination of liver revealed bundles of bacilli within hepatocytes that were weakly Gram negative but strongly positive by Warthin Starry. They were morphologically typical of *Clostridium piliforme*. Nucleic acid extracts were prepared from the liver and also from the livers of seven other similar age otter cubs that had died from unrelated causes. These were incorporated into a PCR assay designed to specifically amplify a 16S rDNA fragment from *C. piliforme* and closely related species. A single amplification product of the anticipated size was obtained from the test case but not from the other seven cubs. The nucleotide base sequence of the product revealed identity with a known *C. piliforme* 16S rDNA sequence. The hepatic pathology in this case was consistent with Tyzzer's disease but the corneal oedema and effusion into the body cavities was atypical and may represent an unusual host response. This is believed to be the first record of Tyzzer's disease in an otter and the first in a wild animal in Britain.

Oral Presentation 25

EWDA Best Student Presentation Award applicant

DOES FELINE LEUKEMIA VIRUS MODIFY PARASITIC FAUNA IN WILD-LIVING CATS?

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Feline Leukaemia Virus (FeLV) is frequently encountered in European wildcats (*Felis silvestris silvestris*) and in domestic free living cats (*Felis catus*). Because FeLV induces immunodeficiency and a high mortality in domestic cats, the question arises of whether wild-living cats suffer from consequences of this infection. A possible effect of FeLV immunodeficiency would be high susceptibility to other parasites. The aim of our study was thus to investigate the relationship between the presence of FeLV and gut parasites and *Toxoplasma gondii* in wild-living cats.

One hundred and six road casualty wild-living cats were sampled between 1996 and 2006 in central and eastern France. Cats were sexed, and ages were determined, as well as their types (domestic cats or wildcats). Blood samples were collected from each cat for serological examinations. Tests for FeLV p27 antigen were made using the ELISA method. Tests for anti-*T.gondii* IgG antibodies were performed, using the modified agglutination test. The entire gastro-intestinal tract of each cat was opened in order to search for worms and to count them. Faeces samples were collected from each cat for coprological examination to determine egg (and oocyst) prevalence.

Relationships between parasite prevalence and FeLV and individual variables were searched for parasites found the most frequently in the cat sample: *Taenia taeniaformis* (worms), *Toxocara cati* (eggs and worms), *Isospora rivolta* (oocysts), and *Capillaria* sp. (eggs). Our study showed that the probability that a cat was infected with FeLV or gut parasites did not depend on individual variables (age, sex, type) in our sample.

Because parasitism can be influenced by local landscape features, the landscape composition of the municipalities where cats were found was characterized with the following ecological variables: urban areas, open fields, forests and an index of landscape diversity (*H*). Multivariate analysis showed that FeLV prevalence was related to the landscape diversity, while prevalence of gut parasites depended on urban or open fields areas.

Lastly, relationship between intensity of gut parasites and FeLV was studied. Taking into account the median intensity in age-sex classes, cats positive for FeLV carried more *T. taeniaformis* worms in their digestive tract than cats negative for FeLV.

We discuss the possible significance of these results for the impact of FeLV on wild-living cats.

This field studies on wildlife was supported by a grant of the "Office National de la Chasse et de la Faune Sauvage" Nr: 2005 /16/6171, entitled: "Etudes et recherches écologiques et épidémiologiques sur la pathologie de la faune sauvage".

Oral Presentation 26

REPORT ON A CANINE DISTEMPER VIRUS EPIZOOTIC IN FOXES (*VULPES VULPES*) AND BADGERS (*MELES MELES*) IN THE ITALIAN EASTERN ALPS

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An epizootic of canine distemper virus (CDV) has been occurring since late spring of 2006 in red fox (*Vulpes vulpes*) and badger (*Meles meles*) populations of Italian Eastern Alps. Early cases were observed along the Italian north-eastern border in the Alto Adige province and Friuli Venezia Giulia region; subsequently the epizootic spread into alpine areas of the Trento province and Veneto region. The aim of this report is to describe the epizootic patterns and discuss possible implications for animal health and conservation.

Since spring of 2006, 278 foxes and 67 badgers have been delivered to the laboratories of the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe). The animals were either found dead or culled, in some cases after the observation of pathological signs and neurological symptoms such as apathy and loss of movement coordination. Neurological symptoms were observed mainly in foxes. Whenever possible, sex and approximate age (juvenile/adult) were recorded. Moreover, the sampling location of each animal was recorded for geographical studies. Carcases were necropsied and samples (mainly brains) were analysed by Reverse Transcriptase PCR for CDV. Analyses for CDV were performed after an immunofluorescence test for rabies virus.

No rabies was found in the samples. Necropsies showed signs of gastroenteritis and pneumonia in a consistent number of cases, but these findings were not specific. 118 (42%) red foxes and 43 (64%) badgers were found to be infected by CDV by RT-PCR. Neither in foxes nor in badgers data analysis showed significant variation in CDV prevalence between genders or age classes. The CDV epizootic appears to move along a south-west direction. On a local scale however, cases can still be detected even 5-6 months after the first occurrence.

Previous data obtained in a sample of 536 foxes and 58 badgers from the Italian Eastern Alps had never shown the evidence of CDV. Thus, the observed occurrence of CDV in wild carnivores of this territory can be considered as a new epidemiological event. In order to prevent transmission to and from domestic dogs, special care has been put into the sensitisation of dog owners to a correct vaccination programme. Due to such animal health implications, and considering that CDV has already caused conservation problems in endangered species of wild carnivores, further epidemiological and phylogenetic studies are needed to define the origin of the epizootic, as well as to understand if wild populations of the area (in particular foxes) could act as a reservoir for CDV as hypothesized in other countries.

Oral Presentation 27

OUTBREAK OF CANINE DISTEMPER IN RACCOONS (*PROCYON LOTOR*) IN GERMANY

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In Germany two major racoon (*Procyon lotor*) population exist, one is located in the centre of the federal republic, the other in Northeastern Germany within the federal state of Mecklenburg-Pomerania. During a long term study of the Mecklenburg' raccoon population concerning ecology, distribution and social behaviour of this species, a total of 65 individual animals were tracked by radio collar so far. During a time period from May 2007 until May 2008 14 of these animals died, but due to close monitoring almost all were recovered soon after death and were submitted for necropsy to the Leibniz Institute for Zoo and Wildlife Research. The first seven animals died during May until August 2007. Clinically, five of these animals revealed abnormal behaviour patterns associated with neurological symptoms. At the time of death, these animals were solely found hidden in impassable moor or swamp areas. Microscopic investigations gave evidence of pathological lesions within lung and/or brain tissue were found consistent with canine distemper. One adult male had also hyperkeratosis of the paw pads (hard pad disease). Analysis of matched tissue samples by PCR revealed infection with canine distemper virus (CDV) of these five individuals, while the other two animals were negative for CDV infection. Subsequent sequencing comprised up to 98.4% similarity with canine distemper virus compared with virus strains from domestic dogs published in Genbank. From August to October 2007 five animals were live captured for blood sampling of which three animals had high antibody titres against canine distemper virus, one animal had a moderate titre, while one animal's serum sample was negative. Of the remaining seven animals which were investigated from October 2007 until May 2008 neither histological nor molecular evidence of CDV was found. Three raccoons were killed or succumbed severe injuries. Two individuals had severe eosinophilic infiltrations in multiple organs suggestive of systemic parasitic infection, while the cause of two further animals could not clearly be determined. This is the first report of an outbreak of CDV in a free-ranging population of raccoons in Europe. As this species effortlessly enters urban habitats, raccoons moving between human communities and nature reserves could play an important role as a reservoir host and even more as a transmission vector for CDV infection between domestic animals and endangered wildlife species.

Oral Presentation 28

THE INFLUENCE OF HUMAN VISITOR ACTIVITY ON SPATIAL PATTERNS OF PARASITE INFECTION

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Human recreation can directly influence wildlife species distributions, but little is known about indirect cascading effects on parasites. Parasites with complex life cycles, that span multiple trophic levels and move from vertebrates to invertebrates and back in a single generation, have the potential to be greatly influenced by human recreation patterns. High human use levels can artificially concentrate parasite hosts, thus potentially influencing transmission of wildlife diseases. In this study we link human recreation at a National Park in Arizona to wildlife disease patterns. We test the hypotheses that human recreation affects spatial patterns of waterfowl habitat use, and that this in turn affects the prevalence of a trematode parasite in an endangered intermediate (amphipod) invertebrate host. An observational study and a randomized experiment supported our hypothesis that human recreation directly affects the foraging location of waterfowl. We found that waterfowl chose to forage in areas more distant from visitor use paths at a desert spring pond, and that these areas supported greater rates of amphipod parasite infection in the initial year of our study. In the second year, when visitor use was reduced, waterfowl did not show as strong a pattern of differential space use and amphipod parasite prevalence was reduced. Our findings clearly demonstrate that human recreation can indirectly affect spatial patterns of parasite abundance and wildlife disease. These results have important implications for the management of areas that are maintained for human leisure activities and wildlife, especially in systems where disease is ecologically important.

Saturday, 4 October

Oral Presentation 29

Plenary talk

CETACEAN MORTALITY ALONG THE CROATIAN COASTLINE

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Systematic research on cetacean mortality in the Croatian waters of the Adriatic Sea was initiated through the Faculty of Veterinary Medicine, Zagreb in 1990, five years prior to the legislative protection of cetacean species in this region. A total of 173 cetacean strandings were recorded till January 2008. An attempt was made to investigate every stranding report received, but 22 carcasses were not examined because of, e.g. difficult field and weather conditions. Post-mortem examinations were performed on 151 cetacean carcasses consisting of 120 bottlenose dolphins (*Tursiops truncatus*), 17 striped dolphins (*Stenella coeruleoalba*), nine Risso's dolphins (*Grampus griseus*), three Cuvier's beaked whales (*Ziphius cavirostris*), and two fin whales (*Balaenoptera physalus*).

The post-mortem examination included determination of species, sex, body mass, external measurements and a patho-anatomical dissection according to a standard protocol. Tissue samples of each necropsied cetacean were stored frozen or preserved in 10% formalin and 90% ethyl alcohol solution in the cetacean tissue bank for further morphological, genetic and toxicological analyses. The age of necropsied bottlenose dolphins and striped dolphins was estimated by counting growth layer groups in dentine, while in other species radiological analysis of epiphiseal fusion of pectoral fin bones was used for age class estimation. Skeletons of the examined cetaceans were stored as well. During the study period the most often recovered cetacean species was the bottlenose dolphin which is also considered the only resident marine mammal in the Adriatic Sea. The striped dolphin was the most often encountered non-residential species. From the geographical point of view the bottlenose dolphin carcasses were evenly distributed along the Croatian coast, whereas the non-residential species were more often recovered in the Southern Adriatic. August is the most fatal month for the bottlenose dolphin. On the other hand, none of the non-residential specimens was recovered during late summer/early autumn months when the human activities in the Adriatic Sea are the most intense. The mortality among the bottlenose dolphin sexes is equally distributed, while in striped dolphins and Risso'dolphins male carcasses were recovered predominantly. The highest mortality in bottlenose dolphins is recorded in the newborns. The newborns are found dead only from April till August. Our findings indicate that these months represent the calving season of the bottlenose dolphin in the Adriatic Sea.

Out of 151 post-mortally examined cetacean carcasses in total, 62 carcasses were in advanced state of decomposition, mummified or represented only by the skeletal remains. In the other 89 specimens non-fatal and fatal conditions (parasitism, trauma, congenital defects, neoplasia, etc.) were determined, which were induced by human activities in 51 (57.3%) cetaceans. By-catch is the most often human induced fatal condition and was determined in 33 cetacean carcasses.

Long-term and systematic investigations of the cetacean mortality provide the important data on their distribution, abundance and health status, as well as threats to their health and survival. The obtained results should prove valuable for the conservation and management of these endangered and protected animal species.

Oral Presentation 30

A REVIEW OF MARINE MAMMAL *BRUCELLA* IN BRITISH WATERS 1994 2007

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Brucellosis is an important zoonotic disease usually associated with cattle, sheep, goats and pigs. In 1994 *Brucella* sp. was isolated from four common seals (*Phoca vitulina*), two harbour porpoises (*Phocoena phocoena*) and one common dolphin (*Delphinus delphis*) in Scotland, UK and a captive bottlenose dolphin (*Tursiops truncatus*) in the USA. These isolates were found to be morphologically and molecularly different to the terrestrial strains and have been proposed the names *B. ceti* and *B. pinnipedialis*.

Since the first isolations, the Veterinary Laboratories Agency (VLA) have been involved in the examination of marine mammals both serologically and with the culture and molecular typing of *Brucella* isolated from various tissues. Up to the end of 2007, we have examined 150 *Brucella* isolations from eight species of cetacea, three species of pinniped and a single European otter (*Lutra lutra*) found around our coast, mainly from Scotland and Southwest England waters. We have tested over 2800 serological samples, both captive and wild animals, by three different ELISA techniques. The serological samples comprise nine species of pinniped, 17 species of cetacea and European otters making a total of 24% of the mammals tested resulting as sero-positive to one or more of the ELISA methods.

In addition to the marine mammals, seven marine turtles (two species) have been tested serologically and culturally with no evidence of *Brucella* being found.

This talk summarises brucellosis in marine mammals in British waters and shows the results obtained from work done at the VLA.

Acknowledgments: Geoff Foster SAC Veterinary Services, Inverness, and Nick Davison Veterinary Laboratories Agency, Truro.

Oral Presentation 31

THE DYNAMICS OF PHOCINE DISTEMPER VIRUS EPIZOOTICS IN EUROPEAN HARBOR SEALS (*PHOCA VITULINA*)

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Phocine Distemper Virus caused mass die-offs of European harbor seals (Phoca vitulina) in 1988 and 2002. An interesting feature of these outbreaks is large variation in seal mortalities among different regions. The Dutch, German, Danish, and Swedish populations experienced much higher mortalities (50 60 %) than populations of England, Scotland and Ireland (10 20 %). Differences in mortalities have been linked to pollution (e.g. PCBs), and to genetic differentiation of harbor seals. The combination of the seasonal behavior of seals and the timing of the virus introduction alone can explain the large differences in mortality among regions. If the virus is introduced to the population in the winter when the population levels on land are low, there will be a small outbreak and the population will suffer low mortality. A large outbreak is most probable in the summer, before the number of the seals on land peaks.

Oral Presentation 32

HOST SPECIFICITY BASED ON A THREE-DIMENSIONAL STRUCTURE MODEL OF MARINE MAMMAL SIGNALLING LYMPHOCYTE ACTIVATION MOLECULE (SLAM), A RECEPTOR OF MORBILLIVIRUS

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Morbilliviruses (family Paramyxoviridae) cause devastating diseases in humans and animals. Four virus species, measles virus (MV), rinderpest virus (RPV), peste des petits ruminants virus (PPRV), and canine distemper virus (CDV) have been identified in terrestrial mammals. In addition, morbilliviruses were recently reported to cause mass mortalities among marine mammals. Two novel virus species, cetacean morbillivirus (CMV) and phocine distemper virus (PDV) have been isolated from the dead animals. A notable biological feature of morbilliviruses is their high-host specificity. Cellular receptors are one of the major determinants of the host specificity and tissue tropism of viruses. Recently, signalling lymphocyte activation molecule (SLAM) was shown to be a primary cellular receptor for viruses in humans, cows and dogs. In order to understand host-specificities of morbilliviruses of marine mammals, we firstly determined the sequences of SLAM cDNA from two cetaceans: Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) and killer whale (*Orcinus orca*), from two pinnipeds: spotted seal (*Phoca largha*) and walrus (*Odobenus rosmarus*), and from one sirenian: American manatee (*Trichechus manatus*), as well as from Indian elephant (*Elephs maximusn bengalensis*). Furthermore, for understanding of virus-receptor interaction, we generated three-dimensional (3-D) models of killer whale, spotted seal, and manatee using a 3-D structure of NTB-A molecule, a member of SLAM family, as a template. The SLAMs of marine mammal and elephant were found to be composed of 336-339 amino acids. They contained the immunoreceptor tyrosine-based switch motifs (ITSMs) in the intracellular region, which were involved in tyrosine-based signalling, and immunoglobulin-like V and C2 (IgV and IgC2) domains in the intracellular region of the SLAMs. Six b-strands of IgV domain form a front b-sheet, which was considered to be an interface to morbilliviruses. Twenty-one residues with protruding side-chains from the interface were thought to potentially bind the viral protein. Among these twenty-one residues, we found eight residues were different among the three marine mammal species. They seemed to correspond to the morbillivirus species. The eight residue of set appears to determine host-virus specificity and may be useful for risk estimation for morbilliviruses.

Oral Presentation 33

THE “NO VISIBLE LESION” FORM OF *MYCOBACTERIUM BOVIS* INFECTION IN A WIDE RANGE OF WILDLIFE HOSTS

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Bovine tuberculosis (TB) is an important re-emerging zoonotic disease caused by infection with *Mycobacterium bovis*, and affects a wide taxonomic range of hosts. However the propensity to develop severe disease varies among species. For wildlife surveillance applications and diagnostic purposes it is important to understand how infection is manifested in the different species. For example, carnivores in general have an apparent low susceptibility to overt disease and low rate of gross lesions. A form of *M. bovis* infection easily overlooked is the “no visible lesion” (NVL) presentation, i.e. infection in the absence of gross lesions. The purpose of this study was to describe the pathology, frequency and diagnostic implications of NVL TB in wild animals. NVL TB occurred in 30-80% of Eurasian badgers (*Meles meles*) in the UK, in 27.8% of feral ferrets (*Mustela furo*) in New Zealand, and has also been reported with high rates in red foxes

(*Vulpes vulpes*), red deer (*Cervus elaphus*), European wild boar (*Sus scrofa*), and other species. Histopathological examination revealed that most NVL infected individuals had tuberculous lesions, but which were so small that they were not detected at necropsy. Microscopic findings included early lesions as aggregations of macrophages, minute active granulomas, and fibrotic calcified nodules. Mycobacterial culture of pools of lymph nodes and oropharyngeal tonsils provides a sensitive detection of infection in the absence of lesions. In conclusion, high proportions of wildlife populations may be infected by M. *bovis* without showing clinical and pathological manifestations. The true prevalence of tuberculosis in wildlife populations may be underestimated if the NVL rate for the species is high.

Oral Presentation 34

COMPARATIVE DIAGNOSIS OF BOVINE TUBERCULOSIS IN WILD UNGULATES FROM SPAIN

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Bovine tuberculosis (bTB) is a worldwide disease that affects a wide range of domestic and wildlife animals and humans. Wild ungulates from Donana National Park (DNP), Southwest Spain, display high prevalences of bTB: European wild boar (*Sus scrofa*), 52.4%; red deer (*Cervus elaphus*), 27.4%; and fallow deer (*Dama dama*), 18.5%. Here we report the histopathological findings associated to bTB and the agreement of lesions with mycobacterial culture in those species. We sampled 124 European wild boar, 95 red deer, and 100 fallow from April 2006 to April 2007. Detailed necropsies were carried out in deer species, while only the head was available for inspection in wild boar. Head lymph node and tonsil samples from all animals were taken for microbiological culture, and samples of all inspected organs were fixed in 10% neutral buffered formalin and routinely processed for histopathological examination.

In wild boar, *Mycobacterium bovis* was isolated more frequently from the mandibular lymph nodes (43 cases) than from the tonsils (31 cases), in 9 cases the agent was only detected in tonsils. Therefore, missing this sample would have significantly reduced the prevalence estimate. In red and fallow deer, M. *bovis* was detected in 19 and 7 cases in the retropharyngeal lymph nodes and in 11 and 5 cases in the tonsils, respectively. Only once the agent was detected exclusively in the tonsil in red deer, and only twice in fallow deer.

Macroscopic TB-compatible lesions (MTB-c) were detected in 63.6% wild boar, 32.3% red deer, and 20.3% fallow deer. Microscopic TB-compatible lesions (mTB-c) but not MTB-c were observed in one third of the culture-positive deer. Generalised TB was frequent in both deer species (5 of 17 red deer and 4 of 9 fallow deer). Six of 43 culture positive wild boar showed no macroscopic lesions, but had microscopic ones.

The kappa agreement coefficient between the isolation of M. *bovis* and the detection of MTB-c mTB-c respectively were 0.22 and 0.39 for wild boar, 0.62 and 0.57 for red deer, and 0.58 and 0.61 for fallow deer. The largest disagreement between lesion detection and microbiology occured in the group of “MTB-c positive culture negative” wild boar.

Our results evidenced that pathology and microbiology need to be combined in detailed studies on wild ungulate bTB. Sampling head lymph nodes and not the tonsils may lead to an underestimation of true bTB prevalence.

Oral Presentation 35

EXPERIMENTAL INFECTION OF EUROPEAN WILD BOAR (*SUS SCROFA*) WITH *MYCOBACTERIUM AVIUM AVIUM* AND *MYCOBACTERIUM BOVIS*

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Mycobacterial diseases are re-emerging worldwide. Among other wildlife, the European wild boar (*Sus scrofa*) is increasingly relevant as a host for several pathogenic mycobacteria. In the Mediterranean habitats of central and southern Spain, characterized by an artificial wildlife management and overabundant wild ungulate populations, the wild boar is considered a reservoir for bovine tuberculosis. The aim of this study was to develop an infection procedure for the study of *Mycobacterium avium avium* and *Mycobacterium bovis* infection in wild boar and for future challenge experiments in vaccination trials. We performed three experiments, two with M. *avium* and one with M. *bovis*. In the first experiment, carried out in summer 2007, wild boar were anaesthetised and 5 ml of mycobacterial suspension were administered by the tonsilar route. Three concentrations of M. *avium* suspension were used; 1E+02 colony forming units (cfu), 1E+04 cfu, and 1E+06 cfu. Two 3-4 month old wild boar were used for each dose. In the second experiment, carried out in autumn 2007, 10 ml of mycobacterial suspension containing 2.5E+08 cfu were administered to two 4 month old wild boars each by the tonsilar and the nasal routes, respectively. All wild boars were euthanized 15 weeks post infection (pi) and at the post mortem examination a wide range of tissues were examined for gross and histopathological lesions of tuberculosis and cultured for mycobacteriae. None of the wild boar of experiment 1 showed any visible or microscopic TB-compatible lesions. However, M. *avium* was isolated from the mandibular lymph node of one of the wild boar. Experiment 2, using a higher dose and two infection routes (tonsilar vs. nasal) yielded more gross and histopathological lesions of tuberculosis at post mortem examination. Both wild boar inoculated by the tonsilar route had TB compatible visible and microscopic lesions in the tonsils and in both mandibular lymph nodes. Interestingly, only one wild boar inoculated by the nasal route displayed visible lesions, and these were only found in the left bronchial lymph node, but not in the mandibular ones nor in the tonsil. As in previous analysis of M. *bovis* infected wild boar, RT-PCR data revealed that three genes, complement component C3, IFN-gamma and RANTES were down regulated in infected animals ($P<0.05$). Hence, we decided to use only the tonsilar route for the M. *bovis* infection experiments. The 6 wild boar inoculated with M. *bovis* are scheduled for necropsy in August and results will be reported at the EWDA meeting in October.

Oral Presentation 36

PROGRESS TOWARDS LICENSED TUBERCULOSIS VACCINES FOR EURASIAN BADGERS (*MELES MELES*)

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Increased incidence of bovine tuberculosis (TB) in the United Kingdom caused by infection with *Mycobacterium bovis* is a cause of considerable economic loss to farmers and Government. The Eurasian badger (*Meles meles*) represents a wildlife source of recurrent M. *bovis* infection to cattle in the UK and their vaccination against TB with M. *bovis* Bacille Calmette-Guérin (BCG) is an attractive disease control option. Longer term, delivery of BCG in oral bait holds the best prospect for vaccinating badgers over a wide geographical area but is technically challenging with no guarantee of a successful outcome. In the more immediate future it should be possible to license the use of BCG for intramuscular inoculation, which could be a practical means of delivery for vaccination on a smaller scale. Use of parenteral BCG vaccine in this way would provide crucial data to gauge the requirements for and likely success of the oral vaccine. In order to license intramuscular BCG vaccine for badgers we have conducted safety studies on captive and free-ranging badgers and demonstrated efficacy against experimental pulmonary infection with M. *bovis*. Evaluation of the efficacy of the vaccine in a field setting is currently ongoing. A review of the studies using BCG in badgers will be given, along with details on progress towards the development of an oral formulation of BCG and identification of suitable bait for its delivery to badgers.

Oral Presentation 37

EWDA Best Student Presentation Award applicant

DIAGNOSING BOVINE TUBERCULOSIS IN LIVE-SAMPLED WILD ANIMALS: JUST HOW ACCURATE ARE THE TESTS?

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Accurate diagnosis of *Mycobacterium bovis* infection (bovine tuberculosis: bTB) in live animals is notoriously problematic due to variations in disease pathogenesis, prolonged incubation periods and intermittent shedding of mycobacteria. Serological-based assays for the detection of bTB present potential advantages over mycobacterial culture in terms of logistics, cost and ease of use. However, the successful application of these tests is limited by delayed seroconversion, cross-reactivity with environmental mycobacteria and an inherent trade-off between test sensitivity and specificity. The aim of the present study was to evaluate the diagnostic potential of two new serological tests (multiple antigen print immunoassay (Mapia) and a lateral flow immunoassay rapid test) in comparison with mycobacterial culture of tracheal washes for determining M. *bovis* infection status in a population of wild meerkats (*Suricata suricatta*). Individual meerkats were sampled up to eight times under anaesthesia every three months during a longitudinal study conducted over 30 months. Diagnostic accuracy was determined through Bayesian and Maximum Likelihood estimations of sensitivity, specificity and likelihood ratios for each diagnostic test when used independently and in parallel, to classify the disease status of individual meerkats in the absence of a gold standard reference test. Culture of tracheal washes was highly specific but of low sensitivity for diagnosing individuals shedding M. *bovis*. The longitudinal nature of the study with repeated sampling of the same individuals served to simultaneously improve the likelihood of detection of infection and increase confidence in a negative result in individuals repeatedly tested negative. Whilst the rapid test and Mapia were individually of limited diagnostic use, interpreting the results of these two tests in parallel produced estimates of sensitivity and specificity that were high enough to usefully inform decision-making when determining exposure to bTB in wild meerkats and potentially other species in which bTB poses a diagnostic challenge.

Oral Presentation 38

EWDA Best Student Presentation Award applicant

PREVALENCE OF *MYCOBACTERIUM AVIUM PARATUBERCULOSIS* IN WILD RUMINANTS (*CERVUS ELAPHUS, DAMA DAMA,* AND *SUS SCROFA*) FROM DONANA NATIONAL PARK

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Paratuberculosis (Johne's disease) is a chronic infectious disease of ruminants and other mammals caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). It is one of the most important diseases in domestic ruminants today. Its distribution is worldwide and the disease is causing severe financial losses among cattle producers. We studied the particular situation in Donana National Park (DNP), in southern Spain. There are three continuous areas with a different ecology: Coto del Rey (CR) in the north has high wild ungulate densities and no cattle; the Biological Reserve (BR) in the centre has both high wild ungulate and high cattle densities; and Marismillas (MA) in the south has less cattle and low wild ungulate densities. Red deer (*Cervus elaphus*) is the predominant ungulate species, followed by fallow deer (*Dama dama*) and Euroepan wild boar (*Sus scrofa*). We studied 95 red deer, 101 fallow deer and 125 wild boar shot by DNP keepers during 2006 and 2007 in a survey on bovine tuberculosis. Serum samples, tissue samples of the mesenteric lymphnodes and ileocaecal valve, and faeces were fozen for MAP detection by ELISA and PCR, respectively. Fresh faecal samples were also used for parasitological studies. Direct IS900 PCR was performed on tissues, while real time PCR was used on the faecal samples. MAP tested positive for 42.6% (32.6-52.6) of red deer, 65.1% (55.1-75.1) of fallow deer and none of the wild boar. However, 25.7% (17.6-33.8) of the wild boar tested positive in the ELISA. The inter-species differences in MAP PCR prevalence were significant. Our results also revealed a North-South gradient (Y coordinates) for red deer, the predominant species across the study area, whereas no spatial pattern was evidenced for fallow deer. Higher prevalence in fallow deer may be the result of higher spatial aggregation of this species. The MAP prevalence pattern among deer was independent of cattle distribution in DNP, suggesting that deer species are able to maintain MAP in DNP.

Oral Presentation 39

MANAGING TB IN BADGERS (*MELES MELES*): A LESSON IN THE ROLE OF ECOLOGICAL COMPLEXITY IN THE TRANSMISSION OF DISEASE BETWEEN WILDLIFE AND DOMESTIC ANIMALS

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The development of sustainable approaches to the management of diseases transmitted between domestic animals and wildlife is a major global challenge. Success in this endeavour will require advances in our understanding of the impact of management interventions on wildlife populations. There is a growing body of evidence on the potentially profound effects of demographic processes, social structure and behaviour of wildlife populations on disease dynamics and management outcomes. In the UK Eurasian badgers (*Meles meles*) are implicated as a source of bovine tuberculosis (bTB) infection in cattle. Consequently, badgers have been culled under a variety of strategies in the past, whilst infection in cattle has persisted. The results of several observational field studies of badger behaviour and a large-scale field experiment suggest that the disturbance of badger population structure and behaviour caused by culling may in some cases have counter-productive epidemiological consequences. Such effects may be expected in other wildlife populations where disease control interventions could elicit similar behavioural responses. Alternative options for the management of bTB transmission to cattle include vaccinating badgers, and using changes to cattle husbandry practices to minimise interactions.

Oral Presentation 40

VETERINARY RISK ASSESSMENT - LIKELIHOOD AND IMPACTS OF TRANSMISSION OF SELECTED INFECTIOUS DISEASES BETWEEN FREE-RANGING WILD BOAR *(SUS SCROFA)* , HUMANS AND DOMESTIC LIVESTOCK IN ENGLAND

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The wild boar became extinct in the United Kingdom in the 17th Century. Through subsequent re-introductions and escapes from farms, small populations totalling 500 - 1,000 animals have become established in southern England. Department of Environment, Food and Rural Affairs (Defra) has therefore had to develop a suitable management policy for these animals balancing the potential benefits of re-introducing a previously native species and therefore increasing UK biodiversity and the potential negative impacts on forest habitats, agricultural damage, public safety and road traffic accidents. One of the most significant concerns relate to the potential impacts on infectious disease transmission and control.

In order to develop a scientific evidence base for policy development a series of risk assessments for the impacts of wild boar were developed. One of these was qualitative veterinary risk assessment for a range of infectious diseases.

Wild boars are susceptible to the same diseases as domestic pigs and therefore have the potential to impact on infectious disease epidemiology and control. Diseases in wild boar have been studied across the world with focus on the economically important diseases such as Classical Swine Fever (CSF), Foot and Mouth Disease Virus (FMDV) and Aujesky's Disease. Wild boar management and disease control is common across Europe however this information cannot be directly transferred to the UK.

The risk assessment considers four key scenarios in which wild boar may influence infectious disease epidemiology:

- Incursion of exotic diseases directly into the free-ranging wild boar population.
- Impact on effective diseases control following transmission of exotic disease to wild boar following incursion into domestic livestock.
- Impact on disease management of endemic diseases common to wild boar and domestic livestock.
- Zoonotic disease risk. (Risk of disease transmission to humans from infected wild boar or wild boar products.

The findings of the risk assessment will be presented and the recommendations made to policy makers discussed.

Oral Presentation 41

BRUCELLOSIS IN WAPITI (*CERVUS ELAPHUS*) AND BISON (*BISON BISON*) IN THE UNITED STATES: A CLASSIC WILDLIFE-HUMAN-LIVESTOCK PROBLEM

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Brucellosis is a zoonotic disease caused by the bacterium, *Brucella abortus*, that is primarily characterized by first-term abortions. For more than 70 years, federal and state governments have tried to eradicate brucellosis in the United States, spending over US$3.5 billion dollars in the process. These efforts have been largely successful for domestic cattle. However, brucellosis still exists in wapiti (*Cervus elaphus*) and bison (*Bison bison*) populations in the Greater Yellowstone Area (GYA) in the western United States. Brucellosis was probably introduced into wild populations as a result of introducing infected bison into the ecosystem in the early 20th century. Efforts to prevent cattle from contracting brucellosis from wildlife have included feedgrounds, vaccination, habitat improvement, and prevention of commingling between cattle and wildlife. Despite these efforts, cattle herds in Wyoming and Idaho were recently diagnosed with brucellosis, most likely from contact with infected wapiti or bison. These cattle outbreaks have resulted in the loss of the states' "brucellosis-free" status, resulting in restrictions on cattle movement and economic penalties. Other management actions that could be considered include: (1) test and slaughter; (2) shutting down elk feedgrounds to reduce disease transmission; (3) removing cattle from public lands; (4) developing and implementing more efficacious brucellosis vaccines for elk and bison; (5) managing cattle through vaccination and physical separation from elk and bison; and (6) using contraceptives to decrease pregnancies and, thus, abortions. Regardless of what, if any, actions are employed, the public will probably have to accept fewer elk numbers which will mean less hunting and less opportunities for viewing wildlife. All solutions will take decades to implement and become effective. Brucellosis in wildlife represents a classic example of the wildlife-human-livestock interface and conflict resolution.

Oral Presentation 42

EVALUATING RISKS ASSOCIATED WITH THE TRANSMISSION OF BOVINE TUBERCULOSIS FROM WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) TO CATTLE IN MICHIGAN, USA: PRELIMINARY RESULTS FROM YEAR ONE

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Bovine tuberculosis (bTB) is a contagious disease caused by the bacterium *Mycobacterium bovis* and may infect humans, wildlife and livestock. While once common in US cattle, the disease has historically been rare in wildlife. In 1994 bTB was found to be endemic in free ranging white tailed deer (*Odocoileus virginianus*) populations in Michigan's Northeastern Lower Peninsula (NELP) and evidence suggests transmission to cattle. A key component to transmission of bTB between deer and livestock appears to be shared resources. To evaluate the extent to which deer and livestock share resources, and thus the risk of bTB transmission, we tracked deer movements on 4 beef cattle farms in the NELP. In addition, we collected data on farming practices employed at study sites and nearby farms frequented by deer as well as land cover data. Between January and March 2007 and 2008 we fitted 27 adult does with GPS collars programmed to record locations every 2 hours for one year. To date 11 collars from 2007 have been recovered, with a total of over 36,000 data points. Analysis is underway to describe co-use of pasture by deer and cattle, and proximity of deer habitat use to stored cattle feed. Preliminary gross analysis suggest up to 30% of recorded deer locations are in areas of cattle use (feeding sites, grazing pastures, water sources, etc), cultivated crops and hay fields. Of these locations, 38% took place in spring, with an additional 21% in the fall. However, use varied by land type. Deer use of areas classified as "cattle use" peaked in the spring, with 46% of use occurring in March, April and May. Similar trends occur for deer use of farm yards (32%), hay storage areas (73%) and hay fields (42%). Higher deer use of cattle feeding areas and pastures in the spring runs counterintuitive to the notion that use of such areas would peak during winter, when resources are scarce. We hope to use the final results to recommend mitigating measures for livestock owners and government agencies responsible for bTB management to reduce the risk of exposing livestock to bTB.

Oral Presentation 43

SUSPECTED SPILL-OVER INFECTIONS WITH GARDEN BIRD ASSOCIATED SALMONELLAE TO OTHER SPECIES

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The VLA Diseases of Wildlife Scheme (VLA DoWS) was set up in 1998 with the Department of Environment and Rural Affairs (Defra) to provide surveillance for disease in wild vertebrates in England and Wales. Salmonella surveillance was targeted in wild birds and mammals by selective culture of intestinal content from all wildlife submissions. Results from VLADoWS, and elsewhere, indicate that certain *Salmonella* Typhimurium DTs (determinative types) are probably host adapted to garden birds in the UK. Infection with these salmonella DTs is frequent in these groups of birds with transmission occurring most frequently at garden bird feeding sites. We present evidence from other VLA projects together with Health Protection Agency data, to show that infection of other wild animals, farm animals, pets and humans occurs each year in the UK and is probably the result of spill-over of salmonella infection from these garden birds. Evidence of infection in a wide range of other species is widespread and reflects the frequency of infection in wild birds and the frequency of exposure through environmental contamination by the birds, however associated disease in other species currently appears to be infrequent.

Oral Presentation 44

DEVELOPING THE METHODOLOGY FOR DISEASE RISK ANALYSIS FOR TRANSLOCATIONS UNDERTAKEN FOR BIODIVERSITY CONSERVATION

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The anthropogenic destruction of wild animal habitat concomitant with an ever increasing human population is leading to an unprecedented number of extinctions. The human response to this has included a burgeoning of translocation (reintroduction and restocking) programmes for animal conservation. There are disease risks in undertaking these translocations because the ensuing changes in host-parasite interactions may lead to catastrophic epidemic disease and threaten both the translocated animals and the recipient population/s. The rinderpest pandemic in Africa which followed the introduction of cattle harbouring the rinderpest virus in the late 19th century, and has caused significant long-term changes to the ecosystem, is a good example of this phenomenon.

There is a compelling need to assess the disease risks of translocations, and modify translocation protocols, through the process of disease risk analysis (DRA). DRAs were first conducted for wild animal translocations in the 1980s, techniques have since been improved but further advances are required. The World Organization for Animal Health (OIE) published an advanced methodology for disease risk analysis for *domestic* animal imports in 2004 which we have used as a framework for disease risk analysis for wild animal translocations. However, there are important differences in conducting DRA for biodiversity conservation, namely, (i) destination hazards (infectious agents present in the recipient populations) may be of equal concern to source hazards (infectious agents harboured by the translocated animals), (ii) the identity and pathogenicity of the parasites of many wild animals is barely known, and (iii) consideration must be given to conserving native parasites for the maintenance of biodiversity.

Translocations have been undertaken frequently as a conservation tool for Natural England's Species Recovery Programme. A qualitative DRA has been undertaken prior to each translocation commenced since 1999. For example, in carrying out hazard identification for a field cricket (*Gryllus campestris*) reintroduction, a potentially alien eugregarine parasite was identified and infected crickets were excluded from the reintroduction. Disease risk assessment undertaken for the translocation of pool frogs (*Rana lessonae*) from Sweden to England identified the chytrid fungus *Batrachochytrium dendrobatidis* as the chief concern. Risk management for the cirl bunting (*Emberiza cirlus*) reintroduction dictated a need to change the location for the rearing of these birds from one close to exotic passerines in a zoological collection to an alternative site close to the reintroduction setting.

In refining DRA methods for wild animal translocations and developing a consistent approach we can safeguard biodiversity and improve conservation outcomes.

Oral Presentation 45

LJUNGAN VIRUS (A POSSIBLE CAUSE OF SEVERAL HUMAN HEALTH CONDITIONS) FOUND IN BANK VOLES (*MYODES GLAREOLUS*) AND YELLOW-NECKED MICE (*APODEMUS FLAVICOLLIS*) FOR THE FIRST TIME IN NORTHERN ITALY

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The Ljungan (picorna) virus (LV) was first isolated in 1998 from Swedish bank voles (*Myodes glareolus*). After clinically recognizable type-1 diabetes-like symptoms were noted in this species in Denmark. LV has subsequently been shown to induce type-2-like diabetes, as well as uterine resorptions, malformations, and neonatal death in CD-1 laboratory house mice, and has been found in wild voles in the USA, as well as in lemmings and laboratory rats. Intriguingly, the incidence of type-1 diabetes, Guillain-Barré syndrome and myocarditis in the human population is correlated with rodent population cycles in Sweden. In addition, LV has been associated with intrauterine fetal death in Sweden, and a preliminary study showed that children newly diagnosed with type-1 diabetes have significantly increased levels of LV antibodies compared to controls. It has been hypothesized that the bank vole as well as other small rodents could act as reservoirs and/or vectors of LV that may be a zoonotic agent several human diseases or pathologies, including economically important type-1 diabetes. Consequently, knowledge of the distribution LV among wild and domestic mammal species is crucial to assess its potential importance as a human pathogen, identify possible zoonotic sources of the virus and lay groundwork for possible vaccine development. Here we provide a review of the current understanding of the ecopathology of LV and present the first results from southern Europe. Using Real Time RT PCR, LV was confirmed in 50% (10/20) of bank voles and in 10% (2/20) of yellow-necked mice (*Apodemus flavicollis*) collected from an alpine meadow in northern Italy during 2006. LV-positive animals included males and females, adults and subadults. This is the first time LV has been reported in Italy and in yellow-necked mice. These results significantly increase the geographical and species range of LV. We believe the global distribution of this picornavirus, and its role as a zoonotic agent, deserve further attention.

Oral Presentation 46

INVASIVE SPECIES-ASSOCIATED EMERGING DISEASES IN WATER BIRDS IN NORTH AMERICA (BOTULISM TYPE E AND TREMATODIASIS)

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Non-indigenous invasive species are an increasing problem in many North American ecosystems, including in the Great Lakes region. Besides the direct threats these species can pose when they alter ecosystems, invasive species can also contribute to significant wildlife disease outbreaks. Recently identified examples of this phenomenon include the re-emergence of botulism type E as a cause of water bird mortality and the emergence of trematodes as a significant mortality factor for duck and other waterfowl species. Type E botulism has been implicated in water bird die-offs on the Great Lakes since the 1960's and has been responsible for the deaths of thousands of birds including common loons (*Gavia immer)*, Laridae species, and migratory waterfowl. In the last decade, these mortality events have been increasing in frequency, scale and scope; amongst other ecologic factors, this re-emergence has been related to the proliferation of non-indigenous Dreissenid mussels and fish (*Neogobius melanostomus)*. Large-scale mortality events for American coot (*Fulica americana)* and lesser scaup (*Aythya affinis*) from infection with trematodes (*Sphaeridiotrema globulus*, *Cyathocotyle bushiensis*, *Leyogonimus polyoon)* have been documented over the last decade during spring and fall migrations on the Mississippi River and in a few other lakes in North America. This emerging parasitic disease problem is associated with a non-indigenous aquatic snail (*Bithnyia tentaculata*), which is an intermediate host for the disease-causing trematodes. Trematodiasis continues to cause significant waterfowl mortality, with thousands of ducks dying every year, and appears to be spreading. Attempts to manage trematodiasis and botulism type E, by control of the aquatic invasive species, have not been successful.

Oral Presentation 47

LEAD INTOXICATION IN WHITE-TAILED SEA EAGLES (*HALIAEETUS ALBICIALLA*) A JOINT PROJECT ON THE CAUSES AND APPROACHES TO SOLUTIONS IN GERMANY

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The goals of our joint project are to identify the causes of oral lead intoxications of the white-tailed sea eagle (WTSE) as an umbrella species for other scavenging birds and to test potential solutions. In this dialogue-oriented and communicative project the involved stakeholders from hunting organisations, ammunition industry, ammunition dealers, foresters, and nature conservationists are integrated and informed on a regular basis. In the natural scientific part the causes of lead intoxication are elucidated by investigating the sources of lead poisoning, the feeding behaviour of the WTSE, and the toxicity of lead in an avian model. In addition the home-range size and habitat use and consequences on population level will be computed.

In the social scientific part the information use of the stakeholders and attitudes of hunters as well as conflicts between social actors will be analysed. This knowledge is quickly transferred to the relevant stakeholders. The social scientists also mediate between the different parties involved.

Previously, post mortem examinations of more than 391 WTSEs from Germany performed at the IZW revealed that lead intoxications are the most important cause of death (23% of mortality).

In this study we identified the potential sources of lead intoxications for WTSEs being waterfowl such as geese and carcasses of game animals or their remains (gut piles) shot with lead containing bullets.

To test the toxicity of bullet metals (Pb, Zn, Cu) feeding experiments on ducks were performed showing that the highest bioavailability and organ accumulation was found for lead in livers, kidneys and brain tissue of Peking ducks.

We examined if lead-free bullets perform as well as lead containing bullets concerning hunting/ killing efficiency. Expanding bullets made of copper or its alloys represent a possibility of harvesting game that is not contaminated with bullet remains and therefore pose no risk of intoxications to humans and wildlife.

Our results together with the field tests performed by pioneer hunters using lead-free ammunition show that the use of lead-free ammunition is possible in hunting practice. The process of reducing lead intoxications in wildlife by changing to lead-free ammunition among hunters greatly depends on the involvement of all relevant stakeholders and a broad information campaign which we tried to realize by producing a leaflet, an internet page (www.seeadlerforschung.de) and organizing several workshops. So far lead-free bullets are used for hunting in two large associations, and eight forestry districts in four federal states and in one National Park in Germany.

Oral Presentation 48

ENVIRONMENTAL TOXINS: A THREAT TO REINTRODUCED RED KITES (*MILVUS MILVUS*) IN ENGLAND (1989-2007)

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The red kite (*Milvus milvus*) (Falconiformes: Accipitridae) was formerly common throughout the UK, but became extinct in England by the end of the 1880s due to persistent persecution. In response, the RSPB and Natural England commenced a project in 1989 to reintroduce the red kite to England, for which the Zoological Society of London has provided health surveillance.

During pre- and post-release health surveillance, 163 red kite carcases (146 free-living birds were found dead and 17 died in captivity) were examined *post mortem* in order to evaluate the disease risks to kites that arose from the reintroduction programme.

Non-infectious disease was the most common pathological finding (n=93, 57%), of which trauma, mostly due to probable involvement in car or train collisions, occurred in 29 birds (18%). Extensive haemorrhage without evidence of trauma was present in 21 birds (13%) and anticoagulant rodenticides, such as bromadiolone, difenacoum and/or brodifacoum, were detected in tissue samples from 10 of these birds (12 birds examined). Twenty red kites (12%) in good to very good body condition that had just eaten and in which there was no gross evidence of disease were believed to have died suddenly. Tissues of 12 of these birds were examined for a range of toxic substances and mevinphos, alpha-chloralose, carbofuran, aldicarb or strychnine was detected in all 12 cases (100% of the birds examined). Liver lead levels of more than 15 mg/kg dry weight, compatible with fatal lead poisoning, were found in six of 44 (14%) red kites examined and the source of lead was confirmed to be ingested lead shot. Pathological lesions consistent with electrocution (n=8) and the presence of lead pellets in tissues (evidence of shooting incidents) (n=7) were other common pathological findings. Metabolic bone disease was seen in five red kites.

These findings show that environmental toxins represent a threat to free-living red kites in England. Anticoagulant rodenticide poisoning is most likely secondary to the consumption of poisoned rodents and could be prevented in some cases through regular removal of dead rodents followed by burning and burying of the bodies. Red kites were also victims of baits laced with pesticides and, although these baits may have been laid to target other species, measures to reduce pesticide abuse are warranted. And finally, the ingestion and accumulation of harmful lead in red kites could be reduced by the use of alternatives to lead shot for the killing of terrestrial mammals and birds. Despite these threats, the population of red kites in England continues to rise.

Oral Presentation 49

TREATMENT OF CHYTRID (*BATRACHOCHYTRIUM DENDROBATIDIS*) INFECTION IN FROGS AT PERTH ZOO - FOUR CASE REPORTS

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Chytridiomycosis is now recognised as a significant threat to both wild and captive frog populations around the world. In Australia, the disease has been reported in wild frogs from eastern, southern and south-western Australia, and has affected species such as the Eungella day frog (*Taudactylus eungellensis*), the Stoney Creek frog (*Litoria wilcoxi*) and the Western green and gold bell frog (*L. moorei*) (Dazsak *et al,* 1999; Retallick *et al*, 2004).

Various methods of treatment for chytrid infection have been attempted, including use of formalin/malachite green mixtures (Parker *et al,* 2002), elevated body temperatures (Woodhams *et al,* 2008), and oral terbinafine (McMeekin *et al*, 2007); however probably the most recognised and used method of treatment is the use of 0.01% itraconazole baths, as described by Nichols and Lamirande (2001).

This presentation will discuss four case studies where 0.01% itraconazole baths were used to treat cutaneous chytrid infections in four different species of frogs found in the south-west of Western Australia (namely *L. moorei, L. adelaidensis, Geocrinia vitellina* and *Spicospina flammocaerulea*). Evidence of infection was determined using Taqman PCR assays (Boyle *et al*, 2004), and affected frogs treated using daily 0.01% itraconazole baths for a period of 8-10 days. Monitoring for evidence of infection continued during and after treatment in most cases using Taqman PCR. The following conclusions are able to be drawn from the experiences of these case studies:

- These cases expand the list of frog species currently reported as having been affected by chytrid fungus, and demonstrate that this disease poses a real risk for wild frog populations in south-western Australia, including for the threatened species *Geocrinia vitellina* and *Spicospina flammocaerulea.*
- Itraconazole baths were successful in clearing cutaneous chytrid infections in these four species, and surviving treated frogs are able to maintain a negative chytrid status subsequent to treatment.
- Regular substrate changes during the period of treatment is an important adjunct to the success of itraconazole baths in clearing chytrid infection.
- A number of deaths occurred during the treatment course for two species (*L. adelaidensis* and *G. vitellina*), however the cause of these deaths appears variable and it is unclear whether there is a risk of toxicity from itraconazole.

Abstracts

Poster Presentations

Poster Presentations

Friday, 3 October & Saturday, 4 October

Poster Presentation 1

HYPERTROPHIC OSTEOPATHY IN A ROE DEER (*CAPREOLUS CAPREOLUS*) IN SWEDEN

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This report presents the first diagnosed case of hypertrophic osteopathy (HO) in a roe deer (*Capreolus capreolus*) in Sweden. An aged (> 8 year-old) male roe deer was observed wandering in a private garden in Rosersberg in Uppland, Sweden, and was euthanized with a gun shot as it appeared ill. The carcass was submitted to the National Veterinary Institute (SVA) in Uppsala, for necropsy at the department of wildlife diseases. The roe deer was emaciated and there was marked dental attrition. There was irregular thickening of all four limbs, especially obvious around the fetlock and pastern joints. The macerated skeletal specimens showed irregular periosteal bony proliferation with perpendicular growing confluent osteophytes on the cortical surfaces of all bones of the four limbs. The lesions were more severe in the distal bones, and extended from the phalanges to the scapulae on forelimbs and to the femurs on hindlimbs. The other important lesion present was suppurative and atelectic changes in most of the lung tissue, diagnosed at histopathology as chronic pneumonia and acute diffuse extensive suppurative bronchopneumonia. *Pasteurella multocida* was isolated from lung and spleen tissue. This report describes a typical presentation of HO, with a significant primary lung lesion and the accompanying secondary bony periosteal proliferation on the bones of the limbs. Previous reports or descriptions of HO in roe deer have, so far, been found from Germany (one case), Switzerland (one case), and Austria (four cases). This case and the few reported described single cases of HO in roe deer and in other various wildlife species seem to follow the pattern of the HO disease syndrome described in domestic species and in humans.

Poster Presentation 2

EWDA Best Student Presentation Award applicant

INFECTION WITH *TAENIA* SPP. IN RODENTS IN DANISH WOODLANDS

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Taenia spp. infection in wildlife develops into hydatid cysts in various tissues of mammal intermediate hosts. Little is known about the ecology of *Taenia* spp. infection in woodland rodents. One reason for this is the lack of morphological characteristics in cysts of different *Taenia* spp. It is recognized that the dynamics of *Taenia* infection in woodland rodents is complex and involves different factors that determine the spatial distribution of the disease. The presence of *Taenia* spp. infection in rodents in areas of active public attendance may raise question about risk of spread of similar cestodes e.g. *Echinococcus multilocularis*. It is important to have a differential diagnosis of cysts of *Taenia* spp. and the potentially lethal *E. multilocularis*.

Rodents of several species were trapped in 8 woodlands in and around Copenhagen greater area. Three types of live-traps were used in grids of 5 by 10 trapping stations. Cysts or white spots in the liver and/or viscera were verified as *Taenia* spp. by PCR.

Seven species of rodents were caught. The species composite varied depending on season of the year which also influenced age composition of the individual species. Yellow-necked mice were more predominant at winter and spring while Bank voles were more captured at summer and autumn. *Taenia* spp. were detected in Bank voles (19/296 n/total), Yellow-necked mice (9/91) and Field voles (4/14). The infection was highly prevalent in adult rodents trapped in winter and spring. The majority of infected Bank voles were found in areas of high rodent trapping rate, in contrast to Yellow-necked mice. *Taenia* spp. infection varied in woodlands from 0-14 infected individuals/locality. No infection with *Taenia* spp. was recorded in any of the Water shrews, Common shrews and Pygmy shrews (n=31 totally) nor Wood mouse (n=1). The infection rate was more frequently observed in woodlands more distant to rural areas.

This study shows that the life cycle of *Taenia* spp. is established in rodents in woodlands in and around Copenhagen greater area. PCR is a good alternative for the less specific morphologic identification of *Taenia* spp. in wildlife, which facilitates studies into the ecology and epidemiology of wildlife diseases.

Poster Presentation 3

DYNAMICS OF AN OUTBREAK OF INFECTIOUS KERATOCONJUNCTIVITIS (*MYCOPLASMA CONJUNCTIVAE*) IN PYRENEAN CHAMOIS (*RUPICAPRA PYRENAICA*) IN ARAGON, SPAIN: PRELIMINARY DATA

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The infectious keratoconjunctivitis by *Mycoplasma conjunctivae* has been described in wild ruminants, affecting the Alpine ibex (*Capra ibex*), Alpine chamois (*Rupicapra rupicapra*) and the mouflon (*Ovis aries*). The disease is characterized by inflammation of the conjunctiva and cornea, with severe cicatricial lesions on the cornea. The appearance of this disease in wild ruminants is epidemic and usually courses with high morbidity and mortality. Recently, one prolonged outbreak of keratoconjunctivitis in Pyrenean chamois was observed in the Central and Western Pyrenees, affecting this species in Aragon, were about 15,000 chamois live in 16 different mountain massifs, and are part of a demographic and sanitary monitoring program since 1997. The first cases observed were in the spring of 2006 in the Monte Perdido massif. Afterwards, the outbreak spread to the E to Liena massif until February 2007. In the early spring of 2007, more cases of keratoconjunctivitis were detected in the Vinamala massif W from Monte Perdido, until February 2008. The disease was progressed to the W, affecting other massifs (Anayet in August 2007; Visaurin and Pena-Forca during 2008 spring) where the disease is still present. In parallel, sick animals appeared in the E massifs: Posets massif at the end of August 2007, where the outbreak is still active, and Maladeta massif in October 2007.

In 2008, from W to E, affected animals have been observed in the borders of its spread: Pena-Forca, Visaurin, Anayet, Posets and Maladeta massifs, leaving the central massifs with no recent cases (Monte Perdido, Vinamala and Liena). The counting of dead animals showed that for the whole area in 2006 (when the outbreak started) rangers found 31 dead chamois and 220 in 2007, 7 times more.

The disease affects, kids, yearlings and adults of both sexes. We performed the necropsy of 52 Pyrenean chamois (2 hunted, 15 dead and 35 found alive) and 21 heads were also examined. All animals had ocular changes involving one or both eyes. General lesions were conjunctivitis, ocular discharge and severe keratitis. In more cases purulent keratitis with opacity and perforation of the cornea were observed.

DNA was extracted from the conjunctiva tissue of 20 affected animals and PCR to detect mycoplasmas was realized. The presence of *Mycoplasma* spp. was demonstrated in 19 chamois and *M. conjunctivae* was finally identified in 14 of them.

The disease is still present and the regular censuses performed in spring and autumn will allow quantifying the level of mortality of different populations.

Poster Presentation 4

VIRUS OR PARASITE: WHAT KILLED ROE DEER (*CAPREOLUS CAPREOLUS*) IN FRANCE?

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For more than 10 years, hunters have reported localized Roe Deer mass mortality outbreaks. It remains unclear if the finding is “real” (i.e. mortality increase) or biased (i.e. increase in human awareness regarding deer mortality). Under the first hypothesis, it is a need to clarify the origin of this mortality; then is the question whether this mortality is “additive” (i.e. not density dependent) or not additive (i.e. restricted by a density dependent process).
Yet, only scattered and mostly anecdotic data are available to document this frequently mentioned phenomenon.
During two short term research programs, carried out with the support of the National game and wildlife agency (ONCFS) and by the hunter federations, field data have been collected. The aim of these studies has been mostly to test if struck deer populations expressed more exposure rate to a yet not identified pestivirus, or another infectious agent. A serological examination of blood samples from hunted and living deer has been used in addition to antigen identification on tissue samples.
Two set of data have been available. The first study has been carried out in 2004 on 189 living deer captured in two game reserves, 170 dead shot deer sampled after hunting and some carcasses collected by the SAGIR network. A second study has been carried out in 2005 on 151 blood samples collected from dead animals. Examination for antibodies or antigen has been carried out at successively the veterinary laboratory of “Haut Rhin” and “Savoie”.
Evidence of morbidity was only observed in two animals found dead in the department of “Yonne” in 2004. Antibodies against a pestivirus were observed in 19% of the samples from the reserve of “Trois Fontaine” in 2004. No other evidence of a circulation of a pestivirus has been recorded so far.
Other infectious hypothesis can be considered, but argument in favour of a mortality caused by “macroparasites” should be as well explored. The poster will compare these options; discuss quality of the results and present recommendations for further studies.

This field studies on wildlife was supported by a grant of the "Office National de la Chasse et de la Faune Sauvage" (ONCFS) Nr: 2005 /16/6171, entitled: "Etudes et recherches écologiques et épidémiologiques sur la pathologie de la faune sauvage". We thank the hunters, hunter federations of Haut-Rhin, Jura, Loire, Marne, Nièvre, Oise, Rhône, Yonne, their technicians (namely Adrien Bauer & François Bride), the diagnostic veterinary laboratories of Colmar-Haut Rhin (Dr C. Mansion), Jura (Dr F. Pozet & A. Viry), Loire (Dr Ph. Béal), Rhône (Dr J. Vialard) and Chambéry-Savoie (Mme Y. Game, Dr Le Tallec & P. Revelli), the SAGIR Network (Dr M.E. Terrier) for their support and collaboration.

Poster Presentation 5

DEVELOPMENT OF SEMI-QUANTITATIVE RT-PCR CYTOKINE ASSAYS FOR THE EURASIAN BADGER (*MELES MELES*)

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Information regarding the fundamental immunology of a disease is essential in understanding its epidemiology and in the development of vaccination strategies. In the case of many wildlife species, however, the immunological reagents required to generate these data are lacking. In such cases semi-quantitative RT-PCR techniques may provide a viable alternative. We present data concerning the development of semi-quantitative RT-PCR assays for four cytokines (interleukin-2, interleukin-4, interleukin-10 and interferon-gamma) in the Eurasian badger (*Meles meles*), a wildlife reservoir of bovine tuberculosis in the UK. These assays will be used in future studies to investigate immunological responses in badgers following BCG vaccination and/or exposure to bovine tuberculosis.

Poster Presentation 6

EWDA Best Student Presentation Award applicant

PREPARATION AND CHARACTERIZATION OF BAIT FOR THE ORAL IMMUNIZATION OF THE EUROPEAN WILD BOAR (*SUS SCROFA*)

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The European wild boar (*Sus scrofa*) is extensively distributed in Europe. Overabundant populations may cause agricultural crop damage, negative effects on other wildlife, and sanitary problems. The European wild boar is a reservoir for pathogens such as *Mycobacterium bovis* (bovine tuberculosis), classical swine fever virus (hog cholera) and porcine herpesvirus (pseudorabies) that affect humans or domestic animals. The eradication of these diseases requires the development of control strategies that reduce pathogen transmission among wild boar. One such control strategy is oral vaccination. An effective oral vaccination program in wild boar populations requires the development of baits that can successfully deliver the vaccine to piglets. The aim of this study was to develop new baits for the oral delivery of vaccine preparations to wild boar piglets. Baits were composed of wild boar feed, wheat flour, paraffin, sacarose, and cinnamon-truffle powder attractant with capsules dipped into the matrix to introduce the vaccine formulation. Physical stability studies showed that baits were stable for at least three days at temperatures as high as 42 ºC. Recombinant *Escherichia coli* expressing a membrane-displayed protein were used to test bacterial viability in the baits and the antibody response in orally immunized captive wild boar. The *E. coli* viability was not affected after bait incubation at 25 and 37 ºC for 96 hours. Two field trials were conducted in a hunting estate to determine the species-specific visitation and bait removal rates. In one trial, baits were placed in track stations set up randomly. In the second trial, baits were placed inside selective feeders for wild boar piglets. The results of these experiments support the use of selective feeders for oral delivery of vaccine formulations to 2-4 month-old wild boar piglets, the preferred age for vaccination. Iophenoxic acid (IPA) is an organic biomarker used to investigate baiting strategies. It marks the serum or plasma of animals that consume labelled baits by elevating plasma iodine levels. A trial was conducted in which two different doses of biomarker (5 and 15 mg/kg) were administered to five wild boars each and one animal was used as an untreated control. Samples of blood from each animal were taken periodically to analyze serum samples by high-performance liquid chromatography at days 1, 15, 30 and 60 after IPA ingestion. Iodine levels were detected in all samples but decreased with time. In summary, the results of this study indicate that the baits could be used for the oral immunization of wild boar piglets during any time of year and at least in places where artificial feeders are used feed wild boar.

Poster Presentation 7

FIRST REPORT OF RANAVIRUS IN THE COMMON MIDWIFE TOAD (*ALYTES OBSTETRICANS*) IN SPAIN

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In this report we describe the pathology, isolation and characterization of a novel systemic haemorrhagic ranavirus responsible for a disease outbreak with a high mortality rate affecting tadpoles of the common midwife toad (*Alytes obstetricans*) in the National Park of the "Picos de Europa" in Northern Spain. Macroscopically, systemic haemorrhages were observed, mainly around the eyes and within the skin, gills and internal organs. Microscopic lesions were similar to those described for the systemic haemorrhagic from of ranavirus disease in frogs and other amphibians, consisting primarily of variably sized foci of necrosis throughout most organ systems. Round, intracytoplasmic, basophilic inclusions, consistent with ranaviral inclusions, were present in the skin, liver, kidney and gastrointestinal tract, accompanied by varying degrees of necrosis. Cell nuclei contained condensed chromatin and vacuoles which frequently showed small round viral inclusions commonly located in the periphery. Histological lesions were not observed in the nervous system or in skeletal muscle. Virus was isolated from total homogenates of diseased toad tadpoles following inoculation of epithelioma papilloma cyprini cells. Molecular characterization of the virus, including partial sequence analysis of the DNA polymerase gene, confirmed that the isolated virus was a ranavirus distinct from other members of the genus *Ranavirus* such as tiger frog virus (TFV), frog virus 3 (FV3), Ambystoma tigrinum stebbensi virus (ATSV) and Regina ranavirus (RRV), we have provisionally named the virus common midwife toad virus (CMTV). A rabbit anti-serum raised against purified virions was prepared and used to definitively demonstrate systemic distribution of the virus, indicating that the isolated virus was the primary pathogen. Immunolabelling for CMTV was observed within the skin, liver, kidney and gastrointestinal tract. The antigen labelling in the epidermis and dermis was focal. Hepatocytes and hematopoietic tissue subjacent to the hepatic capsule frequently contained viral inclusions which were commonly associated with areas of necrosis and corresponding immunolabelling was observed in this organ. Inclusions and necrotic cells were observed in renal glomeruli and intense immunolabelling for CMTV was present within these glomeruli.

Poster Presentation 8

SEROPREVALENCE AND IDENTIFICATION OF *BABESIA* SPECIES INFECTING ROE DEER (*CAPREOLUS CAPREOLUS*)

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In 2004, a serological survey by indirect immunofluorescence on hunted roe deer in Western France revealed seropositivity towards *Babesia divergens* in 75,5% of the animals (268/355). Subsequently, a longitudinal study of roe deer captured for monitoring protocols in protected areas between 2004 and 2008 allowed cultivation and PCR detection of *Babesia capreoli, Babesia sp*. EU1 or both, from 41% of blood samples (83/202). None of the samples revealed infection by *B. divergens*. *Babesia sp*. EU1, (*Babesia venatorum nsp*), is known as potentially zoonotic. *B. capreoli* is very close to *B. divergens*, yet has distinct biological features. The three species probably share the same vector *Ixodes ricinus*, which has a very broad range of vertebrate hosts. These results indicate widespread infection of roe deer by *Babesia spp*., but show the necessity of caution for the interpretation of serological data. We tested cross-reactions of sera towards *B. divergens, B. capreoli and B. venatorum nsp* (EU1) antigens. Extensive cross-reactions occur for a majority of sera.

Poster Presentation 9

PRESENCE OF THE EASTERN COTTONTAIL (*SYLVILAGUS FLORIDANUS*) IN THE PROVINCE OF PERUGIA, ITALY

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The eastern cottontail (Sylvilagus floridanus) is a lagomorph indigenous to the American continent. The species was introduced in Perugia province (central Italy), around 1970s (Castelvieto's area, lat.43,113; long. 12,262), finding a suitable habitat of bushy areas, fields, woods, wetlands and groves. The aim of this work is to value the Sylvilagus floridanus population trend and their distribution on province area.

We considered six areas: Fontignano (lat. 43,026; lat. 12,191) Buchignano (lat. 43,168; long. 12,279), Poggio Montorio (lat. 43,061; long. 12,198), Sant'Apollinare (lat. 42,990; long. 12,264), Pietrafitta (lat. 42,990; long. 12,211), Castel del Piano (lat. 43,060; long. 12,306). In these areas hunting is forbidden and local hunters' associations used them for pheasants' and brown hares' production (ZRC).

From 1999 to 2007, in springtime and autumn, we used night time census with spotlights method to evaluate the diffusion of these species. The collected data have been expressed by an index of point abundance (IPA), which is the total number of contacts divided by the total number of points sampled.

On the basis of the collected results, we noticed a progressive expansion of the species from Castelvieto to a wide area between Perugia and Trasimeno Lake: Fontignano (2000), ZRC Sant'Apollinare (2004), ZRC Pietrafitta (2005), ZRC Castel del Piano (2006).

From March to June 2007 we analyzed 322 adult animals, killed within species' control plans authorized by the province (of whom 56% males, 22% non pregnant females and 22% pregnant females). Their sizes (length 347 - 455 mm; weight

of males 0.900 1.282 kilograms; weight of non pregnant females 0.957 1.411 kilograms; weight of pregnant females 0.978 1.526 kilograms, number of foetus 2 6) are not different from the sizes related by other authors.
In conclusion, we believe that, subsequently Eastern cottontail expansion, it is necessary to implement intervention plans in order to limit species diffusion in this area, considering also their rule in infective and parasitic diseases spread and the possibility of a competition with other species, particularly hares.

Poster Presentation 10

HEALTH STATUS OF THE EASTERN COTTONTAIL (*SYLVILAGUS FLORIDANUS)* IN THE PROVINCE OF PERUGIA: RESULTS OF A SEROLOGICAL SURVEY

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The eastern cottontail (*Silvilagus floridanus*), an indigenous American lagomorph, has been introduced to some different European countries: France (1953), Spain 1980), Italy (1966). This species has been introduced in Perugia's area around 1970s'. Afterwards they propagated in the surround areas with a strong demographic increase. The occupation of new ecological niches could represent a risk for the autocton species. The aim of this work is to value the presence of infective agents among the eastern cottontails and their role in the diffusion of pathogen that could infect hares.
We considered one area in Perugia (Montepetriolo) This area presented a high density of S. *floridanus*, a contemporary presence of brown hares and no hunting activity. Between March 2007 and December 2007 we collected 245 animals in total. We tested their serum in order to point out the presence of antibodies against the Brown Hare Syndrome (EBHS), Haemorrhagic Disease (RDH), Leptospirosis, Brucellosis, Tularaemia and Toxoplasmosis.
We did not detected antibodies against *Brucella* spp, *F. tularensis*, *L. interrogans*; the results obtained induce to exclude a relevant role of S. floridanus in the dissemination and transmission of infectious agents to hares.
The low prevalence resulted for *T. gondi* shows that some subjects could survive to this infection usually lethal, suggesting that this microorganism has a low circulation in the considered area.
Antibodies against EBHS were present in 37 of 245 blood samples (15.1%); 49,5% was positive up to a dilution of 1:20, 34.1% up to 1:80 and 5.4% up to 1.320.
Antibodies against RHDV were present in 57 of 245 blood samples (23.2%); a relevant number (84.2%) of sera had low titres, with the exception of two sera that had high titres up a 1:320.
These results indicate that some of the S. floridanus could have been naturally infected by EBHS, developing good immunity. Even in absence of clinically manifest disease, we can not exclude that cottontails could be infected by EBHS and could play a role in disease's epidemiology On the other hand, titres for RHDV antibodies were always too low to be considered specific or directly induced by one of the two known calicivirus of lagomorphs.
The results points out some important aspects concerning sanitary role that cottontails could play in transmitting EBHS to hares. However it is important to underline that to get wild animals free should be discouraged when they are allochthonous and in any case appropriate studies about sanitary impacts on autochthonous population must go ahead of any action.

Poster Presentation 11

Student Presentation

RED DEER (*CERVUS ELAPHUS*) ARE NOT A PERFECT HOST FOR *FASCIOLOIDES MAGNA*: EVIDENCE FROM A HISTOPATHOLOGICAL STUDY

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Fascioloides magna is one of the most important parasites of a variety of wild ruminants in North America and Europe. The only indigenous primary definitive host of *F. magna* is white-tailed deer (*Odocelius virginianus*) where fluke are encapsulated in thin-walled fibrous capsules and eggs passed out through the bile system. Common definitive hosts are other cervids including *Cervus elaphus canadensis*, *Rangifer tarandus*, *Cevus elaphus*, *Dama dama* and *Capreolus capreolus*. In dead end host, such as large bovines, llamas and horses infection is characterized by excessive fibrosis, thick-walled encapsulation and black pigmentation. In this study samples of 30 livers infected with *F. magna* were analyzed histopathologically in order to evaluate the interaction between parasite and red deer (*Cervus elaphus*). The main histopathological finding is replacement of hepatic tissues with fibrovascular proliferations. They form bridging portal and linear parenchymal fibrosis associated with migratory tracts, concentric hepatic fibrosis around necrotic areas or around flukes forming cyst walls. Iron-porphyrin depositions (parasite hematin) were present inside macrophages trapped within collagen in chronic lesions. This extracellular black pigment was also evident mixed with necrotic debris or within lymph vessels and biliary ducts. Disruption of liver parenchyma resulted with impairment of normal liver blood drainage resulting in arterial smooth muscles hypertrophy and dilatation of central veins, lymph vessels and sinusoids. Acute destructions of parenchyma were characterized with hemorrhage and eosinophylic hepatitis. One of the most common findings in chronic lesions was linear or perivascular inflammatory cell infiltrations of plasma cells and scattered eosinophyls. Multinucleated giant cells of foreign body type were present around dead fluke adults or within cyst walls. Biliary ducts were lined with hyperplastic epithelia containing bile, iron-porphyrin, trematode eggs, and adult flukes in rare cases. High numbers of trematode eggs were found trapped in connective tissue of cyst walls, migratory tracts and especially within granulomas. Most histopathological lesions found in this study suggest that *Cervus elaphus* develops lesions which resemble bovine lesions. This observation was also supported by the absence of eggs in fecal samples in 40% of animals.

Poster Presentation 12

EPIZOOTIOLOGIC SURVEY FOR ZOONOTIC *BABESIA* ISOLATES AMONG MOUSE-LIKE RODENTS AND CERVIDS IN CROATIA

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Tick-borne protozoan parasites from the genus *Babesia* continuously emerge worldwide and are recognized as a substantional public health threat for humans. The main causative agents of human babesiosis in Europe are *Babesia divergens*, *B. divergens*-like organism, provisionally designated as EU-1, and recently *B. microti*. In order to estimate the presence of zoonotic *B. microti* in Croatia we have analyzed 120 spleen samples from small mouse-like rodents (48 woodmice *Apodemus sylvaticus*, 37 yellow-necked mice *Apodemus flavicolis*, 2 striped field mice *Apodemus agrarius* and 33 bank voles *Clethrionomys glareolus)* which serve as its reservoir. To determine the role of cervids as potential *Babesia* reservoirs, spleens of 55 red deer (*Cervus elaphus*) and a single roe deer (*Capreolus capreolus*) were also analyzed by PCR

and subsequent sequencing of the portion of 18S rDNA. *B. microti* was detected in 6 yellow-necked mice and 2 bank voles (6.6%). Five of these isolates, detected in both rodent species, have partial rDNA sequence identical to that of the human Jena/Germany strain (Acc.No. EF413181). The other two isolates were identical to Munich *B .microti* strain (Acc.No. AB071177) isolated from *Mus musculus*. In 63.63 % of deer samples only *Theileria* sp., 99.6% identical to Spanish isolate 3185/02 (Acc.No. DQ866842) was identified. In the single roe deer *Babesia* was detected that is 100% identical to *B. capreoli* (AY726010) from roe deer and *B. divergens* (Acc.No. AY098643) from reindeer (*Rangifer tarandus tarandus*). The results of this study indicate the presence of zoonotic *B. microti* organisms in mouse-like rodents *Apodemus flavicolis* and *Clethrionomys glareolus* and, at the same time, the absence of zoonotic *B. divergens* isolates from cervids.

Poster Presentation 13

EVALUATING BARRIERS AND CORRIDORS TO RACCOON (*PROCYON LOTOR*) STRAIN RABIES MOVEMENT IN CLEVELAND, OHIO, USA: RESEARCH UPDATE

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Raccoon variant rabies occurs throughout the eastern, southeastern and northeastern United States. Westward spread of the disease has traditionally been prevented by geographic barriers and distribution of oral rabies vaccine (ORV) baits. In 2004 raccoon variant rabies was found in northeastern Ohio, representing a localized breach in the ORV barrier between Pennsylvania and Ohio. Modelling research suggests the topography of Ohio could lend itself to rapid spread of raccoon variant rabies if unimpeded by vaccinations or naturally occurring geographic barriers. Because there are few naturally occurring barriers in Ohio, we postulate that urban centers, highways, and developed areas may act as barriers to raccoon movements, while greenbelts and parks may act as movement corridors. We are radio tracking raccoons in rural and suburban areas of Cleveland, Ohio to evaluate whether such barriers or corridors exist. In addition, we are conducting genetic analysis on tissue samples from raccoons in seven counties within and surrounding the vaccination and outbreak zones. The relatedness and distance between raccoon populations will allow us to estimate movement rates, and thus the potential for rabies spread. The locations of barriers or corridors to raccoon movement may provide focal points for rabies management. The current study has been extended through September, 2009 with expanded objectives to include abdominal VHF transmitter implants in juveniles and GPS collars on adult raccoons in heavily urbanized areas. Furthermore, genetic sampling will be expanded to include raccoons from counties outside the initial 7-county sampling zone. Preliminary results suggest most raccoons are remaining within their home ranges, although some individuals have moved up to 2 km across highways and into urbanized areas before returning. A more complete understanding of raccoon movements in rural, suburban and urban environments will allow researchers to make recommendations to Wildlife Services Operations as to the location of ORV bait distribution and trap-vaccinate-release (TVR) strategies.

Poster Presentation 14

PHYLOGENETIC CHARACTERIZATION OF CANINE DISTEMPER VIRUS (CDV) FROM RED FOXES (*VULPES VULPES*) IN GREECE

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Canine distemper virus (CDV), a member of the genus *Morbillivirus* in the *Paramyxoviridae* family, is the causative agent of a systemic, often lethal, disease of wild and domestic *Canidae*. Serological investigations for CDV in the fox populations have revealed a prevalence of about 4-11% and distemper-like neurological signs and histological lesions in the grey fox have been described. These data suggest that the fox may also be a natural host for CDV and that infected dogs might serve as sources of virus for wildlife species or vice versa. In this study, phylogenetic characterization on complete H gene from the first three CDV isolates from foxes in Greece was performed. The extent of genetic variation among these isolates and all other known CDV isolates was analyzed.Tissue samples of twelve red foxes, that were found dead, were submitted to our laboratory. Sequence analysis was performed on complete H gene. Nucleotide sequences from the other CDV isolates were retrieved from the EMBL database. Phylogenetic and molecular evolutionary analyses

were conducted using program MEGA 3.1. Extracted RNA from pooled tissue samples from three foxes was positive for CDV. A different virus strain was isolated from each fox. Sequence analysis showed that the complete haemagglutinin (H) gene was 1824 bp in length in the three Greek canine distemper viruses from foxes. Phylogenetic analysis was performed on the complete H gene of 55 CDV isolates, including the three Greek isolates described in this study. This analysis revealed that the homology of the nucleotide and amino acid sequences between the three Greek isolates was 97.1-99.4% and 95.9-98.5%, respectively. The homology of the nucleotide and amino acid sequences between the three Greek isolates from foxes and six Greek isolates from dogs was 96.9-99.2% and 95.6-99.3%, respectively. In addition, the homology of the nucleotide and amino acid sequences between the Greek isolates and the vaccine strains was 91.4-92.3% and 90.6-91.5%, respectively. The maximum nucleotide and amino acid variation amongst CDV isolates worldwide was 10% and 11.2%, respectively. The two Greek CDV isolates from foxes clustered together with the recent European strains from dogs. The third fox isolates, along with the ferret strain 1493 and the mink strain DK86 (Denmark), was more distantly related to the major European lineage. As previously reported, the vaccine strains were separated from the group of recent field isolates. The detection in red foxes CDV isolates similar to CDVs from dogs might indicate that interspecies circulation between dogs and wild carnivores may occur. The possibility that, under particular conditions, wildlife species might act as a reservoir of CDV infection for domestic dogs or vice versa, should be better addressed by analysis of additional CDVs from wildlife animals.

Poster Presentation 15

DETECTION AND GENETIC CHARACTERIZATION OF THE FIRST ENCEPHALOMYOCARDITIS (EMC) ISOLATES FROM WILD BOAR (*SUS SCROFA*) IN GREECE

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Encephalomyocarditis virus (EMCV) has been recognized in domestic pigs, either as a cause of mortality in young pigs, due to acute myocarditis, or of reproductive failure in sows. Each form of the disease in pigs seems to be restricted to certain geographical areas, probably reflecting the character of viral strains originating from local rodent populations. Rodents are considered to be the natural hosts. Several other species, both domestic and wild, are susceptible to virus. It is so far unknown how pig infection really occurs in the field and what wildlife species are involved. The likely role of wild boars as temporary hosts for EMCV is in agreement with previous studies in which positive samples from wild boars were found in Italy, Belgium, France and Luxembourg. Encephalomyocarditis virus (EMCV) belongs to the genus *Cardiovirus* within the family *Picornaviridae*. It is a single-stranded positive-sense RNA virus with a genome of about 7.8 kb. Previous phylogenetic studies performed on part of the polymerase gene (3D gene) and of the CCR (VP3/VP1 region) grouped the EMCV isolates in two clusters A and B, with a similar tree topology for both genome regions. Those analyses revealed a correlation between the sub-clustering and the place of isolation, but no link has been established with the clinical picture induced in pigs. The objective of this study was (a) to evaluate whether EMCV infects Greek wild boars, and (b) to genetically characterize Greek EMCV strains from wild boars. Thirty-five frozen heart tissue samples from wild boars were submitted to our laboratory by Handing Federations. Six of these heart samples were positive for EMCV. Sequence analysis was performed on the complete CCR gene. Nucleotide sequences from the other EMCV isolates were retrieved from the EMBL database. Phylogenetic and molecular evolutionary analyses were conducted using program MEGA 3.1. A different virus strain was genetically characterized from each of the six wild boars. The entire CCR (2502 nt) has been completely sequenced. The difference between the two genotype (A and B) was clear at both nt and aa level. VP4 and VP1 were the most variable regions. Phylogenetic analysis was performed on the CCR and showed a similar clustering than the one described for the VP3/VP1 junction region. The tree was characterized by high bootstrap values. The Greek strains from wild boars fell on a single lineage, which included all isolates from genotype B (99,3-91,6% nucleotide similarity). From this study it is clear that EMCV can circulate in wild boar populations. However, the question that arises is whether wild boars play the role of hosts or carriers for EMCV infection of domestic pigs. Further studies are needed, in order to clarify the extent of EMCV infection in wild boar population.

Poster Presentation 16

EWDA Best Student Presentation Award applicant

BORDER DISEASE VIRUS SHEDDING AND DETECTION IN ORGANS OF NATURALLY-INFECTED SOUTHERN CHAMOIS (*RUPICAPRA PYRENACIA*)

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Southern chamois (*Rupicapra pyrenaica*) populations of the central and eastern Pyrenees have been affected by severe outbreaks associated with a Border Disease Virus (BDV) for the last 7 years. There is as yet scant information about the pathogenesis of this new disease and its routes of viral excretion, since it has been described only in this species.

Eight Southern chamois (seven males and one pregnant female) from one to eight years old with clinical signs consistent with BDV infection were studied. At necropsy, whole blood, tissue samples (spleen, kidney, brain, lymph node, skin, thyroid gland, intestine, liver, lung, bone marrow and testicle) and nasal, oral, rectal and urine swabs were obtained. The foetus from the pregnant female was also studied.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was used to detect the virus in all samples. Leucocytes and tissue samples were positive in all chamois. Nasal, oral, rectal and urine swabs were positive in almost all samples, indicating that the virus is being excreted via the main routes. In addition, sera were tested for BDV antibodies using ELISA and seroneutralization techniques, with negative results. Sequence analysis in the 5' untranslated region (5'-UTR) in 7 of the chamois confirmed that the virus is grouped into the BDV-4 genotype, the same BDV described in previous outbreaks.

The present results confirm that: 1) the diseased chamois studied were viraemic, being the virus detected in most organs; 2) the viraemic chamois shed the BDV through the four main routes of excretion: nasal and oral discharge, faeces and urine; 3) vertical transmission has been demonstrated as the female and its foetus have been RT-PCR positive. Therefore, as occurs with Bovine Viral Diarrhoea (BVD) and Border Disease (BD) in sheep, persistently infected (PI) individuals could be the main source of the virus in the population.

Poster Presentation 17

RABIES MONITORING IN CROATIAN WILDLIFE

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Rabies is fatal zoonotic disease with serious public health impact in number of countries throughout the world. As in many other European countries, the disease in Croatia is strictly in silvatic form, where red fox (*Vulpes vulpes*) has the main role as reservoir and propagator of virus among wildlife. Although perpetuating the disease mostly among them, foxes occasionally transmit disease to other wild and domestic animal species. Starting at spring 1977 when first rabies case was diagnosed on a fox from Koprivnica-Krizevci county until the end of year 1994, cases of rabies in foxes have been diagnosed with some differences in incidence from every county, covering entire territory of Croatia. In the past 10 years the disease has become enzootic in many counties and only islands have been spared until now. During the last thirty years of rabies monitoring at the Croatian Veterinary Institute and its 5 regional subsidiaries a total number of 55397 fox materials were submited for rabies diagnostics. Out of that number 12963, or 23,4% where positive for rabies. In addition to foxes rabies has been diagnosed in 16 other species of wildlife. Most frequently ferrets (77), badgers (54), roe deer (42) and wild boar (29), and less frequently in wild cats (15), jackal (10), hare (10) ,wolf (10), weasel (9) and polecat (8), and only occasionally in otter (2), rat (2), bear, red deer, lynx or hedgehog (1).

Poster Presentation 18

EWDA Best Student Presentation Award applicant

SCAVENGING ON UNGULATE CARCASSES IN MEDITERRANEAN HABITATS

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The inter- and intra-specific interactions during the consumption of infected animal material, by means of predation or scavenging, can contribute to disease transmission and maintenance. In particular, wild ungulates are potential reservoirs of tuberculosis (TB) in Mediterranean Spain, and availability of infected ungulate carcasses may play a role in disease transmission. The use of camera traps for studying terrestrial mammals has increased in recent years as camera technology has improved and equipment costs have decreased. In this study, infrared camera trap technology has been applied to visualize the natural scavenging. This is relevant to develop sanitary policies and conservation practices. The study was carried in two regions of South Central Spain. The first is Montes de Toledo (Central Spain), which included two localities: Cabaneros National Park, and Quintos de Mora, a big hunting Estate. The second area is Donana National Park, where wildlife and livestock coexist. The design has been based on the continued observation of 60 carrions: 20 red deer, 20 fallow deer and 20 wild boar. Among mammal species, this study revealed that wild boar frequently consumed carrion both at the interspecific and intraspecific level, suggesting that infected carrion convey an important risk for disease transmission. When wild boar detected the carrion, they used it during the following days up to its disappearance. Together with the wild boar, red fox was the dominant mammal species, though wild boar was dominant in the interspecific interactions. In open areas of Montes de Toledo, but not in Donana, probably due to seasonal differences in vulture abundance, we detected a high consumption of carrion by vultures, certifying their role as a natural sanitary filter. We conclude that it is necessary to develop selective methodologies to dispose carrion for the Mediterranean scavengers which should prevent the access of mammals.

Poster Presentation 19

THE RELATIONSHIP BETWEEN ENVIRONMENTAL CONTAMINATION AND PATHOLOGY IN STRANDED CETACEANS (*ODONTOCETES*) IN THE CANARY INLANDS

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Marine mammals are at the top of the food chain, they accumulate high levels of environmental pollutant, especially organochlorine compounds in lipid stores and heavy metals in other tissues. They have integrated environmental contamination for longer period of time and chronic intoxication can success. The exposure at these toxic compounds has been associated with different adverse effects.

In this study we report the results of statistical analyses to test the relationship between contaminants and histopathological findings reported by the Unit of Histology and Pathology of the Las Palmas University.

Organochlorine contaminants (PCBs, DDTs and chlordanes) in blubber and heavy metals (Cd, Hg, Pb and Al) in liver or/and kidneys were considered. Presence of infectious agents, especially internal parasites and viruses (morbillivirus and herpesvirus) has also been taking into account in this study.

A transversal study approach, from 29 cetacean odontocetes stranded in Canary Island between 1997 and 2005 were made. Stranding causes were classified into anthropogenic (n=16) (naval and fishering activities) and no-anthropogenic causes (n=13). Main lesions reported were: nonspecific chronic reactive hepatitis, glomerulonephritis and verminous pneumonia. The different variables were compared by a binary logistic regression in which dependent variables were the reported lesions and stranding causes.

The result support that contaminant bubbler level of PCB up to 15 ppm lipid weight increase 10 times the possibility of presence of hepatic lesion (OR was 10, 95% CI, 1,22 -81,8, p=0,03) in the cetaceans studied. No statistical significance relationship was found for other variables. Increasing the number of samples is needed to confirm the impacts of PCBs on of internal parasites and other pathological relevant effects.

This study was supported by the project RTA 2006-00168.

Poster Presentation 20

WEST NILE VIRUS: TWO YEARS OF ORNITHOLOGICAL AND ENTOMOLOGICAL SURVEILLANCE IN PADULE DI FUCECCHIO, TUSCANY, ITALY

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West Nile virus (WNV) is a mosquito-borne virus belonging to the Flaviviridae family. Neuropathogenic for birds, horses and humans WNV is maintained in nature in an enzootic cycle between birds and ornithophilic mosquitoes, predominantly Culex species, whereas horses and humans are considered incidental hosts.
The ornithological and entomological activities have been planned in the context of the EDEN project (Emerging Diseases in a changing European environment, funded by EU grant GOCE-2003-010284 EDEN), focused to improve the understanding of the natural history of the virus and its vectors in Europe and to assess the potential for transmission under future conditions of climate and environmental change. Activities have been conducted in the Padule di Fucecchio area where, in the late summer of 1998, an outbreak of WNV occurred for the first time in Italy, in horses.
Entomology: five catching sites have been selected: four of these are located inside horse farms and one inside the protected area of the Padule di Fucecchio.
Entomological specimens come from catches carried out fortnightly from 24th April to 20th October in 2006 and from 4th April to 9th October in 2007. Mosquitoes were collected using 3 different methods: bird baited traps, CDC light/CO_2 traps and manual catches into animal shelters.
Ornithology: bird trapping was carried in the same period described above. Trapping was conducted fortnightly according to the EDEN trapping protocol. 108 meters of mist nets were located along water canals.
A total of 20.789 mosquito females were collected in 2006 and 16.222 in 2007. Vectors were identified to belong to 12 species and 6 genera. Our results showed that Culex pipiens represented the predominant species (97,84% of the total sample N= 36213) in the 5 sites and using the three catching methods.
A total of 944 birds were trapped in 2006 and 867 in 2007. The majority of the trapped bird were passeriformes. In the two years 1180 bird blood samples were totally collected and laboratory tested for serum neutralization (SN). In 2006 one adult Italian sparrow resulted weak SN-positive while in 2007 the only positive bird was an adult European nightjar. Both birds were captured in June.
Considering the presence of competent vectors and wild birds, with a similar Abundance Index (AI), the findings of one residence and one migratory SN positive bird and the previous WN outbreak in the 1998 in the same area, the risk of new introduction for WNV seems to be a realistic hypothesis. The results of this inquiry represent a part of the whole ongoing surveillance system that is aimed at improving the understanding of the ecology of WNV in Italy. Additional studies about the seroprevalence in other species of birds are needed to evaluate the presence of virus.

Poster Presentation 21

EWDA Best Student Presentation Award applicant

CLINICAL INFLUENZA TYPE A IN FERRETS (*MUSTELA PUTORIUS FURO*)

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Two ferrets with history of anorexia, sneezing and serous nasal discharge were presented to the Ambulant for birds, rabbits, rodents and reptiles, Institute for Poultry health, Veterinary Faculty, University of Ljubljana. Clinical examination revealed ocular discharge in both ferrets and fever (39,1 °C) in one of them. Regarding the owner of ferrets

was diagnosed with (confirmed with tests) Influenza type A, the transmission of the virus to both of the ferrets was suspected. Radiographs were taken to exclude possible complication with pneumonia. Both animals were put on preventive therapy with amoxicilin with clavulanic acid (Synulox®, 12,5 mg/kg).
Naso-pharyngeal swabs were taken from both affected animals. Real time reverse-transcription polymerase chain reaction (RRT-PCR) targeting matrix (M) gene was used to detect influenza A viral RNA. Viral nucleic acid was detected by RRT-PCR in both samples. All attempts of isolation of the virus on chicken embryos were negative.
High susceptivity of ferrets to human Influenza types A and B and similar biological response to the infection as the one in humans, makes them important as experimental models for influenza virus pathogenesis and immunity studies. With more and more ferrets living in close contact with their owners, the risk of mutual infection between ferrets and humans has been increasing considerably. Control and prevention of the transsmission between ferrets and humans rests mainly on preventing exposure of ferrets to infected individuals. With significantly growing number of ferrets in Slovenia more clincal cases are expected to be seen and more reserach on the subject should be done in the future.

Poster Presentation 22

EWDA Best Student Presentation Award applicant

SALMONELLA INFECTION IN UK WILD BIRDS

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Salmonella in wild bird populations has been linked to infection in humans (Palmgren *et al.* 2006), pets (Philbey *et al.* 2008) and livestock (Palmgren *et al.* 2006; Daniels *et al.* 2003). Previous analyses indicate, at least for garden birds, possible seasonal trends in salmonella prevalence, with peaks in the winter, possibly associated with anthropogenic food sources (Pennycott *et al.* 2005). We attempt to identify wider trends in, and possible causes of, salmonella infection in the British avifauna.
Between 2002 and 2006, over 5000 wild bird carcasses were submitted to 14 Veterinary Laboratories Agency (VLA) regional labs in England and Wales and subsequently tested for Salmonella serovars. Of these, 384 tested positive for the presence of salmonella. As identification at the species level was not always possible, samples were assigned to their relevant family, of which 43 were represented. Garden bird families, such as finches, made up the bulk of our samples however, other groups, notably gulls, waterfowl and pigeons were also well represented. As the data were split by lab, month and year, they were sparsely distributed. This, in addition to the inherent biases associated with collecting data on expired wild organisms, meant that many of the more traditional analytical techniques were not appropriate for dealing with these data. We consider the utility of Cox-proportional hazard models in determining the extent to which the presence of salmonella infected individuals can be explained in terms of measured covariates. Incidents of infection were treated as ordered events, and analysed using a marginal models approach. We then compare our baseline Cox model with an Anderson-Gill model, which treats multiple events as independent of each other.
The infection rate was highest in finches (n=588 tested, 248 positive), with 42% of the individuals examined positive, and lowest in birds of prey (n=148), with none of the individuals examined found to be positive. We discuss underlying spatial and temporal trends in salmonella infection in wild birds and show how these are related to differences in climate, land use and species composition.

Poster Presentation 23

A SURVEY OF THE HEALTH STATUS OF HOODED CROW (*CORVUS CORNIX*) AND MAGPIE (*PICA PICA*) POPULATIONS IN CENTRAL ITALY

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Hooded crow (*Corvus coronae cornix*) is one of the avian species that in few years has colonized rural and suburban environment other than urban areas such as parks and rivers course. This species has gradually lost own migratory attitude, in order to assume a sedentary behaviour because of the climatic changes and large food availability of the urban centres.

In Umbria the hooded crow distribution covers all the regional territory. Magpie (*Pica pica*) is a species extremely diffused in Umbria, too. Its distribution interests two homogenous regions: the hilly relieves of the High Tiber Valley and the western part of Umbria Region, among the Trasimeno Lake, the Orvieto territory and the Low Tiber Valley. This aspect has resulted in an increasing of zootechnical and agricultural productions damages. To control the numbers of these species in the territory were adopted some control plans. These plans normally use methods of numerical limitation.

Despite their close association with the human population, little is know about the healthy status of these species and their role as possible carriers of zoonosis.

The aim of this work is to investigate the diffusion of some pathogens in magpies and hooded crows captured during the selective programmes (2006-2007).

Using Larsen type cages, 131 birds were captured in two years time (60 hooded crows and 71 magpies).

Blood samples were taken from the heart of each bird before the animals were euthanized (131 blood samples). At the anatomo-pathological examination, trachea and lungs were taken for PCR in 74 birds (33 hooded crows and 41 magpies).

Sera were tested in Rapid Serum Agglutination (RSA) for antibodies against Mycoplasma gallisepticum and Mycoplasma synoviae and in Complement Fixation Test for antibodies against Chlamydophila psittaci; Haemoagglutination Inibition Test was used for detection of antibodies against Avian Influenza Virus (H5 and H7) and Newcastle Disease Virus.

Serological results showed antibodies absence for Avian Influence Virus, Newcastle Disease Virus and Chlamydophila in both species. RSA for Mycoplasma gallisepticum and Mycoplasma synoviae were positive in 35,9% and 13% of animals, respectively.

PCR results were negative for Chlamydophila psittaci in both species; 1,4% and 16,2% of the tested samples were positive for Mycoplasma gallisepticum and Mycoplasma synoviae, respectively.

This work represents the first check up for magpie and hooded crowed populations in Umbria region. The monitored territory was the humid zone of the Trasimeno Lake, important site for the avian ecology of several aquatic and migratory species that share the same areas with magpies and hooded crows.

Even if, these two species can act as “ring of conjunction” between migratory-tank species and the domestic ones, our results seems to suggest the absence of the examined pathogens spreading in the controlled territory.

Poster Presentation 24

EPIDEMIOLOGY OF RESPIRATORY DISEASES IN CHAMOIS (*RUPICAPRA RUPICAPRA*) IN TRENTINO, NORTH-EASTERN ITALY

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During 2005-2006 was carried out a research on respiratory diseases of chamois, of which we know the possible impact on dynamic population. The study was done in several areas of Trentino: one located in the western part (Maddalene faunal area) and four located in the eastern part (Val di Fassa, Val di Fiemme, Primiero e Tesino). In this period postmortem examination on 55 dead found chamois (delivered in Istitute during 2005-2006), anatomical-pathologist exams on 306 lungs of shotted animals and bacteriological exams on lungs which showed lesions were performed. In the same period we conducted a serological survey on 131 sera of shotted chamois and a serological survey on sympatric domestic ruminants (333 goats and 271 sheep) in order to research antibodies anti *Pestivirus* and anti Respiratory Syncytial Virus (RSV).

The anatomical-pathologist exams pointed out severe inflammatory lesions (pneumonia, pleurisy, fibrinous or fibronecrotic pleuropneumonia, catarrhal and fibrinous bronchopneumonia) in 74.54% of dead found chamois and in 37.91% of shotted chamois. From lungs of dead found and shotted chamois there were isolated bacteria of secondary eruption, so we could suppose a viral etiology at the origin of pulmonary lesions detected.

The serological study has highlighted a prevalence for *Pestivirus* of 44.27% in chamois, of 16.52% in goats and of 35.79% in sheep. In this research the infection of *Pestivirus* was present in all of studied areas, even if positive serological cases in chamois were detected only in Val di Fassa and Val di Fiemme. Although the level of prevalence showed significant difference between the five studied areas no differences regarding age and gender were noted in the distribution of infection in chamois. As regard RSV, only chamois showed serological positiveness (47.33%), without differences between age and gender. Since our research didn’t found serological positive RSV cases in sheep and goats, the infection seemed to be absent in these species. So we could suppose that chamois maintain the infection regardless its presence in domestic ruminants. In this study and in the areas where sarcoptic mange was absent, respiratory diseases seemed to be the first cause of death rate. Since high positive rate of serological exams for *Pestivirus* and RSV and frequent bacteriological isolation from lungs of secondary erupting bacteria were found in chamois, we can suppose that there was a possible viral etiology for the respiratory diseases detected so far. Only further virological studies, aimed to isolate and typify viruses from chamois and domestic ruminants, will allow us to investigate the importance of serological positivity, the possible impact of infection on dynamic population and the likelihood of viral transmission between wild and domestic animals.

Poster Presentation 25

EWDA Best Student Presentation Award applicant

OVARIAN CARCINOMA IN A GREAT BUSTARD (*OTIS TARDA*)

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Neoplasic disease in wild birds is found infrequently. Here we report a case of an ovaric carcinoma in a Great bustard (*Otis tarda)*.

An adult female great bustard was found dead in the province of Ciudad Real in South-central Spain and submitted for necropsy to a Wildlife Rehabilitation Centre. Upon examination the bird was severely emaciated, weighing only 1.3kg. The cause of death was determined to be trauma from gunshot.

A mass of approximately 4 cm in diameter was located adjacent to the medial portion of the left kidney. The mass was of cream-white colour, firm and viscose consistence and cauliflower-like shape. White, firm foci of viscose consistence ranging in size from 2 to 8 mm in diameter were also found in the liver. The portion of kidney that had been located underneath the mass showed loss of its normal structure, and a jelly-like appearance. The rest of renal parenchyma showed numerous lesions similar to those described in the liver. The left lung was haemorrhagic, while the right lung had been replaced by material of a jelly-like consistence.

Lesions observed in other organs included hydropericardium, blood in the coelomic cavity and a massive parasitation by taenia in the duodenum.

Tissue samples of the tumour and organs were fixed in 10% neutral buffered formalin and processed routinely in order to obtain H&E stained sections.

Histopathologically the mass contained glands and papillae with epithelial cell atypia, reflecting the complexity of the architecture of the glands. In the affected organs the lesions locally invaded the parenchyma, extensive deposits of intra and extracellular mucin and frequent calcification were observed. These features lead to the diagnosis of a mucinous cystadenocarcinoma with malignant features.

Little is known about neoplasia in wild birds, generally because these do not survive to old age. In these species the appearance of tumoral processes may be more related to the influence of viral agents or ambient toxins.

Poster Presentation 26

TRICHINELLA NATIVA MUSCLE LARVAE FROM FOXES (*VULPES VULPES*) ARE ABLE TO WITHSTAND REPEATED FREEZE/THAW EPISODES WITH LITTLE EFFECT ON INFECTIVITY

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Trichinella nativa is the most frequent *Trichinella* species in arctic wildlife and also the predominating species seen in Norwegian wild fauna. The adaptation of *T. nativa* to a cold climate is reflected by the well documented freeze tolerance of its muscle larvae. The ability of the larvae to survive repeated freezing and thawing events has not however been elucidated and was investigated in the present study, using an Alaskan isolate and two isolates from coastal and inland Norway respectively. Minced fox muscle containing *T. nativa* after standard infection trials was subjected to two different freezing protocols: either continuous freezing (-5°C) for up to seven weeks or freezing (-5°C) for up to seven weeks with seven overnight (+21°C) thawing events. Once a week a bag was removed from each group, the meat was digested and muscle larvae isolated. *In vitro* (based on larval motility and morphology); and *in vivo* assessment of larval viability (bio-assay in mice) was carried out. The mice were euthanized four weeks post inoculation, the muscle digested

and larvae per gram (lpg) and reproductive capacity index (RCI) were calculated. During the second part of the study, some of the minced fox muscle, exposed to the initial freeze protocol, was stored for a further 23 weeks at -18°C prior to *in vitro* and *in vivo* assessment of larval viability and infectivity.
It was demonstrated that larval morphology and motility are not suitable for the assessment of infectivity of *Trichinella* larvae. It was concluded that bio-assay in mice was the only suitable method currently available for assessing larval viability. The study demonstrated that *Trichinella nativa* isolates originating from carnivores from Alaska expressed highest tolerance to freezing compared to the Norwegian isolates. The inland Norwegian *T. nativa* isolate showed higher freeze tolerance than the coastal isolate. Temperature fluctuations around freezing point, for up to seven weeks, had little effect on larval infectivity. A negative effect of the initial repeated freeze-thaw events could be demonstrated once the larvae were exposed to longer periods of subsequent deep freezing.

Poster Presentation 27

PRESENCE OF *BABESIA* SPP. IN THE CROATIAN RED FOX (*VULPES VULPES*) POPULATION

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The red fox (*Vulpes vulpes*) is the most disseminated predator of the northern hemisphere, which has resulted from its extraordinary ability to adapt to every kind of living conditions. It can be found in woods, grasslands, agricultural and suburban areas. Red foxes are also known as carriers of various parasitic diseases. Within the period of six months (autumn 2007 spring 2008), 191 foxes were comprised by sampling on various locations in Croatia. Our research was focused on determination of the presence of *Babesia* spp. in foxes. Presence was proved by analyzing spleen samples using polymerase chain reaction with *Babesia*-specific primers. Due to the fact that babesiosis represents a serious parasitic disease both for domestic animals and humans, with possible death, data considering *Babesia* presence in wild animals are of high epidemiologic importance. Out of 191 samples, 13 (6,8%) foxes were affected by *Babesia*. The positive findings include the County of Krapina Zagorje 2 (1,04%), the County of Osijek Baranja 1 (0,52%), the County of Zagreb 4 (2,09%), the County of Primorje Gorski Kotar 3 (1,57 %) and the County of Istra 3 (1,57%). Obtained data confirm the presence of *Babesia* spp. in surveyed foxes and implicate the role of the population of red foxes as the reservoir of *Babesia* spp. in wildlife.

Poster Presentation 28

EWDA Best Student Presentation Award applicant

RISKS OF GAME FARMING: ENTEROBACTERIAS IN FARMED AND RESTOCKED RED LEGGED PARTRIDGES (*ALECTORIS RUFA*) IN SPAIN

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Enteric pathogens, namely *Salmonella*, *Campylobacter* and *Escherichia coli* are still the most important agents of food-borne zoonosis, and an important problem for the livestock industry, especially poultry production. Salmonella has been the causative agent in numerous mortality events especially among songbirds. Domestic and wild animals are generally considered as a reservoir of human infections, and intensive production systems have been shown to favour the maintenance of enteric pathogens.
According to official data, more than four million red-legged partridges (*Alectoris rufa*) are hunted yearly in Spain of which more than three million have been farm reared and released just prior to the hunting season. In the hunting areas where restocking is carried out, farm-reared partridges share habitat with free-living red-legged partridges and other steppe birds, some of which are endangered. Because of the potential risk of contamination of natural populations, and the potential risk for humans from the consumption of restocked, hunted red-legged partridges we initiated a preliminary study on the prevalence of enteric pathogens in farmed, restocked and free-living red-legged partridges.

We compared the prevalence of *Escherichia coli*, *Salmonella* and *Campylobacter* spp. in 188 red-legged partridges in south central Spain. Of these, 94 were farm-reared from 5 different farms, 62 were hunted from 2 hunting areas where realeses are currently conducted and 32 were from an area where restocking has not been carried out for at least ten years. Cloacal swabs where cultured using standard methods for the isolation of *Salmonella*, *Campylobacter* and other *enterobacteriaceae*. *Salmonella* was detected in one of the farms, while none of the restocked or free-living partridges tested positive for *Salmonella*. *E. coli* was found in 15.97% (15 out of 94) of the farmed birds, while 25.8% (16 out of 62) of the restocked hunter-harvested partridges tested positive. Only one of the free-living partridges from areas without restocking tested positive for *E. coli* (1 out of 32, 3,12%), however this bird was considered to possibly be a restocked individual from a nearby area due to the bad condition of its feathers. *Campylobacter* was only detected in farmed (12 out of 94 samples, 17.76%) and restocked (24 out of 62 samples, 38.71%) partridges.
These preliminary results suggest that the production of red-legged partridges in farms and consequent restocking may pose a risk for both native wild birds and the consumers of this gamebird, as hunter-harvested partridges undergo no sanitary control. The higher prevalence of the investigated bacteria among restocked partridges may be related to aggregation at feeding points in the hunting states, where contamination between birds may be possible and because of the stress caused by the release and adaptation to the new habitat.

Poster Presentation 29

HISTOPATHOLOGICAL FINDINGS FROM THE SUB-MANDIBULAR LYMPH-NODES OF RED DEER (*CERVUS ELAPHUS*)

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During 2007 in Italy, as in other EU countries, a control plan for Chronic Wasting Disease (CWD) in red deer (*Cervus elaphus*) was initiated. The programme involved the sampling of brain stem and retropharyngeal lymph-nodes from shot animals in order to search for prion proteins; this analysis was performed by different regional labs of Istituti Zooprofilattici Sperimentali. Besides these organs, we also organized the sampling of sub-mandibular lymph-nodes in order to identify incidental lesions consistent with diffusive diseases.
In this work we present the main pathological observations and the results of some microbiological examinations performed on the most interesting lesions. We have considered the sub-mandibular lymph-nodes from 275 deer, fixed in 4% formalin. At the CeRMAS' labs (National Reference Centre for Wildlife Diseases of Aosta) we have performed the macroscopic exam by cutting lymph-node tissue in serial sections of 0.5 mm thickness. Afterwards, the sections were treated for histological analysis in accordance with routine method (Hematoxilin-Eosine stain).
Here we catalogue the different histopathological patterns followed by the number of cases and % prevalence.
No lesions: 85 (30.91%); Fibrosis: 7 (2.55%); Hemorrhages/hyperemia: 20 (7.27%); Hyperplastic lymphadenitis: 109 (29.64%); Hyperplastic lymphadenitis with hemorrhages/hyperemia: 33 (12%); Eosinophilic lymphadenitis: 9 (3.27%); and Granulomatous lymphadenitis: 12 (4.36%).
Considering the different types of lesions, we can put forward the following conclusions. Hyperplastic lymphadenitis can be regarded as physiologic reaction to different injuries (such as micro-trauma of the buccal mucosa) considering that sub-mandibular lymph-nodes partially drain the oral cavity and neck tissues. Eosinophylic lymphadenitis is probably a reaction to some parasitic diseases (passage of pulmonary nematode larvae through the tracheal lumen).
Hemorrhages/hyperemia can result from the hypertension caused by firearm trauma. Evaluation of granulomatous lesions is more interesting. All observed granulomas were formed by eosinophilic deposits with radiate/club borders surrounded by macrophage reaction with neutrophils and occasional Langhans giant cells. These aspects, described as Splendore-Hoepply phenomenon or "botriomicosis", are generally linked to bacteria such as *Staphylococcus* spp., *Pseudomonas aeruginosa*, *E.coli*, alpha-hemolitic streptococci, *Actinobacillus* spp. and others. According to bibliographic references, *Staphylococcus xylosus* was cultivated from suppurative lesions observed in one of the checked lymph nodes.
Nevertheless, considering some typical features of TB granulomas (epithelioid macrophages and Langhans cells), it is not possible to exclude a contemporary presence of mycobacteria for which it will be necessary to carry out further confirmatory analyses.

Poster Presentation 30

SURVEILLANCE FOR SALMONELLAE IN WILD BIRDS IN ENGLAND AND WALES

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Over 5000 submissions of wild birds, of 128 species from all regions of England and Wales were submitted to the VLA Diseases of Wildlife Scheme (VLADoWS) between 1998 and 2006. As part of scanning surveillance, intestinal content from at least one bird per submission were cultured by selenite and brilliant green agar.

There were 255 salmonella isolates between 1998-2006 from over 20 different species.

The majority of isolates, 223, were from birds found in British gardens, typically passerines. Salmonellosis was found to cause disease and death in garden birds but there was little evidence of clinical salmonellosis in the other species of wild bird.

In Britain the wild bird feed industry is worth more than £25 million a year and feeding of garden birds is a common practice. Salmonellosis in species of birds using garden bird feeders occurs relatively frequently.

Wild species may be infected with *Salmonella* sp., usually at low prevalences. Within certain ecological niches however, certain salmonellae have become host-adapted to wild species and in these scenarios, can cause infection and disease at higher prevalences.

Poster Presentation 31

MANAGING THE BIOSECURITY OF AN AMPHIBIAN BREEDING AND RESEARCH PROGRAMME, PERTH ZOO, AUSTRALIA

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The Amphibian Breeding and Research Centre was established at Perth Zoo, Western Australia, in response to a request by the WA Department of Environment and Conservation to assist with the conservation of native frog species, particularly from the south-west of WA, an internationally recognized biodiversity hotspot. The program has four main areas of focus for research:

- Captive husbandry for native frog species, including nutrition
- Captive breeding, growth and development
- Assisted reproduction, including induction of ovulation and cryopreservation of reproductive materials
- Health and disease monitoring

Frogs involved in this program are typically sourced from wild populations and brought into captivity to facilitate this research. Maintenance of adequate biosecurity is considered of high importance in managing this program, in order to prevent outbreaks of emerging and novel diseases, and prevention of transmission of potential pathogens from one captive population to another. Biosecurity protocols have been developed for use in the field, when collecting frogs for the program, and for captivity, and will be discussed as part of this presentation. These protocols have proved invaluable in managing biosecurity issues, and are likely to be of considerable value in the event of a catastrophic disease outbreak in a wild frog population in Western Australia. Some aspects have proved particularly challenging and have resulted in the conceptual development of unique solutions.

Poster Presentation 32

HERPES-SIMPLEX LIKE SEQUENCES IN STRANDED *DELPHINIDAE* SPP. IN THE CANARY ISLANDS

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Herpesviral infections are very common in a wide variety of hosts, mainly vertebrates, including birds, reptiles and mammals. Herpesviruses are double stranded DNA viruses, with an icosaedrical form and a genome length that varies from 120 to 230 kbp.

There are few reports of herpesviral infections in marine mammals. Since no cetacean herpesvirus have been isolated on cell culture, molecular diagnostic tools are essential to establish their taxonomic classification.

A retrospective study was performed to detect herpesviral sequences in banked tissue of stranded bottlenose dolphins and short finned pilot whales on the Canary Islands. To achieve this objective amplification of conserved DNA region within the herpesvirus polymerase gene was made. Sequencing and further comparison with the Genbank database were made in all positive samples.

Two bottlenose dolphins and one short-finned pilot whale exhibit sequences with high homology to herpes simplex virus (HSV) (p-distance: 0.00-0.02). One dolphin presented the herpesviral sequence in lung, whereas the other bottlenose dolphin and the short-pilot whale presented it in the brain. Histopathologically, these two individuals showed mild encephalitis, lesions previously reported by the Department of Morphology (Veterinary School -University of Las Palmas de Gran Canaria).

Although it is widely demonstrated that viruses are species-specific, alpha herpesviruses are considered the less specific among the Herpesviridae family. Most of the reports about HSV in other species different than humans are restricted to non human primates, such as chimpanzees, gorillas, common marmosets, saki monkeys, a gibbon and an orangutan. Few descriptions are available about HSV infections in non primate hosts. These are in a chinchilla, a rabbit and an African pygmy hedgehog.

The presence of a "HSV-like" in cetaceans of possible human origin has never been described before. The small continental shelf of the Canary Archipelago favours the proximity of different cetacean populations to the coast, such as bottlenose dolphins, and this could predispose them to greater exposure to pesticides, industrial pollutants and urban wastes. As the human population rises, the environmental release of human microorganisms through urban sewages also increases, as it has been demonstrated for enterovirus and norovirus.

Although an "HSV-like" sequence has been detected in the brain of a stranded bottlenose dolphin, the role of this virus as etiologic agent of the mild non-suppurative encephalitis has not been proved yet. To elucidate this question, immunochemical or TEM detection would be performed. The presence of herpesviruses in stranded cetaceans from Canary Islands and their role on the cetacean health status and stranding causes will be evaluated in further studies.

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Poster Presentation 33

ADENOVIRUS AND MYCOPLASMA INFECTION IN AN ORNATE BOX TURTLE (*TERRAPENA ORNATA*)

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An ornate box turtle died soon after hibernation showing clinical signs of upper respiratory tract disease (URTD). Mucopurulent nasal discharge, appearance of foaming fluid in the oral cavity and lethargy was observed.

After gross necropsy of the turtles main parenchymal organ samples were fixed in 4% buffered formalin and processed for further histopathologic examination. Samples of the liver, lung and intestines were collected for virus isolation and PCR. After DNA extraction nested PCR - designed to amplify partial sequence of the adenoviral DNA-polymerase gene - was carried out. For detection of mycoplasma infection a general mycoplasma PCR was used. The samples were also

screened for chelonian herpesviruses, invertebrate and vertebrate iridoviruses by PCR. PCR products were directly sequenced. Phylogenetic calculations were based on the new sequence data and sequences available in the GenBank.
At necropsy medium amount of mucopurulent exsudate was observed in the nasal cavity and the trachea. Congestion of the organs was determined. Liver had a yellowish-brownish discoloration, and signs of fatty infiltration could be seen. Unfortunately histopathologic examination was unsuccessful, because of the autolysis of the organs.
Mycoplasma sp. infection was confirmed by PCR targeting the 16S ribosomal RNA gene. Mycoplasma spp. are very common in tortoises causing URTD.
Attempts for virus isolation were unsuccessful. Adenovirus-like particles were detected in different snake and lizard species, also in Nile crocodile and leopard tortoise (*Geochelone pardalis*). Based on sequence analyses and phylogenetic calculations, all snake and lizard adenovirus types investigated so far proved to be a member of the genus *Atadenovirus*. On the unrooted phylogenetic tree of the DNA-polymerase gene four clearly separated groups of the family *Adenoviridae* could be distinguished and the adenovirus originating from the box turtle was a clearly separate branch. Interestingly the adenovirus originating from a tortoise (*Indotestudo Forsteni*) seems to be a new a member of the genus *Siadenovirus* (unpublished data).Comparison of the box turtle adenovirus sequence with other adenovirus sequences also indicates that it is a new virus. Further investigations are needed, including more DNA sequence data, to establish the taxonomic position of this new virus. The role of the Mycoplasma sp. and the adenovirus in causing the death of the turtle is not clarified yet.

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Poster Presentation 34

EWDA Best Student Presentation Award applicant

HEMATOLOGY AND SERUM CHEMISTRY IN SOUTHERN CHAMOIS (*RUPICAPRA PYRENAICA*) NATURALLY INFECTED WITH PESTIVIRUS

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In 2005 and 2006 an outbreak of disease asssociated to a Border Disease Virus (BDV) caused a high mortality in the Southern Chamois (*Rupicapra pyrenaica*) in the Cadí National Hunting Reserve in the Catalan Pyrenees (NE Spain). The aim of this study was to describe the values of different hematological and serum biochemical parameters of chamois affected by the disease and compare them with those obtained from healthy chamois.
Blood samples were taken from 32 free-ranging chamois with clinical signs consistent with pestivirus infection, and 12 hematological and 16 serum biochemical parameters were analyzed. In all diseased chamois pestivirus infection was confirmed by RT-PCR. Clinically, all diseased chamois had depression, weakness and movement difficulties, 13 chamois presented abnormal behavior, and 23 showed different degrees of alopecia with skin hyperpigmentation. At necropsy cachexia was observed in all animals, 14 had pneumonia, two had supurative lymphadenitis and one had pyoderma.
The chamois affected by the BDV-associated disease showed a lower red blood cell count, hemoglobin concentration, packed cell volume, mean corpuscular volume (MCV) and lymphocyte counts while the neutrophil and platelet counts were higher. Glucose, triglycerides, creatinine, total protein concentrations and alkaline phosphatase (ALP) activity were lower in the diseased chamois, whereas the concentrations of total bilirubin, urea and aspartate aminotransferase (AST) activity were higher.
The anemia, neutrophilia, and higher AST activity observed in the diseased chamois could be related to the inflammatory process, although anemia could also be influenced by undernutrition. The lymphopenia suggests that the lymphocytes are cellular targets of BDV or bone marrow dysfunction caused by the virus. The higher platelet count could be explained as a reactive thrombocytosis, due to the inflammatory process or to a previous BDV-thrombocytopenia. The decrease in creatinine concentration is attributable to a reduced muscle mass. The increase of total bilirubin and urea concentrations, the lower glucose and triglycerides concentrations and the reduced ALP activity suggest endogenous catabolism, due to a reduced food intake or to cachexia.

Poster Presentation 35

LEISHMANIA INFANTUM IN FOXES (*VULPES VULPES*) FROM THE PROVINCE OF IMPERIA, NORTH-WEST ITALY

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Leishmania infantum is a protozoan parasite responsible of human and canine visceral leishmaniasis in the Mediterranean area (Gradoni et *al.*, 1983). Dog is the domestic reservoir host of leishmaniasis and the infection is transmitted to vertebrate hosts by phlebotomine sandflies (Lainson and Shaw, 1987). Mancianti *et al.* (1994) proposed that infected fox could introduce the infection in *Leishmania*-free territories, and recently other studies suggest that wild carnivores could be considered the reservoirs of visceral leishmaniasis in areas where sporadic cases of disease were found (Mohebali *et al.*, 2005). Unfortunately data concerning the presence of leishmaniasis in wild canid have been seldom collected in Europe (Criado-Fornelio et *al.*, 2000). In Spain Sobrino et al. (2008) found a high prevalence of infection in foxes and compared strains from foxes and dogs suggesting that foxes can be a reservoir of the pathogen.

L. infantum infection has been signalled in the Liguria region in the North of Italy since 1994 (Mancianti et *al.*1994) with a seroprevalence of 18%. Our aim was to evaluate by PCR the *L. infantum* infection status in 90 foxes culled in the province of Imperia (Liguria region) during 2006-07 hunting seasons, and to compare their PCR-RFLP strains with those found in 30 infected (PCR positive) dogs living in the area.

PCR and RFLP were carried out on foxes' spleen and lymph nodes and dogs' blood according to the protocol described in Ferroglio et *al.* (2006).

Twenty-eight out of the 90 foxes (31.1%) were positive at PCR. The RFLP analysis of the amplified evidenced the presence of 10 RFLP strains in the foxes and 19 RFLP strains in the 30 PCR positive dogs. Foxes culled in the same area showed similar RFLP patterns, but we did not evidence overlap of fox and dog strains. Our results suggest that a high number of fox is infected by *L.infantum* and that strains in fox populations differ from the ones found in dog. A wider number of samples must be tested to confirm that foxes can maintain and circulate *L.infantum* within their population, without the presence of dogs.

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Poster Presentation 36

PHYSALOPTERA SIBIRICA (NEMATODA) IN FOXES (*VULPES VULPES*) AND BADGERS (*MELES MELES*) FROM THE NORTH WESTERN ALPS, ITALY

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Physaloptera sibirica (Petrow & Gorbunow, 1931) is a Spirurid worm belonging to *Physalopterinae* subfamily, characterized by an indirect transmission cycle with carnivores (i.e. fox, badger, linx) as definitive host and coleopteran (i.e. beetles, cricket and cockroach) as intermediate host. This nematode seems to be limited to Siberia, Afghanistan and the far east of Asia (Tenora and Barus, 1968, Acta Univ.Agric. 16: 327-336). However in the last thirsty years *P. sibirica* has been signalled in Europe in fox (*Vulpes vulpes*), badger (*Meles meles*) and dormice (*Elyomis quercinus*) from Spain (Miguel et al., 1996, Wiadomoscì Parazytologiczne, 42: 435-442) and in dormice in the Alps (Quentin & Biocca, 1976, Ann Parasitol Hum comp 51: 255-262). We deemed it interesting to investigate the nematode presence in foxes (*Vulpes vulpes)* and badgers (*Meles meles*) from Piedmont and Aosta Valley (NW Italy), and to evaluate its distribution and the association between the parasite and abiotic factors (i.e. altitudes and cold climates). From all Provinces of Piedmont and from the Aosta Valley, a total of 608 foxes, harvested or road killed, and 39 road killed badgers were examined from 1996 to 2008 for the presence of nematodes in the digestive tract. The digestive tract (stomach) of the animals was dissected and examined for the presence of the parasite. Nematodes were collected and stored in 70° ethyl alcohol and lately counted, sexed and classified according to the keys described in Quentin and Biocca (1976). Prevalence and 95% exact binomial confidence intervals (CIs) were calculated using R software (Binom.test function, R Software). In relation to habitat, prevalence (P) was significantly higher (X-squared=16.3638, $p \leq 0.05$) in mountain foxes (P=6.43%, 95%Cs: 3.25-11.22) respect to both hills (P=2.22, 95%Cs: 0.46-6.36) and irrigated plains foxes (P=0.34, 95%Cs: 0.01-1.90). There were no significantly differences between sexes and age. Prevalence in badger is 2.6% (1/39). This represents the first report of the presence of *P. sibirica* in fox and badger in the Alps. Our results suggest that the fox is a frequent host of *P. sibirica* in mountainous areas and is present from the Cottian to the Pennine Alps. According to previous study this parasite is associated, probably due to its intermediate host's biology, with high altitudes.

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Poster Presentation 37

DETECTION OF *CHLAMYDOPHILA PECORUM* IN THE LUNG OF AN ALPINE CHAMOIS (*RUPICAPRA RUPICAPRA*) IN NORTHERN ITALY

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Chlamydial infections have been widely detected worldwide both in farmed and in free-living animals, including wild ruminants. Several species belonging to the family *Chlamydiaceae* cause major reproductive disease (metritis, infertility and abortion), conjunctivitis, arthritis, encephalomyelitis and pneumonia.

In our study area, a mountain region in the province of Bergamo, Northern Italy, two cases of *Chlamydophila abortus* infection were detected in the last few years respectively in a mummified roe deer foetus and in individuals of an ibex population affected by conjunctivitis. Moreover, *Chlamydophila pecorum* has been isolated several years ago in a chamois affected with pneumonia in another area of the Alps.

We report here the detection of *Chlamydophila pecorum* in a 7 month-old female chamois, submitted to the laboratory by a gamekeeper. The animal came from an area where a remarkable mortality in kids was occurring. At necropsy, the chamois presented a nasal discharge with conglutination of the hair of the nasal region. A fibrinous pericarditis and pleuritis was observed; the lungs were enlarged, oedematous and showed areas of red consolidation in the cranial lobes and at the peripheral sites of the middle and caudal lobes. Wide areas of parasitic nodular lesions were observed in the caudal lobes.

Laboratory investigations for bacteria, including mycoplasmas, were performed by inoculating samples of lungs onto Blood agar and PPLO agar plates. Only *Moraxella* sp., but no mycoplasma, were isolated. Specific PCR-RFLP targeting chlamydiae was carried out and allowed to detect *Chlamydophila pecorum*.

The virological investigation focused on the research of a respiratory syncytial virus, which has been recognized as an agent of pulmonary distress in the area of investigation, but the lung tested negative.

No information other than a marked decrease in the number of kids in the chamois population was reported by the gamekeeper nor by local hunters. Although the role of *Chlamydophila pecorum* and *Moraxella* in determining the death of the kids is unknown, it is suggested that the necropsied chamois had died of acute respiratory distress, caused by *Chlamydophila pecorum* with the superinfection by *Moraxella* sp.

Poster Presentation 38

WILDLIFE HEALTH MONITORING IN BERGAMO PROVINCE, NORTHERN ITALY: A COORDINATED PROGRAM BETWEEN THE OFFICIAL VETERINARY SERVICE, THE PUBLIC ADMINISTRATION AND THE HUNTERS

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Wildlife welfare is a common concern of different stakeholders: the official veterinary service, for the increasing number of infectious diseases shared between wildlife, domestic animals and humans, the public administrators, for management reasons, and the hunters, for a direct interest in hunter-harvesting. Since 1997 a health monitoring on wildlife in Bergamo Province has been performed, with the informal cooperation of the hunters associations. In 2005 an agreement between the official veterinary service, the public administrators and the hunter associations formalised the collaboration between these parties, with the common aim to a better understanding of health and disease in free-ranging wildlife. Through this accord the biological samples, such as blood, faeces and viscera, are collected by the hunters during the hunting seasons and delivered to the laboratory for analysis. Moreover dead animals, recovered in the field by gamekeepers or by hunters, are submitted for necropsy to investigate the death's cause. This network allows to check wildlife morbidity and mortality and to recognize atypical events in the most important wild species of the Province: roe deer, red deer, chamois and wild boar. The presence of some infectious diseases has been investigated through the yearly monitoring program, in particular Brucellosis, Trichinellosis, Classical Swine Fever, Swine Vesicular Disease, Tuberculosis, Johne's disease, Lyme disease, Respiratory Syncytial Virus, Infectious Keratoconjunctivitis, Salmonellosis. Right now we have historic data that can be useful to define the sanitary picture in the wildlife of our Province. Interesting data are recovered by the analysis of both hunted and dead animals; for instance we assessed the presence of M. *microti* in tuberculosis-like lesions in the wild boar population, we studied the circulation of Respiratory Syncytial Virus inside the chamois population and we have been checking through the years for the potential presence of relevant zoonosis, such as Brucellosis and Trichinellosis.

Poster Presentation 39

INTERSPECIES TRANSMISSION OF PATHOGENS BETWEEN DOMESTIC AND WILD UNGULATES CHANGES THEIR BIOLOGICAL PROPERTIES AND THUS EPIDEMIOLOGICAL FEATURES

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Horizontal interspecies transmission is a central mechanism in the emergence of diseases in wild-living populations. Three modalities are studied: (i) transmission involving direct contact (« nose-to-nose ») : bronchopneumonia with *Mannheimia haemolytica*, (ii) transmission with resistant pathogens, involving succession on the same places : brucellosis with *Brucella abortus* and *B. melitensis* , (iii) transmission requiring a social contact (venereal infection, lactation) : Caprine Arthritis Encephalitis Virus.

For each type, biomolecular studies have been carried out on strains isolated from Chamois *Rupicapra rupicapra* (n=566), Ibex *Capra ibex ibex* (n=26) and sheeps coming from 11 study areas. Simultaneously, geographic positioning studies were conducted to estimate contact rates and exposure between wild and domestic species (924 hours of field observation data).

M. *haemolytica* is the main agent responsible for pneumonia in free-ranging Chamois, and frequently infects domestic flocks. Even if common sensibility to this bacterial species is obvious among different animal hosts, strain passage between domestic and wild ungulates is unlikely in field conditions. Thus, only two bacterial strains (among n=384) presented a genetic kinship between wild and domestic ungulates but with no epidemiological relationship; the field risk assessments of interspecies transmission were non-existent in 10 study-areas / 11. Nevertheless, experimental assays, submitting *Mannheimia* strains to binding culture conditions, induced genetic drift. This innovating result in bacterial field might enlighten some peculiar focus of pasteurellosis, where sheep strains over-crossing species barrier to Bighorn or Chamois change their virulence and lead to « die-off » situations.

Regarding *Brucella* sp., the clinical expression of the disease has been radically more violent in the Chamois (n=19) compared to domestic hosts that form the source. The prognosis is mostly lethal, however few individuals have survived to infection, but were excluded from reproduction because of their lesions.

Although most wild species are quite permissive to CAEV infection, cases of natural infection are exceptional. Our data show for the first time infection in a wild species (Ibex) close to caprine species. The large homology between sequences of the virus infecting two ibex, goats and hybrids living on the same site, suggests the passage of CAEV from goats to ibex. The interspecies contamination has taken a minor modality (venereal route), which, despite it is exceptional in domestic epidemiology, becomes the point of rupture of the species barrier. In addition, ibex infection has a high probability of being lethal, as a corollary of changes in the viral genome

These first investigations suggest that the emergence of diseases shared between domestic and wild animals caused by the increased opportunities for contact is likely to lead to unprecedented pathological expressions and justifies specific sanitary monitoring.

Poster Presentation 40

MULTILOCULAR LIVER CYSTS IN OLD-AGED CHAMOIS *(RUPICAPRA RUPICAPRA)* IN AUSTRIA

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Unknown liver alterations were diagnosed in twelve adult free-living chamois *(Rupicapra rupicapra)* originating from alpine regions in the western part of Austria. The animals (eight female, four male), all in advanced age (average 13 years old) were killed by local hunters most of them because of emaciation or abnormal behaviour. The liver of the animals was severely enlarged and contained thin walled multiple cysts of various sizes filled with serous, amber fluid. No evidence for parasites was found in the cysts. Furthermore there were no cystic lesions present in other organs of the carcasses.

Histologically, the cysts were lined by a single layer of flattened or low cuboidal epithelium giving evidence for their biliary origin. Except in one case no fibrosis was found in the surrounding liver tissue. In some cases signs for inflammation and tissue damage caused by parasites suggest that the cysts could be a result of parasitic migration in the liver of old-aged chamois. Although nothing is known regarding heredity, these morphological findings can also indicate an adult type of polycystic disease. To our knowledge, this is the first report describing multilocular liver cysts similar to polycystic liver disease in this species.

Poster Presentation 41

THE PREVALENCE OF CONGENITAL UMBILICAL HERNIA IN COMMON BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) IN THE ADRIATIC

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Congenital umbilical hernia is a malformation of the ventral abdominal wall of animals and humans. In terrestrial species, such malformation causes death in-utero or soon after birth. To our knowledge there are no data on the incidence of this malformation in the bottlenose dolphins (*Tursiops truncatus*) in the literature. In humans, the congenital umbilical hernias appear in 3.5 out of 10,000 cases of normal births. The bottlenose dolphin is the only resident marine mammal species in the Croatian part of the Adriatic Sea, with an estimated number of around 200 adult individuals and around 20 cubs living in the area. It is estimated that between 15 and 20 bottlenose dolphin births occur there annually. We examined 123 carcasses of bottlenose dolphins found in the Croatian part of the Adriatic Sea since 1990. The congenital umbilical hernia was found in 13 cases (five of them were either foetuses found in uteruses of dead mothers or aborted foetuses; while 8 were born alive and lived for various periods of time). The estimated total number of births of bottlenose dolphins in the Croatian part of the Adriatic Sea since 1990 is between 270 and 360. This means that congenital umbilical hernia appears in between 361 and 482 out of 10,000 cases of normal births of bottlenose dolphins in the Adriatic. Our findings indicate that the incidence of congenital umbilical hernia in the Adriatic bottlenose dolphins is more than 100 times higher than that recorded in humans. The cause of such high incidence in the bottlenose dolphin remains unknown. Our future research will focus on determining a degree of relatedness among affected animals using molecular markers, in which we would test the hypothesis of the genetic basis of this malformation in the bottlenose dolphin. Furthermore, we do not know whether such high incidence of congenital umbilical hernia is found only in the Adriatic population of the bottlenose dolphin, or is it inherent to the bottlenose dolphin species.

Poster Presentation 42

EWDA Best Student Presentation Award applicant

EVIDENCE OF EUROPEAN BROWN HARE SYNDROME IN SOUTHERN BELGIUM

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Since a few years, a decline of European brown hares (*Lepus europaeus*) populations has been reported in some regions of Southern Belgium. Different reasons have been suggested to explain this trend to decline such as major changes in agricultural practices, climatic conditions, and increase of predators and/or infectious diseases. In this context, analyses of found dead animals are strategic to determine if infectious diseases may be partly involved in this decline.
In 2007, the Surveillance Network of Wildlife Diseases operating in Wallonia collected 24 hares (21 adults and 3 juveniles less than 2 months old). Animals were found dead or euthanized by hunters for ethic reasons ; the carcasses were frozen before examination. After complete necropsy, livers were systematically tested for European Brown Hare Syndrome virus (EBHSV) by RT-PCR (Dr. G. Le Gall-Reculé, AFSSA, Ploufragan, France). Targeted bacteriologic and parasitologic analyses were also performed according to suggestive gross lesions.
RT-PCR results for EBHS were positive for 4 of the 24 hares tested. The cases concerned 3 adults and one juvenile. All except the juvenile were in good body condition. Gross lesions consisted in mild to severe pulmonary congestion (n = 4), hemorrhagic content in the small intestine (n = 3), discoloration and loss of consistency of the liver (n = 2), hepatic congestion (n = 1) and mild enlargement of the spleen (n = 1). Histopathology was not performed due to the freezing of the carcasses. Five other hares presented lesions suggestive of EBHS (pulmonary congestion or haemorrhages, hepatitis, enlargement of the spleen or the liver and/of haemorrhagic syndrome) but RT-PCR showed negative results for these cases. Since some tissue samples were of poor quality, these negative results could be partly due to the degradation of the viral RNA. Besides EBHS, other pathologies were diagnozed such as pseudotuberculosis, pasteurellosis and coccidiosis.
These preliminary results confirm the presence of EBHS in Southern Belgium but additional data are needed to strengthen the epidemiologic picture of EBHS in hare populations in our country.

Poster Presentation 43

EWDA Best Student Presentation Award applicant

CARBON SOURCE UTILIZATION OF *FRANCISELLA TULARENSIS* STRAINS ISOLATED IN HUNGARY

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Francisella tularensis is a gram-negative intracellular bacterium, the causative agent of tularaemia, which is a highly contagious zoonosis. Fifteen *F. tularensis* strains isolated from brown hares (*Lepus europaeus*), a patas monkey (*Erythrocebus patas*) and a vervet monkey (*Chlorocebus aethiops*) originazing from different regions of Hungary were identified using the Biolog system (Biolog Inc., Ca). This identification system was able to identify the strains not only after 24 hours, but already after 4 hours of incubation. These results were confirmed by PCR and partial sequencing of the 16S rRNA gene. Analysing and comparing the metabolic fingerprint of our strains with the data in the Biolog database, we conclude that there are differences in carbon sources utilisation pattern among our isolates and the Biolog database. We would like to point out that the Biolog program fails to differentiate the highly virulent *F. tularensis* ssp. *tularensis* (glycerol fermentation positive) and the less virulent *F. tularensis* ssp. *holartica* (glycerol fermentation negative) based on the use of glycerol as a carbon source. As none of our strains was able to use glycerol as a carbon source they can be declared as *F. tularensis* ssp. *holarctica*. Showing the metabolic relationship between the fifteen strains on a dendrogram we found that our isolates are similar to each other which correlates with the conservative genetic character of *F. tularensis* ssp. *holarctica*.

Poster Presentation 44

ESTABLISHING ZOO MORTALITY PATTERNS USING ANIMAL RECORD KEEPING SOFTWARE (ARKS) RECORDS

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Many European zoos use the Animal Record Keeping Software (ARKS) produced by The International Species Information System (ISIS) to keep and maintain their records. These data repositories can be utilised to produce reports of deaths and necropsies for all available records. In many cases this information is not utilised to its full potential (or indeed utilised at all). This project examines death and necropsy data for the species held at Twycross zoo to establish baseline data on life span and cause of death. This data will be used to pinpoint common causes of mortality at the zoo and identify unrecognised disease syndromes for follow up investigation or research. Diseases already identified include sporadic outbreaks of challitricid hepatitis, subsequently diagnosed as lymphocytic choriomeningitis virus (LCMV). This project will be expanded to compare data from other zoos to identify differences in mortality patterns.

Poster Presentation 45

WEST NILE FEVER IN EAGLES IN SPAIN

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West Nile Virus is a Flavivirus with a natural cycle between birds and mosquitoes and for which humans and horses are accidental hosts in which it can cause severe meningoencephalitis. Interest into this virus has been triggered by its introduction in 1999 in the North American continent, where it has caused more than 10.000 cases of neuroinvasive disease in humans, including over 1.000 fatalities, and the death of hundreds of horses and thousands of birds. A recrudescence of the virulence of WNV has also been observed in the recent past in Eastern Europe, including outbreaks among humans and horses but also in birds. Evidence for increased West Nile virus (WNV) activity has also been observed in the western Mediterranean region including more recently Spain, with the first human case in 2004, and data that supports circulation of the virus among birds, both in coastal regions and central Spain in the recent past. However WNV had not been isolated thus far. Birds of prey have been among the species most susceptible to the disease in the North American continent, and the limited number of cases of birds reported with clinical disease in Europe has also largely been from raptors. Here we report cases of clinical West Nile Fever in eagles in central Spain and the first isolation of the virus in the country.

A captive bred juvenile golden eagle (*Aquila chrysaetos*) was released into the wild, and followed using terrestrial telemetry. One month after release, the bird was recovered moribund and admitted to a rehabilitation and captive breeding centre for birds of prey, where it died five days later with neurological signs. Two captive eagles showed clinical signs eleven days after admission of the index case, but recovered with treatment. WNV was detected by RRTPCR in the three diseased birds, and virus isolation was achieved on Vero-cells from tissues of the deceased golden eagle and an oropharyngeal swab of the diseased golden eagle. Antibodies against WNV could be demonstrated in the diseased birds and some of the birds that had been in contact with the diseased eagles. The most important lesions observed in the golden eagle were moderate encephalitis, with perineuronal and pervascular edema, gliosis and a moderate mononuclear infiltrate. Previous to this case, WNV had also been detected by molecular methods and immunohistochemistry in Spanish Imperial eagles (*Aquila adalberti*) that had died due to different causes. This evidence for pathogenicity of WNV in raptors is of concern as many of the birds of prey and namely the Spanish Imperial Eagle are endangered.

Poster Presentation 46

USING MOVEMENTS OF AQUATIC WILD BIRDS TO IDENTIFY RISKS OF H5N1 HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) SPREAD AMONGST SPANISH WETLANDS

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Highly Pathogenic Avian Influenza of the subtype H5N1 (H5N1 HPAI) has recently spread in domestic and wild avian populations. Since October 2005, 15 countries from the European Union have been affected by H5N1 HPAI, and many of these cases (85%) have involved mortality in >60 wild bird species. The spreading of H5N1 HPAI has been largely related to aquatic bird migratory movements and their concentration in natural wetlands. The importance of the introduction of H5N1 HPAI into a country from wildbirds is based not only on the risk of contact with domestic birds, but also on the implications for the conservation of several globally-threatened species of waterbirds. For example, one single outbreak in 2005 in Qinhai Lake (PR China) caused mortality of an estimated 10% of the global population of Bar-headed Geese (*Anser indicus*). Spain has one of the richest clusters of wetlands on the planet, is home to a diversity of aquatic bird life, with an annual average of 1,5000,000 wintering waterbirds. There are 391 IBAs (Important Bird Areas) spread over 32% of the surface of the country (16 million Ha). Out of the 278 European species needing conservation measures, 63% are present in Spain. Donana is the most important wetland in Spain with National Park, Natural Park, Biosphere Reserve (UNESCO), World Heritage (UNESCO), Ramsar, IBAs (Importand Bird Areas), Biological Reserve, Natural Monument and European Diplome status.

The recognized mechanism for AIV transmission within wild waterfowl populations is via contaminated water. Therefore, the characteristics of the wetlands will influence virus survival. Recently, Brown et al (2007) have described virus persistence in water, which was found to be inversely correlated with water temperature and salinity.

The objective of this study was 1) to identify risk areas in Spanish wetlands due to their optimal conditions for the survival of AIV and 2) to assess the associations amongst wetlands through aquatic wild bird movements. The risk areas were identified by determining the range of viability of the virus (analysis of salinity, temperature and U.V. exposure conditions) in 113 Spanish wetlands. The data were represented with Arc Gis 9.1.(ESRI ®). Connections amongst wetlands were assessed in relation to bird census and movement data (ringed and recoveries) for 44 waterbirds species (from the Spanish Migration Species Office (SMO)) using network analysis (performed with software PAJEK 1.22 (c) 1996). The knowledge gained from these studies will allow geozones to be identified which represent areas with different susceptibility to AIV and their biological relationships. This will inform the design of active and passive surveillance for AIVs in wild birds.

Poster Presentation 47

HARES AS SENTINELS FOR *YERSINIA PSEUDOTUBERCULOSIS* IN THE ECOSYSTEM

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Yersinia pseudotuberculosis is a zoonotic enterobacterium causing severe disease (pseudotuberculosis) in wild mammals and birds. It has also caused several large outbreaks of food-borne illnesses in humans in Finland in the last decade. These outbreaks have often been associated with fresh produce. The source of bacterial contamination in these cases was suspected to be faeces of wild animals. Reservoir species of *Y. pseudotuberculosis* are not properly known in Finland but rodents, lagomorphs and birds are suspected. During 2000-2007, the Finnish Food Safety Authority Evira examined 1174 hares (641 European brown hares *Lepus europaeus* and 533 mountain hares *L. timidus*) found dead. Of these, 44 (3.7%) were diagnosed with pseudotuberculosis by bacterial culture and pathology. In 16 cases, the bacterial strain was serotyped and 15 of them were type O:1 and one was O:2. The disease typically caused a severe generalised infection characterized by nodular necrotic inflammation of liver, spleen, mesenteric lymph nodes, caecum and various other organs. The bacterium grew abundantly from macroscopically affected organs but it was only relatively rarely isolated from small intestine (15% of cases). The body condition of pseudotuberculotic hares varied from poor to good. Almost all hare cases (93%) were found in the cold season (September to February). Geographically, the proportion of pseudotuberculosis cases in hare material was highest in southwestern part of the country. Isolated cases of pseudotuberculosis were also found in a fox *Vulpes vulpes*, a flying squirrel *Pteromys volans* and three passerine birds (a magpie *Pica pica*, a wagtail *Motacilla alba* and a house martin *Delichon urbica*). These individuals were typically in poor condition with some debilitating factor, such as sarcoptic mange or fowl pox dermatitis. Hares seem to be very susceptible to *Y. pseudotuberculosis*. Even adult individuals in good condition can succumb to the disease. In the epidemiology of *Y. pseudotuberculosis*, hares can be regarded mainly as spill-over hosts that can act as common sentinels for the occurrence of this pathogen in the environment.

Poster Presentation 48

EWDA Best Student Presentation Award applicant

SKIN TEST RESPONSE OF EUROPEAN WILD BOAR (*SUS SCROFA*) TO MYCOBACTERIAL AND NON-MYCOBACTERIAL ANTIGENS

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The European wild boar (Sus scrofa) is considered as the main wildlife reservoir for bovine tuberculosis (bTB) in Mediterranean areas from South Central Spain. Here big game has become an important industry during last decades, and translocation of individuals is a common practice. Comparative skin-testing (CST) with purified mycobacterial protein derived antigens (PPDs) is the standard *in vivo* test diagnostic to detect tuberculosis in domestic animals. Although tested in domestic pigs, there is no data concerning its use in wild boar (*Sus scrofa*). We aimed testing the CST in wild boar in relation to bTB infection, and to compare the response to the mycobacterial antigens (purified protein derivatives, bovine bPPD and avian aPPD), with the response to a mitogen used as positive control, phytohaemagglutinin (PHA). We also used Phosphate Buffered Saline (PBS) as a negative control.

The study included 98 wild boars, 57 farmed, and 41 wild. The antigens and the controls were injected intradermally at the neck and/or inguinal site, and skin-fold thickness increases were measured at 72 hours following injection. As threshold for bTB, we considered positive a bPPD skin fold increase higher than 20 mm, which should be at least 10 mm higher than aPPD. Responses to all three antigens were highly variable between sexes, ages and origins of the tested wild boar. Only part of the tested animals could be necropsied and submitted for culture. However, agreement between the skin test response to bPPD and culture status was poor, only a 57% of the animals with lesions had a bPPD skin fold increase higher than 20 mm, suggesting that skin testing as done in this study is not an appropriate test for bTB in wild boar. Part of the cultures are being processed at the time this abstract was submitted, and results concerning the sensitivity, specificity and logistics of the technique will be reported at the EWDA meeting in October.

Poster Presentation 49

EWDA Best Student Presentation Award applicant

MOLECULAR ANALYSIS OF *MYCOBACTERIUM BOVIS* ISOLATED FROM BUFFALOES (*SYNCERUS CAFFER*) IN HLUHLUWE IMFOLOZI PARK, SOUTH AFRICA

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Bovine tuberculosis (BTB) a chronic disease of mammals is a threat to South African wildlife. The etiological agent of BTB, *Mycobacterium bovis*, has a wide host range including buffalo which is a reservoir host in South African wildlife populations. This study reports the molecular typing of *M. bovis* isolated from buffalo in Hluhluwe iMfolozi Park (HiP) by utilizing tools such as PCR based analysis of deleted genomic regions, Multiple Locus Variable [number of tandem repeats] Analysis (MLVA) and spoligotyping.

Bronchial lymph node tissue samples (n=12) from buffaloes which previously tested positive to the tuberculin skin test were cultured using standard methods. DNA for PCR was obtained by boiling a loop from pure culture colonies at 80 °C for 1 hour. Deletion analysis, amplifying the regions of difference (RD); RD4 and RD9 were done on the isolates. Also, PCR amplifying 16 variable number of tandem repeats (VNTR) loci using previously described conditions and spoligotyping analysis were carried out on the samples.

Four of the 12 were positive after mycobacterial culure. Deletion analysis results confirmed the isolates as M. *bovis* (absence of both regions). After MLVA genotyping, 2 distinctly different VNTR profiles were observed. Similarly two different spoligopatterns belonging to two different families were observed. A novel strain yet to be reported (in either cattle or buffalo at HiP) based on data submitted to International spoligotype database (Spol Db4) was identified in 3 of the 4 isolates.

This study shows that at least two M. *bovis* strains could be characterized in HiP, which means that there has been more than one introduction of BTB in this area. The study emphasizes the need for a more thorough molecular study in order to assess the origin of strains and the routes of transmission between different animal species.

Poster Presentation 50

LJUNGAN VIRUS PREVALENCE IN GERMAN WILD RODENTS

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Ljunganvirus (LV) is a member of the *Picornaviridae*. LV was isolated from various species of voles and lemmings in both Scandinavia and North America, i.e. *Myodes glareolus*, *Myodes rufocanus*, *Myodes gapperi*, *Microtus montanus*, *Microtus agrestis*, *Myopus schistoclor*, *Lemmus lemmus*, suggesting a wide host range and a world wide distribution of this virus. LV causes severe disease in its rodent reservoirs, such as diabetes and myocarditis. In addition, laboratory mice infected with LV suffer from encephalitis and fetal deaths indicating that LV might also induce this disease in the wild. A raised stress level could be shown as essential for disease outcome. It has been proposed that LV infection of rodents may affect their morbidity and mortality rates in the wild. It has further been hypothesised that the combination of disease caused by LV infection and a raised stress level is responsible for the decline of cyclic vole and lemming populations in northern latitudes.

The aim of this study is twofold: to (1) investigate the LV prevalence in German wild rodents; (2) to screen additional rodent species for the presence of LV.

In total, 390 animals belonging to eleven different rodent species were trapped in different urban and silvatic areas of Germany. The brains were taken (a pilot study showed that LV genome copies are most abundant in the brain), RNA was extracted and analysed by a LV specific RT-PCR. RT-PCR products of LV positive samples were subsequently sequenced to determine the LV genotype of the samples.

We analysed 42 of the 390 (10.8 %) animals positive for LV with a slight difference in gender distribution (females: 13,3 % positive; males: 9,6 % positive).

The following species were found positive for LV: bank vole (*Myodes glareolus*) (8 of 97 [8.2 %]), field vole (*Microtus agrestis*) (8 of 48 [16.7 %]), common vole (*Microtus arvalis*) (5 of 26 [19.2 %]), striped field mouse (*Apodemus agrarius*) (4 of 35 [11.4 %]), yellow necked mouse (*Apodemus flavicollis*) (12 of 87 [13.8 %]), long tailed field mouse (*Apodemus sylvaticus*) (1 of 17 [5.9 %)], harvest mouse (*Micromys minutus*) (1 of 2) and house mouse (*Mus musculus)* (1 of 1).

LV positive animals were found in the areas of Baden-Württemberg, Saxony-Anhalt, Brandenburg, Mecklenburg-Western Pomerania and the city of Cologne.

All four known LV strains were present in the different LV positive samples. The LV strain M1146 that was to date only isolated from voles in North America could be associated with Apodemus species.

Our data present a so far unknown picture of LV prevalence in wild rodents also in Central Europe. In addition to species from the *Arvicolinae* subfamily susceptible for LV we could also show that species from the *Murinae* subfamily are LV infected.

Poster Presentation 51

Student Presentation

RETROSPECTIVE ANALYSIS OF THE DEATHS OF THREE SIBERIAN TIGER (*PANTHERA TIGRIS ALTAICA*) CUBS IN ZAGREB ZOO

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Canine distemper is contagious, potentially lethal disease of mainly domestic and wild canids, but also of other mammals including large felids. During the February 2004 three Siberian tiger (*Panthera tigris altaica*) cubs at the age of six months have died in Zagreb Zoo. Animals were presented for necropsy with two day history of severe digestive disorders characterized mainly by haemathemesis. Dissection revealed catarrhal to pseudomembranous gastroenteritis (depending on animal) accompanied with haemorrhagic oedema of the lungs. Necrotic tonsillitis and disseminated depletion of lymphocytes were the most prominent histological findings in all examined animals, while intranuclear inclusion bodies were found in the tongue epithelium of one animal in question. Representative portions of liver, intestines, tonsil's and lymph nodes were submitted for bacteriological and mycological analysis. The presence of *Clostridium* spp., *Campylobacter coli* and *Escherichia coli* was detected in gut samples, coli-like bacteria were found in samples of liver, tonsil's and lymph nodes, while *Candida* sp. was found in the gut and pharynx samples. Toxicological analysis excluded poisoning as the cause of death. After two years, paraffin embedded samples of tiger organs were sent to the University of Bern for additional immunohistochemical analysis (IHC). IHC positive staining was obtained for canine distemper virus. Based on all, epizootiological, clinical and additional findings a canine distemper was recognized as the cause of death of respective animals.

Poster Presentation 52

PATHOLOGY OF POST-WEANING MULTI-SYSTEMIC WASTING SYNDROME IN WILD BOAR (*SUS SCROFA*) IN CROATIA

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Postweaning multisystemic wasting syndrome (PMWS) in the European wild boars was reported 2003 in Spain and Germany. In Croatia the first finding of the disease was reported in 2006. During last two years several piglets approximately 3 to 7 month of age were found dead in the continental region of Croatia. A complete necropsy was performed at the Pathology Department of the Croatian Veterinary Institute. The diagnosis of PMWS was established based on the three key diagnostistic criteria including clinical picture, specific pathomorphologic lesions and detection of the presence of PCV2 antigen in the lymphoid lesions by immunohistochemistry (IHC). The major macroscopical findings were emaciation, splenomegaly, hepatomegaly, generalised increase of lymph node size and catarrhal-purulent bronhopneumonia. Histologically moderate lymphocyte depletion and histiocytic infiltration, and multinucleate giant cells in lymphoid tissues (spleen and lymph nodes) were observed. PCV2 antigen in the follicular (macrophages and dendritic cells) and interfollicular (macrophages) areas of lymph node was detected by IHC.

Poster Presentation 53

SEROLOGICAL SURVEY OF SELECTED INFECTIOUS DISEASES IN MOUFLON (*OVIS ARIES MUSIMON*) FROM SOUTH CENTRAL SPAIN

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Introduced mouflon (*Ovis aries musimon*) populations are widespread throughout Europe, but little information on the prevalence and distribution of infectious diseases among mouflons in Spain is available. The objective of this serosurvey is to determine the seroprevalence of *Anaplasma* spp. *Brucella* spp., bovine viral diarrhea virus (BVDV), *Chlamydophila abortus*, *Coxiella burnetii*, *Mycobacterium avium* ssp *paratuberculosis* (MAP), and Maedi-Visna virus (MVV) in mouflons from south-central Spain. From July 2002 to January 2006, blood samples were obtained from 101 mouflons from three different study areas (Sierra Morena, Montes de Toledo and Guadiana basin) in south-central Spain (38º46'07" - 40º96'30"N, 3º23'15" - 4º55'47"W), and were tested for antibodies against the aforementioned pathogens. The mouflons came either from extensive farms or high ungulate density fenced hunting estates. Antibodies were detected against *Anaplasma* spp. (22.2%), *Coxiella burnetii* (4.0%), *Mycobacterium avium* subspec. *paratuberculosis* (1.0%) and *Chlamydophila abortus* (1.0%). All the serologically positive mouflons to *Coxiella burnetii* (4/55), *Chlamydophila abortus* (1/55) and MAP (1/55) came from the Montes de Toledo area. This suggests that tick-transmitted pathogens, as well as MAP, may be widespread and reach considerable prevalences in this species when circulating within a high density-population kept in private hunting estates. Conversely, no statistically significant differences were found in the prevalence of antibodies against *Anaplasma* spp. among the three study areas, and prevalence was also similar between farmed mouflons (2/12) and mouflons from hunting estates (18/78). To summarize, mouflon does not seem to play an important role in the epidemiology of several infectious diseases in south-central Spain, due either to its natural resistance and/or to its low and disseminated population in private hunting areas. However, it may play a role in the interspecific transmission of vector-borne (particularly tick-borne) diseases, like anaplasmosis or Q fever, which it may share with the more abundant native red deer and wild boar. Further research on other vector-borne diseases in mouflon, like blue tongue, ehrlichiosis, or other rickettsial diseases, would help to clarify the role of this species in the transmission of such diseases.

Poster Presentation 54

EWDA Best Student Presentation Award applicant

CADMIUM CONTAMINATION OF THE FOOD CHAIN IN A FOREST ECOSYSTEM

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The aim of this study was to evaluate the concentration of cadmium in forestry ecosystems.

The study was carried out in two areas: one was15 km from a very polluted area (Forest District C.M., county S.) and the other was in an area known to be unpolluted (Forest District C., county A.) and considered as "control".

Collected samples (seven from each kind of sample/area) consisted of water (from drinking water sources for wild animals), forest soil, from two different depths (20 and 40 cm respectively), snails and earthworms (biomarkers for heavy metal pollution), grass, trees (root, bark, leaves), some organs (kidneys, liver, heart, lungs, spleen, bone and lymphonodes) and muscles from wild boar (*Sus scrofa)* during the hunting period.

Cadmium concentration was determined by Atomic Absorption Spectrophotometer (AA 6650 Shimadzu), after digestion (by CHEM-MARSX 5 microwaves digester) in the Laboratory of Biochemistry, Nutrition and Toxicology in the Faculty of Veterinary Medicine Timisoara.

The data were statistically evaluated by Kruskal-Wallis and Mann-Withney tests.

Cadmium levels in the polluted areas as compared with the "control" areas, were as follows: in drinking water significantly higher ($p<0.001$) (+130.25%); in soil significantly higher ($p<0.001$) at both depths (+166% at 20 cm and +112.32% at 40 cm) but the differences were not statistically significant. Cadmium levels in biomarkers such as snails and earthworms were significantly higher ($p<0.001$) (by +116% and +176% respectively); fodders (grass, trees: root, bark, leaves) (+86.87%, +714.34%, +199.06%, +235%) significantly higher ($p<0.05$ for grass and $p<0.01$ for trees). For grass, roots, soil at 40 cm depth and bark in the "control" area cadmium levels were accordingly to the Romanian statutory acts (1 ppm/soil and 1.6 ppm/grass) within the limits permited.

The descending hierarchy of organs and tissues related to cadmium levels was: kidneys, liver, spleen, muscles, heart, lung, lymph nodes and bone in the "control" area and: kidneys, liver, spleen, lymphonodes, lungs, muscles, heart and bones in the polluted area. With the exception of the bones in the unpolluted area, in all organs and tissues cadmium levels exceeded the admitted limit. Cadmium levels in polluted area were significantly higher ($p<0.05$ / $p<0.01$) in comparison to the "control" area: in kidneys (+221.35%), lymph nodes (+250%) and bone (+250%), but not significantly higher ($p>0.05$), in liver (+33.82%) and spleen (+9.09%). In muscles and heart cadmium levels were higher in the "control" area than in the polluted area but the difference was not statistically significant ($p>0.05$).

Poster Presentation 55

EWDA Best Student Presentation Award applicant

HAEMATOLOGICAL AND BIOCHEMICAL INVESTIGATIONS IN FALLOW DEER (*DAMA DAMA*) FROM WESTERN ROMANIA

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Determination of haematological and biochemical blood parameters is important for diagnosis (positive/ negative diagnosis) medication and health status monitoring of wildlife.

The study was carried out on seven fallow deers does (*Dama dama*) from Forestry District C., county A. We determined the haematological and biochemical blood parameters during the hunting period. The does were healthy from a clinical point on view and were between 1 and 2 years old.

Blood samples were taken from the heart in vacuum tubes with EDTA K3. Haematological examination determined the total number of erythrocytes and leukocytes (E, L), haemoglobin (Hb) level, haematocrit (Ht), erythrocytes (mean erythrocyte volume VEM, mean erytrocyte haemoglobin concentration CHEM) and leukocytic series. Biochemical investigations determined: AST, ALP, ALT, GGT, Ca, Mg, total protein and albumin.

Haematological and biochemical analyses were carried out in the Laboratory of Internal Disease of the Faculty of Veterinary Medicine Timisoara using a Vet-Screen semi-automatic analyzer and leukocytic series was determined using the May-Grunwald-Giemsa method. The obtained results were statistically evaluated with Mann-Withney non parametrical tests.

Compared to the current literature data obtained (English and Lepherd, 1981; Zdravko et *al*., 2000; Vengust and Klinkon, 2002), the hemograme in fallow deer has shown decreased values of erythrocyte number (9,11 mil/mm^3) and mean haemoglobin concentration (16,59 g/dl). Thus regarding the values of haemoglobin (15,06 g/dl), haematocrit (45%) and leucograme (number of leukocytes, leukocytic series) there were no significant differences. Also, total protein values were higher and albumin values were within comparable limits.

Also compared with the data obtained from a study of fallow deer from the Islands of Brijuni (English and Lepherd, 1981), the values of AST were lower and the values of ALT were higher in the present study. Also, GGT activity was lower and calcium registered higher values comparative with data in the literature.

Poster Presentation 56

EWDA Best Student Presentation Award applicant

IMPORTANCE OF LYNX (*LYNX LYNX*) ATTACKS TO LIVESTOCK AS PERCEIVED BY INHABITANTS OF LYNX AREAS IN SLOVENIA AND CROATIA

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Eurasian lynx (*Lynx lynx*), similarly to other large carnivores can cause damage to human property by preying on sheep or goats. This controversial aspect of lynx ecology is one of the reasons why people sometimes oppose its conservation. Lynx population, shared by Slovenia and Croatia, was established following a reintroduction of 6 individuals to southern Slovenia in 1974. In both countries, lynx is listed as an endangered species which is strictly protected by law. Damages caused by lynx are compensated by the governments. The purpose of this paper is to evaluate perceptions of local inhabitants of lynx areas in Slovenia and Croatia about the issue of lynx damages to livestock. We have carried out a public attitude survey in the beginning of 2008. Questionnaire with paid return postage was sent to a randomly selected 1000 households in each country. Questionnaire was followed with a reminder/thank-you card 10 days later. Obtained response rates were 35.3% for Slovenia (SI) and 20.1% for Croatia (HR). We did not find any significant difference on respondents' demographic characteristics among the two countries. Average age of respondents was 52 years for Slovenia and 51 for Croatia. In both countries most of the respondents were male (68.6% in SI and 66.8% in HR). Sheep owners were 4.5% and 4.3% of respondents in Slovenia and Croatia, respectively. There were fewer goat owners (3.1% and 2.9% for SI and HR, respectively). Damage caused by lynx on their livestock experienced 1.1% and 1.0% of respondents in Slovenia and Croatia, respectively. Although majority of respondents in both groups agreed or strongly agreed to the item "It is necessary to conserve lynx population in SI/HR for the future generations", respondents from Croatia scored significantly higher on the item (Mann-Whitney U test, p=0.001), thus demonstrating more positive attitudes toward lynx. Few respondents (9.0% in SI and 11.0% in HR; p=0.863) expressed fear that lynx could cause them financial damage, however almost 20% of respondents from Slovenia and 15.9% from Croatia believed that sheep and goats are the main food for lynx (p=0.035). Approximately one third of respondents (SI: 35.2%; HR: 33.7%; p=0.500) from both countries agreed that damage-causing lynx should be shot. Large majority of all respondents (SI: 90.6%; HR: 97.2%; p=0.011) agreed that the damage caused by lynx should be compensated.

In conclusion, both Slovenian and even more so Croatian respondents were in favour of lynx conservation in their respective countries. Majority of respondents do not fear that lynx could cause them financial damage but relatively high percentage, especially in Slovenia falsely believes that sheep and goats are the main lynx food. The next step is to investigate whether and how do the beliefs about the species impacts to livestock relate to support for conservation of the species.

Poster Presentation 57

RED SHEEP TICK (*HAEMAPHYSALIS PUNCTATA*) IN SCANDINAVIAN MOOSE (*ALCES ALCES*)

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A 5-year old male moose was submitted to the National Veterinary Institute in 2007 for postmortem examination. The moose had been found being unresponsive, laying in a ditch on the same day. It was subsequently euthanized by a local hunter and immediately transported to the Institute for examination. The post-mortem examination did not reveal the cause of the condition in the moose. The moose was in a poor body condition, weighing 280 kg. Several ticks were located in the groins, the axillar regions, and on the head of the moose. Initially, the ticks were presumed to be *Ixodes ricinus*, a tick commonly found in cervids in Sweden. An entomological examination of 20 ticks revealed that they were all *Haemaphysalis punctata*, or the red sheep tick. This is the first report of this tick in moose. It has previously been identified in rodents and birds in Sweden. Being a common vector of several disease agents, such as *Borrelia burgdorferi*, and *Anaplasma phagocytophilum*, and considering the importance of cervids as hosts for ticks, the discovery of *Haemaphysalis punctata* in moose should facilitate an increased awareness and risk evaluation of ticks acting as reservoirs of zoonotic diseases in Scandinavia.

Poster Presentation 58

CHARACTERIZATION AND ANTIBIOTIC SENSITIVITY OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM COTTON-TAIL RABBITS (*SYLVILAGUS FLORIDANUS*) IN UMBRIA, ITALY

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Three hundred and sixty free-ranging cotton-tail rabbits (*Sylvilagus floridanus*), hunted in a game reserve in Umbria region, Italy, were sampled over a 3-month period. Each animal was submitted to necropsy and examined in order to assess their sanitary status. One hundred and ten animals were tested by culture in order to detect *Escherichia coli* in the caecum; 104/110 (94,5%) rabbits resulted positive. For every positive animal, one colony was further characterized. The

E. coli strains were biotyped, according to the Camguilhem and Milon scheme, and checked for antibiotics sensitivity according to the CLSI M31-A2 procedures. The following antimicrobials were tested: *Sulfamethoxazole+Trimethoprim, Flumequine, Cephalexin, Chloramphenicol, Cephtriaxone, Neomycin, Enrofloxacin, Apramycin, Amikacin, Ampicillin, Colistin, Amoxicillin+Clavulanic Acid, Streptomycin, Tetracycline, Nalidixic Acid, Kanamycin, Gentamicin*. Finally, the strains were investigated for the virulence genes coding for intimin and adhesive factors (eae, af/r1, af/r2) by a multiplex PCR.

Fourteen different biotypes were identified: B9 (1 out of 104, 0.96%), B16 (5/104, 4.8%), B17 (5/104, 4.8%), B18 (13/104, 12.5%), B19 (16/104, 15,4%), B20 (1/104, 0.96%), B24 (1/104, 0.96%), B25 (3/104, 2,9%), B26 (3/104, 2,9%), B27(42/104, 40.4%), B28 (2/104, 1.9%), B29 (1/104, 0.96%), B30 (4/104, 3.8%), B31(7/104, 6,7%). Most of the isolates showed large sensitivity to the antibiotics considered, low resistance levels were recorded for Neomycin (2,9%), Cephalexin (0.96%) and Kanamycin (0.96%). The results of molecular characterization showed that only 3 isolates out of 104 (2,8%) belonged to the patotype EPEC, for the presence of the *eae* gene, but all of them lacked the adhesive factors AF/R1 and AF/R2. All the EPEC isolates belonged to the B27 biotype. In cotton-tail rabbits, *E.coli* seems to be a normal component of the alimentary flora (Kozlowsky, Glantz, Anthony, 1976); since germs were collected from rabbits in healthy conditions without any signs of diarrhoea, it seemed quite predictable to isolate only a few numbers of EPEC strains.

Poster Presentation 59

BLOOD LIPIDS AND LIVER FATTY ACID COMPOSITION IN BROWN HARES (*LEPUS EUROPAEUS*) FROM TWO DIFFERENT CROATIAN HABITATS

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The purpose of the study was to examine the concentration of blood lipids and fatty acid composition of liver in autochthonous brown hares (*Lepus europaeus*) from two different ecological habitats in Croatia. The 31 blood and tissue samples were collected during the regular hunting operation in November at two different hunting grounds, island Vir (area 1) located in mid Dalmatia and Medimurje region (area 2) situated in the continental north west Croatia. Both areas are naturally inhabited with brown hares. The body weights (BW), the liver fatty acid composition, as well as plasma total cholesterol and triglycerides concentrations were determined during the same months of hunting period. The significant area differences were observed in body weight and fatty acid composition of liver tissue. The significantly higher BW (3,68 kg *vs.* 2,77 kg) and proportion of miristic (C14:0) and palmitic acid (C16:0) was determined in the liver of hares from area 2, whereas significantly higher content of margaric (C17:0) and stearic acid (C18:0) was observed in the liver of hares inhabited area 1. No area differences were observed in total plasma cholesterol and triglycerides concentration. The Spearman correlation coefficient revealed the positive correlation of total plasma cholesterol level and stearic and margaric acid ($r=0,94$; $p<0,05$), and negative correlation of total plasma cholesterol with palmitoleic acid ($r= - 0,83$; $p<0,05$) in livers of hares. The significant differences of liver fatty acids composition in hares from two different habitats may indicate the difference in fatty acid metabolism regarding the food niches they occupy, but also might have been indirectly related to population density and predation risk that should be further studied.

Poster Presentation 60

BREEDING DISTRIBUTION AND POPULATION SIZE OF THE GREY HERON (*ARDEA CINEREA*) IN SLOVENIA

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Grey Heron (*Ardea cinerea*) is a protected species under the national law in Slovenia. New data regarding the distribution of heronries and number of nests were provided by the national censuses which were carried out during the breeding seasons in the years 2006-2008. The locations of heronries were obtained from previous publications and field observation data of ornithologists, hunters and fishermen. The numbers of nests were counted using optical instruments (binoculars and telescopes). At one occasion, airplane was used as an alternative way of collecting data. We registered 4 single nests and 638 nests in 26 heronries. Most heronries were located in the central and north-eastern part of Slovenia. Our study showed that five colonies disappeared since 1992 and that the breeding population of Grey Heron in Slovenia was stable during in last three years (2006-2008). However, further systematic studies are needed to get more information about long-term changes and population status of this bird in Slovenia.

Poster Presentation 61

EWDA Best Student Presentation Award applicant

SEROLOGICAL SURVEY FOR INFECTIOUS ABORTIVE AGENTS IN FREE-RANGING ALPINE IBEX (*CAPRA IBEX IBEX*) IN SWITZERLAND

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In the past few years, a stagnation or decrease of Alpine ibex populations have been reported in some Swiss colonies. Since no abnormal mortality events have been observed in these colonies, the question was raised whether reproductive problems due to infections with abortive agents could influence the population dynamic. The aim of this study is to describe the serological status of Alpine ibex in selected colonies, and to assess whether infectious abortions might be the cause of the decrease of ibex populations.

Blood samples were collected from captured and shot animals in “problem colonies” and in selected “healthy colonies” (control) between 2006 and 2008. In one control colony, a biological study on the reproductive success of ibex was performed in parallel, thus allowing comparison of serological status and reproductive success of marked adult females. Until June 2008, 318 serum samples were collected (79 captured animals and 239 shot animals). To date, serological analyses were performed for about 140 ibex for *Brucella abortus*, *Coxiella burnetii*, *Leptospira interrogans*, and *Chlamydophila abortus*.

The large majority of the tested samples were negative. Animals with positive results originated from different colonies, both “problem” and control colonies. At individual level, 2 of 10 females did not give birth to a kid either in 2006 or in 2007. They were both seronegative for the tested bacteria.

Preliminary results suggest that infections with the considered abortive agents are not widespread in the studied Alpine ibex populations, and that ibex from “problem colonies” do not seem to be more often infected than those from the control colonies. Samples will also be tested for other abortive agents such as *Salmonella* Abortusovis, ruminant Pestivirus, Herpesvirus, *Toxoplasma gondii*, and *Neospora caninum*. Furthermore, the collection of additional samples during the 2008 hunting season will increase the sample size and allow better interpretation of the results.

Poster Presentation 62

EWDA Best Student Presentation Award applicant

CONSIDERING THE RED DEER (*CERVUS ELAPHUS*) AS A POTENTIAL DEFINITIVE HOST AND RESERVOIR OF LIVER FLUKES (*FASCIOLA HEPATICA*, AND *DICROCOELIUM DENDRITICUM*) IN NORTHWEST SPAIN

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In the north of Spain there are a lot of natural protected areas where wild and domestic animals coexist. They usually share parasites and other infectious agents and sometimes these animals can act as an important reservoir complicating sanitary control. Several studies have investigated nematode and cestode infestations in wildlife in Spain although information about trematodosis in red deer is scarce. In this study we investigate the existence, importance and distribution of liver flukes (*Fasciola hepatica*, *Dicrocoelium dendriticum*) in red deer (*Cervus elaphus*) from the Regional Hunting Reserve of RiaNo (78.000 ha), which is situated in the Cantabrian mountains in the northwest of Spain (Leon province). A total of 110 animals were sampled from March 2005 to March 2007. During the necropsy, the liver was removed and faecal samples were collected directly from the rectum to investigate the relationship between faecal egg counts and liver parasite burden. The big bile ducts were opened longitudinally in order to recover flukes of *Fasciola hepatica* and after that the parenchyma was cut and pressed down to collect *Dicrocoelium dendriticum*. Faeces (5 g from each animal) were processed by means of sedimentation method using McMaster chambers to determinate egg counts (expressed in eggs per gram of faeces, epg). For histopathology, liver lesions were fixed in 10 % neutral buffered formalin and stained by haematoxylin and eosin technique.

61 (55.50 %) animals were parasited by liver flukes. Adults of *Dicrocoelium dendriticum* were found by liver examination with a prevalence of 50 % and, the total number of flukes recovered in each deer at necropsy varied between 1 and 700 (mean, 46.00 ± 76.26). The prevalence and mean intensity of *Fasciola hepatica* was 12.70 % and 2.43 ± 0.97 (1-6) respectively. *D.dendriticum* and *F.hepatica* eggs were found only in 5 (4.50 %) and 6 (5.50 %) animals respectively. Several macroscopic and microscopic hepatic lesions were found in some animals, which were compatible with these parasitic diseases.

These preliminary results showing high prevalence of adult worms in red deer might be related to the concurrence of three factors: 1) environmental conditions favouring the presence of intermediate hosts, 2) the presence of egg-shedding domestic animals and, 3) the presence of abundant receptive wild red deers. Nevertheless, red deer can not yet be considered as an adequate definitive host in this region. This statement is based on the following facts: 1) the low rate of egg elimination, despite the presence of adult worms which were sexually mature and 2) the significant histopathological lesions in some animals even with low parasite burdens.

However, the red deer could become an important definitive host and reservoir of these parasites in the future, posing a risk for transmission among wildlife and livestock coexisting in this area.

Poster Presentation 63

SEROLOGICAL MONITORING OF MOUFLON (*OVIS ORIENTALIS MUSIMON*) IN THE ARCIPELAGO TOSCANO NATIONAL PARK, ITALY

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Mouflon (*Ovis orientalis musimon*) is present in many preserve and natural areas in Central Italy, where the population size is estimated at about 7,500 heads. This species, native to Sardinia and Corsica, is actually distributed all over Europe (about 60,000 animals) where, like in the Arcipelago Toscano National Park, it has been released in the past. In our study we analyzed samples of sera collected from 77 mouflons harvested in The Arcipelago Toscano National Park (Isola d'Elba), Italy.

Considering that, after introduction in the '70, the population remained isolated from other wild and domestic ruminants, we deemed it important to monitor this population for selected pathogens which represent a risk also for livestock. Sera were screened for the presence of antibodies against Bluetongue virus, Visna-Maedi virus, *Toxoplasma gondii, Neospora caninum* and *Brucella abortus/melitensis*.Antibodies against Bluetongue virus (BTV) were searched by a competitive enzyme-linked immunosorbent assay (kit produced by Istituto Zooprofilattico Sperimentale "G. Caporale", Teramo-Italy). Indirect enzyme-linked immunosorbent assays were used to detect antibodies against Visna-Maedi virus (Istitut Pourquier, Montpellier-France), *T. gondii* (Istitut Pourquier, Montpellier-France) and *N. caninum* (IDEXX Laboratories Westbrook, Maine-USA). Sera were screened for *B. abortus/melitensis* with a Complement Fixation assay. All animals resulted seronegative for Bluetongue virus, Visna-Maedi virus, *N. caninum* and *B. abortus/melitensis*. On the contrary antibodies against *T. gondii* were detected in 22% animals (17/77). The present serosurvey suggests an elevate exposure risk to *T. gondii* in the Isola d'Elba mouflons. This may be due to presence of stray cats in many parts of the island, because no other definitive host of this parasite is present in the area. All the other pathogen tested negative and this suggest that in absence of reintroduction/contact with livestock the health status of the population of the island is not at risk.

Due to the number of samples analyse the present results should be considered as preliminary, and more data are needed, also in comparison with other areas, to better define the epidemiological situation of the mouflon population.

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Poster Presentation 64

RESULTS OF A SURVEY FOR CHRONIC WASTING DISEASE IN ITALIAN CERVIDS

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Chronic Wasting Disease (CWD) is a Transmissible Spongiform Encephalopathy (TSE) of mule deer (*Odocoileus hemiosus*), white tailed deer (*Odocoileus virginianus*), Rocky Mountain elk (*Cervus elaphus nelsoni*) and moose (*Alces alces shirasi*), which has been firstly recognized in 1967 in a captive mule deer in Colorado (U.S.A.) as fatal syndrome. To date, CWD has been detected only in cervids from North America where it is well established as an endemic form. CWD is the only TSE known to affect free-ranging wildlife species. The origin of the disease is still unknown and the evidence of the circulation of TSEs in European population of wildlife ungulates does not exist. According to EU Commission decision of 19 March 2007 (2007/182/CE), active surveillance of CWD in cervids became mandatory in EU Member States which should have completed their survey by means of rapid tests within the end of the 2007 hunting season. In accordance with the opinion of the Standing Committee on the Food Chain and Animal Health, this Decision laid down rules for the surveillance programmes to detect CWD in wild and farmed *Cervus elaphus* and/or *Odocoileus virginianus*, which were identified as target species in Europe.

Italy has a free-ranging population of *Cervus elaphus* of about 44,000 heads, whereas farmed animals are very few; *Odocoileus virginianus* is not present. The largest part of this population is distributed in Northern and Central Italy, so the Italian Ministry of Health split the samples among the Regions with congruous target species population to fulfill the required sample size. In accordance with the European Commission decision, on August 2006 the Italian Ministry of Health issued a dedicated regulation in which a CWD surveillance plan was made mandatory.

In this study we present the results of Italian mandatory surveillance programme. According to our legislation, we would have to test 600 *Cervus elaphus* including clinical, road-killed, fallen or healthy animals, older than 18 months. As a matter of fact we tested an overall number of 739 heads by means of Bio-Rad TeSeE test (BIO-RAD, Marnes la Coquette France). On the basis of reliable statistical analysis, the sample size guarantees the detection of the disease, if present, with a prevalence ≥ 0.5% and confidence limit of 95% as advised by the "Report of the EFSA working group on a surveillance program for Chronic Wasting Disease (CWD) in the EU" of 2004. To enhance the sensitivity of the surveillance system, we tested both the obex region of medulla oblongata and the medial retropharyngeal lymph nodes. All samples tested resulted negative on both tissues. Our data show that CWD does not affect the Italian cervid population, or that, in the worst case scenario, if the infection is present it is expected to reach a maximal possible prevalence of 0.4 %.

Poster Presentation 65

LEPTOSPIROSIS IN WILD AND DOMESTIC CARNIVORES IN ANDALUSIA, SPAIN

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Leptospirosis is a zoonosis that affects humans, domestic animals, and wildlife. Carnivores are at the top of the feeding chain, thus being exposed to pathogens through their preys. From June 2004 to April 2007, we analyzed for evidences of contact with 14 serovars of *Leptospira interrogans* serum and urine or kidney samples collected from 201 wild and domestic carnivores, including 26 free-living Iberian lynx (*Lynx pardinus*), 33 red foxes (*Vulpes vulpes*), 33 Egyptian mongooses (*Herpestes ichneumon*), 25 common genets (*Genetta genetta*), two Eurasian badgers (*Meles meles*) and one Eurasian otter (*Lutra lutra*), and 53 free-roaming cats and 28 dogs in protected areas in Andalusia (southern Spain). Twenty-three percent of the animals presented evidences of contact, being the prevalence similar among wild (23.5%) and domestic species (22.2%). Contact with *L. interrogans* was detected in all the species but the otter. Prevalence was: lynx (11% by direct detection, 32% by serology), fox (0%, 47%), mongoose (5%, 20%), genet (0%, 12%), badger (0%, 50%), cat (20%, 14%), dog (only serology: 36%). Serovar Icterohemorragiae accounted for 2/3 of the cases. Serovar Canicola was detected in half of the positive dogs and one lynx. Other serovars detected were Ballum, Sejroe and Australis. No macroscopic lesions were observed in necropsied animals that showed evidence of contact with the agent, although histopathologic lesions (chiefly chronic interstitial nephritis) were observed in 7 out of the 11 microscopically analyzed individuals. Thus, *L. interrogans* may cause previously unrecorded disease in wild carnivores in Spain. Wild and free-roaming carnivores may not act as reservoir of *L. interrogans* but as dead-end hosts, though the dog may act as reservoir of serovar Canicola. Carnivores are apparently good sentinels for the epidemiological monitorization of leptospirosis.

Poster Presentation 66

SEROPREVALENCE OF FELINE VIRUSES IN FREE-LIVING EUROPEAN WILDCATS (*FELIS SILVESTRIS*) IN SPAIN

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Twenty-five serum samples of 22 free-living wildcats (*Felis silvestris*) captured from 1991 to 1993 in central Spain were tested for evidences of exposure to seven feline pathogens. All the wildcats but one (95.4%) presented evidences of contact with any of the agents (mean = 2.2). Evidences of contact with feline leukaemia virus (FeLV) were detected in 81% of the wildcats (antibodies: 77%; antigen p27: 15%). Antibodies to feline calicivirus (FCV, 80%), feline herpesvirus (FHV, 20%), feline parvovirus (FPV, 18%), and *Chlamydophila* sp. (27%) were also detected. Analyses were negative for feline immunodeficiency virus and feline coronavirus. The probability of having antibodies to FPV had an inverse relationship with the concentration of serum cholesterol and with a morphometric index of body condition. Similarity in the composition of antibodies against disease agents (number and identity of detected and undetected antibodies) was significantly higher in pairs of female wildcats than in pairs of males or heterosexual pairs, suggesting that females had a more homogeneous exposure to pathogens. Seroprevalence for FHV was higher in males than in females. Antibodies to FHV and *Chlamydophila* sp. were more frequent in winter than in other seasons. In addition, the mean similarity of the pathogen community between pairs of serum samples was higher if both wildcats were caught during the same season than if they were not. Mean similarity was lowest if serum samples obtained in winter were compared with those from spring or summer. The results suggest that some agents probably had it reservoir in domestic cats and may cause some undetected morbidity/mortality in the studied wildcat population, whereas others, such as FeLV and FCV, were apparently enzootic.

Poster Presentation 67

EWDA Best Student Presentation Award applicant

LEPTOSPIROSIS IN ENDANGERED EUROPEAN MINK (*MUSTELA LUTREOLA*) AND SYMPATRIC MUSTELIDAE AND VIVERRIDAE IN SOUTHWESTERN FRANCE

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The European mink (*Mustela lutreola*) is a highly endangered species, especially in South-Western France. Leptospirosis is known to be severe in some domestic carnivores, but the prevalence and significance of the infection in free-ranging carnivores and the potential epidemiologic role of these predators of rodents are largely unknown.

To better understand the possible role of disease in the decline of the European mink, a survey of seroprevalence and renal carriage of pathogenic leptospires was conducted between 1990 and 2007 on free-ranging Mustelids and Viverrids, and farmed American mink of south-western France.

Four hundred and twenty four samples of sera originating from 79 genets (*Genetta genetta*), 19 stone martens (*Martes foina*), 19 pine martens (*M. martes*), 99 European mink, 133 polecats (*M. putorius*), 74 American mink (*Mustela vison*) and 51 farmed American mink, were submitted to a microagglutination test (MAT) and six different serogroups were tested (Australis, Autumnalis, Icterohaemorrhagiae, Grippotyphosa, Panama, Sejroe). In addition, kidneys from 85 animals (34 European mink, 33 American mink and 18 polecats) found dead or euthanased were submitted to a PCR test specific for pathogenic leptospire DNA.

Genets (35%) and farmed American mink (31%) had significantly lower seroprevalences than all other species (90% in stone martens, 87% in free-ranging American mink, 74% in European mink and pine martens, and 65% in polecat) and American mink had a significantly higher seroprevalence than other *Mustela* species. Australis was the dominant serogroup in all the free-ranging species but Icterohaemorragiae serogroup was also of high prevalence. European mink had a significantly higher seroprevalence of the Grippotyphosa serogroup than the other species. The renal carriage rate was much lower than seroprevalence (20%) and not significantly different between tested animals.

To our knowledge this study represents the first description of leptospiral infection in free-ranging Mustelids and Viverrids in Western Europe. Results are consistent with other studies on free-ranging striped skunk (*Mephitis mephitis*) in northern America, in mongoose (*Herpestes auropunctatus*) in the Carribean and farmed mink and polecats in Argentina. Very high seroprevalences observed in the free-ranging mustelids could be related to habitat use, diet and the presence of known reservoirs in the study area (i.e. coypu (*Myocastor coypu*) and muskrat (*Ondatra zibethicus*)). Renal carriage shows that these Musterlids can shed leptospires at least for a short while, but further investigations regarding how long this shedding lasts are necessary to determine precisely their possible epidemiologic role.

Further studies on the pathological effects of infection in these hosts are needed to assess the potential impact on their demography.

Poster Presentation 68

INDICES OF WELFARE AND HEALTH IN FARMED WILD BOAR (*SUS SCROFA*)

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The farming of wild ungulates for alternative meat production in recent years has become increasingly popular in the Italian agriculture. Wild boar outdoor farming in extended fenced areas is being successfully and profitably run in many regions as long as demand for alternative meat consumption.

Unfavourable environmental conditions can influence the animal welfare and several homeostatic functions, like some immune functions and in particular the non-adaptive immune system. Changes in the immune system, especially non-specific immunity which is the first line of defence of the organism, are markers of a degree of chronic stress response and are predictive of the animal exposure or susceptibility to pathogens. The aim of this work is the evaluation of different reaction of some no specific immunity parameters: haemolytic complement activity, serum lysozyme and serum bactericidal activity in different environmental situation.

We studied two groups of wild boars between January and December 2005 to test non-specific immunity parameters like lysozyme, bactericidal activity and complement to evaluate the influence of the rearing environment and the season.
The first group was formed by a breed of wild boars that are free moving in a 10 hectares fenced area characterized by typical Mediterranean vegetation which is the natural environment for free ranging wild boars in our country. Animals are given supplementary feeding in winter. For the purpose of this study sampling was done on 30 animals during winter and on 30 animals during spring slaughtering. All sampled animals were between 1 and 2 years of age.
The second group was of free ranging wild boars, captured during selective capture programmes in winter or spring. Captured animals, ageing between 1 and 3 years, as determined by hair colour and dentition, were kept in individual boxes for three days before slaughtering, they were apparently healthy and body weight was within normal range. No evidence of pathological lesions was recorded at the P.M. standard inspection procedure. 30 animals were sampled during the winter slaughtering and 30 during the spring slaughtering cycle.
Lysozyme activity resulted significantly higher then normal in winter season in both groups. Bactericidal activity was found below the normal in both wild boars during the winter season. Complement seemed to be less affected by seasonal changes and only in the wild boars.
In the spring season all groups investigated showed parameters within the normal range.
The investigation results provide clear indication that values for lysozyme, bactericidal activity and complement vary according to the winter or spring season when two groups are tested. Instead there isn't important difference between breed wild boars and free wild boars.
Thermal discomfort (cold) compromises their innate immunity status, making them much more susceptible to other stressors or specific disease.

Poster Presentation 69

SERUM BIOCHEMISTRY VALUES OF FALLOW DEER (*DAMA DAMA*) IN CENTRAL ITALY

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Fallow deer is widely spread in the centre of Italy and there is an increasing interest in the farming of this animal both for alimentary use that for hunting. The knowledge of blood biochemical reference values is an important requirement for veterinary health surveillance programmes similar to other livestock species. This study was conducted from November 2005 to March 2006 on thirty-four fallow deer witch were caught in control programmes and other forty-seven bred in a half extensive herds. The animals were clinically healthy and aged between one and three years; they were kept in individual boxes for three days before slaughtering. At slaughter blood was collected in heparinised tubes. Tubes were centrifuged at 2000 rpm for fifteen minutes and collected plasma was kept at -80°C until tested. Glucose, urea, total serum protein (TP), albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), creatinine, calcium, chloride, phosphorus, magnesium, were measuring using Hitachi 704.
Observed average value (means and ± s.d.) are: urea (mmol/L 6.58 ±3.18), TP (g/L 50.43 ± 14.52), albumin (g/L 33.12±4.94), AST (U/L 113.15 ±67.55), ALT (U/L 29.72 ± 14.03), GGT (U/L 37.84 ± 26.25), creatinine (µmol/L 118.31 ±32), calcium (mmol/L2.49 ± 0.80), chloride (mmol/L 105.83 ±9.98), phosphorus (mmol/L 3.90 ± 1.27), magnesium (mmol/L 1.49 ± 0.56).
Biochemical values (means and ± s.d.) of all fallow deer divided into male and female are significantly different (P< 0.05) for urea (♀7.56 ± 3.70, ♂ 5.97 ± 2.65) and creatinine (♀126.86 ± 27.97, ♂ 112.02 ± 33.46)
Biochemical values of all fallow deer divided into young (≤ 1) and old (> 1) are significantly different (P< 0.001) for urea (young 8.33 ± 4, old 5.98 ± 2.6), while GGT (young 27 ± 14.25, old 41.53 ± 28.42) and magnesium (young 1.22 ± 0.44, old 1.59 ± 0.57) have P value <0.05.
Serum AST and ALT activity in this study is lower than the previously reported values by Gorazd (2002) and English (1981). In our study also serum creatinine value is lower than concentration reported by Gorazd (2002). These differences can be due to many factors like environment, nutrition, physiology, health status and genetic. Besides blood sampling and the way to capture can have an impact on the results.
The results presented in this work, in association with values obtained in parallel studies, can provide references values for a wide range of blood biochemical parameters for fallow deer living in our areas.

Poster Presentation 70

REASONS FOR THE PRESENTATION OF BADGER (*MELES MELES*) CASUALTIES TO A VETERINARY HOSPITAL AND OUTCOMES FOLLOWING TREATMENT

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One hundred and forty three adult badger (*Meles meles*) casualties were presented to Quantock Veterinary Hospital (QVH) in South West England, over the period November 2002-July 2006. Many of the casualties came via Secret World Wildlife Rescue (SWWR). Animals considered suitable for rehabilitation were cared for by SWWR following veterinary treatment. All animals were presented members of the general public who provided information in 113 cases as to where the animals were found and their main presenting signs. 40/113 (35%) of casualties were found at the roadside, 23/113 (20%) found in domestic gardens and 19/113 (17%) found in domestic buildings and 19/113 (17%) in farm buildings. 29/113 (26%) were described as 'paraplegic' or 'dragging their legs', 18/113 (16%) had wounds as their main presenting sign, 14/113 (12%) were presumed Road Traffic Accidents (RTA), 13/113 (12%) were found to be in the wrong place in absence of clinical signs, 11/113 (10%) were found recumbent or collapsed. Most of the casualties were presented in March 24/123 (20%) and April 11/123 (9%), with a secondary peak in August 11/123 (9%) and September 11/123 (9%). Where the sex of animals was recorded (110), animals were equally divided between the sexes (55/110). 115 animals had records made regarding the presence of bite wounding; 65/115 (57%) had wounds. 33/65 (51%) of wounds were at multiple sites, most commonly involving the rump, head and neck. 27/65 (42%) of wounded badgers had other concurrent injuries. When it was recorded where the animals were found, animals with bite wounding came more frequently from gardens or buildings 41/58 (71%) than the road side 12/58 (21%). Overall 12/40 (30%) of roadside badgers had wounds compared with 41/61 (67%) of badgers from gardens and buildings. Badgers were clinically examined under sedation using medetomidine and ketamine hydrochloride. Clinical tests (bloods, radiographs, ultrasound) were carried out as medically required and clinical decisions made based upon these findings and an assessment of the animals' likely survival and eventual release back to the wild. Outcome options were euthanasia, rehabilitation at SWWR, hospitalisation at QVH or immediate release. Within the first 24hrs of presentation 48/123 (39%) animals were euthanized, 44/123 (36%) were transferred for rehabilitation, 19/123 (15%) required further hospitalisation, 11/123 (9%) died and 1/123 (1%) was released. Of those animals surviving the first 24hrs (where recorded) 11/59 (19%) were euthanized, 6/59 (10%) died and 42/59 (71%) were released. 5/40 (13%) found at the roadside and 33/61 (54%) of those found in gardens or buildings were released. Of the badgers with bite wounds, 6/65 (9%) with wounds and concurrent problems were released compared with 25/65 (38%) with evidence of wounding only. Time in captivity for all animals eventually released ranged from 1 to 49 days with a median of 17 days.

Poster Presentation 71

A SURVEY FOR DISEASES IN YELLOW-LEGGED GULLS (*LARUS MICHAHELLIS*) FEEDING AT MAJORCA GARBAGE DUMP, SPAIN

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The yellow-legged gull (*Larus michahellis*) has experienced a marked population growth in recent times in Majorca Island, mainly due to the overabundance of human-derived resources. In 2003, about 15000 gulls were counted feeding at the garbage dump of Majorca. In that year, the insular government established a control program of gulls by means of shooting and the use of falconry raptors. From 2003 to 2007, 23968 gulls have been hunted. Of them, 900 (754 shot, 146 preyed by raptors) were selected by random and subjected to necropsy and culture in order to detect infections by *Aspergillus* sp. and *Salmonella* sp. The presence of helminths was also recorded. Lesions of aspergillosis (confirmed by culture) were detected in 36 gulls (4%). No lesions of salmonellosis were observed, but *Salmonella*-positive cultures were obtained from 64 gulls (7%), suggesting that gulls act as asymptomatic carriers. Only one gull presented a mixed infection with *Aspergillus* and *Salmonella*. Helminths were detected in 423 gulls (47.0%): 41.5% were parasitized by cestodes, 1.9% by nematodes, and 3.5% presented mixed parasitizations.

Interestingly, 30.4% of the preyed gulls presented lesions of aspergillosis, whereas only 0.26% of the shot gulls did ($p<0.001$). In other words, gulls presenting lesions of aspergillosis were more frequently killed by raptors (94.4%) than by shot (5.6%). No significant differences were detected in the case of *Salmonella*-infected or helminth-parasitized gulls.

Results suggest that a selective predation of raptors on diseased gulls took place. Aspergillosis may impair the flying capability of infected gulls, affecting predation avoidance.

Poster Presentation 72

MONITORING WILD BIRDS FOR AVIAN INFLUENZA IN NORTH-WESTERN ITALY (2005-2008)

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Wild birds, in particular those living in damp areas are considered a main reservoir of avian flu viruses. In fact they are able to carry the virus asintomatically and it has been shown as they are responsible for introduction of influenza virus among poultry birds along migratory routes.
Since 2005 the European Community has required the Member States for preparing avian influenza surveillance plans on wild birds in order to verify the circulation of the viral strain and to recognize the presence of the subtypes H5 and H7. These subtypes could determinate Low Pathogenicity Avian Influenza, but low pathogenic viruses can undergo mutations or re-assort with viruses in the domestic birds populations until an HPAI virus arises, that causes High Pathogenicity Avian Influenza (HPAI).
The monitoring program carried out in Italy consists of active monitoring on live or hunted birds (cloacal swabs or serum are sampled) and passive monitoring (organs from dead birds are picked up). Samples were collected in North Western Italy from Liguria, Piemonte and Valle d'Aosta regions during the period 2005-2008. The diagnostic tests used are: haemoagglutination inhibition test and ELISA to find antibody anti-influenza; virus isolation on embryonated eggs and RT-PCR to find virus.
Starting from 2005 sample analysed were 1696, 287 coming from Liguria, 25 from Valle d'Aosta and the remaining from Piemonte. Only during 2007 positive samples were found in Liguria, belonging to H7, H10N1 and H3N8 serotypes.
It is important to continue a constant monitoring and data collection on wild birds to prevent any spreading of H5N1 and to predict future outbreaks.

Poster Presentation 73

TISSUE RESPONSES ASSOCIATED WITH ABOMASAL PARASITES IN ROE DEER (*CAPREOLUS CAPREOLUS*)

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The real impact of helminthofauna in wild ruminants is controversial and the ecopathology rarely considers the anatomopathological data. This work describes the abomasal anatomopathological findings in infested roe deer (*Capreolus capreolus capreolus*) and attempts to compare the lesions with the abomasal parasite infrapopulation.
Fifty-one roe deer shot during the period 2004-2007 in the Julian Prealps game reserves, were investigated. Each abomasum (N=44) was opened along the *Curvatura major* and the contents collected for parasitological identification. After macroscopic examination, abomasal standard samples of *Fundus abomasi* and *Pars pylorica* were collected and fixed in 10% buffered formalin for anatomopathological description. From each abomasal standard sample three perpendicular sections to *Plicae gastricae* were obtained. The samples were processed by standard lab techniques. Microscopic evaluations, based on specific scored parameters for the fundic and antral mucosa, were used. The final score is expressed by a number ranging from 1 to 30 for each abomasum.
Prevalence (P), mean abundance (A) and relative abundance (RA) of each parasite species were calculated. From this roe deer population, 53438 nematodes were collected and identified. Fifteen helminth species (an average of six species/animal) were recovered. For the aims of this study data from pairs of recognised polymophic species were analysed together: *Spiculopteragia spiculoptera/mathevossiani* (P=100; A=324; RA=30,9) and *Ostertagia leptospicularis/kolchida* (P=100; A=360; RA=34,3) and they were the abomasal dominant species. Amongst the generalist species, *Haemonchus contortus* showed a high prevalence and abundance (P=74.5; A=294; RA=28).
The fundic inflammatory patterns were: hyperplastic-catarrhal (24%), eosinophilic (20%), superficial simple (18%), deep simple (18%), edematous (11%) and atrophic (7%), in "pure" or "complex" form. The pyloric inflammatory

patterns were: hyperplastic-catarrhal (39%), superficial simple (39%), deep simple (10%), eosinophilic (5%) and atrophic (2%), in "pure" or "complex" form. Lesions referable to the different abomasitis were found in 98% of animals (43/44). Severe lesions (score >15) were observed in 9% of abomasa, appreciable lesions (score ranging from 7 to 15) in 70% and slight lesions (score < 7) in 21%. Fundic lesions were more serious than antral. The frequency of hyperplastic-catarrhal abomasitis confirms the pathogenic role of the abomasal parasites in roe deer, as already described in domestic ruminants.

The abomasal polyparasitism is a normal condition in wild ruminants and therefore it is very complex to discriminate the pathogenicity of the different parasite species. Neverthless, some interesting relationships between abomasitis and parasite species are discussed.

Poster Presentation 74

BLUETONGUE VIRUS IN WILD RUMINANTS: A SEROLOGICAL SURVEY IN PIEDMONT, ITALY

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Bluetongue is caused by an orbivirus of the family Reoviridae. It infects most domestic and wild ruminants, and it is transmitted by arthropods of the genus Culicoides and actually 24 serotypes are described for the bluetongue virus (BTV) serogroup.

Since 1998, Bluetongue was signalled in Southern Europe where 5 serotypes (1, 2, 4, 9 and 16) have been until now described in domestic ruminants. In 2006 a strain of serotype 8 has been reported also in Central Europe and this suggests that the virus can become widespread in many part of Europe.

In Italy BTV was firstly described in August 2000 in Sardinia Island and, since then, the infection, due to serotypes 2, 9, 4 and 16, spread northward involving many regions of the mainland. Since 2000, Bluetongue surveillance measures (clinical, entomological and serological) were established in order to monitoring the presence of vectors, to determine the geographical distribution of the infection and the prevalence in sheep and cattle populations and for the first time in 2008 BTV serotype 8 was found in three cattle herds in the north-east of Italy. Wild ruminants are considered a potential reservoir for BTV and it has been reported in white-tailed deer (*Odocoileus virginianus*) and pronghorn (*Antilocapra americana*) in the USA. In Germany, a study carried out in 2005/06 reported a seroprevalence of 0.09%, 5.7% and 4.9% in red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and mouflon (*Ovis musimon*) respectively. In Belgium, in 2006, out of 684 hunter-killed deer seroprevalence was of 0.58%. A recent study from Spain reports BT seroprevalence in 21.9% of red deer, 35.4% of fallow deer, 5.1% of roe deer, 13.2% of mouflons and 25% barbary sheep (*Ammotragus lervia*).

The aim of this study is to make a preliminary screening for the presence of BTV-antibodies in wild ruminants sera from Piedmont (North West Italy).

During the period 2006-2008 we collected 1083 samples of sera, 589 roe deer, 339 chamois (*Rupicapra rupicapra*), 113 red deer, 37 fallow deer (*Dama dama*), 3 mouflons and 2 alpine ibex (*Capra ibex*), from different areas of Piedmont. Sera were tested by a competitive ELISA kit produced by Istituto Zooprofilattico Sperimentale "G. Caporale", Teramo-Italy (Italian Reference Center for BTV).

All animals tested seronegative, confirm the absence, also in livestock, of BTV in the area. However the survey must continue in order to evaluate the epidemiological pattern of the infection in the case of BTV introduction in the area and to evaluate the possible role of wildlife, especially cervids, as sentinels for BTV surveillance.

Poster Presentation 75
EWDA Best Student Presentation Award applicant

POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN THE BLACK SEA DOLPHINS (*PHOCAENA PHOCAENA* AND *DELPHINUS DELPHIS*) AND IN THE FOOD WEB (SEDIMENT-FISH-DOLPHINS)

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The aim of the present study was to evaluate levels of contamination with polycyclic aromatic hydrocarbons (PAHs) in dolphins and other components of the food web (sediment-fish-dolphins) in the Romanian Black Sea.

Samples from sediment, fish (*Sprattus sprattus*) and dolphins (*Phocaena phocaena* and *Delphinus delphis*) were analysed. Dolphins liver samples were taken from dead stranded cetaceans. The samples were analyzed by gas chromatography. Mean levels found in sediment were: Naphthalene 146,4 ng/g; Acenaphthilene 6,4 ng/g; Acenaphthene 13,9 ng/g; Fluorene 29,2 ng/g; Phenanthrene 21,7 ng/g; Anthracene 22,7 ng/g; Fluoranthene 21,1 ng/g; Pyrene 27,2 ng/g; Benz[a]anthracene 1,6 ng/g; Chrysene 1,2 ng/g.

Mean levels in Black Sea fish samples were: Naphthalene 13,3 ng/g; Acenaphthilene 27,1 ng/g; Acenaphthene 30,8 ng/g; Fluorene 0 ng/g; Phenanthrene 50,7 ng/g; Anthracene 61 ng/g; Fluoranthene 19,2 ng/g; Pyrene 18,6 ng/g; Benz[a]anthracene 73,1 ng/g; Chrysene 89,7 ng/g.

Dolphins mean values were: Naphthalene 1171,6 ng/g; Acenaphthilene 20 ng/g; Acenaphthene 21,4 ng/g; Fluorene 5,3 ng/g; Phenanthrene 57,4 ng/g; Anthracene 30,4 ng/g; Fluoranthene 11,3 ng/g; Pyrene 11,6 ng/g; Benz[a]anthracene 79,8 ng/g; Chrysene 111,8 ng/g.

There was no evidence of biomagnification of PAHs in the sediment. Even though there are no published data for contamination levels in dolphin liver samples we were able to make a comparison with available data on contamination in muscle. The results of this study indicate very high levels of PAHs in dolphins from the Black Sea: Naphthalene 1171,6 ng/g compared with 0,7 -16 ng/g; Phenanthrene 57,4 ng/g compared with 0,8 -5 ng/g; Anthracene 30,4 ng/g compared with 0,1 -3,6 ng/g; Fluoranthene 11,3 ng/g compared with 0,5 -6 ng/g; Pyrene 11,6 ng/g compared with 0,5 - 7,2 ng/g; Chrysene 111,8 ng/g compared with 0,11 -0,56 ng/g in the reference literature. We suggest that PAHs are likely to cause chemical stress and health problems in the cetaceans of this basin.

Poster Presentation 76

PATHOGEN SURVEILLANCE IN WILD BIRDS IN ZAGREB

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Wild birds can carry a wide range of viral, bacterial, fungal and protozoan zoonotic agents, which may be transmitted to humans. Many of them cover long distances during annual migrations, and can potentially play a role in the epidemiology of zoonoses.

In the locations around City of Zagreb during 2004 and 2006, 73 and 273 wild birds of different species were captured, respectively, and pharyngeal and cloacal swabs were taken. Birds were belonging to the species: Blackcap (*Sylvia atricapilla*), Robin (*Erithacus rubecula*), Chiffchaff (*Phylloscopus collybita*), Blackbird (*Turdus merula*), Garden Warbler (*Sylvia borin*), Sedge Warbler (*Acrocephalus schoenobaenus*), Great Tit (*Parus major*), Nightingale (*Erithacus megarhynchos*), Great Reed Warbler (*Acrocephalus arundinaceus*), Pied Flycatcher (*Ficedula hypoleuca*), Wren (*Troglodytes troglodytes*), Song Thrush (*Turdus philomelos*), Greenfinch (*Carduelis chloris*), and Goldcrest (*Regulus regulus*).

All swabs were streaked directly on nutrient agar (Becton Dickinson, Cockeysville, USA) BPLS-Agar (Merck, Darmstadt, Germany), and Sabouraud Dextrose Agar (Becton Dickinson, Cockeysville, USA). Also, cloacal swabs were examined for the presence of *Campylobacter* spp. The taxonomic classification of isolated bacteria was based on colony morphology, microscopically and biochemical characteristic, mostly using API 20 E and API 20 NE strips (BioMerieux, Lyon, France). Fungi were identified macroscopically, based on colony morphology and microscopically. Isolation of avian influenza (AI) and Newcastle disease (ND) viruses was performed by inoculation of 0.2 ml of the sample into allantoic cavity of 10 days old chicken embryos.

From pharyngeal swabs bacteria *E. coli*, *Staphylococcus* spp., *Streptococcus* spp., and *Bacillus* sp., and fungi *Candida* spp., *Cladosporium* sp., *Mucor* sp., *Alternaria* sp., *Aspergillus* (*A.) flavus*, and *Penicillium* sp. were isolated. From cloacal swabs bacteria *Bacillus* sp., *E. coli*, *Staphylococcus* spp., *Streptococcus* spp., and *Pseudomonas* sp., and fungi *A. fumigatus*, *A. niger*, *Candida* sp., *C. albicans*, *Cladosporium* sp., *Mucor* sp., *Penicillium* sp., *Alternaria* sp., *Scapulariopsis* sp. and *Rodotorula rubra* were isolated. All examined cloacal swabs were *Campylobacter*, as well as AIV and NDV negative.

Comparing the results from two different years, similarities of bacterial and fungal species and their number is evident, even on higher number of the samples. All isolated bacterial and fungal species are opportunistic, but they can also be obligatory pathogens, especially *E. coli* and *Aspergillus* species, and may cause diseases in poultry and humans, especially in elderly or immunocompromised ones. The results obtained by these two investigations revealed the need for permanent monitoring of wild bird’s health status, especially the species that are more infected and are in the closer contact with the humans.

Poster Presentation 77

AVIAN PARAMYXOVIRUS SUBTYPE 2 (APMV 2) IN ROBINS (*ERITHACUS RUBECULA*) IN SLOVENIA

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Avian paramyxoviruses (APMV) infect and cause disease in wild birds. Of these avian paramyxovirus type 1 (APMV 1) is the best studied. Much less is known about other eight avian paramyxoviruses (APMV 2 to APMV 9) and none of this viruses has been recognized to cause disease in free-living wild birds, but they are assumed to persist in wild bird populations. In the study a total of 670 cloacal swabs were taken from 37 species of wild living passerine birds in years 2004, 2005 and 2006. Isolation of avian paramyxoviruses (APMV) was done on chicken embryos. Propagation on embryonated chicken eggs resulted in isolation attempts that were mostly negative. Only one amnioallantoic fluid had haemagglutinating activity, and was positive for APMV 2 using the haemagglutination inhibition assay. The positive sample was from a robin (*Erithacus rubecula*) captured in autumn 2005. There are some reports about APMV 2 isolations from captured wild living passerine birds. The literature data and our successful isolation of APMV 2 from a robin reflect apparently endemic association of viruses of this serotype with passerines. There is currently no evidence that APMV 2 causes disease in wild birds, and the APMV 2 positive robin appeared clinically healthy. On the other hand it is known that APMV 2 can cause egg production problems and respiratory diseases in turkeys and other poultry. In light of the presented results and literature data, passerines appear to play a minor role as potential disseminators of different paramyxoviruses; however it seems that they could act as occasional carriers.

Poster Presentation 78

EVALUATION OF A MITOCHONDRIAL GENETIC MARKER FOR TERRESTRIAL AND MARINE WILD MAMMAL SPECIES IDENTIFICATION

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Species identification proves to be important and fundamental to resolve suspected caseworks as trade in endangered species, illegal import, wildlife poaching, and food fraud. Wildlife species discrimination with a traditional morphological approach is not always sufficient, for instance when only a part of an illegally hunted animal has to be analysed or in case of stranded cetaceans found in bad conservation conditions. In all these cases the investigation of genetic markers can be useful.

Aim of this work is to set up and evaluate a method using the mitochondrial cytochrome *b* gene (*cytb*) as genetic marker for species identification and discrimination. A case report is also described.

DNA from six terrestrial (*Capreolus capreolus*, *Cervus elaphus*, *Dama dama*, *Lepus europaeus*, *Rupicapra rupicapra*, *Sus scrofa*) and eight marine (*Balaenoptera physalus*, *Delphinus delphis*, *Globicephala melas*, *Grampus griseus*, *Stenella coeruleoalba*, *Steno bredanensis*, *Tursiops truncatus*, *Ziphius cavirostris*) wild animals were analysed by automatic DNA sequencing of *cytb* marker. Obtained sequences were analysed and aligned with SeqMan and have been compared with those deposited in GenBank database using MegaBlast program.

For the case report we also performed an alignment with MegAlign program to carry out a direct comparison between the sample and the sequences of *Sylvilagus floridanus* and *Oryctolagus cuniculus* that were the most suspected involved species. The genetic distance was calculated and a phylogenetic tree was constructed. The bootstrap test confidence was used to determine the statistical significance of the species assignment. Bootstrap proportions ≥70% usually correspond to a probability ≥95% that the corresponding cluster is real.

Obtained results revealed that c*ytb* is an effective marker for species identification and is a promising tool for genetic purposes involving terrestrial and marine wild mammals. Preliminary data showed the possibility to investigate highly

degraded or limited samples. *Cytb* presented also a good discrimination power regarding phylogenetically close species like cetaceans.

The analysis protocol developed for the case report appeared a good approach which can be used in forensic investigations for wildlife protection.

Poster Presentation 79

MONITORING OF *SALMONELLA* SPP. IN WILD ANIMALS IN PIEDMONT, LIGURIA AND VALLE D'AOSTA IN THE PERIOD 2007/2008

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The European Food Safety Authority (EFSA) has recently published data on the prevalence of *Salmonella* spp. in slaughtered domestic pigs (10.3%) and turkey flocks (13.6%) in the European Union. The results indicate that despite the adoption of appropriate hygiene measures, *Salmonella* spp. is highly prevalent and this is a constant risk to human health.

Although the European Community continues to highlight *Salmonella* spp. In domestic animals, monitoring in wild animals is insufficient. Wild animals may play a part in the complex cycle of *Salmonella* spp. as healthy carriers. Today little is known about *Salmonella* spp. in wild mammals while some studies have been carried out on wild birds. The presence of *S. typhimurium* was identified in the guts of wild birds gut as a consequence of consuming infected carcasses. Given the potential role of wildlife in the epidemiology of *Salmonella* spp., Ce.R.M.A.S. (The National Reference Centre for Diseases of Wild Animals) continually monitors bacterial infection in carnivorous wild mammals, mustelids, ungulates and wild birds.

In 2007 and in the first five months of 2008 we tested 201 and 161 animals, respectively. The specimens were mainly recovered dead as a consequence of trauma or car accident, and in smaller numbers were hunted within regional or provincial plans. Gut samples of wild birds and mesenteric lymph nodes from wild boar were examined, and when possible we took samples of feaces and mesenteric lymph nodes from foxes and mustelids.

Salmonella spp. isolation was performed by the classical method composed by four phases: pre-enrichment, enrichment, isolation and biochemical/serological identification. Subsequently, colonies identified as *Salmonella* spp. were sent to the Food Control Laboratory of Turin IZS for serotype definition.

During 2007 *Salmonella* spp. were isolated from 7 foxes, 6 of which were from Liguria and the remainder from Piedmont. Also, one positive pigeon was found in Valle d'Aosta.

In the first five months of 2008 we detected *Salmonella* spp in six foxes and four wild boar from Liguria and in two wild birds from Piedmont. No cases were confirmed in Valle d'Aosta.

It is interesting to notice that we found some serotypes such as *S. typhimurium*, *S. veneziana*, *S. thompson*, *S. kottbus* that are responsible for human infection which confirms the potential risk of wild animals spreading *Salmonella* spp.

Poster Presentation 80

PATHOLOGICAL FINDINGS IN SOME PREDATORY BIRDS

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The aim of this study was to describe four pathological case reports on predatory birds of the Northern Western Alps. Case 1 was an adult male Golden eagle (*Aquila chrysaetos*) which died in a recovery centre for wild animals following surgical admission for permanent decubitus. Post mortem examination showed undigested food in the crop and glandular stomach, polyserositis with severe fibrinous pericarditis linked to mild pericardic effusion, and fracture of the thigh-bone. Histological examination found fibrinous pericarditis associated to neovascularization, active fibroblastic component and neutrophil granulocytes. Bacteriological examination (pericardial exudate) revealed *Staphylococcus xylosus*. Parasitological examination of a fecal sample was negative, although *Salmonella ohio* was detected. Examination of pectoral muscles for *Trichinella* spp. was negative. Case 2 was an adult female Common buzzard (*Buteo buteo*) that was found dead in the environment. Post mortem examination showed cachexia, multiple granulomatous-like nodular lesions (caseous-calcific contents) located in the air sac and lung serosa. Histological examination showed nodules consisting of neutrophil granulocytes surrounded by fibrous capsule, invaded by fungal hyphae (PAS positive). Ziehl-Neelsen staining of nodule exudate was negative. Bacteriological examination was negative. Mycologic examination identified *Aspergillus* sp. *Salmonella* spp. were not detected in the organs, and examination of the pectoral muscles for *Trichinella* spp. was negative. Case 3 was an adult female Tawny owl (*Strix aluco*) found dead in the environment. Post mortem examination showed cachexia, and fracture of the left wing. *Salmonella enteritidis* was detected in the organs but examination of the pectoral muscles for *Trichinella* spp. was negative. Case 4 was an adult female Common kestrel (*Falcus tinnunculus*) found dead in the environment. Post mortem examination showed fracture of the humerus; and multiple fractures in the right tarsus-metatarsus region associated with necrosis of the claw. *Salmonella* spp. *brancaster* was detected in the organs but examination of the pectoral muscles for *Trichinella* spp. was negative.

Poster Presentation 81

Student Presentation

AVIAN TUBERCULOSIS IN WILD BIRDS IN SOUTH CENTRAL SPAIN

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Avian tuberculosis is a potential zoonotic disease of domestic and wild birds. It is a bacterial disease caused by intracellular acid-fast bacilli most commonly *Mycobacterium avium* complex and M.*genavense*. The unknown prevalence of the disease in wild avian species, variation of clinical presentation and the persistence of infective forms make the diagnosis of avian tuberculosis a challenge in wildlife rehabilitation centers. The gross necropsy and histopathologic findings of 13 cases of tuberculosis caused by *Mycobacterium spp*. in birds admitted between 1999 and 2007 to 3 Wildlife Rehabilitation Centres of the Community of Castilla La Mancha, in South central Spain, are presented. The species affected include 6 common kestrels *(Falco tinnunculus)*, 2 griffon vultures *(Gyps fulvus)*, 1 golden eagle *(Aquila chrysaetos)*, 1 common buzzard *(Buteo buteo)*, 1 cormorant *(Phalacrocorax carbo)* and 1 Cattle egret *(Bubulcus ibis)* received as wildlife casualties, and a peregrine falcon *(Falco peregrinus)* from the breeding stock of one of the wildlife rehabilitation centres. The birds were found dead, died or were euthanized due to their physical condition. In all cases, mycobacterial infection was diagnosed preliminarily by the detection of acid-fast bacilli in tissue sections and imprints. All birds were emaciated or cachectic, 5/13 had subcutaneous nodules, 9/13 had lesions in the liver, 6/13 presented lesions in both liver and spleen and 9/13 were considered generalized. Osseous lesions were observed in two cases: In the cattle egret the main lesion was a large granuloma affecting the right tibiotarsus, while the main lesion in the peregrine falcon affected a portion of the sternum. Secondary pathogens as *Aspergillus spp* and yeasts where isolated from different tissues in several of the cases. In two cases the presence of M.*avium* was confirmed by culture in Loewenstein Jensen medium, while in PCR was used to confirm the rest of the cases. The most consistent histopathologic finding were granulomas with central caseous necrosis surrounded by heterophils, lymphocytes, macrophages and giant cells. A capsule was generally not prominent. There is little information about the prevalence of avian tuberculosis in the wild avian species in Spain, but the disease should be considered in the differential diagnosis of wild birds that present with chronic weight loss, inability to fly, inflammatory leukogram, subcutaneous nodules, abdominal distension, or exostosis.

Poster Presentation 82

GENETIC CHARACTERIZATION OF *TRICHOMONAS GALLINAE* INDICATES CORRELATION WITH ITS PATHOGENICITY

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Avian trichomonosis is a parasitic disease caused by *Trichomonas gallinae*, a flagellated protozoan which can cause lethal caseous lesions on the mouth and the oesophagus. Parasite main hosts are Columbiformes, especially rock pigeon

(*Columba livia*), which is blamed to be the responsible of the worldwide distribution of the parasite. Although many avian families can be parasitized by the flagellated, birds of prey are the principal affected group, overall ornitophagous species which feed regularly on pigeons. It has been demonstrated that broods of raptor species which have been forced to nest near urban areas have highest prevalence and incidence of trichomonosis than broods located far from the city. Strain differences in pathogenicity have been reported, but there are few studies considering genetic and pathogenic characteristics of the parasite. Our objective in this study was to characterize several *T. gallinae* strains obtained from different raptor species in order to asses the genetic diversity of the parasite in an open avian population. Also, the relationship between the presence of lesions and the genetic pattern has been investigated.

We have characterized 35 strains of *T. gallinae* obtained from five species of birds of prey, showing or not clinical signs. The strains were obtained by culture in TYM medium at 37°C. We have employed two techniques for genetic characterization of the strains: Restriction Fragment Length Polymorphism (RFLP) and Random Amplified Polymorphic DNA (RAPD). A Restriction Map was generated after PCR and sequencing using 5.8S rDNA and the surrounding ITS regions and *Hae III* was selected to differentiate between strain types. RAPD was made using four different primers (OPD 3, 5, 7 and 8) that have been successfully employed to differentiate among *Trichomonas gallinae* strains.

RFLP analysis showed two different patterns: A and B. Sixty five point seven percent of the samples (n=23) showed pattern A, while 34.3% (n=12) showed genotype B. All the strains isolated from animals having oral lesions belonged to this last one genotype.

RAPD analysis displayed 4 to 5 different patterns with each primer. These results show a great heterogeneity of the parasite not related to the host species.

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Poster Presentation 83

MORPHOLOGICAL AND MOLECULAR ANALYSIS OF MAMMALIAN HAIR: A FORENSIC APPROACH

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We investigated a case of pet animal abuse that allowed us to attempt identification of small-sized mammalian species by morphological analysis and single nucleotide polymorphism (SNP) typing of the cytochrome *b* gene using guard hair as an analytical material.

This case involved an owner of small pet mammals, who had recorded on video his manual strangulation of ferrets (*Mustela putorius furo*) and uploaded the videos to the bulletin board of a pet lovers' Internet website. The suspect had subsequently been arrested for violating the protection and control of animal law. No carcasses had been found at the suspect's home because all of them had been disposed of with the household garbage. We decided to conduct analysis of the crime scene by identification of ferret guard hair from bulk samples of hair collected from a carpet that had appeared in one of the videos uploaded by the suspect, who owned about 30 small mammals other than ferrets.

Guard hair samples from domestic dog, cat, ferret, Japanese weasel, rabbit, gerbil, degu, Djungarian hamster and macaque monkey were measured for length, width, medulla formation, and cuticle scale pattern by light microscopy or scanning electron microscopy. These samples were also analyzed for SNPs in the cytochrome *b* gene using a multiplex single-base primer extension reaction.

Morphological analysis using cuticle scale pattern and medulla formation was able to discriminate ferret hairs from the bulk hair samples including rabbit, gerbil, degu and Djungarian hamster. However, this also revealed similarity between the subspecies ferret and Japanese weasel. Although three sites of nucleotide colored signals of SNPs in the cytochrome *b* gene were able to discriminate completely among human, dog and gerbil, the signals for cat, ferret and Japanese weasel were demonstrated at the same nucleotide sites. Unfortunately, no signals were obtained from Djungarian hamster and rabbit. Although the discriminated hair samples were 100% homologous to those of ferret, there was a 5% difference from Japanese weasel (*Mustela itatsi*) in the partial sequence of the cytochrome *b* gene.

The present study demonstrated the feasibility of hair analysis by using numerical morphology and SNP typing of the cytochrome *b* gene for mammalian species identification. The slight difference in the cytochrome *b* gene sequence between ferret and Japanese weasel was thought to have arisen through mutation at the time when European polecat (*Mustela putorius*) became the domestic ferret. A comparative suborder study between human and macaque monkey focusing on the cytochrome *b* gene is currently in progress.

Construction of a database of mammalian hairs would be useful not only in forensic science, but also for investigation of smuggling of endangered species in contravention of the Washington convention.

Poster Presentation 84

Student Presentation

OFFSPRING MORTALITIES IN CAPTIVE-BRED BROWN HARES (*LEPUS EUROPAEUS*) CAUSED BY *BORDETELLA BRONCHISEPTICA*

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Diseases of the upper respiratory tract as well as alimentary tract are the most common diseases in intensive breeding of both rabbits and European brown hare. Diseases of the upper respiratory tract are most frequently caused by *Pasteurella multocida*, but other pathogens like *Bordetella bronchiseptica* and *Staphylococcus* spp. are occasionally involved. This paper presents results of pathohistological and bacteriological examination of brown hare died in cage breeding. Within the outbreak, eight out of twelve offspring died. Four carcasses were presented to the Department for Game Biology, Pathology and Breeding for necropsy. Lung, stomach, intestine and liver samples were taken for histopathological examination, while liver, spleen and lung were submitted to Department for Microbiology and Infectious Diseases, for bacteriological examination. *Bordetella bronchiseptica* was isolated from liver and lung of two animals. Immediately after necropsy anti-bacterial therapy, based on results of antimicrobial susceptibility testing, was prescribed in order to prevent further mortalities. The obtained results and observed non-adequate breeding technology indicate bordetellosis as the most probable cause of the mortalities in question.

Poster Presentation 85

EWDA Best Student Presentation Award applicant

MORTALITY OF REINTRODUCED EURASIAN LYNX (*LYNX LYNX*) IN CROATIA

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On March 2nd 2008 we have acknowledged the 35th anniversary of Eurasian lynx reintroduction to Dinaric Mountains. Six animals from Slovakia, released in 1973 in Slovenia, founded one of the most successfully reintroduced European lynx populations. Lack of prey and human prosecution led to extermination of the autochthonous lynx in Croatia around the year 1903. Reintroduced animals exhibited pronounced population growth and range expansion during initial 15 years, followed by a period of stabilization. During the last 15 years the Dinaric lynx population is decreasing. Eurasian lynx live at low densities occupying large areas; have low reproductive and population growth rates. As such, their populations are unusually sensitive to increase in mortality and decreases in reproduction. Mortality of individuals is one of the basic parameters of population dynamics. General knowledge about the distribution and trend of mortality is important, because excessive mortality can have negative impacts. The aim of this study was to provide quantitative information on causes of lynx mortality in Croatia and to evaluate their effects on long-term population stability. During the 30-year period (1978-2007) a total mortality of 227 animals has been recorded. Yearly mortality ranged 1 17 animals, with 7.56 animals per year in average. First animal killed after the reintroduction was in 1978, and without protection until 1982, a total of 29 animals have been killed. Then lynx became protected and limited yearly hunting quota was issued. Last permit was issued in 1998 and after that mortality data were very sparse (total of 27 records), with 1 5 dead animals recorded per year, most of them being unproven, anonymous poaching reports. Out of all 227 mortality cases, only 2 (0.88%) were not due to human related causes (1 animal drowned in the sea and one kitten died of pneumonia), and in 10 cases (4.4%) cause of death was not identified. Recorded mortality was primarily due to human related causes: shooting (76.21%), traffic accidents (8.37%) and other (snares, poison, killing with rock, axe, wooden or metal poles, land mine) (9.69%). Until 2000 a total of 17 poaching cases were recorded (8.06%), while in the 2000-2007 period poaching caused 81.25% of the total mortality. Age was not determined for 15 animals (6.6%), 148 were older than one year (65.20%) and 64 younger than one year (28.20%).

We suspect that overall lynx mortality in Croatia during this period was probably higher, but we were not able to record all cases. Especially poaching and infectious diseases are likely to be under-represented. It can be concluded that the high percentage of human induced mortality and especially the adult mortality could have played a mayor role in the current population decline which can threaten its survival and therefore should be closely monitored.

Poster Presentation 86

MOLECULAR ANALYSIS OF H5N1 HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS (HPAIV) ISOLATES FROM SLOVENIA IN 2006

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During an outbreak of HPAIV in Slovenia in 2006, viruses of subtype H5N1 were detected in 48 birds by one-step RT-PCR using primers for genes coding matrix protein (M), nucleoprotein (NP), haemagglutnine (HA) H5 and H7 and neuraminidase (NA) N1, respectively. Virus was isolated by egg inoculation (Anon. 2006) in 23 out of the 48 RT-PCR positive samples. All H5N1 positive birds, forty-four mute swans, two grey herons, one pintail and one mallard, were found in the same geographically region near the Drava River in the Northeast part of Slovenia from middle of February to the end of March 2006 (Racnik, 2008).
Molecular characterization of five H5N1 isolates obtained from four different bird species (mute swan, grey heron, pintail and mallard) should contribute to more knowledge on similarities or potential differences between virus isolates. In our study, HA and NA complete sequences were compared to the first H5N1 virus isolated from mute swan in Slovenia (A/swan/Slovenia/760/2006) and to further sequences available from GenBank which were included in phylogenetic analyses of European and African influenza H5N1 viruses (Salzberg et al., 2007). Sequencing reactions were performed as described previously (Starick et al., 2008). Analysis of nucleotide and predicted amino acid sequences were conducted using MEGA v. 4.0, NCBI Blast program and ClastalW v. 1.8 software.
Analyses showed 100 % identity for the nucleotide sequences of the NA gene of all five investigated isolates. The phylogenetic tree constructed by using neighbour-joining method with 1000 bootstrap replicates clustered our isolate in EMA clade 1, close to Italian isolates *Cygnus olor*/Italy/808/06 and Mallard/Italy/835/2006 (Salzberg et al., 2007). Multiple sequence alignments of the HA gene sequences showed 16 mutation when comparing four later isolated viruses to the first isolate A/swan/Slovenia/760/2006. However, only three of them were non-synonymous. The virus obtained from a pintail has Glycine at position 239, while the other isolates carry Serine. The isolate from the mallard has two amino acid substitutions. Interestingly, the first substitution at position 5 where Methionine replaces Valine is located in the signal peptide coding region. However, more evident is the mutation at position 341 where Arginine is replaced by Glycine so reducing the number of basic amino acids in the HA_0 cleavage site. An identical cleavage site PQGEGRRKKR*GLF have isolates from Sudan (e.g. A/chicken/Sudan/1784-7/2006). In general, phylogenetic analysis of HA gene sequences clustered our isolates in clade EMA 1 as it could be shown for NA, too. However, the isolate from the mallard is more closely related to Sudan isolates, while the others are closely related to Italian isolates as for NA genes.
Our results seem to indicate that two different H5N1 viruses were introduced into Slovenia in 2006 although the duration of HPAI outbreak in Slovenia was relatively short.

Poster Presentation 87

WILD BOAR (*SUS SCROFA*) AND DOMESTIC PIG (*SUS SCROFA SCROFA*) INBREEDING PROBLEM IN CROATIA

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Wild boar, with its firm morphological characteristics is indigenous to the territory of Croatia before since remote millennia. In the past the size of population fluctuated considerably, mostly due to feed availability during favourable years and outbreaks of infectious diseases, notoriously classical swine fever. In the past decades the population maintained a stabile number counting some 16 - 17000 animals until a sudden drop to 7000 animals during the

Homeland war in 1991. The number started to recover, and by 2001 the initial number of 16500 animals as before 1991 was reached. Even more so, the population is in vigorous increase, and despite every year's higher hunting quotas the number of wild boar is continuously increasing. Simultaneous with the unusually fast increase in number of free living wild boars is the percentage of inbred wild and domestic pig individuals observed in hunting grounds as well as in National parks and protected areas. Considering the sample of 526 wild boar carcasses, most of which were harvested during regular hunts, the objective evaluation of percentage of inbreds was determined for the first time on a large sample number, and the most frequent exterior characteristics are described. The results showed that nearly 40% of the wild boar harvested as pure-breed, were indeed crossbreds. The mechanisms leading to logarithmic perpetuation of the crossbreed were studied and the significance of the phenomenon and possible repercussions on genuine wild boar population are postulated, as well as solution of the problem.

Poster Presentation 88

TOXIC METAL CONCENTRATIONS IN TISSUES OF WILD BOAR (*SUS SCROFA*) FROM LOWLAND CROATIA

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Results presented are part of an ongoing comprehensive investigation on the toxic heavy metals (Hg, Pb, Cd) contamination in game animals in Croatia. During regular hunting procedures samples (muscle, liver, kidney) of wild boar of 3 different age groups (piglets, < 1 year, n = 17; middle-aged animals, around 2 years, n =12; adults, >3 years, n =11) from the low land area of Croatia were collected. Concentrations of metals were analyzed using atomic absorption spectrometry and are expressed in µg/g wet weight. Statistical evaluation of results was made by calculating medians and mean values to enable further statistical procedure. In muscle tissue the lowest median concentration was measured for Cd (0.0019 µg/g), lead was present in slightly higher median concentration (0.002 µg/g) while median Hg concentration was the highest (0.009 µg/g). Statistically significant different Cd concentrations were determined in piglets comparing to middle-aged and adults animals ($p<0.05$). There was no statistically significant difference of Pb concentrations between all age groups. Mercury concentrations were significant different comparing piglets and middle-aged animals to adults ($p<0.05$). In liver tissue the lowest median concentration was measured for Pb (0.024 µg/g), median Hg concentration was double (0.047 µg/g), while median Cd concentration was much higher (0.099 µg/g). For Pb and Cd concentrations there were no statistically significant differences between all age groups. Significant difference of Cd concentrations were determined between all age groups ($p<0.01$; $p<0.05$). In kidney tissue the lowest median concentration was measured for Pb (0.022 µg/g), Hg follows (0.088 µg/g), the highest median concentration was found for Cd (1.209 µg/g). There was no statistically significant difference of Pb concentrations between all age groups. Significant differences of Hg concentrations were determined between piglets and adults as well as between middle-aged and adults ($p<0.05$). There was significant difference of Cd concentrations between all age groups ($p<0.01$; $p<0.05$). Amount of investigated metals in muscle tissues did not exceed concentration recommended by Croatian regulations, except in one case of lead in piglet, what is probably the consequence of lead contamination from the bullet. Some samples of liver and more samples of kidney especially of adult animals contained Cd and Hg amounts above the recommended concentrations established by Croatian regulations. From the ecotoxicological point of view it can be concluded that the determined amounts of heavy metals in tissues of wild boars are primarily the consequences of the naturally present metals in the environment.

Poster Presentation 89

EWDA Best Student Presentation Award applicant

PRESENCE OF *TRICHINELLA* SPP. IN SWEDISH LYNX (*LYNX LYNX*)

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Thirty six lynxes (*Lynx lynx*) were investigated for *Trichinella* spp. using artificial digestion, the magnetic stirrer method. The animals were of both sexes, various ages and collected during the first half of 2004 at different locations in Sweden. Three animals (8.3%), all adult males from the county of Jamtland, were found to be infected with *Trichinella* spp. The level of infection was in the different cases 0.054, 0.063 and 0.12 larvae per gram muscle tissue, (lpg). Identification of *Trichinella* species was successfully confirmed by polymerase chain reaction, PCR, in one of the lynxes. The species identified was *T. spiralis*. Low expected frequencies precluded statistical analysis. The differences in frequency of trichinellosis in Swedish lynx, red fox (3.3%) and brown bear (0%) are discussed. The differences in *Trichinella* infection in Swedish wildlife is compared to considerably higher frequencies in Finland.

Poster Presentation 90

IMPROVEMENT OF THE ORGANISATION OF WILDLIFE DISEASE SURVEILLANCE IN BELGIUM: THE *WILDSURV* PROJECT

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In Belgium, like in other countries, surveillance in domestic animals used to be limited to agents with a known economic or zoonotic impact while pathogen surveillance in wildlife was little intensive to non-existent until recently. The scattering of expertise in wildlife disease surveillance among different institutes hampered the set-up of integrated surveillance schemes as well as the cooperation among private (nature and hunter associations) and public instances (regional nature and forest departments). The exception is the regional government funded "surveillance network of wildlife diseases" operating since 2001 in Wallonia.

Growing international awareness of wild living animals being a possible source of emerging diseases with zoonotic, veterinary and conservational impact boosted the interest in the study of pathogenic agents in wildlife. With the aim of improving the organisation of wildlife disease surveillance in Belgium, a federal government funded two-year project "WILDSURV" was started in March 2007. The main objective of this pilot-project is to suggest, specifically for Belgium, a basic structure for wildlife disease surveillance, suited to sustain a more complete integrated network in the future. Ideally this basic structure should permit to implement the first steps of a risk analysis, being "hazard identification" and "release assessment" as defined by the OIE, for a range of prioritized pathogenic agents.

First, a broad literature study will identify the relevant pathogenic agents and animal species for the Belgian context. Next, through an inquiry, the necessary resources for a surveillance network will be inventoried, including organisations and institutes competent in sampling, sample transport, and diagnostic procedures. This step will afford a view on the existing surveillance schemes and detect the most important gaps in wildlife diseases surveillance in Belgium.

A prioritization of surveillance themes will be based on a choice of simple qualitative criteria referring to interfaces between pathogenic agents and sensitive species, known disease occurrences, known trends in disease occurrences, and available resources. These qualitative criteria will be integrated in "WILDTOOL", a new database that is meant to manage the abundance of information in order to determine surveillance priorities. Taking into account the thus determined priorities, a basic surveillance network will be set up for a limited number of selected topics. Ultimately the usefulness of this network will be tested and evaluated.

WILDSURV should be considered as an exploratory and early architectural stage of a wildlife disease surveillance structure for federal and regional Belgium and, depending on policy decisions, as a base for further project proposals on specific topics.

Poster Presentation 91

EWDA Best Student Presentation Award applicant

DEVELOPMENT OF AN ENZYME LINKED IMMUNOSORBENT ASSAY FOR THE METASTRONGYLOID NEMATODE, *OTOSTRONGYLUS CIRCUMLITUS,* AND ITS APPLICATION TO PINNIPEDS OF UNKNOWN INFECTION STATUS

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Otostrongylus circumlitus, a lungworm with a circumpolar distribution, is responsible for morbidity and mortality in many pinniped species. In this report, we describe the development of an enzyme-linked immunosorbent assay (ELISA) using whole nematode extract, H49A monoclonal mouse anti-seal IgG and goat anti-mouse IgG-HRP to detect O. *circumlitus* in northern elephant (NES, *Mirounga angustirostris*) and Pacific harbour seals (PHS, *Phoca vitulina richardsi*).

Sera from infected (n=33) and uninfected (n=11) NES, and infected and uninfected (n=13 each) PHS were evaluated using this assay. Antibody levels between uninfected and infected individuals were significantly different for NES ($p<0.001$) but not for PHS. In both species, cut-off points for seropositivity were established which gave test sensitivities of 96.8 % (NES) and 76.9% (PHS) and test specificities of 91.7% (NES) and 100% (PHS). The lower sensitivity of the PHS assay was associated with a high proportion of false negatives. The occurrence of these false negatives means that, while the assay is able to identify infected animals with 100% confidence, there exists an ambiguous titre zone containing both infected and uninfected animals. It is recommended that animals with anti-*Otostrongylus* antibody levels falling within this zone should be retested in 3 to 4 weeks for signs of active seroconversion.

Following validation, the assay was used to test NES and PHS of unknown infection status. The results of these studies raise some interesting questions regarding the transmission of this parasite. In future studies the utility of this assay in identifying the infection status of individuals in clinical settings and in steering anthelmintic therapy will be examined. In addition, it will be used in studies into the natural history of this parasite in free-ranging pinniped populations.

Poster Presentation 92

Student Presentation

A SURVEY FOR EUROPEAN BROWN HARE SYNDROME IN FRIULI VENEZIA GIULIA REGION, NORTH-EASTERN ITALY

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A European Brown Hare Syndrome outbreak was recorded in hares (*Lepus europaeus*) from Trieste province in the autumn 2004. EBHS is considered endemic in Italy, but a constant sanitary monitoring is needed for a correct management of hare populations. In the Friuli Venezia Giulia Region hare densities are average-high.

The project is an observational study carried out between the 2005/2006 and 2006/2007 hunting periods.

A survey was activated in both hunted and found dead hares, in collaboration with the Region administration and hunting districts. During the hunting seasons samples from 569 brown hares were collected. A sandwich ELISA system was used to detect EBHS virus antigen on liver and spleen samples and sera were tested for specific antibodies by means of a competitive ELISA. Mortality was not recorded during the study period. EBHS virus was detected from only 0,9% healthy hares (2/ 222) the first year of survey from only one hunting district; recorded seroprevalence was 67,3%. In the second year 1% (3/279) of samples were EBHSV positive, from the same and a further hunting district; seroprevalence was 78,5%. In both hunting seasons, titres were mainly low-medium and serological results showed a variable antibody titre in the population. Our results showed EBHSV positive hares in two hunting districts where different titres were observed in seroreactors.

There is evidence of a recent virus circulation in the neighbouring areas to the first outbreak and a good immunity level, in high-density populations. Hares from two hunting districts resulted to have an increasing seroprevalence during the two studied seasons. Variability of virus spread correlated to hare densities should be taken into account for the management of this species at local scale. The high seroprevalence and the low number of viral positive hares allow confirming that EBHSV has a general endemic condition in Friuli Venezia Giulia Region and that there is virus circulation in populations with diffused immunity and average-high hare density.

Poster Presentation 93

Student presentation

THE HEALTH STATUS OF WILD UNGULATES IN THE FRIULI VENEZIA GIULIA REGION OF THE ITALIAN-SLOVENIAN BORDER

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The objective of this study was to set up a health surveillance network in the Friuli Venezia Giulia Region along the border with Slovenia, an area characterized by spread and migration of wildlife populations. Samples were collected from 1048 ungulates during 2005-06 and 2006-07 hunting seasons: roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), chamois (*Rupicapra rupicapra*) and wild boar (*Sus scrofa*). Different issues were considered: species conservation (Sarcoptic mange, Pestiviruses, Chronic Wasting Disease), domestic livestock and wildlife interaction (Brucellosis, Aujeszky's Disease, Classical Swine Fever, Paratuberculosis) and zoonotic and emerging diseases (Tick Borne Encephalitis, Lyme's Disease, verocitotoxic *Escherichia coli* O157, *Salmonella*, *Campylobacter*).
Species conservation. The results point out the presence of sarcoptic mange in chamois (4,3% mite detection, 4,8% seropositivity - ELISA), in a population where *Sarcoptes scabiei* appears to have reached an endemic stability. The search for CWD was negative, according with other studies in Europe. No specific antibodies for pestiviruses were detected, suggesting chamois populations to be free, while low seroprevalence in wild boar (0,2%) and in red deer (1%) need further studies in order to clarify possible origin.
Livestock-wildlife interaction. Antibodies for brucellosis and CSF were not present, accordingly with the status of officially free region. The lack of antibodies for Aujeszky's disease was consistent with the situation in the Slovenian border areas but was different from other Italian and European studies. Paratuberculosis appears to have a limited spread in cervid populations (5,2% seroprevalence red deer - ELISA; 1% roe deer - PCR IS900).
Zoonotic and emerging diseases. The sentinel role of seroreactors for TBE (5,1% of roe deer and 7,1% of red deer) and Lyme disease (6,3% in chamois, 19,2% in roe deer and 9,1% in red deer) gave us useful information to identify risk areas. *Salmonella* (1,7%) and *Campylobacter* (0,8%) strains were isolated only from wild boars and verocitotoxic *E. coli* O157 were not identified, suggesting a low risk related to these food-borne pathogens.
This project allowed obtaining relevant data on the sanitary status of wild ungulates in the study area, a first step towards constant monitoring of wildlife diseases as a tool for wildlife and environment management.

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Poster Presentation 94

EWDA Best Student Presentation Award applicant

INTERPRETATION OF AN APPARENT TRANSIENT SEROPOSITIVE RESPONSE TO *MYCOBACTERIUM BOVIS* INFECTION IN EURASIAN BADGERS (*MELES MELES*)

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The interpretation of diagnostic test results associated with infectious diseases, whilst being critical to our understanding of disease dynamics in a population, is often challenging. The diagnosis of *Mycobacterium bovis* infection in live Eurasian badgers (*Meles meles*) is a case in point. We investigated the nature of serological responses to *M. bovis* in badgers, and in particular found that apparently transient serological responses were not isolated to cubs and maybe artefacts of test specificity.
Since the publication of a small-scale descriptive analysis of diagnostic test results from a single social group of wild badgers over a 5 year period, there has been ongoing debate over the epidemiological significance of transient

seropositive test results in cubs. Most importantly, it was suggested that transient seropositivity in cubs might in some way be protective, as measured either by a reduced incidence or a delay in subsequent detection of M. *bovis* excretion. One suggested mechanism for transient seropositivity was passive transfer of maternal antibodies. In the present study we examined historical diagnostic test result data collected during a long-term intensive study of the epidemiology of M. *bovis* infection in 40 badger social groups in South West England, to assess the evidence for 'transient seropositivity' in cubs and to identify its epidemiological correlates.

This study identified a substantial proportion of animals that could be described as exhibiting a transient seropositive response. However, this phenomenon was not restricted to cubs and there was no significant statistical association between the age at which a badger was first detected as seropositive and the likelihood that the response would be transient or maintained. Although animals exhibiting 'transient seropositivity' had a lower probability of subsequent detection of M. *bovis* excretion, this effect was greater in adults.

These recent analyses suggest that transient seropositivity is not especially associated with cubs and most likely arises from the fact that the serological test is not 100% specific. The possibility that transient seropositivity might also represent exposure to M. *bovis* without establishing true infection will be discussed. Consistent with the nature of the protective immune response against tuberculosis, there was no evidence to support the hypothesis that maternally derived antibody confers protection from imminent or subsequent M. *bovis* excretion, since the apparent protective effect of transient seropositivity is more marked in adults than cubs.

Poster Presentation 95

A SURVEY FOR SEVERAL ZOONOTIC PATHOGENS IN FREE-LIVING FERAL PIGEONS (*COLUMBIA LIVIA*) IN MADRID

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Public gardens and parks host many populations of birds. One of the most abundant specie is the feral pigeon (*Columbia livia*), whom's population densities can reach above 2,000 individuals/km^2. Due to the close relationship between pigeon and human populations, and the fact that wild birds are vectors of zoonotic agents, they constitute a potential risk on public health. In adult and immunocompetent humans direct risk for feral bird-human transmission of pathogen agents is considered low. However, this risk may be much higher on children, elder people and immunodepressed individuals. In the present work a sampling study to detect zoonotic agents in feral pigeons from a single area of the city of Madrid has been made for two consecutive years. The selected zoonotic agents were: *Salmonella spp*., *Campylobacter jejuni*, *C. coli*, *Chlamydophila psittaci* and Avian Influenza virus (AIV).

During 2006-2007 118 free-living feral pigeons have been captured and sampled. Three different samplings were made: November 2006 (n=62), May 2007 (n=27) and November 2007 (n=29). Body weight and wing length were sized and ringing was made in all individuals. A tracheal swab and a cloacal enema were sampled for each individual. Venipunction and blood storage in K3 EDTA was also made, to confirm possible AIV positives.

AIV and *Chlamydophila psittaci* were detected from cloacal enemas by real time RT-PCR and conventional PCR, respectively. Salmonella was isolated from cloacal enemas in specific media (XLD agar, Oxoid) by previous enrichment on Rappaport broth, and identified by biochemical strips (RapId One, Oxoid) and a universal PCR method for *Salmonella spp*. previously described. Finally, *Campylobacter jejuni* and *C. coli* were also detected from cloacal enemas by a duplex PCR by previous enrichment in Bolton broth in microaerophilic conditions. All PCR positive samples were confirmed by sequencing and comparing to the Genbank database.

High percentages of animals were positive for *Campylobacter jejuni* (59.32% overall- (77.4% nov06; 86.4%may07; 35.7% nov07-) and for *Chlamydophila psittaci (52.54% overall)* (59.7% nov06; 37%may07; 51.8% nov07). In contrast low percentages were obtained for *Salmonella spp* (2.54% overall) (4.8% nov06; 0% may07; 0% nov07) and for *C. coli* (0.84% overall) (0% nov06; 3.7% mayo07; 0% nov07). None individuals were positive to AIV.

The results confirm the presence of zoonotic agents in feral pigeons, mainly *Campylobacter jejuni* and *Chlamydophila psittaci*. The temporal trends of positive sample percentages and the role of feral pigeons as reservoirs of these agents will be evaluated in further studies.

Poster Presentation 96

THE TRANSMISSION OF THE LARGE AMERICAN LIVER FLUKE (*FASCIOLOIDES MAGNA*) FROM RED DEER (*CERVUS ELAPHUS*) TO DOMESTIC ANIMALS IN CROATIA

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Fascioloides magna or the large American liver fluke as an aetiological agent causing liver fascioloidiasis in deer has been recognized and described in Europe more than 130 years ago. In Croatia however, it has not been diagnosed in red deer until the past decade, and there are convincing evidence that this was the first introduction of the parasite to the Croatian territory. It seems that the Croatian outbreak is a part of much larger epidemics involving several neighbouring countries. Practically the entire Danube basin deer habitat. Even before the determination of the parasite in red deer, a progressive, debilitating and fatal disease of several sheep flocks from the same area in close vicinity of Kopacki rit National park in Baranja was noted. Sheep pastured during the day on the fields which have been grazed during nights by deer herds. The aetiology of the disease in sheep remained unrecognized for several years and all intended erratic treatment proved inefficient. This situation prompted thorough diagnostics and after several initial necropsies the problem was aetiologically elucidated as fascioloidiasis. The phenomenon has been consecutively studied epidemiologically, pathomphologically and from the aspect of purposeful therapy. During the screening of the local domestic herbivore pathology, fascioloidiasis was further confirmed in goats and cattle. Through an insight of the observed epizootics during several years a detailed pathomorphological picture, basic parasite features as well as the significance of those findings were given.

Poster Presentation 97

EWDA Best Student Presentation Award applicant

A SURVEY FOR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES IN EUROPEAN MOUFLON (*OVIS GMELINI MUSIMON*) IN GERMANY

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Proteinaceous, infectious particles (prions) are supposed to be the causative agents of transmissible spongiform encephalopathies (TSEs). Prion diseases represent a group of fatal neurodegenerative disorders in humans and several free-ranging and captive mammalian species with a sporadic, inherited or infectious background. These include bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in North American cervids and scrapie in sheep and goats. Sequence analysis of the prion protein gene (PRNP) in sheep has shown that polymorphisms in this gene are related to susceptibility to scrapie. A report of scrapie in European mouflon from England and references of identical PRNP sequences in mouflon and sheep indicate that mouflon are susceptible to TSE. Mouflon may be infected by (1) transmission of scrapie from sheep by direct contact, (2) transmission of scrapie from sheep by sharing habitats, (3) exposure to feeds containing prion contaminated material, or (4) transmission of a spontaneous form of TSE between mouflon.

This study aims to examine the risk of the occurrence of TSE in mouflon in Germany, including a population screening for prion diseases and genotyping of PRNP.

All administrative districts in Germany were divided into four risk categories defined by three factors: the abundance of sheep, the number of hunted mouflon per year (as an index of population density), and the number of reported cases of scrapie in domestic sheep. Samples were taken from mouflon older than 18 months (age estimation by teeth characteristics) collected mainly from hunting bags during the hunting seasons 2006/2007 and 2007/2008. In order to detect preclinical typical and atypical scrapie cases, samples were taken from brain stem, cerebellum and retropharyngeal lymph nodes. These were analysed by a highly sensitive enzyme immuno assay (IDEXX HerdChek BSE-scrapie)**.** Brain tissue samples were used for DNA extraction and subsequent PCR amplification and sequencing to conduct prion protein genotyping.

Altogether 822 mouflon were sampled; the majority were females (n=549). Age distribution ranged from 18-24 months old (n=142), 2-4 years old (n=326) and 4-6 years old (n=257) to over 6 years old (n=97). 53 tested mouflon showed clinical signs or pathological findings such as foot root and claw disorders (n=20), lameness (n=8), emaciation (n=5), apathy (n=5), CNS disorders (n=3) and endoparasites (n=2). No protease resistant prion protein (PrP^{res}) was detected in any of the 2450 examined brain and lymph node samples. The sequence analysis for the genotyping is still ongoing. At present, this survey provides no evidence of prion diseases in the German mouflon population. Nevertheless, there remains a chance that mouflon may be affected by TSEs at very low level of prevalence. In addition to that the results of the PRNP genotyping will permit a more detailed risk assessment for prion diseases in mouflon.

Poster Presentation 98

DEVELOPMENT OF A ONE-STEP, REAL TIME PCR ASSAY FOR RAPID DETECTION OF AVIAN INFLUENZA VIRUS SUBTYPE H7

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Among the different hemagglutinin (HA) subtypes of Avian Influenza viruses (AIV), H7 is one of major interest because of the serious consequences of both poultry industry and wild birds and the increasing frequency of direct transmission of this virus to humans. The availability of new tools to rapidly detect and subtype the influenza viruses can enable the immediate application of measures to prevent the widespread transmission of the infection. In this work, a novel fast one-step real-time reverse transcription-PCR (RRT-PCR) was developed to specifically detect H7 subtype of AIV.
A total of 11 complete gene HA sequences of AIV subtype H7 (6 Eurasian isolates and 5 American isolates) were downloaded from Genebank and aligned using ClustalW. Conserved regions were identified and a *TaqMan* probe and primers were defined with the help of Primer Express software. The specificity to detect H7 was confirmed doing a Blast search. A panel of 9 AIV (including 1 H7), 1 Equine IV, 1 Newcastle disease virus (NDV) and a total of 96 oropharyngeal and cloacal swabs from pigeons and seagulls were employed in specificity assays. The sensitivity of the RRT-PCR assay was determined using 3 replicates of 10-fold dilutions of a viral suspension of A/Tk/It/2676/99 H7N1 ($6.02x10^4$ EID_{50}/ml), and compared with the sensitivity of the generic influenza A (M1)[1].
Nucleic acids were automatically extracted with *Biosprint 15 (Qiagen)*, using *Biosprint DNA blood kit,* with specific modifications for RNA purification.
The fast RRT-PCR method was performed in a one-step using *QuantiFast Probe RT-PCR kit (Qiagen)*.
Results showed that $1.806x10^{-4}$ EID_{50}/ml of AIV H7 can be detected. A standard curve was constructed based on the observed Ct values for each viral dilution, showing a linear relationship of 7 orders of magnitude. The new assay proved slightly more sensitivity compared to M1[1] ($1.806x10^{-3}$).
The analytical specificity of the developed RRT-PCR was confirmed as no signal was detected in any of the 9 Influenza A non-H7 strains or NDV tested. Similarly, all avian field samples analysed remained negative, as expected.
This work describes a fast RRT-PCR method for the rapid detection of AIV subtype H7, that employs a specific primer set and a *TaqMan-* probe conserved for both Eurasian and American isolates. The completed studies prove the RRT-PCR as a highly sensitive and specific method, which only takes 80 minutes. This, together with the automation of the RNA extraction procedure, makes possible to determine the presence of AIV H7 in less than 3 hours.

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Poster Presentation 99

ISOLATION AND CHARACTERIZATION OF *SALMONELLA* STRAINS FROM WILD BOAR (*SUS SCROFA*) IN NORTHERN ITALY

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In order to investigate the role of wild boar as a reservoir of *Salmonella spp.* we controlled, during the 2006 and 2007 hunting season, 786 animals from a mountain area in Brescia Province, Northern Italy.

Cecal content were sampled and cultured according to ISO 6579: 2002 Annex D and, at the same time, to an home made method using Rappaport Vassilidais Broth as enrichment and Hektoen Enteric Agar as differential selective medium.
217 samples (27,61%) resulted positive showing that the most prevalent serotypes, covering 76,03% of the positive isolates, were *Salmonella* Coeln (45 isolates) *Salmonella* Ball (34), *Salmonella* Typhimurium (29), *Salmonella enterica* subsp. *diarizonae* 50:r:1,5,[7] (23), *Salmonella* Thompson (19) and *Salmonella* Veneziana (15). Among the other serotypes, 4 isolates of *Salmonella* Enteritidis were recovered.
Phagetyping of *Salmonella* Typhimurium revealed the prevalence of phagetype DT104 (17 strains), DT193 (2), U302 (2), DT2 (1); among *Salmonella* Enteritidis, the only phagetype present was PT4 (3 strains).
Strains of *Salmonella* Typhimurium were also tested for antimicrobial resistance using a panel of 16 antibiotics: 25 strains resulted multi resistant (resistant to 4 or more antibiotics) and among them, all phagetype DT104 strains were resistant from 6 to 10 antibiotics with a basic profile of resistance for amoxicillin, chloramfenicol, spectinomycin, streptomycin, sulphonamides and tetracycline. Strains of *Salmonella* Enteritidis did not show multiresistance against tested antibiotics.

Poster Presentation 100

PARASITES OF *KNIPOWITSCHIA CROATICA* AN ENDANGERED ENDEMIC CROATIAN FISH

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Endemic species *Knipowitschia croatica* is according to the IUCN status listed as critically endangered fish species in Croatia. This is a short-lived species with a maximum life span of two years. It becomes sexually mature in its first year and spawns several times over the course of the reproductive season. *K. croatica* shows spawning behaviour typical of the sand goby group of species, in which the male guards the eggs laid in a nest he previously digged. In this study performed during the research of biological and ecological characteristics of this endemic species we provided the first data on occurence of parasites on *K. croatica*. Totally we examined 42 specimens out of which 34 were infected by parasites. Ectoparasitic infections caused by ciliates *Trichodina* sp. was noticed in 29/42 specimen, *Ichthyopthyrius* sp. in 11/42, while monogenean *Gyrodactylus* sp. was recorded in 4/42 specimen. In body cavity of 2 specimen nematod parasite *Raphidascaris* sp. was noticed. The most interesting was isolated finding of the cyst on the top of the head consisted of numerous spores of myxosporean from the genus *Sphaerospora*. The more extensive sampling should be performed on the precise sampling points to study the proportion of the parasite presence as well as their influence on the biology of the endangered species.

Poster Presentation 101

A COGNITIVE APPROACH TO RISK ASSESSMENT IN WILDLIFE WELFARE

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In these last decades, the human/animals relationship has seen such an evolution that as a result Animal Welfare, including wildlife, has become an argument of primary importance not only for motivations ethics, economic, lawyers, but also for sanitary reasons. However, although the increasing needs to understand this phenomenon, a proper method for its assessment still does not exist. Similarly to what is happening in other fields of biomedical sciences, the use of Risk Assessment (RA) is considerably increasing also in the wildlife welfare analysis. RA is normally used to describe and to quantify the risk of infections and toxi-infections introduction as a result of the import of live animals or their products; it also comes applied in the choice of the better protocols for the control of the endemic or epidemic diseases (Muller-Graf et al., 2007; OIE Terrestrial Animal Health Code, 2007). Unfortunately, it does not exist up to the present specific guidelines and precise protocols for the RA applied to the animal welfare, including wildlife. This lack has a deep motivation, tied to the difficulty to identify in univocal way the risks and the needs of the animals. Generally speaking, the RA can be done using qualitative or quantitative methods or both. Among the several welfare indexes used, the study of the cognitive dimension of animals, is acquiring an always greater importance in the process of RA.

For instance, *to see* the cage bars does not mean to *perceive* them as a real obstacle; galliform birds see an obstacle represented by a vertical bar barrier (i.e. the slabs of a cage) but they do not perceive it as a real obstacle. They treat thin, vertically elongated patterns as grass (and thus attempting to pass through them) while, in contrast, young gulls consider the vertical pattern as a rigid obstacle but show the same perceptual problem with horizontal and grid patterns (Zucca et al., 2004). Wild animals should be kept in cages that they succeed to see and also to perceive like such. Also in the field of social recognition, the cognitive approach to RA in wildlife animal welfare, allows to obtain useful information from the animal perspective of the problem. For instance, quails use their left visual hemifield when approaching a companion and their right visual hemifield when approaching a stranger. Simply observing which eye a quail observes a cospecific with, it can be inferred if the other subject is considered a friend or an enemy (Zucca and Sovrano, 2008).

Poster Presentation 102 - Addendum 1

Student Presentation

IMPACT OF INFECTIOUS DISEASES ON THE MANAGEMENT AND CONSERVATION OF URSIDS

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The objective of this study was to evaluate the impact of infectious diseases on the management and conservation of *ursids*. The presence of selected infectious agents and causes of mortality in several *ursid* species was studied as well as the risk of cross-infection to canine and ruminant species especially with the aspect of possible zoonotic and/or emerging disease.

A bibliographic revision of infectious disease linked deaths reported in 8 different species of captive and wild *ursids* were studied where at least one individual succumbed. Diseases were descriminated by bear species and frequencies were determined.

From the total number of infectious agents reported, on the basis of the variables described, 39 were selected. In total, the selected agents were isolated in 30,8%. The species in which those selected agents prevailed to a great extent were *Ursus americanus* (66,7%) and *U. arctos* (51,3%), while those in which a lesser number of reported aetiological agents was presented were *U. malayanus* (10,3%) and *U. ursinus* (7,7%). The main infectious agents were bacteria (35,9%), viruses (28,2%), protozoa (23,1%) and helmints (12,8%). Investigations into *U. ameri*canus and *U. arctos* reported the highest percentage of reported virus infections, 82% and 73% respectively, whereas *U. thibetanus* were presented with the highest percentage of the helmints reported (80%). Of all the retrospectively studied causes of death in the reported *ursids* species, 67% were caused by protozoa. Leptospira was reported in 75%, whereas *Clostridium* spp. and CDV were reported in 62,5%. In order to associate the risk of those infectious diseases with other groups of mammals, a retrospective review had shown 79,5% of these agents were reported in canine species and 48,7% for ruminants. Further epidemiology analysis regarding the influence on the management of u*rsids* had shown that 69,2% of those agents were of zoonotic importance while further 38,5% had shown a potential risk of an emerging disease.

Infectious diseases can represent a risk factor in the management and conservation of the u*rsid* species in general. Further biomedical studies are needed however, to evaluate the presence of infectious aetiology in *U. ursinus*, *U. malayanus*, *Tremarctos ornatus* and *U. thibetanus* taking into consideration the presented model.

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8th CONFERENCE of the
ewda
EUROPEAN WILDLIFE DISEASE ASSOCIATION
ROVINJ - CROATIA
2-5 October, 2008

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European
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Time	**Thursday** **October 2**	Time	**Friday** **October 3**	Time	**Saturday** **October 4**
09:00	Welcome and opening ceremony	09:15	Wildlife handling and immobilization	09 00	Marine mammals and health of marine ecosystems
09:30	Wildlife disease reporting and surveillance In Focus: European wildlife disease monitoring by the O E	09:15	**Keynote address** Dr. Terry J. Kreeger Chemical immobilization in the 21st century: the future or back to the future?	09 00	**Plenary talk** The Croatian Marine Mammal Research Group Cetacean mortality along the Croatian Adriatic coastline
09:30	**Keynote address** Dr. Torsten Morner Important diseases in European wildlife - the past, present and future	10:15	*Refreshment break* and Poster presentations	09 55	In Focus: Morbiliviridae of marine mammals – Phocine distemper virus
10:45	*Refreshment break*	10:45	Diseases of ungulates	10 25	*Refreshment break* and Poster presentations
11:15	Wildlife disease reporting and surveillance - continued	12 00	*Lunch*	10 55	Mycobacterial diseases In Focusing: TB as re-emerging disease of wildlife
12:15	*Lunch*	13:15	Diseases and preservation of carnivores	12 25	*Lunch*
13:30	Emerging diseases and the role of wildlife In Focus: Bluetongue in Europe, a serious threat with critical questions	13:15	**Plenary speaker** Dr. Djuro Huber Biology and health of Croatian large carnivore populations	13:40	Disease issues at the Wildlife-human-livestock interface In Focus: translocation, cohabitation, and human intervention
13:30	**Keynote address** Dr. Philip Mellor Expansion of bluetongue in Europe and climate change	14 50	In Focus: Canine distemper in canids, mustelids and procyonids	15 25	*Refreshment break*
14:45	Avian influenza	15 30	**EWDA Auction** and a visit to the medievial town of Motovun Buses depart in front of Hotel Eden	15:45	Birds and Amphibians
15:15	*Refreshment break*			16:45	Closing remarks
15:45	West Nile fever and exotic diseases of public concern			17 00	EWDA activities discussion meeting Best student presentations recognition awards
15:45	**Keynote address** Dr. Rosella Lelli West Nile fever: the European situation and the role of wildlife			19 00	**Banquet** (*optional*)
16:55	Epidemiology and environmental drivers of wildlife diseases Diseases caused by protozoa				**Sunday** **October 5** 08 30 **Field trip** to Brijuni National Park ***optional***) Buses depart in front of Hotel Eden
18:00	**Welcome party** – Hotel Eden, Terrace in front of the Conference hall				