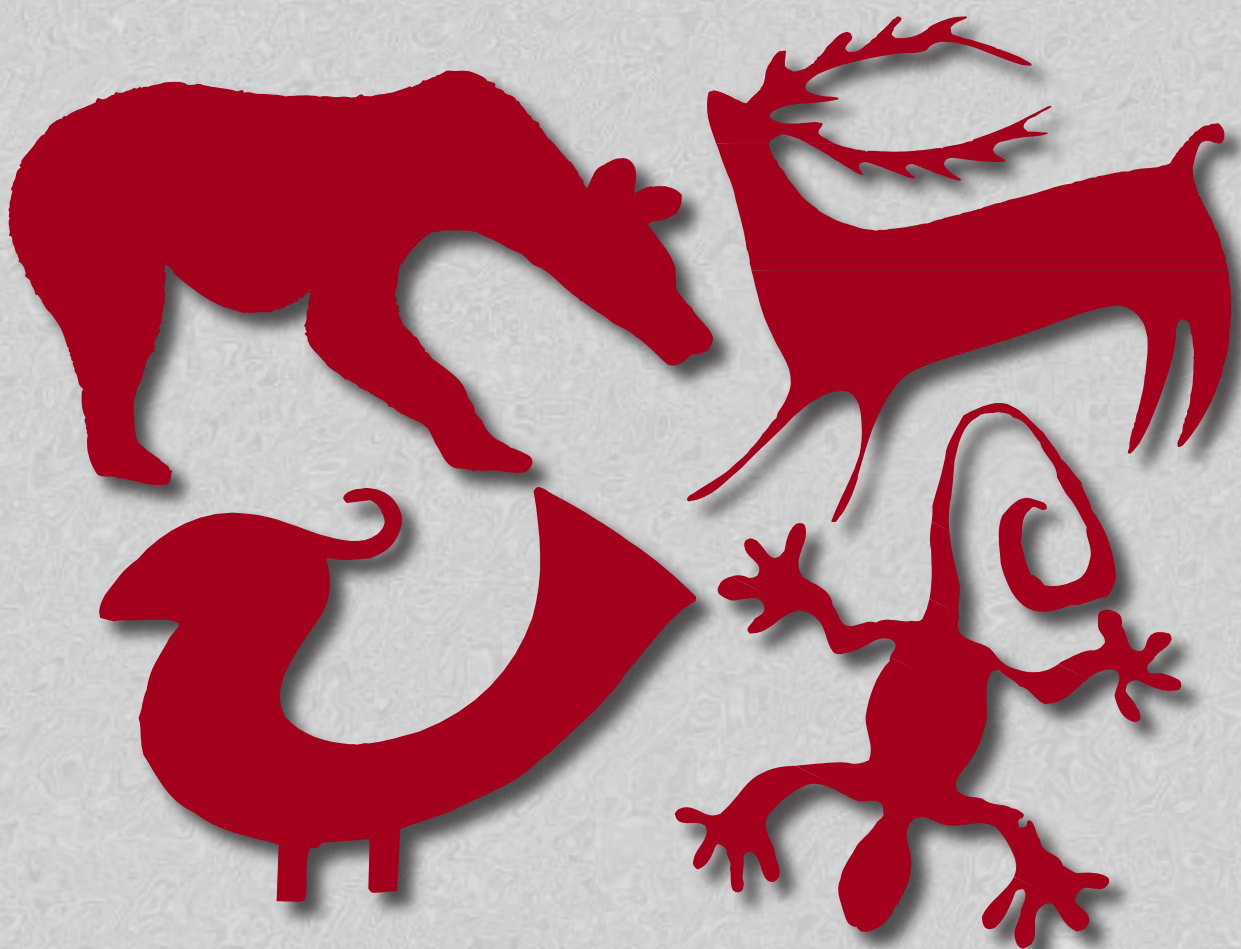


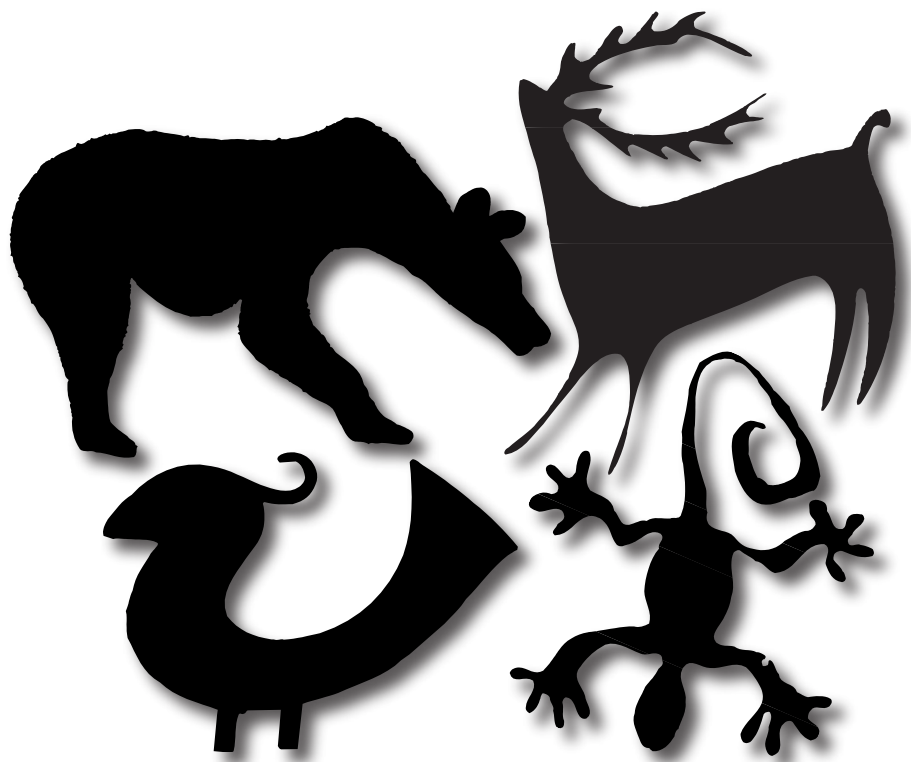
The Sixth Conference of the European Wildlife Disease Association TSE and CWD Workshop

8-12 September 2004
Uppsala, SWEDEN



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Welcome!

On behalf of the National Veterinary Institute and the Wildlife Disease Association it is our great pleasure to welcome you to Uppsala and the sixth conference of the European section of the Wildlife Disease Association.

September in Sweden is one of the most fantastic times of the year. The summer is almost at its end and the cool clear air from the arctic is starting to come down to our latitudes. The nights are cool but the days can be sunny and warm. At this time of the year the number of birds are at their annual peak and a lot of migrating birds are moving southwards. The number of mammals such as moose, deer, wild boar or hares is also at their annual peak and it is common to see wildlife at dawn or dusk.

Welcome to a conference with a full and varied scientific program with lots of interesting presentations of high quality. Around 120 scientists from all over the world will attend the conference.

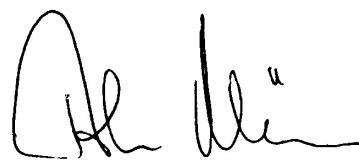
The program will start with the TSE and CWD workshop on Wednesday and will be followed by the EWDA conference for four days. There will be more than 50 presentations and 40 posters.

Wildlife diseases and the care of the environment are of great importance to Sweden and is also an important part of the work at the National Veterinary Institute. That includes all kind of wildlife diseases as well as zoonoses and emerging diseases. We are honoured to host this conference of high quality and are convinced it will give all participants an interesting week.

We hope you will enjoy your stay here and the conference, the old historic Uppsala and its surroundings.

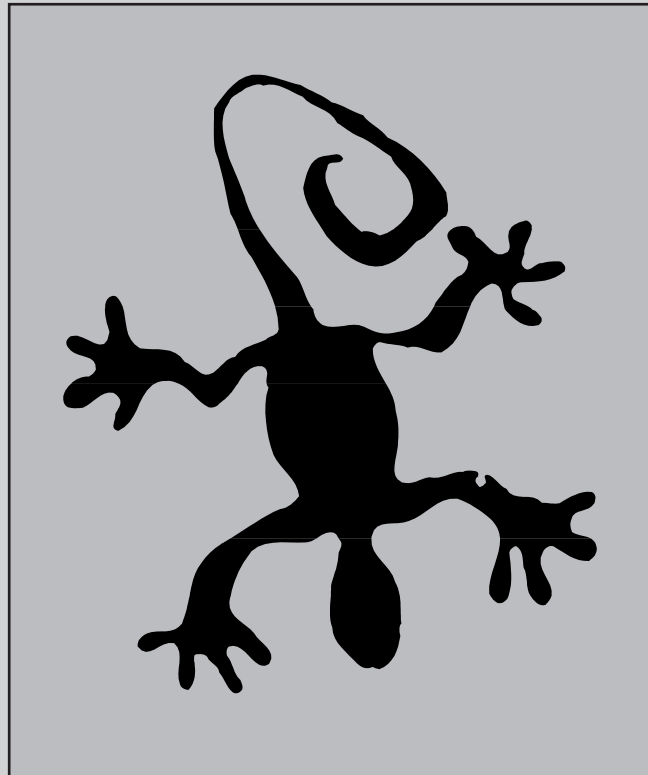


Anders Engvall
General Director
National Veterinary Institute



Torsten Mörner
President
Wildlife Disease Association

Programme



TSE and CWD Workshop Programme

Wednesday, September 8th 2004

Place: Loftets stora sal and hörsal, SLU, Uppsala

| | | |
|---|--|-------------------------|
| Session I: Transmissible Spongiform Encephalopathies (TSEs) in Europe | | |
| 09.00-9.50 | TSEs – an evolving diagnostic challenge | Dr Marion Simmons |
| 09.50-10.15 | The new European Structure for Risk Assessment: Biological Hazards Area | Dr Bart Goossens |
| 10.15-10.35 | Study on TSE (CWD, BSE and Scrapie) in cervids from Germany | Dr Elvira Schettler |
| 10.35-11.00 | Break | |
| Session II: CWD- North America | | |
| 11.00-11.50 | Chronic Wasting Disease: History, Epidemiology, and Views from the Field | Dr Elizabeth Williams |
| 11.50-12.20 | Chronic Wasting Disease (CWD) - Current Situation in Canada | Dr Aru Balachandran |
| 12.20-13.10 | Lunch | |
| 13.10-14.00 | Chronic Wasting Disease in cervids | Dr Terry Spraker |
| Session III: Atypical TSEs | | |
| 14.00-14.10 | The histopathological and immunohistochemical features of Bovine Amyloidotic Spongiform Encephalopathy (BASE) | Dr Cristina Casalone |
| 14.10-14.20 | The histopathological and immunohistochemical features of the Nor98 variant of scrapie | Dr Sylvie Benestad |
| Session IV: Histopathology in parallel with poster display, presentation of rapid tests and coffee | | |
| 14.20-17.25 | Discussion at the microscopes for the participants with the guidance of the speakers: BSE, BASE, Scrapie, Nor98 (atypical scrapie), CWD, FSE, and others. Histopathology and immunohistochemistry. Commercial companies will show their rapid tests for TSE/CWD. | |
| 17.25-17.30 | Concluding remarks | Dr Dolores Gavier-Widén |
| 17.30 | Assembly of Neoprion-Cervids group (members only) | |

Conference Programme

Thursday, September 9th 2004

Place: Loftets hörsal, SLU, Uppsala

| Registration from 08.00 | | |
|-------------------------|---|--|
| 09.15-09.45 | Opening ceremony and Introduction to the Conference | Chair: Torsten Mörner Speakers: Prof. Anders Engvall , General Director, National Veterinary Institute (SVA) Marc Artois , president of EWDA Torsten Mörner , president of WDA |
| 09.45-10.45 | Session: disease interaction between predators and prey animals and emerging diseases | Chair: Torsten Mörner Co-chair: Marc Artois |
| 09.45-10.45 | Anthrax and tuberculosis as examples of disease interaction between predators and prey animals | KEY NOTE SPEAKER: DR ROY BENGIS |
| 10.45-11.05 | Break | |
| 11.05-12.20 | Session: Tuberculosis and testing | Chair: Christian Gortázar Co-chair: Dolores Gavier-Widen |
| 11.05-11.20 | The epidemiological characteristics of <i>Mycobacterium bovis</i> infection in badgers (1) | Richard J. Delahay |
| 11.20-11.35 | Rapid test for serological detection of tuberculosis in multiple animal species (2) | Konstantin Lyashchenko , Rena Greenwald, Javan Esfandiari, Ray Waters, Mitch Palmer, Diana Whipple, John Olsen, Ray Ball, John Pollock and Peter Andersen |
| 11.35-11.50 | Improved Serodetection of <i>Mycobacterium bovis</i> Infection in Badgers (<i>Meles meles</i>) using Multi-Antigen Test Formats (3) | Rena Greenwald, Javan Esfandiari, Konstantin Lyashchenko, Sandrine Lesellier, R.Glyn Hewinson Mark Chambers , Raymond Houghton, John Pollock, Claus Aagaard and Peter Andersen |
| 11.50-12.05 | Use of phytohaemagglutinin as positive control in tuberculosis skin testing in wild mammals (4) | Isabel G. Fernández de Mera , Ursula Höfle, Joaquín Vicente, Andrés García, José Antonio Ortiz, Yolanda Fierro, Julián Garde and Christian Gortázar |
| 12.05-12.20 | Tuberculosis in wild mammals in south-west England: results of a recent survey (5) | Richard J. Delahay |
| 12.20-13.15 | Lunch | |
| 13.15-14.15 | Session: Mycobacteria in deer and wildboar | Chair: Richard Delahay Co-chair: Mark Chambers |
| 13.15-13.30 | <i>Mycobacterium bovis</i> infection in free-living wild boars (<i>Sus scrofa</i>) and red deer (<i>Cervus elaphus</i>) in France (6) | J. Hars , M.L. Boschirolì, B. Garin-Bastuji, J.R. Gaillet, MF. Thorel |
| 13.30-13.45 | Diseases of the wild boar: final report of a survey on epidemiological risk factors in Spain (7) | Christian Gortazar , Joaquin Vicente, Ursula Höfle, Isabel G. Fernández –de-Mera, Pelayo Acevedo, Fran Ruiz-Fons, M ^a Paz Martín, Diego Villanúa, Vanesa Alzaga, Dolo Vidal and Vidal Montoro |
| 13.45-14.00 | Sanitary risk factors associated to the management of Iberian red deer for hunting purposes in South-Central Spain (8) | Joaquín Vicente , Ursula Höfle, Isabel G. Fernández-de-Mera, M ^a Paz Martín, Yolanda Fierro and Christian Gortázar |
| 14.00-14.15 | Monitoring of wild cervids paratuberculosis (<i>Mycobacterium avium</i> subspecies <i>paratuberculosis</i>) in southern Belgium (9) | Annick Linden |
| 14.15-15.15 | Poster session and coffee | |
| 15.15 | Departure to boat trip to Skokloster | |

Friday, September 10th 2004

| | | |
|-------------|--|---|
| 09.00-10.30 | Session Ungulates: cervids | Chair: Dolores Gavier-Widen Co-chair: Kai Frölich |
| 09.00-09.15 | Causes of mortality and neurological diseases in farmed deer in Switzerland (10) | V. Sieber , N. Robert, C. Botteron, and M-P. Ryser-DeGiorgis |
| 09.15-09.30 | Evaluation of the Prionics®-Check WESTERN and LIA for chronic wasting disease in deer and elk (11) | Müller, P., Schacher, P., Miglino, N., Buchmann, A., Struckmeyer, T., Huber-Musahl, C., Raeber, A.J. and B. Oesch |
| 09.30-09.45 | The national health surveillance program for wild cervids in Norway (12) | Turid Vikøren , Atle Lillehaug and Kjell Handeland |
| 09.45-10.00 | Fascioloidosis of red deer (<i>Cervus elaphus elaphus</i>) – an emerging disease in Croatia (13) | Ivan Vickovic , Branko Sostaric, Radovan Fuchs, Zoran Lipej, Josip Toncic, Ivan Tarnaj, |
| 10.00-10.15 | Chorioptic ear mange in Moose (<i>Alces alces</i>). A pilot prevalence study in the Swedish wild moose population (14) | S. Bornstein , D. Gavier-Widen, Bröjer C. Uhlhorn H. and Ågren E. |
| 10.15-10.30 | Gangrenous ergotism in wild-living moose and roe deer in Norway (15) | Kjell Handeland and Turid Vikoeren |
| 10.30-10.50 | Break | |
| 10.50-11.45 | Session: pestivirus in chamois | Chair: Jean Roch Gaillet Co-chair: to be announced |
| 10.50-11.05 | First evidence for a novel pestivirus in Pyrenean chamois (<i>Rupicapra pyrenaica pyrenaica</i>) from France (16) | Kai Frölich , Sandra Jung, Arne Ludwig, Dietmar Lieckfeldt, Philippe Gibert, Dominique Gauthier and Jean Hars |
| 11.05-11.20 | Pestivirus infection in Pyrenean chamois (<i>Rupicapra pyrenaica</i>): isolation and characterisation by monoclonal antibodies and cross-neutralization (17) | Ignasi Marco , Rosa Rosell, Jorge Ramon Lopez-Olvera, Manel Beneria and Santiago Lavín |
| 11.20-11.35 | Pestivirus disease and seroprevalence in Pyrenean chamois (<i>Rupicapra pyrenaica pyrenaica</i>) (18) | Arnal, M.C. , Fernández-de-Luco, D., Riba, L., Maley, M., Gilray, J. Willoughby, K., Vilcek, S. and Nettleton, P.F |
| 11.35-11.45 | Discussion | |
| 11.45-12.05 | Session: Training | |
| 11.45-12.05 | Training veterinary students about diseases of wild carnivores, a EWDA international project. State of the art (19) | Artois, Marc , Gortázar, Christian and Frölich, Kai |
| 12.05-13.05 | Lunch | |
| 13.05-14.35 | Session Ungulates: chamois, ibex, wild boar | Chair: Carl Hård Co-chair: Jean Hars |
| 13.05-13.20 | Do abortive diseases persist in populations of chamois (<i>Rupicapra rupicapra</i>)? (20) | Emmanuelle Fromont , Maryline Pioz, Philippe Gibert, Jean Hars and Dominique Gauthier |
| 13.20-13.35 | Impact of three abortive infections on the fecundity of chamois (<i>Rupicapra rupicapra</i>) in the alpine massif of Bauges (France) (21) | Pioz, Maryline , Artois, Marc, Gauthier, Dominique, Gibbert Philippe and Fromont, Emmanuelle |
| 13.35-13.50 | Classical swine fever in free ranging wild boar: a European problem (22) | S. Rossi , J. Hars, J. Barrat, MF. Lepotier A., Mesplede M., Artois, E., Fromont N., Masse-Provin and Y. Louguet |
| 13.50-14.05 | Geographic distribution and host specificity of ticks of wild ungulates in Spain: preliminary results (23) | J. Francisco Ruiz-Fons , J. Vicente, I. Fernandez de Mera, U. Höfle, C. Gortázar |
| 14.05-14.20 | Effect of capture method on haematological and serum biochemical parameters of Spanish ibex (<i>Capra pyrenaica</i>): preliminary results (24) | Encarna Casa-Díaz, Jorge R. López-Olvera , Gregorio Mentaberre, Ignasi Marco and Santiago Lavín |
| 14.20-14.35 | Modulation of transport stress in Southern chamois (<i>Rupicapra pyrenaica</i>) using a short acting neuroleptic (25) | Jorge R. López-Olvera , Ignasi Marco, Jordi Montané, Encarna Casas-Díaz and Santiago Lavín |

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| 14.35-14.55 | Break | |
| 14.55-15.45 | Session: lagomorphs and other species | Chair: Kai Frölich Co-chair: To be announced |
| 14.55-15.10 | Emerging infections with tularaemia in Sweden (26) | Torsten Mörner , Roland Mattsson, Erik Ågren, Karin Bernodt, Henrik Uhlhorn and Dolores Gavier-Widén |
| 15.10-15.25 | Rabbit haemorrhagic disease in wild European rabbit (<i>Oryctolagus cuniculus</i>) populations in Hungary (27) | Karoly Erdelyi , Krisztina Ursu and Katalin Matiz |
| 15.25-15.45 | Neospora caninum in wild animals: a review (28) | E. Ferroglio and A. Trisciuglio |
| 15.45-16.45 | General Assembly of EWDA for all the participants | Marc Artois, Kai Frölich and Torsten Mörner |
| 20.00-23.00 | Auction | |

Saturday, September 11th 2004

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|-------------|--|---|
| 09.15-10.30 | Session: Carnivores (foxes) | Chair: Tony Sainsbury Co-chair: Marc Artois |
| 09.15-09.30 | Pathology of necrotizing encephalitis in Swedish arctic foxes (<i>Alopex lagopus</i>) (30) | Erik Ågren , Dolores Gavier-Widén, Kristina Nilsson, Torsten Mörner, Anna-Lena Berg |
| 09.30-09.45 | Detection of herpesvirus by PCR in Arctic foxes (<i>Alopex lagopus</i>) with encephalitis in Sweden (31) | Frederik Widén and Mikael Berg |
| 09.45-10.00 | Helminths of the predator-prey system red fox – wild rodent and effects of urbanization (32) | Leslie A. Reperant , Claude Fischer, Daniel Hegglin, Jean-Marc Weber and Peter Deplazes |
| 10.00-10.15 | Courtship and mortality in foxes (33) | Duff, J.Paul |
| 10.15-10.30 | Diseases found in French carnivores according to SAGIR database – global analysis | Marie-Eve Terrier , Jean-Roch Gaillet, Florence Cliquet and Jacques Barrat |
| 10.30-10.50 | Break | |
| 10.50-12.05 | Session: Carnivores (others) | Chair: Enzo Ferroglio Co-chair: Henrik Uhlhorn |
| 10.50-11.05 | A novel <i>Hepatozoon</i> sp. causing myocarditis in pine martens (<i>Martes martes</i>) in Scotland (34) | Vic Simpson , Roger J. Panciera, Judith Hargreaves, John W. McGarry, Sandra F. Scholes, Kevin J. Boms and Richard J. Birtles |
| 11.05-11.20 | Disease risk analysis for a reintroduction programme: common dormice (<i>Muscardinus avellanarius</i>) (35) | Anthony W. Sainsbury , Edmund J. Flach, Ghislaine Sayers, Andrew A. Cunningham, Shaheed K. MacGregor and Mark T. Fox |
| 11.20-11.35 | Rabies and racoons-Biology and behaviour (36) | Debra Bourne , Suzanne I. Boardman |
| 11.35-11.50 | The role of veterinary medicine in the Iberian lynx conservation program (37) | Fernando Martínez and Astrid Vargas |
| 11.50-12.05 | Detection of prey mtDNA in carnivore scat by polymerase chain reaction (38) | Anne Acton |
| 12.05-13.00 | Lunch | |
| 13.00-14.00 | Session: newly emerging diseases and the role of wildlife | Chair: Thomas Muller Co-chair: To be announced |
| 13.00-14.00 | SARS and influenza: newly emerging diseases and the role of wildlife | KEY NOTE SPEAKER: DR THIJS KUIKEN |
| 14.00-14.45 | Session: Birds-viruses | Chair: Thijs Kuiken Co-chair: Frederik Widén |
| 14.00-14.15 | The epidemiology of West Nile virus within wildlife populations and the potential risk of introduction and spread to the UK (39) | Mike Taylor and Colin Morgan |
| 14.15-14.30 | Usutu virus spread in Austria monitored by dead bird surveillance (40) | Herbert Weissenböck , Sonja Chvala, Tamás Bakonyi, Bernhard Seidel and Norbert Nowotny |

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| 14.30-14.45 | Pathology and viral distribution in Usutu virus infections of birds (41) | Sonja Chvala , Herbert Weissenböck, Tamás Bakonyi and Norbert Nowotny |
| 14.45-15.45 | Poster session with coffee | |
| 15.45-16.45 | EWDA Board meeting | |
| 19.00 | Banquet | |

Sunday, September 12th 2004

| | | |
|-------------|--|--|
| 09.15-10.30 | Session: birds | Chair: Karoly Erdelyi Co-chair: Vic Simpson |
| 09.15-09.30 | A virological survey in migrating waders and waterfowl in Germany (42) | Thomas Müller , Ortrud Werner, Anja Globig, Elke Starik, Horst Schirrmeier, Franz J. Conraths, Ralf-Udo Mühle, Dieter Wallschläger; Sonja Linke, Georg Pauli, Matthias Niedrig, Heinz Ellerbrok, Andreas Hlinak, Helmut Kruckenberg, Axel Degen, Melanie Buss, Johann J. Seeger |
| 09.30-09.45 | <i>T.gallinae</i> and trichomoniasis in wintering woodpigeons (<i>Columba palombus</i>) in South-Central Spain: sublethal effects and implications for endangered birds of prey (44) | Diego Villanúa , Ursula Höfle and Christian Gortázar |
| 09.45-10.00 | Avian trichomoniasis in birds of prey: emerging disease, emerging reservoirs of bioindicator? (45) | Ursula Höfle , Juan Manuel Blanco, Diego Villanúa, Birgit Knispel, Erhard F. Kaleta |
| 10.00-10.15 | Histological lesions provoked by hydrocarbons to oiled seabirds (46) | S. Le Dréan-Quéneç'hdu , E. Risi, A. Poncet, A. Frère, M. L'Hostis. |
| 10.15-10.30 | Bacterial and fungal contaminants in raptor ejaculates and their survival to sperm cryopreservation protocols (47) | Juan Manuel Blanco , Ursula Höfle |
| 10.30-10.50 | Break | |
| 10.50-11.20 | Session: exotics | Chair: Erik Ågren Co-chair: Ignasi Marco |
| 10.50-11.05 | Chronic hyperplastic gastropathy with cyst formation in 13 green iguanas (<i>Iguana iguana</i>): a preliminary study (48) | Berrocal, A |
| 11.05-11.20 | Acute death due to infectious diseases in five monkeys (49) | Berrocal, A |
| 11.20-12.00 | Discussion: The future of wildlife disease surveillance in a European Community of 25 countries. Report from round table on 7 Sept 2004 | Marc Artois |
| 12.00-12.15 | Closure of the Conference | Marc Artois Torsten Mörner |
| 12.30 | Departure to Skansen | |

Poster Programme

Thursday, September 9th 2004

Place: Loftets stora sal, SLU, Uppsala

| | | |
|----|--|---|
| 1 | A case of simultaneous cerebral setariosis and nodular cutaneous onchocercosis in red deer (<i>Cervus elaphus hippelaphus</i>) | Christoph Beiglböck , Theodora Steineck, Herbert T Feuchter, Armin Deutz |
| 2 | Comparison of the efficacy of two methods of capturing Spanish ibex (<i>Capra pyrenaica</i>): drive-net and box trap | Encarna Casas-Díaz , Jorge R. Lopez-Olvera, Gregorio Mentaberre, Ignasi Marco and Santiago Lavin |
| 3 | Mycoplasmas carried by Spanish ibex (<i>Capra pyrenaica</i>) in southern Spain: frequency and risk factors associated | Monica González Candela, Gisele Verbisck Bucker, Pablo Martin Atance, María Jose Cubero Pablo and Luis León Vizcaíno |
| 4 | Effect of acepromazine on drive-net capture stress in Southern chamois (<i>Rupicapra pyrenaica</i>) | Jorge R. López-Olvera , Ignasi Marco, Jordi Montane, Encarna Casas-Díaz and Santiago Lavin |
| 5 | Time trends of metals in organs of moose (<i>Alces alces</i>) from Sweden | Tjelvar Odsjö, Anders Bignert, Sofia Baez, Vera Galgan, Lars Petersson, Torsten Mörner, Henrik Uhlhorn |
| 6 | A case of swine pox in a wild boar (<i>Sus scrofa</i>) in Austria | Theodora Steineck , Jolanta Kolodziejek, Norbert Nowotny, Franz Schilcher |
| 7 | Antibiotic resistance in wildlife | Marianne Sunde, Jannice Schau, Atle Lillehaug and Kjell Handeland , Jon Martin Arnemo |
| 8 | Searching for TSE diseases in reindeer in Norway and Iceland | Sigurdur Sigurdarson |
| 9 | Rapid histological exam applied to the diagnosis of wild animal diseases: cases report | Domenis L., Robetto S., Pepe E., Navarretta R., Orusa R |
| 10 | Wildlife disease surveillance in Asturias (Northern Spain) | Diego Villanúa , Alberto Espi, Ursula Höfle, José Miguel Prieto, Ana Balseira, Pelayo Acevedo, Vanesa Álzaga, and Christian Gortázar |
| 11 | <i>Mycoplasma agalactiae</i> infection in Spanish ibex (<i>Capra pyrenaica</i>) populations from Andalusia, Spain | Gisele Verbisck Bucker, Monica González Candela, Maria Jose Cubero Pablo and Luis León Vizcaíno |
| 12 | Sanitary survey of the Iberian lynx (<i>Lynx pardinus</i>) and associate species | Javier Millán |
| 13 | Distribution of allelic forms at the porcine Natural Resistance-Associated Macrophage locus (Nramp1) among west European populations of wild and domestic pigs | Thomas A., Vanden Bergh P., Linden A. , Desmecht D |
| 14 | Analysis of prion protein gene (PRNP) sequence variation in Italian wild ruminants | S. Peletto , L. Sbaiz, A. Ferrari, S. Gennero, E. Ferroglio, R. Orusa, M. Caramelli, P.L. Acutis |
| 15 | Diagnosis of TSE strains using the prion specific antibody 15B3 | Kuhn F., Dietrich A., Pürro M., Zwald. D., Schmid J., Biffiger K., Oesch B. , and A.J. Raeber |
| 16 | Novel rapid TSE diagnostic tests | Price, P., Nayki, I., Iswanti-Stanek, D., Meier, M., Stadelmann, M., Buchmann, A., Biffiger, K., Kübler, E., Meissner, K., Sidler, M. and B. Oesch |
| 17 | Analysis of the Prion Protein of Big Game Cervids in Sweden Indicate Susceptibility to Chronic Wasting Disease | Magnus Simonsson, Mikael Klingeborn, Tommy Linné |
| 18 | Western blot characterisation of a Swedish case of Nor98 | Therese Ottinger, Magnus Simonsson, Mikael Klingeborn , Tommy Linné |
| 19 | Shedding of the bovine prion protein | Mikael Klingeborn , Hongxing Zhao, Magnus Simonsson, Tommy Linné |

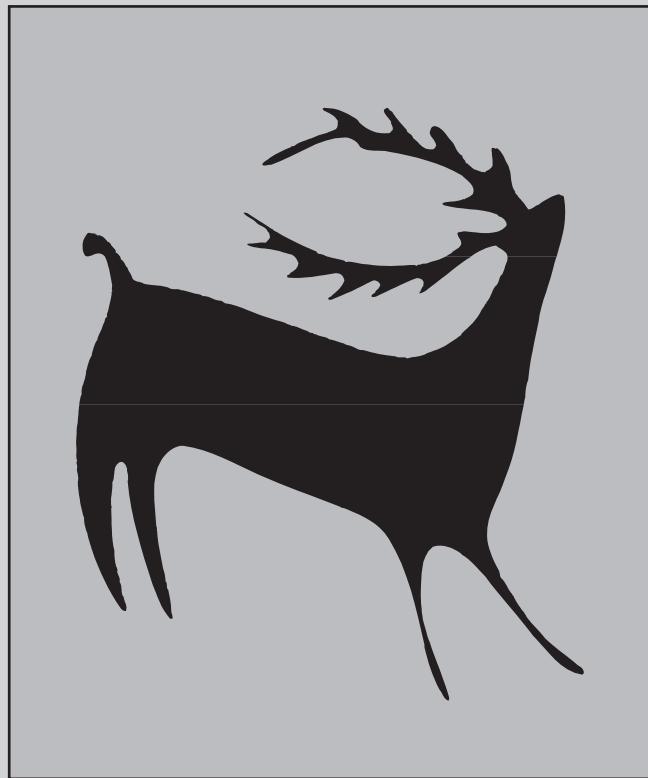
Saturday, September 11th 2004

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| 20 | Vanadium content of oiled birds: is it a good marker for oil exposition? | Kammerer M., Le Dréan-Quenech'du S. , Pouliquen H., Larhantec M |
| 21 | Risk assessment of lead poisoning in white-tailed sea eagles from Germany | Norbert Kenntner, Oliver Krone , Frieda Tataruch |
| 22 | Detection of organochlorides in marine turtles stranded in the canary | Patricia Monagas , Jorge Oros, Javier Arana and Óscar M. González-Díaz |
| 23 | An outbreak of cutaneous poxvirus infection in stone-curlews (<i>Burhinus oedicephalus</i>) in Gran Canaria island, Spain | Patricia Monagas , Jorge Oros, Ana B. Casal and Francisco Freire |
| 24 | Impact of the human activities on the mortality of sea turtles stranded in the Canary Islands, Spain (2003-2004) | Jorge Oros, Patricia Monagas , Ana B. Casal, Pascual Calabuig |
| 25 | Detection of metals in tissues of loggerhead turtles (<i>Caretta caretta</i>) stranded in the Canary Islands, Spain | Ayose Torrent, Patricia Monagas , Óscar M. Gonzalez-Diaz and Jorge Oros |
| 26 | Zinc phosphide- A risk for granivorous birds | Frieda Tataruch, Theodora Steineck , Oliver Krone |
| 27 | <i>Leucocytozoon ziemanni</i> infection in a wild population of little owl (<i>Athene noctua</i>) in Southern Portugal | Santos, N. , Tomé, R. Cardia, P. Ferrand, N. Korpimäki, E. |
| 28 | Wild carnivores as bioindicators of <i>Salmonella sp.</i> infections (Aosta Valley, NW Italy) | Orusa R. , Lo Valvo T., Balestrieri A., Ferrari N., Remonti L., Ferrari A., Robetto S., Domenis L. |
| 29 | <i>Trichinella spp.</i> In large predators in Finland | Marja Isomursu , Antti Oksanen and Tania Mikkonen |
| 30 | Mixed hemoprotozoa infection causing disease in an injured, wild Iberian wolf (<i>Canis lupus signatus</i>) | Santos, N.G. ; Madeira de Carvalho, L.M.; Pacheco, C. and Fazendeiro |
| 31 | Wild birds and West Nile virus in Camargue, Southern France | Elsa Jourdain , Michael Gauthier-Clerc, Dominique Bicout, Yann Toussaint, Yves Kayser, Phillippe Sabatier |
| 32 | Lead intoxication by ingestion of lead shot in racing pigeons (<i>Columba livia</i>) | Paul Tavernier , Stefan Roels, K. Baert, K. Hermans, F. Pasmans |
| 33 | Diseases found in French carnivores according to SAGIR database – interesting cases | Marie-Eve Terrier , Jean-Roch Gaillet, Florence Cliquet and Jacques Barrat |

Abstracts

TSE and CWD Workshop

September 8th



Wednesday 8 September

Oral Presentation 1

TSES – AN EVOLVING DIAGNOSTIC CHALLENGE

MARION M SIMMONS, Department of Pathology, Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey, UK.

It is now well established that transmissible spongiform encephalopathies (TSE) are a group of invariably fatal diseases with long incubation periods, recorded forms of which have occurred naturally in some species – notably as scrapie in sheep – for over 250 years. In 1986, an apparently novel TSE, which became known as bovine spongiform encephalopathy (BSE) was reported in cattle in the UK. The subsequent, epidemiologically linked TSE in domestic cats (FSE), zoo collections of exotic ungulates and big cats and most recently a variant of Creutzfeldt-Jakob disease in man, have resulted in these diseases being extensively researched worldwide. Exhaustive active surveillance programmes with a public health focus have also been implemented, through both European and national legislation, to guard the human food-chain throughout the EU and elsewhere. Despite this extensive effort, most of the many fundamental questions regarding the nature and transmissibility of the causal agent, its zoonotic potential and possible therapeutic strategies remain unanswered.

The histopathological changes associated with these diseases are confined to the central nervous system, and are not apparent until late in the incubation period. However, an abnormal isoform of a host-encoded protein (PrP) invariably accumulates in association with these diseases, and it is hypothesised that this protein is in fact the causal agent. Certainly, the presence of host PrP has been shown to be a pre-requisite for effective experimental transmission of disease, and polymorphisms in the host PrP gene in sheep have been clearly linked to relative susceptibility to natural scrapie. The phenomenon of genetic susceptibility in sheep is so pronounced that it forms the basis of current disease control strategies for scrapie in a number of European countries.

Abnormal protein can be detected in advance of the histopathological changes and (in some circumstances) it is present in readily detectable quantities in the lymphoreticular system, a feature which has been exploited for diagnostic purposes in the live animal. The immunochemical detection of this abnormal protein forms the basis of all the methods currently used for surveillance of both healthy and suspect populations for evidence of infection and confirmation of disease.

There is a marked diversity in both the biological and molecular characteristics of these diseases, and much current research is focussed on attempts to classify isolates into 'strains' based on these different parameters. Indeed, it was the similarity in biological characteristics of isolates from a variety of species when transmitted to mice that first supported the epidemiological links between BSE in cattle and its subsequent identification in a range of other host species.

As more active surveillance programmes are implemented our appreciation of the range of this diversity in natural disease is growing, and challenging and exceeding our current definitions and categorisations. It is of particular concern that indications of infection have now been reported in a small but growing number of sheep of the most resistant genotypes, raising doubts about disease control strategies and sub-clinical or carrier states. A lack of knowledge about which aspects of any isolate determine its pathogenicity, transmissibility and (most particularly) its zoonotic potential means that approaches to strain typing of these previously unrecorded isolates, and understanding their basic biology, is currently one of the most pressing areas of TSE research.

This presentation will review the pathology of known TSEs in a range of species, describe the current research being undertaken at the VLA into phenotypic diversity, and discuss the diagnostic and political problems arising from the increased number of previously uncharacterised isolates being identified by current surveillance programmes within the EU.

Oral Presentation 2

THE NEW EUROPEAN STRUCTURE FOR RISK ASSESSMENT: BIOLOGICAL HAZARDS AREA

DR. BART GOOSSENS, EFSA, Scientific Panel on Biological Hazards

The European Food Safety Authority (EFSA) is the keystone of the European food safety network. The Authority is committed to providing scientific advice of the highest possible quality and clear communication of existing and emerging risk. It provides risk assessments on all matters linked to food and feed safety, including animal health and welfare and plant protection. In addition, the Authority provides scientific advice on nutrition in relation to Community legislation.

Five principal objectives underlie EFSA's scientific activities and work program:

1. To provide scientific opinions and advice in response to questions related to food safety issues formally addressed to the Authority by the European Commission, the European Parliament, the Member States or by the Authority itself (ie through "self-tasking).
2. To assess the risk of, and as appropriate, propose risk-related factors for specific groups of regulated substances, following notification procedures and time schedules defined by legislation (e.g. food additives, food flavorings, feed additives including medicinal products, pesticides, GMOs and novel food ingredients).
3. To monitor specific risk factors and diseases and provide scientific opinions on tests and other tools to control these risk factors and diseases (for instance, the geographical BSE risk assessment or the monitoring of zoonoses and other food-borne zoonotic agents).
4. To prepare work for the future evaluation of health claims made for food products. Depending on the final text of the legislation currently being discussed in Council and Parliament, this could include the development of proposals for and guidance on nutritional profiles which must be respected or on the nature and extent of scientific data required to substantiate a given claim.
5. To apply and promote new and harmonized scientific approaches and methodologies for hazard and risk assessment of food and feed.

EFSA's risk assessments are carried out by its Scientific Committee and eight Scientific Panels specialized in the following areas:

Panel on food additives, flavorings, processing aids and materials in contact with food (AFC)

Panel on additives and products or substances used in animal feed (FEEDAP)

Panel on plant health, plant protection products and their residues (PPR)

Panel on genetically modified organisms (GMO)

Panel on dietetic products, nutrition and allergies (NDA)

Panel on biological hazards (BIOHAZ)

Panel on contaminants in the food chain (CONTAM)

Panel on animal health and welfare (AHAW)

The Panels are made up of leading independent scientists coming from all over Europe and even in a few cases from beyond Europe, and were appointed following an open call for expression of interest. The Scientific Committee co-ordinates the work of the Panels, proposes common methodology and guidance in carrying out risk assessments, and addresses transversal issues common to all Panels (for instance, exposure assessment).

The Scientific Committee and Expert Panels are supported by EFSA's own scientific staff. In addition, the Authority expects to reinforce its Science department by creating a series of expert service "teams" each dedicated to a specific area of risk assessment (eg data collection, epidemiology and exposure...).

In total EFSA's team of highly qualified scientists, experts in their respective fields, and support staff is expected to number 70 in 2004, representing approximately 50% of total headcount.

Finally, the Authority will also seek to build scientific networks involving Community institutions, national food safety authorities and scientific institutions in and outside the EU as well as international organizations in order to: facilitate exchange of information and expertise; evaluate possible collaboration in areas of mutual interest and continuously improve its own scientific knowledge and expertise.

More in particular, the Scientific Panel on Biological Hazards replies to questions related to Biological Hazards for human health and food safety. It provides scientific based risk assessments (opinion) on biological hazards relating to food safety and food-borne disease, including food-borne zoonoses and transmissible spongiform encephalopathy (TSE), microbiology, food hygiene and associated waste management.

As compared to the structure previously under the Commission services, the Scientific Panel on Biological Hazards combines the TSE/BSE ad hoc group of the Scientific Steering Committee (SSC) and the Scientific Committee on Veterinary Measures related to Public Health (SCVMPH).

Oral Presentation 3

STUDY ON TSE (CWD, BSE AND SCRAPIE) IN CERVIDS FROM GERMANY

- SURVEY METHOD AND SAMPLING DESIGN, PRELIMINARY RESULTS, DIFFICULTIES AND RECOMMENDATIONS FOR FUTURE SURVEYS-

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Chronic wasting disease (CWD) spread within North America during the last 30 years and scrapie is present for more than 200 years in Germany. Not surprisingly, after the German BSE crisis in November 2000, the question arose if transmissible

spongiform encephalopathies (TSEs) could also affect wild ruminants within Germany since approximately 1.2 million cervids are hunted annually in Germany and around 30,000 tons of venison originating from German game are consumed each year. The most important species hunted are 1.1 million roe deer (*Capreolus capreolus*), 60,000 red deer (*Cervus elaphus elaphus*) and 52,000 fallow deer (*Cervus dama dama*). Thus far, there is no information that TSEs existed in cervids from Germany or any other European country. It is, however, theoretically possible that TSEs (BSE, CWD or scrapie) were introduced to German cervid populations in the past. Therefore, a study was set up to determine the occurrence of TSEs in cervids from Germany with a 95% probability of detecting at least one positive case at a prevalence of $\geq 0,5\%$. To reach this goal, the required sample sizes were statistically estimated at 3000 roe deer, 2000 red deer and 2000 fallow deer (free-living cervids) and at 1000 (captive cervids) considering also the below listed risk factors. Cervids included into the study are older than 18 months and brain (obex region) and retropharyngeal lymph nodes of each individual are tested by Biorad ELISA. Moreover, immunohistochemistry (IHC) is performed from at least 1000 selected brain and lymph node samples. Due to the fact that several risk factors may affect a possible transmission of BSE, CWD and scrapie a stratified sampling procedure was set up. Sampling methods are based on hunter harvested as well as targeted survey. For each risk category, hunter harvested samples are collected proportionally to bags in each region within Germany. Moreover, determined risk factors are (1) regions where BSE incidence in cattle is higher than average BSE incidence in Germany, (2) sick animals with clinical signs like cachexia or CNS disorders as well as road kills, (3) species of higher risk: European red deer is considered more likely than other species to be susceptible to CWD, because zoologically closely related to Wapiti (*Cervus elaphus nelsoni*), (4) age, and (5) captive cervids (there is a higher chance that captive cervids have come into contact with meat or bone meal than free-living cervids).

Preliminary results: Since August 2002, more than 4,000 samples from German cervids have been tested negative for TSEs using Biorad ELISA. A limited number of samples were also analysed by IHC. However, the detection limits are not yet sufficient to draw any final conclusion.

Scientific surveys in different European countries would be advisable to gain further knowledge on susceptibility and pathogenesis of TSE infections in further European cervid species and regions. In this context, it should be recognized that difficulties may arise e.g. with local authorities or hunters especially regarding the collection of samples from wildlife. It should be taken into consideration that all EU member states have to follow regulations of the decree V (EG) 999/2001 on TSE prevention. Even though cervids are not explicitly mentioned, questions (e.g. regarding the consumption of venison before test results) might be controversially discussed between local authorities and could consequently arise difficulties for studies on TSE in cervids. Moreover, in many European countries game farmers might not receive reimbursement payments in case of a positive testing.

Oral Presentation 4

CHRONIC WASTING DISEASE: HISTORY, EPIDEMIOLOGY, AND VIEWS FROM THE FIELD

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Chronic wasting disease (CWD) is not a new disease; it has been present in populations of deer for decades. But following recognition of bovine spongiform encephalopathy and its relationship to variant Creutzfeldt-Jakob disease, the need to have a better understanding of CWD was apparent. Chronic wasting disease was first identified in captive mule deer (*Odocoileus hemionus*) in the late 1960s but evidence suggests that it was present in populations of free-ranging deer prior to that recognition. Its geographic distribution, although apparently expanding, is still relatively limited in western North America with foci in midwestern states.

Chronic wasting disease transmission is being studied at two levels: among individual animals and among populations. Horizontal transmission and indirect transmission associated with environmental contamination appear to be significant in CWD; however, specifics of how transmission occurs remain to be determined. Movement of CWD in populations of deer and elk depends upon how these populations are managed. Chronic wasting disease in farmed cervids (primarily elk [*Cervus elaphus nelsoni*]) has been moved among jurisdictions with live animals transported between farms and ranches prior to institution of state, provincial, and federal CWD control programs. Surveillance, development of herd plans, quarantine, and depopulation of more than 10,000 elk have resulted in little known CWD in the captive cervid industries in North America at this time. The presence of CWD in free-ranging mule and white-tailed deer (*Odocoileus virginianus*) and elk is complicated by difficulties in surveillance, movement of deer and elk under little human influence, and lack of proven techniques to control the disease. This likely will result in continued slow spread of CWD among free-ranging cervid populations in North America.

CHRONIC WASTING DISEASE (CWD) - CURRENT SITUATION IN CANADA

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Chronic wasting disease (CWD) is a newly- recognized disease of cervids with the potential to affect free living and captive populations of deer and elk in North America. Canada had three cases of CWD prior to 2000. In 1974, CWD was diagnosed in a mule deer at the Toronto Metropolitan Zoo. In 1996 and 1998 CWD was confirmed in farmed elk from Saskatchewan. In both cases a single elk was diagnosed with CWD.

In 2000, the Canadian Food Inspection Agency (CFIA) confirmed CWD in another infected elk on the same premises as the elk found to be infected in 1998. When the CFIA established that all three cases (1996, 1998, 2000) had originated from a single premises (source herd), CFIA took immediate action to trace the movement of all elk from this farm and implemented a national program to eradicate CWD in October 2000. A regulation making CWD a Reportable Disease under the *Health of Animals Act* came into effect in April 2001.

Since 1996, over 9000 farmed cervids (elk and deer) from 41 infected premises in Saskatchewan and 2 premises in Alberta have been destroyed in Canada for disease eradication purposes. All cervids except very young animals were tested, 232 of which were infected, 31 of them considered clinical. Diagnosis and confirmation of the "diseased" state was made by several methods:

1. Presence of clinical signs (emaciation, pneumonia etc.) leading to suggestion of disease and a clinical diagnosis.
2. Presence of bilaterally symmetrical microscopic lesions of spongiform encephalopathy leading to a morphological diagnosis supplemented by the IHC demonstration of PrP^{CWD} in brain and/or in germinal centers of lymphoid follicles.
3. Using extracts of unfixed brainstem (caudal to the obex) samples were separated for the detection of PrP^{CWD} either by WB or ELISA and in index cases by disease -specific aggregated fibrils. The fibrils are shown following treatment with proteinase-K, negative staining and examination by electron microscopy (EM).

In contrast to the relatively predictable distribution of PrP^{CWD} in the brain and cranial lymph nodes of sheep and mule deer, elk demonstrated a more variable pattern unrelated to genotype. There was no detectable infectivity in tonsils or retropharyngeal nodes of 14% of the elk with PrP^{CWD} in the brain. This distribution patterns in elk suggest that various routes of infection or different strains of the agent could be involved in the pathogenesis.

Genetic analysis of the open reading frame of the cervid PrP gene was done on 105 of the 230 test positive elk and 101 of 105 were homozygous for 132M. Four animals were heterozygous LM and CWD was not detected in elk homozygous for 132L. Molecular analysis of PrP derived from different TSEs has been regarded as a valuable aid to confirm the etiological diagnosis.

A collaborative study conducted at the VLA laboratory, Weybridge, U.K using 2 monoclonal antibodies (mAbs) examined the molecular profile of PrP^{res} from both elk and white-tailed deer in comparison to PrP^{res} obtained from other animal TSEs. The PrP^{res} isolated from CWD infected cervids had a higher molecular weight profile than did the corresponding PrP^{res} from the scrapie and BSE isolates. The PrP^{res} from CWD cases cross reacted with both mAbs, a property shared with PrP^{res} in isolates from scrapie but not with PrP^{res} isolates from BSE or sheep experimentally infected with BSE. The results from this study seem to indicate that the PrP^{res} from CWD cases differs in its molecular and immunological characteristics from the BSE and scrapie cases studied.

All Saskatchewan cases have been in elk and most are epidemiologically linked to the source herd which imported from a US premise known to be infected. There has not been a farmed infected animal found in Saskatchewan since March 2002. The infected elk in Alberta was detected on a farm that was established from US elk. Through slaughter surveillance an infected white-tailed deer was found in Alberta in late 2002. No new infected farm deer herds have been discovered since, despite extensive tracing and destruction of animals from that premise.

Mandatory provincial CWD surveillance in the provinces of Alberta, Saskatchewan, Manitoba and the territory of the Yukon requires that all cervids that die, whether at slaughter or otherwise, over one year of age be tested for CWD. The provincial governments are also responsible for testing wild cervids for CWD. In wild deer populations CWD was first detected in mule deer in 2000 with 34 confirmed cases to date in three focal areas in Saskatchewan, majority of them detected in an area called "Saskatchewan Landing" in the south of the province.

The eradication policy applies to all cervidae in zoos and on farms. The federal and provincial governments, the cervid industry, veterinary colleges and veterinary practitioners are cooperating in the national program to eradicate this disease. The national CWD eradication policy requires the destruction of all exposed cervids on an infected premise and any exposed cervids which have moved off the premises in the 36 months prior to the detection of infection. Animals that are ordered destroyed are buried or incinerated. Carcasses and products from known infected animals are not permitted to enter the food chain. A voluntary herd certification program is being implemented in all provinces, based on standards developed by the CFIA which requires submission of CWD samples from animals that die on the premises over a 5 years period, as well as restrictions on acquisitions, after which time the herd is considered free from the disease.

August 2004

Oral Presentation 6

CHRONIC WASTING DISEASE IN CERVIDS

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Spongiform encephalopathies are a family of neurodegenerative diseases that occur in humans and animals. These diseases appear to be progressive with long incubation periods. The spongiform encephalopathies are characterized by histological lesions confined to the brain and include a spongiform degeneration of the neuropil, neuronal vacuolization, and degeneration with mild astrocytosis. The spongiform encephalopathies that have been identified in man include Kuru of Papua, New Guinea; Creutzfeldt-Jakob disease; variant Creutzfeldt-Jakob disease; fatal familial insomnia; and Gerstmann-Sträussler-Scheinker disease. The spongiform encephalopathies described in animals include scrapie of domestic sheep and goats, transmissible mink encephalopathy (TME), chronic wasting disease (CWD) of deer and elk, bovine spongiform encephalopathy (BSE), BSE in exotic ruminants, and feline spongiform encephalopathy. All of these diseases cause similar spongiform degenerative changes in the brain and spinal cord of the species they affect. This presentation will be limited to Chronic Wasting Disease of deer and Rocky Mountain elk.

Clinical Signs: Early cases of CWD are not detectable clinically because symptoms do not appear until several months before death. The primary clinical signs of animals with CWD include weight loss that progresses to emaciation; excessive salivation; abnormal behavior, including loss of fear of humans; and mild ataxia. Captive animals drink excessive amounts of water and are usually polyuric. Sometimes the animals will not shed their winter hair coat in the spring. Occasionally because of atrophy of muscles of the head, the eyes tend to bulge and one can visualize more of the sclera. Age of onset of clinical signs varies, but in free-ranging animals cases occur in deer and elk most commonly between 3-5 years of age.

Gross Lesions: There are usually no gross lesions in early cases of CWD. The primary gross lesions in advanced cases of CWD in deer and elk include weight loss, emaciation, and loss of abdominal and subcutaneous adipose tissue. The adipose tissue behind the eye; around the spinal cord; within the bone marrow, joints, and pelvis of the kidney; and around the coronary vessels is characterized by serous atrophy. Adrenal gland enlargement may be noted especially in mule deer. Aspiration pneumonia is often observed and is more common in elk than deer. Occasionally, abomasal ulcers with or without localized peritonitis can be found in deer, but is not a common finding in elk. In captive mule deer the rumen may be filled with a light-green to gray watery froth, but this is rare in free-ranging deer and elk.

Differential Diagnosis: Using only clinical signs any disease that can cause debilitation can be confused for CWD. The most common conditions confused with CWD in captive cervids includes but is not limited to: abnormal or worn dentition, tooth abscess, hardware disease, and ingestion of foreign material such as baling twine, rope, or plastic. Other conditions that may be confused with CWD on clinical examination include neoplasia and internal abscesses. One of the most important conditions observed clinically is bronchopneumonia. This condition is rare in deer and elk, but when present is commonly associated with CWD. There has been several times where CWD was missed on gross examination because the animals had a severe bronchopneumonia and the brain was taken but not examined. Ulceration of the abomasums is occasional seen in deer. It is not uncommon for these ulcers to perforate the wall and cause localized peritonitis. Therefore if bronchopneumonia or ulcerative adomasitis are found the brain should be examined carefully for spongiform encephalopathy. Chronic Bluetongue or Epizootic Hemorrhage Disease may be confused with CWD in deer. There are numerous other lesions with these two diseases that are not found in CWD including chronic ulcers of the rumen (not abomasal ulcers), ulcers and erosions of the mouth, hemorrhage diarrhea, and hemorrhages on the pulmonary artery.

Pathogenesis: The proposed pathogenesis of CWD is similar to the other TSE's. It is believed that a rogue protease-resistant prion protein gains access to a susceptible host, probably via ingestion. Once in the susceptible host, the abnormal prion protein enters lymphoid tissues, then by an unknown route gains access to the brain. Following entrances of the abnormal prion into the lymphoid tissues and the brain, it has the ability to convert normal prion protein to the aberrant protease-resistant (PrP^{res}) conformation. Even though nearly all tissue types contain the normal isoform of the prion protein; the

tissues of the body in which the PrP^{res} accumulates to the highest degree and appears to cause the most damage is the brain. The pathogenesis in which the PrP^{res} causes disruption of neurological function is not known. Typical histological lesions of spongiform encephalopathy (spongiform degeneration of the neuropil, neuronal vacuolization and degeneration, and astrocytosis) follow the accumulation of the PrP^{res}. The protein appears to be sticky and clumps in neural and lymphoid tissues. These plaques may be visualized with various immunohistochemical staining techniques that are used commonly in diagnosing and confirming these diseases.

In domestic sheep with scrapie and in deer with CWD, PrP^{res} appears initially in lymphoid tissues after infection, but the greatest concentration develops later in the brain where it causes the most damage, resulting in clinical signs. In elk early data shows that the majority of the time the abnormal prion infects the lymphoid tissue first, but occasionally the lymphoid tissues seem to be bypassed and the abnormal prion go straight to the brain.

Histological Diagnosis and Limitations: The primary histological lesions found in deer and elk with CWD are limited to the nervous system and are typical of the spongiform encephalopathies of the other animals and man. The lesions are characterized by microcavitation of neuropil, intraneuronal vacuolation, neuronal degeneration and loss with mild astrocytosis. The spongiform degeneration appears to be first in the neuropil; vacuolation of neurons follows the lesions within the neuropil. Lymphoid depletion of the tonsils, lymph nodes, and spleen also occurs in most animals in the terminal stages of the disease, but not in the early cases. Microscopic examination can be used to confirm secondary gross lesions seen with the disease, such as bronchopneumonia, gastric ulceration and peritonitis, hypertrophy of the adrenal cortex, and serous atrophy of fat.

The major limitations of histopathology are the quality of the sample and the correct neuroanatomical location submitted for examination. Samples should be fresh and fixed well in 10-15% neutral buffered formalin. Autolysis causes the most problems. With autolysis there are numerous holes throughout the neuropil; neurons and astrocytes shrink and are surrounded by a clear zone. The nuclei of the neurons and astrocytes are not crisp as in fresh samples of the brain. Vacuolation can be found in normal neurons, but is usually not a feature of autolysis. Occasionally vacuolated neurons can be found in any of the nuclei of the thalamus, hypothalamus, or brainstem, but are especially common in the Red Nucleus. Vacuolated neurons also are common in the trigeminal ganglia.

Immunohistochemical staining: Because the abnormal prion is present in the brain before histological changes of spongiform degeneration occur, immunohistochemical stains designed to detect abnormal prion proteins are helpful in detecting early (preclinical) cases of CWD. These stains also are useful for confirming cases when freezing or autolysis of tissues has made histological evaluation impossible. Several immunohistochemical stains are available to demonstrate PrP^{CWD} in deer and elk. Currently the most commonly used monoclonal antibody in the United States and Canada for CWD and scrapie is MAb F99/97.6.1 produced by Katherine O'Rourke, Agricultural Research Service, U.S. Department of Agriculture, Pullman, Washington. This particular monoclonal antibody reacts with a conserved epitope (residues QYQRES) on the normal cellular prion protein of mule deer, white-tailed deer, Rocky Mountain elk, domestic sheep, and cattle. This monoclonal antibody is specific for the prion protein and is effective on fresh and formalin fixed tissues. This stain allows the detection of early infections with CWD using brain or lymphoid tissues in mule deer, white-tailed deer, and elk.

The appearance of PrP^{CWD} in lymphoid tissues is characterized by coarse bright red granular material filling the lymphoid follicles. In deer usually the entire follicles are filled. However, in some elk the positive red chromogen forms a crescent shaped area filling approximately half to three-quarters of the individual follicles. In deer and elk the medial retropharyngeal lymph node is the most commonly used lymph node. In deer most all of the follicles of the medial retropharyngeal lymph node and tonsil are filled with PrP^{CWD}. In elk it is common to see only a few lymphoid follicles stain for PrP^{CWD}; therefore, multiple sections of lymphoid tissues are sometimes necessary to confirm CWD in elk using lymphoid tissue. PrP^{CWD} is more consistently found in the medial retropharyngeal lymph node as compared to the palatine tonsil in elk. Deer seem to have similar amounts of PrP^{CWD} in the medial retropharyngeal lymph node as in the palatine tonsil. Lymphoid follicles free of IHC staining have a pale blue background.

The appearance of PrP^{CWD} in the brain is a coarse red granular material. The positive granular PrP^{CWD} often surrounded neurons appearing to be on their cell surface and scattered throughout the neuropil. Intracellular staining is rare. In many areas plaques of PrP^{CWD} can be found. Negative brain tissues are a pale blue.

In addition, because this stain also can detect abnormal prion protein in lymphoid tissues and because tonsil and retropharyngeal lymph nodes typically become positive before brain tissues in mule deer and white-tailed deer, this antibody may be used on tonsil biopsies for early preclinical testing in deer for CWD. Unfortunately, this staining pattern has not been as consistent in Rocky Mountain elk, limiting the usefulness of the test on tonsil biopsies in this species.

Mapping/Distribution of lesions and prion accumulation:

Detailed mapping of accumulation of PrP^{CWD} and spongiform degeneration has been done in mule deer and is presently being done in Rocky Mountain elk. One important feature is that the spongiform degeneration follows the accumulation of PrP^{CWD}. This pattern occurs in all of the 130 or so neuroanatomical sites examined in mule deer. The retropharyngeal lymph node and palatine tonsil are some of the first tissues to accumulate PrP^{CWD} to levels that can be visualized with immunohistochemistry.

The spread of PrP^{CWD} has been examined in mule deer. The first neuroanatomical area of the brain to accumulate PrP^{CWD}

is the lateral aspect of the middle one-third of the vagus nucleus. The PrP^{CWD} then spreads throughout this nucleus. The next location that accumulates PrP^{CWD} is the hypothalamus. The PrP^{CWD} then spreads from the vagus nucleus throughout the brain stem, thalamus, and olfactory cortex: then to the cerebrum. The cerebellum is the last region to be affected. The spread of PrP^{CWD} in elk is currently being studied and it appears to be similar as described in mule deer.

Rapid tests with methods of confirmation used in Colorado: Recently three ELISA based tests (Bio-Rad ELISA, IDEXX HerdChek CWD Test, and VMRD:Chronic Wasting Disease Antigen Test Kit, dbELISA) have been approved in the United States to be used for rapid screening of lymphoid tissues for CWD. Two of these tests were used last year for screening CWD in the United States and Canada. The Bio-Rad ELISA CWD assay was validated for mule deer, white-tailed deer, and Rocky Mountain elk at Colorado State University Veterinary Diagnostic Laboratories in 2001. Last year (hunting season 2003-2004) the Bio-Rad CWD test was used in 10 states and the IDEXX test was used in one state. Colorado State University tested over 17,000 deer and elk using Bio-Rad. Over the last 2 hunting seasons nearly 50,000 animals have been tested. The sensitivity and specificity of the Bio-Rad test as compared to IHC is 99.99 percent. Currently the Bio-Rad test is the most widely used screening test for CWD. Immunohistochemical staining is used for confirmation of these rapid ELISA screening tests.

Oral Presentation 7

THE HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES OF BOVINE AMYLOIDOTIC SPONGIFORM ENCEPHALOPATHY (BASE)

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A new BSE phenotype has been recently identified in Italy in a systematic study carried out on the whole brain from eight cattle that were positive to the rapid test (1). All these cases were identified through active surveillance and detailed clinical informations were not available. The new BSE variant was found in two animals and differed from typical BSE for the presence of PrP amyloid plaques and a PK-resistant prion protein (PrP^{res}) faster in migration and with predominance of the "mono-glycosylated" pattern of glycosylation. In addition, PrP^{res} had a different brain regional distribution, being most abundant in the forebrain as opposed to the predominance of the abnormal protein in the brainstem of typical BSE cases. This "Amyloidotic" BSE variant was named BASE. Actually, the neuropathologic hallmark of BASE was the occurrence of dense uni- and multi-centric PrP amyloid plaques, particularly in the white matter of the olfactory bulb, and frontal and parietal cortices. By contrast, typical BSE cases were characterized by the presence of granular-, linear- and glial-type PrP deposits in the olfactory bulb, and glial-type in the grey matter of the cerebral cortex. In the thalamus, BASE was marked by co-occurrence of PrP-plaques and granular- and glial-type PrP deposits, while the brainstem showed only a weak aggregates of PrP. The presence of early autolysis precluded an accurate study of the lesion profile. Although spongiosis was not found consistently in the brainstem, mild vacuolar changes were observed in the nucleus of the spinal tract of the trigeminal nerve and nucleus of the solitary tract. No vacuolation was detected in the olfactory bulb, piriform cortex, hippocampus, and frontal, parietal and occipital cortices. To verify the prevalence of BASE in Italy, we re-evaluated the immunohistochemical and immunoblot profile of all BSE cases detected to date. Although the retrospective analysis could be carried out only at the level of the obex due to unavailability of the whole brain in most instances, no additional cases with features of BASE were found. To investigate whether BASE phenotype is related to a prion strain distinct from the BSE strain, experimental transmission experiments have been set up in wild-type mice (SJL, C57Bl, RIII, VM), transgenic mice, monkeys, cattle and sheep. At the time of writing, preliminary data are available only for wild-type mice. All SJL mice infected with BSE and VCJD have developed clinical signs of disease with a mean incubation period of 274 and 266 days, respectively, while mice challenged with BASE are still free of clinical signs. C57Bl mice inoculated with BSE are developing neurological disturbances starting from 400 days after infection, while BASE-inoculated mice are still healthy. These data support the view that BSE and BASE are caused by prion strains with different biological properties.

1. Casalone C, Zanusso G, Acutis P, Ferrari S, Capucci L, Tagliavini F, Monaco S & Caramelli M (2004) Proc Natl Acad Sci USA, Mar 2; 101(9):3065-70

SCRAPIE NOR98

SYLSVIE.L. BENESTAD¹, P. SARRADIN² AND B.BRATBERG¹

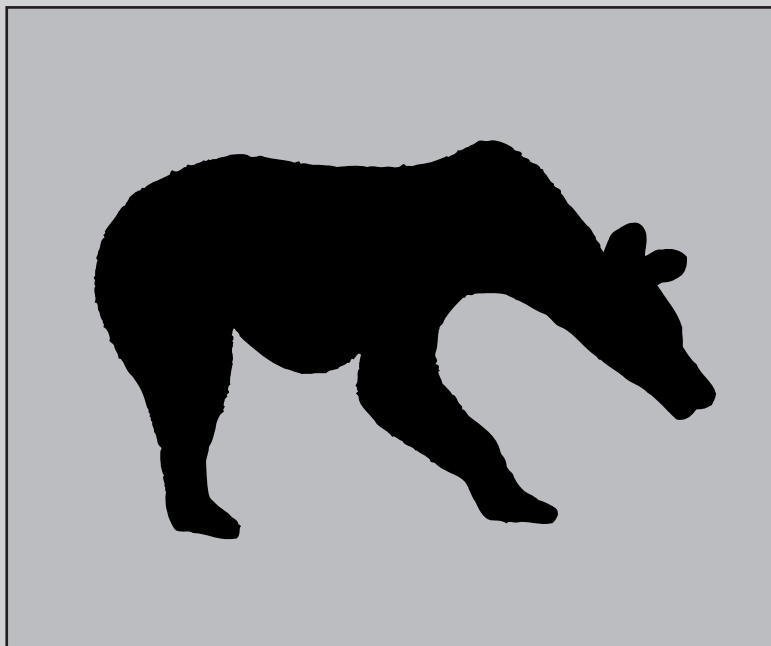
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Since 1998 38 atypical scrapie cases, designated Nor98, have been identified in Norway. The clinical signs, if present, were dominated by ataxia. Brain histopathology revealed neuropil vacuolisation essentially in the cerebellar and cerebral cortices, less in brainstem, and no lesions were found at the level of the obex . PrP^{Sc} deposits were mainly in the cortex of the cerebellum and cerebrum. Western blot analysis showed a glycoform different from other known scrapie strains and from BSE. No PrP^{Sc} was detected in the lymphoid tissues investigated.

Abstracts

Oral Presentations

September 9th-11th



Oral Presentation 1

THE EPIDEMIOLOGICAL CHARACTERISTICS OF *MYCOBACTERIUM BOVIS* INFECTION IN BADGERS.

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In the UK the European badger (*Meles meles*) is implicated in the transmission of *Mycobacterium bovis* to cattle. Understanding the underlying causes of the distribution of bovine TB in badgers is important in the context of effective management of the disease in cattle. The epidemiological characteristics of *M. bovis* infection have been studied using data from a long term epidemiological and ecological study of a wild badger population in South West England. The probability of individual animals being allocated to different infection classes varied with age. Live-sampling indicated that bacilli were most likely to be isolated from sputum although faeces, urine, bite wounds and abscesses were also infectious. Spatio-temporal analyses of disease patterns showed that infection persisted over time in specific localities, related to the social structure of the population. Movement of animals between social groups was correlated with increased incidence of infection. The results are discussed in relation to management strategies for reducing the risks of transmission from badgers to cattle.

Oral Presentation 2

RAPID TEST FOR SEROLOGICAL DETECTION OF TUBERCULOSIS IN MULTIPLE ANIMAL SPECIES.

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Tuberculosis remains a serious re-emerging disease in wildlife and zoo animals, as various species are susceptible to *Mycobacterium bovis* and/or *M. tuberculosis*. To improve tuberculosis control, new diagnostic tools that would be rapid, accurate, and host species-independent are needed. We propose novel serological methods, MAPIA (MultiAntigen Print ImmunoAssay) and the lateral-flow technology, for specific antibody detection in tuberculosis. The humoral immune responses against multiple proteins during either experimental or natural infection were characterized by MAPIA in several animal species including white-tailed deer, elephant, and gazelle. The results demonstrated the remarkable heterogeneity of antigen recognition during disease in various species. Positive associations between antibody responses and the degree of pathological change due to tuberculosis infection were detected. Serological markers of diagnostic importance were identified for each host. Elephant antibody kinetics data suggested that the MAPIA could be used for monitoring treatment in these animals. Using selected antigens, a lateral-flow test was developed for rapid antibody detection in multiple species. The test can use serum, plasma, or whole blood and provides results within 15 minutes. Advantages of the proposed serological approach for tuberculosis detection in multiple animal species will be presented.

Oral Presentation 3

IMPROVED SERODETECTION OF *MYCOBACTERIUM BOVIS* INFECTION IN BADGERS (*MELES MELES*) USING MULTI-ANTIGEN TEST FORMATS.

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Despite attempts to control bovine tuberculosis, the incidence of disease in Great Britain continues to rise. In GB, the European badger (*Meles meles*) is a reservoir of infection with *Mycobacterium bovis*. In an effort to improve the serodetection of badger tuberculosis, we examined sera from *M. bovis* culture-positive and culture-negative badgers for their ability to recognise *M. bovis* antigens, using a multi-antigen print immunoassay (MAPIA). Depending on the antigens used in

the MAPIA, the assay had a sensitivity of 49-55% and a specificity of 84-88%. Results from the MAPIA were used to select antigens for the development of a lateral-flow immunoassay. This so-called 'Rapid Test' used 5µl of serum and gave unambiguous results within 10 minutes. When applied to 178 badger sera, the Rapid Test had a sensitivity of 53% and a specificity of 95%. This represented an improvement over the performance of the existing ELISA Test, which had a sensitivity of 47% and a specificity of 89% on the same sera. Two formats of 'Rapid Test' are currently undergoing validation on approximately 1500 badger sera. The performance of each test format will be presented at this meeting. These diagnostic tests for badger tuberculosis are the first that can be performed alongside the captive animal and open up new options for the diagnosis and control of tuberculosis in wildlife species.

Oral Presentation 4

USE OF PHYTOHAEMAGGLUTININ AS POSITIVE CONTROL IN TUBERCULOSIS SKIN TESTING IN WILD MAMMALS.

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Skin-testing with purified mycobacterial protein derived antigens (PPDs) is still the standard test for tuberculosis. In immunocompromised patients, other antigens have been used as positive controls in order to detect anergic reactors in the skin test, however, this has never been applied to wild mammals. Wild reservoirs are known to be related to the failure of TB-eradication campaigns, and skin testing could be useful in cases where animals are captured for different reasons. We used phytohaemagglutinin (PHA) as positive control in tuberculosis skin-testing using Iberian red deer (*Cervus elaphus hispanicus*) as a model species. A first experiment was used to determine the optimum concentration and time of evaluation of skin-fold thickness. Second, we tested for the influence of sex, age class and body condition on immune reaction, and for differences in reactivity between wild and captive animals. The reaction to PHA was more intense, longer and less variable with a concentration of 2500 µg PHA per ml and reading was optimal at 72 h post injection. According to our results, the response to the PHA injection is influenced by sex and age class. The increase in the measurement of skin-fold thickness was higher in males than in females and in adult than in younger deer, the latter being stronger in the case of males. The captive and wild deer populations studied differed significantly in body condition and reaction to PHA injection. This indicates that significant variation in immune reactivity between populations of one singular species exist, even among individuals of the same sex and age class. No relations between the response to PHA and individual parameters of body condition were found. Also, handling stress may affect immune reactivity in wild animals more than in captive individuals. The results suggest that the nonspecific stimulant PHA may be a useful tool as positive control in order to adjust skin-testing criteria and that, in addition, could help to detect anergic reactors.

Oral Presentation 5

RESULTS OF A UK SURVEY TO ASSESS THE RISK TO CATTLE OF *MYCOBACTERIUM BOVIS* INFECTION FROM WILD MAMMALS IN AREAS OF HIGH CATTLE HERD BREAKDOWN RISK.

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Bovine TB is endemic in badger (*Meles meles*) populations in the UK, which are suspected to be the main wildlife reservoir of infection for cattle. However, there is scant data on the potential role of other wild mammals as previous to the present study only limited work had been undertaken. This paper describes the largest systematic survey to collect data on the distribution, frequency and pathology of *M. bovis* infection in a range of wild mammals in south-west England. Carcasses were collected and subjected to post mortem examination, microbiological culture of tissues and spoligotyping of isolates. A total of 4714 mammal carcasses were examined and tissue samples cultured. Infection was confirmed in foxes (3.2% of 756), stoat (*Mustela erminea*) (3.9% of 78), polecat (*Mustela putorius*) (4.2% of 24), common shrew (*Sorex araneus*) (2.4% of 41), yellow-necked mouse (*Apodemus flavicollis*) (2.8% of 36), wood mouse (*Apodemus sylvaticus*) (0.6% of 333), field vole (1.5% of 67), grey squirrel (*Sciurus carolinensis*) (0.4% of 450), roe deer (1.0% of 885), red deer (1.0% of 196), fallow deer (4.4% of 504) and muntjac (*Muntiacus reevesi*) (5.2% of 58). Sample sizes varied widely between species and consequently so did confidence limits associated with prevalence estimates. Lesions were only found in a single fox, stoat and muntjac, but were present in a high proportion of positive fallow, red and roe deer. In these deer species principal sites of infection were in the lungs and the associated lymph nodes, consistent with infection by inhalation and the potential for onward transmission. The isolation of common *M. bovis* spoligotypes from a variety of wild mammal species and cattle is consistent with inter-species transmission. A qualitative risk assessment of the likelihood of transmission to cattle identified red and

fallow deer as representing the greatest relative risk, although in most other infected species the risks appeared relatively low. Substantially higher levels of infection have been historically observed in badgers, which can also excrete potentially large numbers of bacilli and will forage on pasture and in buildings used by cattle. Nevertheless, deer should be considered as a potential, although probably localised, source of infection for UK cattle.

Oral Presentation 6

MYCOBACTERIUM BOVIS INFECTION IN FREE-LIVING WILD BOARS (*SUS SCROFA*)°AND RED DEER (*CERVUS ELAPHUS*) IN FRANCE

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Since 1965, the French bovine TB eradication plan in domestic cattle has been based on a test and slaughter program. This plan proved to be efficient since France was declared «officially free of bovine tuberculosis» by the European Commission in December 2000 (prevalence < 0.1 %).

Until that date, *Mycobacterium bovis* had never been found in free-living wildlife in France.

However, in January 2001, a wild red deer, hunted-killed in a Norman forest (in the *département* of Seine-Maritime in the North West of France), disclosed typical lung and mesenteric tuberculous lesions from which *Mycobacterium bovis* was isolated. Moreover, four cattle outbreaks had recently been reported in this area. During the 2001-2002 hunting season, an epidemiological survey was carried out in the respective forest. Lung, liver, tonsil samples and retropharyngeal, hepatic and tracheo-bronchial lymph nodes were collected from 84 wild boars, 77 red deers and 37 roe deers and processed for mycobacterial culture. *M. bovis* was isolated from 24 wild boars (apparent prevalence: 28.5%) and 11 red deers (apparent prevalence = 14%) but none from roe deer. Most of them did not present visible macroscopic lesions at hunters' first inspection.

During the 2003-2004 hunting season, two red deers (one fawn and one adult) were found dead with disseminated TB lesions and a very poor physical condition. Among 80 hunted-killed red deers, *M. bovis* was isolated from 14 animals presenting numerous macroscopic lesions (pulmonary, pleural, mesenteric and nodal abscess). Moreover, *M. bovis* was also isolated from at least 6 wild boars out of 110 inspected (partial results at abstract submission), but none from 35 examined badgers. These results would indicate an aggravation of the epidemiological situation from 2001 to 2004, particularly in the red deer population.

Genotyping by spoligotyping and different VNTR-PCRs was performed. The same genotype was found in isolates obtained from wild boars, red deers and the four cattle outbreaks occurred two years before. Although a direct link between wild and domestic TB could be logically assumed, the moment and the origin of transmission remain unknown.

In 2003-2004, *M. bovis* infection was detected in hunted-killed wild ungulates in three additional French *départements*:

- In one red deer and one wild boar in the *département* of Côte d'Or (Burgundy region) in an area where eight cattle outbreaks (1600 animals slaughtered) were reported in 2002-2003.
- In one wild boar in the *département* of Savoy (Alps region)
- In three wild boars in the *département* of Haute-Corse

In the Seine-Maritime and the Côte d'Or, veterinary services took preventive measures in the infected area not only to avoid contamination of cattle and humans but also to reduce transmission among wildlife. The measures implemented were: to reduce wildlife densities by increasing hunting pressure, to ban artificial game feeding, to destroy killed animals viscera, to fence cattle pastures neighbouring the forest, to inspect wild boars and deer carcasses and to reinforce TB cattle testing.

These results raised the question of the emergence of TB in wildlife, while this disease is almost eradicated in cattle. The prior existence of a latent reservoir in wildlife and the dramatic increase in wildlife density, that favours spreading of the disease, could be a first answer to this question.

DISEASES OF THE WILD BOAR: FINAL REPORT OF A SURVEY ON EPIDEMIOLOGICAL RISK FACTORS IN SPAIN *

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The European wild boar (*Sus scrofa*) is the most widely distributed ungulate in the Spanish mainland, and its range and densities have largely increased in the last three decades. In order to increase hunting harvest, populations are managed by high-wire fencing, artificial feeding, and restocking with farm-bred individuals. These changes in wildlife management have already risen concerns regarding the control of infectious and parasitic diseases. This paper describes the results of the first nationwide survey on the health status of the wild boar from Spain and studies the role of individual, geographical, and management-related factors generating heterogeneity of infection.

Between 2000 and 2004, biometrical data and samples were collected from more than 1,000 hunter-harvested wild boar in 56 Spanish localities. Samples were biased towards the main hunting season (October to February), and towards the Mediterranean shrub-lands of the central and southern regions, where hunting activities are most important. The survey included serological screening for viral, bacterial and parasitic diseases, the study of TB-compatible lesions, and helminthological investigations, among others.

Results include a preliminary general serosurvey, a case report on an Aujeszky outbreak, large-scale cross-sectional studies on Aujeszky's disease and porcine circovirus 2, the description of TB pathology, a comparison between helminths of autochthonous and imported wild boars, and an anthelmintic treatment assay, as well as papers in preparation. Aujeszky's disease and tuberculosis are among the most problematic diseases found. Their prevalence is highest in south central Spain, where large game species are increasingly managed for hunting. More intensively managed populations had higher prevalences than wild boar living in natural situations.

*The study was supported by project AGL2001-3947, Ministerio de Ciencia y Tecnología and FEDER.

Oral Presentation 8

SANITARY RISK FACTORS ASSOCIATED TO THE MANAGEMENT OF IBERIAN RED DEER FOR HUNTING PURPOSES IN SOUTH CENTRAL SPAIN*

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Differences in exposition to infectious pathogens may generate differences in the dissemination of such agent across wild population. Once an infectious agent has been encountered by the host, within-host parasite dynamics depends on the parasite immunogenicity and on the host immune response.

We have evaluated the epidemiology and risk factors associated to a bacterial microparasite (*Mycobacterium tuberculosis* Complex, TB) and a helminth macroparasite (*Elaphostrongylus cervi*) infecting populations of red deer (*Cervus elaphus hispanicus*) through South Central Spain, mainly managed for hunting purposes. In addition to the environment, we considered demographic and host aggregation parameters (including those of other potential reservoir species: the wild boar), both highly dependent on human management. All were tested against the presence and abundance (in the case of *E. cervi*) of the pathogens by means of GLMs.

In these Mediterranean habitats, aggregation of hosts at waterholes seems to be a crucial factor for micobacteria and *E. cervi* transmission. Whereas a pattern of acquired resistance seems to operate in parasitic diseases, the risk to exposition, thus the age, mainly shapes the TB infection profile. This research rises concerns regarding the control of infectious and parasitic diseases in managed ungulate populations.

*The study was partly supported by project AGL2001-3947, Ministerio de Ciencia y Tecnología and FEDER. This is a contribution to the agreements between Yolanda Fierro and UCLM, and between CSIC and Principado de Asturias.

MONITORING OF WILD CERVIDS PARATUBERCULOSIS (*MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS*) IN SOUTHERN BELGIUM

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Paratuberculosis (*Mycobacterium avium* subsp *paratuberculosis* - *Map*) is a chronic granulomatous enteritis of ruminants responsible for significant economic losses in cattle industry. The existence of wildlife reservoirs of *Map* has to be considered in the epidemiology of paratuberculosis. This disease has been reported in wild ruminants in several European countries, including Belgium. The purposes of this study were (1) to determine the prevalence of paratuberculosis in wild cervids and (2) to put forward strategies aimed at controlling wildlife reservoirs of *Map*.

In 2001 and 2002, a total of 1118 wild cervids (*Cervus elaphus* and *Capreolus capreolus*), either hunter-killed (n = 1055, group 1) or found dead (n = 63, group 2) were investigated. A complete necropsy was conducted on each animal (sampling of serum, spleen, mesenteric lymph nodes, ileocaecal junction and feces). Formalin-fixed and frozen tissues were collected for histopathology and bacteriologic examinations. Indirect (ELISA, Herd Check ; IDEXX) and direct (bacterioscopy, culture and PCR IS900/IS901) diagnostics were performed.

In group 1, overall seroprevalences were 7.26 % (95 IC = 4.52-10.00) in red deer (n = 358) and 1.13 % (95 IC = 0.01-2.82) in roe deer (n = 353). Macroscopic examination of roe deer viscera never revealed any change compatible with paratuberculosis. On the other hand, 20 of 661 red deers presented suggestive lesions, such as mesenteric lymphadenitis (hypertrophy and/or purulent discharge after cross-section). Amongst these 20 suspicions, 14 were confirmed *Map* positive (culture and PCR). These results indicate that 4.3 % of (sub)adults red deers and 0.4% of fawns display macroscopic lesions attributable to *Map*. In group 2, principal mortality causes of wild cervids were paratuberculosis (17/63), polyparasitism (15/63) and traumatic lesions (15/63). Some geographic areas were more concerned than others by paratuberculosis.

On the basis of these results, an official decree was published in October 2003, according to which any cervidae showing obvious signs suggestive of emaciation and/or diarrhoea could be culled (even outside hunting seasons). All these animals have to be systematically necropsied, sampled and analysed by the Veterinary Faculty to confirm/reject the diagnosis of paratuberculosis. Until now (October 2003 to June 2004), 23 wild cervids were culled, among which 12 were *Map* positive (by PCR). It is concluded that, in addition to implementation of classic control strategies (decrease of cervid densities and management of winter feeding), a selective culling would probably drastically improve the control of the disease.

This study was supported by funds from the Minister of Agriculture – Region of Wallonia – Belgium

Friday 10 September

CAUSES OF MORTALITY AND NEUROLOGICAL DISEASES IN FARMED DEER IN SWITZERLAND

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In Switzerland, deer farming is an alternative to cattle, especially for the traditional small farms up in the hills. The Swiss captive deer population numbers about 8000-9000 animals: 85% are fallow deer (*Dama dama*), 10% red deer (*Cervus elaphus*) and about 5% sika deer (*Cervus nippon*). Chronic Wasting Disease (CWD) is a transmissible spongiform encephalopathy found in farmed and free-ranging deer in the United States and in Canada. A surveillance program for Bovine Spongiform Encephalopathy (BSE) has been implemented in Switzerland several years ago, however, no investigations had ever been performed regarding presence and prevalence of CWD in farmed or free-ranging deer species.

Therefore, a research project on prevalence and aetiology of neurological diseases in farmed deer was initiated at the FIWI on behalf of the FVO. Furthermore, the study should include other reportable diseases, with special attention to infectious agents causing abortion. And investigation of causes of mortality, in particular for the fawn age class. The aims of

the study is to collect at least 200 heads of deer older than 2-yrs- (sick or healthy ones) for CWD testing, and as many animals (found dead or shot for sanitary reasons) and aborted fetuses as possible over a period of 2 years for post-mortem investigations

All registered deer breeders were informed by mail about the project and asked to send material. Dead animals are necropsied according to a standard protocol including macroscopic examination, histology, bacteriology and parasitology. Shipping and all examinations are done at no cost for the breeders and the study is anonymous.

In 2003, we examined 125 deer: 1 aborted fetus, 37 fawns, 14 subadults (>6-mo- to <2-yr-old), and 72 adults (2-yr-old or older). 56 out of 72 adults were healthy slaughtered animals; in these cases, we investigated the head only. The analyses revealed specific problems for two age classes: 63% of the necropsied fawns died of necrobacillosis, and 78% of the subadults were severely infested with endoparasites. In adults, miscellaneous causes of mortality were diagnosed: pneumonia, ruminal acidosis, neoplasia and septicaemia. Examination of the heads of adult deer revealed no particular pathological changes attributable to any specific disease. Only one aborted fetus was sent for investigation: It was diagnosed with multiple malformations (i.e. Palatoschisis). Regarding the reportable diseases, only one case of Yersiniosis (*Yersinia pseudotuberculosis*) was diagnosed in a fawn.

The investigations performed during the first year clearly revealed that necrobacillosis and endoparasites represent the major sanitary problems in deer farming in Switzerland. In adult animals, no relevant disease could be identified. Causes of abortion remain unknown since almost no material was sent in. Concerning CWD, histological investigations did not reveal any case so far. However, the sample size is still very small, especially if we consider the very low prevalence that can be expected for this disease. Furthermore, immunohistological investigations are still pending.

The low number of carcasses and heads obtained for examination is the major problem encountered in this study: many deer breeders are indeed afraid about the possible discovery of a case of CWD on their farm and do not want to participate to the survey; death of a single individual is often considered as a "normal" event and such cases are usually not sent for examination. In addition, it seems that some farmers are simply not interested in a research project about deer mortality. Efforts in motivating deer farmers are therefore essential for the success of the project.

Oral Presentation 11

EVALUATION OF THE PRIONICS®-CHECK WESTERN AND LIA FOR CHRONIC WASTING DISEASE IN DEER AND ELK

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Chronic wasting disease (CWD) was first described in the US in a captive herd of mule deer in the 1960s. In recent years, local outbreaks of CWD in free-ranging and captive deer and elk have been described in several midwestern and western states and also in Canada. In contrast to BSE, CWD has been shown to spread efficiently between animals of the same species but it is unclear whether the CWD agent can be transmitted to humans following oral uptake or to ruminants by exposure to infectious material on the pasture. Routine laboratory diagnosis of CWD is based on immunohistochemical analysis (IHC) of the protease resistant PrP (PrP^{CWD}) in the obex and lymphoid tissue of affected animals. However IHC is time consuming, standardized protocols are not available and therefore interpretation of results are subjective. We have evaluated two rapid screening tests, Prionics®-Check LIA and Prionics®-Check WESTERN (WB) for the diagnosis of CWD in obex and lymph nodes of white tail deer, rocky mountain elk and mule deer. We analysed more than 700 lymph node and obex samples from deer and elk with the Prionics WB. In addition, more than 1600 lymph node and obex samples were analysed with the Prionics LIA. The test results on both tissues were compared to IHC as the reference test. On lymph nodes, both tests showed an excellent performance with specificities of >99% and sensitivities of >92%. Collection of lymph nodes can easily be done by hunters suggesting that testing of lymph nodes may be a good surveillance strategy for CWD. We conclude that, compared to IHC, both rapid diagnostic test show excellent results and could be used to determine the prevalence of CWD in deer and elk.

Oral Presentation 12

THE NATIONAL HEALTH SURVEILLANCE PROGRAM FOR WILD CERVIDS IN NORWAY

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Norway has a large population of wild cervids and, of these, approximately 37,000 moose (*Alces alces*), 30,000 roe deer (*Capreolus capreolus*), 24,000 red deer (*Cervus elaphus*) and 6,500 reindeer (*Rangifer tarandus*) are shot during the hunting season each year. The distributions of the species vary with red deer predominating along the west coast whereas moose and roe deer mainly inhabit other areas of the country, with the exception of the most northerly parts. The wild reindeer live in dispersed populations in separate high mountain areas in southern Norway.

In 1998, a National Health Surveillance Program for Wild Cervids (Helseovervåkingsprogrammet for hjortevilt or HOP in Norwegian) was established in Norway. This program is coordinated by a secretariat following directions from a steering group with representatives from the Directorate for Nature Management, the Norwegian Food Safety Authority, the National Veterinary Institute (NVI) and the Norwegian Institute for Nature Research. The HOP-secretariat is located at the National Veterinary Institute in Oslo, in the Section for Wildlife Diseases.

The main aim of HOP is to generate systematic and updated information on the health of wild cervids for the interest and benefit of the animal health and wildlife management authorities. The program comprises of the monitoring and surveillance of infections and other diseases as well as the causes of death in wild moose, red deer, roe deer and reindeer. In 2004 the musk ox (*Ovibus moschatus*) was also included in this list of species. The scope of the program is to reveal the causes of disease in individual animals in addition to the detection of epizootics or increased mortality in populations during the early phase of an outbreak. Various health data are generated by the systematic collection and screening of serum and tissue-samples. Other objectives are the study of infections that might spread between cervids and farm animals and the clarification of the role cervids play as reservoirs of zoonotic agents.

The tools used in the gathering of data in HOP are: 1) systematic registration of diseases and causes of death in cervids from 65 selected municipalities which report quarterly to the HOP-secretariat; 2) systematic sampling of specimens during ordinary hunting, for screening purposes; 3) diagnostic work, and 4) research. The first tool requires close cooperation between local wildlife management officials and veterinary surgeons which is clearly detailed in the handbook for HOP-personnel. In cases where the cause of disease or death is unknown, a local veterinary surgeon may perform a field post mortem and, where necessary, submit specimens to the NVI for laboratory examination in order to establish a diagnosis. The NVI offers a free diagnostic service with regard to wild cervids to all municipalities in the country.

The HOP-secretariat records all reported cases and their diagnoses in a central database. It also maintains an archive of specimens, mainly serum, from cervids. The occurrence of diseases in wild cervids and the causes of death are summarised in an annual report. The HOP program is funded by the Ministry of the Environment and the Ministry of Agriculture.

Oral Presentation 13

FASCIOLOIDOSIS OF RED DEER (*CERVUS ELAPHUS ELAPHUS*) - AN EMERGING DISEASE IN CROATIA

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Before 1991, the red deer (*Cervus elaphus elaphus*) population in Croatia counted more than 15 000 animals. The vast majority (some 70%) of those animals occupied a relatively small area of the north-eastern region of the country, Baranja, enclosed by the Drava and Danube river valleys and bordering Hungary and Serbia. The game in Baranja was managed within strictly controlled government hunting grounds, and the cadavers of all shot animals were subject to veterinary control. There are no records of liver lesions suggestive of fascioloidosis during that time. At the beginning of war in 1991, the region of Baranja was occupied and sequestered from Croatia and was not reintegrated until 1996. When counting of animals was resumed during 1997, the population was estimated to be 3500. In the following years it was noticed that deer herds were in generally poor condition: many animals were emaciated, there was an increased mortality rate and poor fawn progression along with a lowering of antler trophy values. During the years 1999 – 2000, peculiar liver lesions were observed by technical personnel and specimens of unusually large liver flukes were occasionally collected in several exenterated animals in the field after the hunt. Some specimens were submitted for parasitological evaluation to the licensed laboratory and were determined as the Large American Liver Fluke (*Fascioloides magna*). Those findings initiated an extensive research during the 2002/03 and 2003/04 hunting seasons in which the eviscerated internal organs of 755 deer of different ages and of both sexes derived from hunting grounds throughout Croatia were collected and submitted as frozen specimens for pathomorphologic and laboratory diagnostic procedures.

Reflecting the population density, 555 or some 75% of animals were derived from Baranja while the rest were samples representing all other hunting grounds of Croatia. A very high percentage (88%) of examined deer livers from Baranja exhibited pathomorphological lesions compatible with fascioloidosis and the diagnosis was confirmed by observing different numbers of adult parasites as well as their positive identification by means of parasitological methods. In grading the severity of the liver involvement among positive animals from Baranja, it was observed that more than half the animals exhibited very seriously compromised livers, while for roughly 1/3 of involved animals recuperation would be questionable even after triclabendazol treatment. The 200 examined organs derived from other areas of the country showed that a much lower percentage (some 25%) of animals was positive for fascioloidosis.

The animals from hunting grounds closer to Baranja were affected with a higher incidence and the lesions were more severe in degree, so it was concluded that the disease was being propagated out of Baranja to the rest of the country. At the moment it is not clear when the parasitosis first started in Baranja or from where it was introduced. However, since neighboring countries detected the same problem on their own deer population, it is postulated that the appearance of the disease, due to the migratory character of deer, is part of a larger natural phenomenon evolving in Danube river basin.

CHORIOPTIC EAR MANGE IN MOOSE (*ALCES ALCES*). A PILOT PREVALENCE STUDY IN THE SWEDISH WILD MOOSE POPULATION

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Mites of *Chorioptes* species are a common cause of mange in domestic ruminants and horses worldwide. They also occurs in wild ungulates but has not been reported as a cause of otitis in wildlife. The finding of a *Chorioptes* species that was verified by A. Baker (The Natural History Museum, London, UK) as *C. texanus*, a species that had been reported infesting cattle, goats, reindeer, roe-deer, red deer and elk, inspired a pilot survey of this primarily ectoparasitic mite dwelling mostly on the body skin surface. *Chorioptes texanus* has previously been isolated from ears of reindeer in Canada. A generalized mange and excess production of cerumen have been attributed to infestations. Visible symptoms have never been reported.

Specimens of the right and left outer ear canals of 20 moose that had been subjected to routine diagnostic post mortem examination at the National Veterinary Institute of Sweden between 1997 and 2004 were investigated. The external ear canals were inspected under a low power microscope and if no mites were seen, scrapings from the epithelium lining the ear canals were treated with 10% KOH for 5 hours and there-after the scrapings were searched for mites. The mites were identified morphologically and by gene sequencing of the ITS-2 region of the rDNA.

Skin samples of the external auditory meatus (external ear canal) were examined histopathologically. In the samples from the moose infested with a *Chorioptes* sp. there was hyperplasia and hyperkeratosis of the epithelium, with formation of rete pegs in the more severe cases. Crusts composed of exfoliated debris and exudate over the surface of the epithelium were commonly observed, in some cases including embedded mites and mite debris. There was a variable infiltration of inflammatory cells in the dermis underlying the epithelium and in deeper layers, consisting mostly of lymphocytes, plasma cells, macrophages and a few eosinophils.

The isolated *Chorioptes* species of the moose differed slightly morphologically from *C. texanus* in the length and shape of the outer opisthosomal seta. Phylogenetic analysis indicates that there is separation between the *Chorioptes* sp. from the moose and the genotypes of *C. bovis* and *C. texanus*.

Chorioptes sp. was isolated from the ears of 17 moose (90 %) and in two animals no mites were found (10 %).

Oral Presentation 15

GANGRENOUS ERGOTISM IN WILD-LIVING MOOSE AND ROE DEER IN NORWAY

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Ergotism is the name given to several clinical syndromes of intoxication with alkaloids of the parasitic fungus *Claviceps purpurea*. Intoxication follows the ingestion of alkaloid-containing sclerotia (ergots) that are present in the seed heads of grasses and cereals during autumn. The classical manifestation of ergotism, characterised by gangrene of the extremities, specifically the limbs, ears and tail, is well recognised in domestic ruminants worldwide. In wild-living animals, however, reports of ergotism seem to be restricted to a single case of gangrenous ergotism in a roe deer (*Capreolus capreolus*) from England.

Suspected gangrenous ergotism in ten moose (*Alces alces*) and one roe deer in Norway is reported. Three of the moose came from the Holmestrand municipality in south-eastern Norway where the disease occurred as a cluster during 1996. The remaining moose represent solitary or sporadic cases diagnosed in a total of four municipalities (Halsa, Hemne, Molde and Sunndalsøra) in north-western Norway between 1996 and 2004. The moose, seven calves and three yearlings, were found at various times between October and June. Two of the animals were found dead whilst the remainder were destroyed, on humane grounds, because of their severe locomotory disturbances. All of the moose showed distal limb lesion, from one to three of the limbs being affected. Most commonly the hind limbs were affected, usually bilaterally. The lesions in the moose found during October and November presented as dry gangrene, whilst moose seen between December and June presented with amputations or open lesions close to amputation. The distal limb lesions seen in seven of the moose extended from the distal phalanx up to the fetlock, whereas the remaining three moose had lesions extending as high as the mid metacarpus/metatarsus. The site of amputation was either at the joints themselves or at the physal lines. Four of the moose also had bilateral ear lesions affecting the outer third of the pinna. One of the moose presented had gangrenous and clearly demarcated ear-tips, whilst the remainder had sloughed ear-tips.

A retrospective diagnosis of ergotism was made in the case of a roe deer from the Tingvoll municipality in north-western Norway, dating from June 1981. The one year old animal showed amputation of all four limbs distal to the fetlock.

FIRST EVIDENCE FOR A NOVEL PESTIVIRUS IN PYRENEAN CHAMOIS (*RUPICAPRA PYRENAICA PYRENAICA*) FROM FRANCE.

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Pestiviruses are important pathogens of cattle, sheep, and pigs and cause significant economic losses worldwide. The impact of pestivirus-associated diseases on the health of free-ranging wild ruminants and the interactions between wild ungulates and livestock are still not well understood. Nearly 25,000 individuals of Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) occur currently in the French part of the Pyrenean range. A regular health monitoring scheme for ungulates in the east of the Pyrenean mountain range ("Orlu Reserve") has been ensured more than 15 years. Although no unusual mortality in Pyrenean chamois was observed in the "Orlu Reserve", a high seroprevalence for pestiviruses (up to 90%) has been detected in this population since 1995. Moreover, several sick and dead chamois, with clinical signs of cachexia and alopecia have been observed since 2000 from other parts of the French and the Spanish Pyrenees. However, due to the regular health monitoring scheme fifty spleen samples were taken from chamois hunted in the "Orlu Reserve" between 2001 and 2002. Two of them were identified as pestivirus positive in RT-PCR. Phylogenetic evaluation of the pestivirus was done based on a fragment from the 5' noncoding region. We used 20 published nucleotide sequences of different pestivirus strains for comparison. The new strain grouped within the clade of border disease viruses (BDV). However, it had an intermediate position between clade BDV and classical swine fever viruses (CSFV). The phylogenetic analysis of this new pestivirus isolate indicated that Pyrenean chamois harbor a pestivirus distinct from the established pestivirus species representing a basal position to BDV strains of domestic sheep. Based on the phylogenetic grouping of pestivirus isolates, a postulated cross-species transmission of pestivirus from domestic sheep to chamois via shared pastures is unlikely.

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PESTIVIRUS INFECTION IN PYRENEAN CHAMOIS (*RUPICAPRA PYRENAICA*): ISOLATION AND CHARACTERISATION BY MONOCLONAL ANTIBODIES AND CROSS-NEUTRALIZATION

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A Pestivirus not previously recorded, was isolated from 5 chamois from the Catalan Pyrenees (NE Spain), affected by an outbreak of disease in 2001 and 2002. The reactivity of these isolates against a panel of specific Pestivirus monoclonal antibodies, showed a positive reaction against BD genotype. Virus neutralisation test (VN) was performed on 23 sera from healthy chamois from the same area, positive to an ELISA antibody test. 6 Pestivirus strains were used: Esp. 97 BD genotype (aveyron like), Moredum UK-76 BD genotype, 137/4 BD genotype, NADL BVDI genotype, pestivirus isolate from a cell line BVDII genotype and chamois isolated strain. The sera show a high reactivity against Esp.97 BD and chamois BD, medium against 137/4 BD genotype strains and a very low reactivity against BVDI and BVDII genotype strains. These results also confirm that the Pestivirus isolated strain in the affected chamois is a BD genotype and due to the high VN titres obtained against this strain, it is probably that this virus is circulating in the chamois population.

To investigate if the Pestivirus isolated from the chamois has infected domestic ruminants that share the same habitat, a VN test was performed in 55 ovines and 32 bovines. 38/55 of the ovine sera were positive against at least one of the 6 strains tested and showed a high reactivity against the BD Esp97 and medium against BD 137/4 and a very low reactivity to the rest of the strains. 25/32 of the bovine sera were positive against at least one of the 6 strains tested and showed a high reactivity against the NADL BVDI genotype and a very low reactivity to the rest of the strains. The low reactivity to the chamois strain in the bovine and ovine sera tested indicates that this virus has not been transmitted to domestic ruminants. However, due to the VN results against the Esp97 BD strain obtained from the ovine and chamois populations, it is possible that BD strain affecting domestic ruminants has been transmitted to the chamois population.

PESTIVIRUS DISEASE AND SEROPREVALENCE IN PYRENEAN CHAMOIS (*RUPICAPRA PYRENAICA PYRENAICA*)

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A decrease in Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) population in the central Pyrenees associated with pestivirus has been described. A study to know the seroprevalence and the disease in Pyrenean chamois from the Principality of Andorra and Aragon (Spain) has been carried out.

Twenty-one animals found dead during 2002-03 and 145 hunted animals were analysed for virus detection and a total of 394 sera from these and other chamois hunted during 2000-03 were examined for anti-pestivirus antibody.

Pestivirus was demonstrated by immunohistochemistry (IHC) in several tissues from 2 of the 21 animals found dead but not in the 145 hunted animals. The presence of pestivirus in one of the two dead chamois (H2121) was confirmed by RT-PCR and by virus isolation from the spleen, skin and serum. The isolated virus, containing cytopathic and non-cytopathic variants, was characterised further by analysing the genetic sequence of the 5'UTR region and the N^{pro} and E2 genes.

Sequence and phylogenetic analysis revealed that the chamois pestivirus was closely related to border disease virus (BDV), and probably represents a new genotype, BDV-4. Animal H2121 was negative for anti-pestivirus antibody by ELISA, but such antibody was detected in 55 of 394 (14 %) sera. Antibody prevalence was significantly higher ($\chi^2=25,36$, d.f.=1, $p<0,001$) in chamois from the two regions (Andorra and Benasque) closest to the Central Pyrenees compared with those from the 3 regions further west. This study has provided evidence of pestivirus disease in Pyrenean chamois. Animal H2121, which was approximately 9 years old, died following apparent persistent infection with a virus related to BDV.

Oral Presentation 19

TRAINING VETERINARY STUDENTS ABOUT DISEASES OF WILD CARNIVORES, A EWDA INTERNATIONAL PROJECT. STATE OF THE ART

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A joint venture between IREC (Instituto de Investigación en Recursos) in Spain, Institute for Zoo biology and wildlife research in Germany and Lyon Veterinary school in France started some time ago. Purpose has been to provide EWDA (European section of WDA) with a comprehensive package of training material, namely slides, related with origin, diagnosis, ecological impact, management of all kind of diseases of wild Carnivores in Europe and worldwide.

The presentation will introduce the current state of the project and recent uses of it; prospects for an appropriate spread of the presentation will be examined and deadline for completion, suggested.

Oral Presentation 20

DO ABORTIVE DISEASES PERSIST IN POPULATIONS OF CHAMOIS (*RUPICAPRA RUPICAPRA*)?

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One strategy for parasites to survive on the long term is to infect alternative hosts. Here we test whether chamois may serve as an alternative host for three bacterial abortive diseases using the results from a long-term serological survey that has been performed since 1986 in the population of chamois (*Rupicapra rupicapra*) living in two sites of the alpine massif of Bauges. The survey measured serological responses of 692 chamois against the abortive bacteria *Chlamydomphila abortus*, *Coxiella burnetii* and *Salmonella Abortusovis*. We test whether the serological responses observed are consistent with the epidemiology of the corresponding diseases in sheep and goats. Using either a multinomial model or a proportional-odds model when appropriate, we show that all three serological responses vary according to age, year and site. Moreover, a within-year dynamics is observed for *Chlamydomphila* and *Salmonella*. The dynamics of responses is synchronous in both sites, suggesting a rapid propagation between sites, however the two sites play distinct roles, one of them possibly being a reservoir.

This pattern is consistent with a long-term persistence of the corresponding diseases in the chamois population. We thus demonstrate that germs homologous to *Chlamydothyla*, *Coxiella* and *Salmonella* have dynamical propagation in the chamois population. It is noteworthy that, although the bacteria have been searched for, we have not identified the pathogens that cause the serological responses, probably because of the difficulty to obtain appropriate samples. Nevertheless, this raises the question of whether inter-species transmission occurs. We plan to model the dynamics of diseases in natural populations and test whether infections may persist in wild populations alone or if external supply is required.

Oral Presentation 21

IMPACT OF THREE ABORTIVE INFECTIONS ON THE FECUNDITY OF CHAMOIS (*RUPICAPRA RUPICAPRA*) IN THE ALPINE MASSIF OF BAUGES (FRANCE)

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Infectious diseases of reproductive system may limit reproductive success and survival of animals in wild populations and so may affect the dynamic of these populations. Importance of these factors are well-known in domestic flocks, but is scarcely supported among wild populations. In order to get a first approach of this phenomenon on chamois population (*Rupicapra rupicapra*) in massif of Bauges (North Prealps, France), we survey three common bacterial abortive infections in domestic ruminants: Q fever caused by *Coxiella burnetii*, chlamydial infections caused by *Chlamydothyla abortus* and salmonellosis caused by *Salmonella Abortusovis*.

This chamois population have been surveyed for 25 years for demographical studies (thanks to marked individuals (N= 657)) and sanitary investigations (N= 252 samples of blood for serology from 1980 to 2003). Serological methods used to test the three abortive infections are the same as those employed on domestics ungulates (complement fixation for Q fever and chlamydial infection and micro titration for salmonellosis). The reproductive success of females is got either during their capture (lactation status) or by the longitudinal observation of their offspring in wild.

We analysed the impact of these diseases on reproductive success at the individual level and at the population level. For the former, we used logistic regression on 252 females caught from 1980 to 2003 and tested for the three abortive infections. First we tested factors that influence reproductive success: age, season and year of capture, then we added serological status of animal. For the latter, we recognized serological events (increase of prevalence characterised by space and time items), and used logistic regression on 1017 data of reproductive success (female-year observations) according to the same process as above.

Whereas Q fever and chlamydial infections do not alter individual reproductive success of females, salmonellosis significantly reduces it. Adult females with titter of 320 or more against salmonellosis have 2.8 (between 1.7 and 4.4) more risk to fail in her reproductive event. This risk increases with females having titter higher than 640 (which is the threshold usually admitted for sheep and goat clinical abortive salmonellosis): they have 44.4 (between 13.5 and 146.3) more risk not to have a kid. Moreover when prevalence of salmonellosis titter 320 increases by 10 % in a given population, females belonging to this population have 1.2 (between 1.01 and 1.42) more risk to fail in their reproductive event.

These results clearly show that females with a positive serological test against *Salmonella Abortusovis* are affected in their reproductive performances, although the real causative agent have not been yet isolated. One opened question is to know whether it is related to contact with infected domestic sheep or an indigenous strain spreading into wild populations.

Oral Presentation 22

CLASSICAL SWINE FEVER IN FREE RANGING WILD BOAR: A EUROPEAN PROBLEM

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Classical Swine Fever (CSF) is a viral disease affecting wild and domestic *Suidae* worldwide. Outbreaks occurring in domestic pigs entail severe losses to pork industry because the virus is highly contagious and some strains can cause up to 90% mortality. Moreover, in the E.C., massive slaughtering is required to eradicate the disease and CSF-free countries restrict pig trading during outbreaks.

The European wild boar (*Sus scrofa sp.*) is regarded as a potential reservoir of CSF. Cross-contamination is supposed to occur either through direct contact between infected wild boar and inappropriately restricted domestic swine or through introduction of infected feed, the CSF virus being able to survive in fomites and meat during several months. In the last twenty years, several outbreaks of CSF occurred in European populations of wild boar. Some of them were self-limited as they go extinct after few months. Others persisted for years or became enzootic. Subsequently, the monitoring of CSF epizootics has become obligatory in the E.C.

In the present communication, we propose a review of the monitoring and management measures implemented in France regarding CSF in wild boar. We first describe the evolution of two CSF outbreaks, which emerged respectively in April 2002 and April 2003 in North-eastern France. In both situations, CSF spread naturally from Germany and Luxembourg over hundreds kilometres. In the first outbreak, CSF virus has been only isolated over a few months, and the epizootic apparently went rapidly to extinction. In the second epizootic, CSF is still spreading in a large area, and is attempted to persist many years. Analyzing differences between that two epizootics, we discuss the factors favouring CSF emergence, spreading and persistence in wild boar. We also discuss the efficiency of management strategies. Finally, we open the discussion on future measures to be intended regarding CSF monitoring and management at the European level.

Oral Presentation 23

GEOGRAPHIC DISTRIBUTION AND HOST SPECIFICITY OF TICKS OF WILD UNGULATES IN SPAIN: PRELIMINARY RESULTS

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Oral Presentation 24

EFFECT OF CAPTURE METHOD ON HAEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS OF SPANISH IBEX (*CAPRA PYRENAICA*): PRELIMINARY RESULTS

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Physical capture of wild animals causes strong physiological changes due to stress. Capture and translocation are increasing practices in wildlife management, and determining the less stressful method is a matter of concern related to animal welfare and survival.

Different capture methods (snares, corral-traps, box-traps) have been used to capture Spanish ibex (*Capra pyrenaica*), and drive-nets are being used since 2002. In this study, physiological changes in animals captured with box-trap and drive-nets were compared.

Animals were driven to the net by a line of beaters. Once an animal fell into the net, it was unwrapped, blindfolded, their legs tied and put in a net bag. Box traps were baited regularly two weeks before their activation. When a Spanish ibex was captured in a box, a net was put in the entrance of the box to physically restrain the animals. Once in the net, the animals were handled in the same way that drive-net animals. Blood samples for haematology and serum biochemistry were taken from the jugular vein as soon as the animals were immobilized in a net bag. Samples from 31 animals captured with drive-nets and 26 captured with box-traps were obtained.

Red blood cell count, haematocrit, haemoglobin concentration, MCV, MCH, MCHC, white blood cell total and differential counts and platelet count were determined from whole blood. Serum biochemistry parameters analyzed included cortisol, glucose, muscular enzymes (AST, ALT, CK, LDH and ALP), metabolites (bilirubin, lactate, urea and creatinine), lipids (cholesterol and triglycerides), total protein and protein electrophoretic fractions, electrolytes (chloride, sodium and potassium).

MODULATION OF TRANSPORT STRESS IN SOUTHERN CHAMOIS (*RUPICAPRA PYRENAICA*) USING A SHORT ACTING NEUROLEPTIC.

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Animal translocation is an increasing practice involving their capture and transport. Southern chamois (*Rupicapra pyrenaica*) translocations have been carried out with restocking purposes, but no studies on the effect of transport stress have been conducted up to now.

21 free-ranging Southern chamois (*Rupicapra pyrenaica*) captured by means of drive-nets were physically restrained for 100 minutes and then transported during approximately 100 minutes. 11 randomly selected animals received 0.1 ± 0.02 mg/kg of acepromazine maleate intramuscularly at the moment of capture, whereas the remaining 10 animals received 0.5 mL of saline, acting as controls. Clinical parameters (heart rate and temperature) were registered throughout the study period. Haematological and serum biochemical parameters were analyzed from blood samples obtained at capture, just before transport and just after transport. Treatment effects on the evolution of the parameters analysed were studied.

Heart rate decreased during the first 10 minutes and then stabilised in both groups during the pretransport period. During transport, heart rate remained stable in the treated group, whereas it increased in the control group, being statistically higher than in the treated group. Body temperature also decreased over time in both groups, but it was statistically higher in control animals from the beginning of the monitoring to minute 85 and stabilised earlier in the treated group (15 minutes) than in the control group (75 minutes).

RBC, haemoglobin concentration and haematocrit decreased during the pretransport period and then stabilised during transport in both groups. Control animals showed higher values immediately before transport for RBC, haemoglobin concentration and MCHC. Monocytes, band neutrophils, cortisol, and urea increased over time in the control group, remaining stable in the treated group. Control animals had higher monocytes, band neutrophils, cortisol, total bilirubin, ALT, AST, CK and LDH after transport than treated animals. Creatinine and sodium decreased before transport only in the treated group. Both creatinine and potassium were statistically lower in the treated group immediately before and after transport.

Stress response in Southern chamois is stronger during transport than during physical restraint, and it can be modulated by the use of acepromazine.

Oral Presentation 26

EMERGING INFECTIONS WITH TULAREMIA IN SWEDEN

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Abstract: Tularemia has since it was discovered in Sweden in 1931 regularly appeared mainly as a disease with recurring epidemic outbreaks in humans and varying hares (*Lepus timidus*). Tularemia was for many years predominantly seen in the middle and northern parts of Sweden, north of LAT 59°. In recent years, an increased number of human and animal cases have been observed, and the disease is now found in areas in the south where it has not been seen before. In 2000 there was a large outbreak with more than 400 human cases and with simultaneously a large mortality among hares. More than 500 human cases were recorded in Sweden in 2003.

Interestingly, in the late nineties a new picture of the tularemia ecology has been observed where the disease now besides found in the varying hare also occurs in the European brown hare (*Lepus europaeus*).

The pathological picture of tularemia in the European brown hare is in many cases different from the picture seen in varying hares, with a more chronic feature and granulomatous reactions in the lungs and kidneys.

The reason for the expansion of tularemia is not known, but could maybe be linked to the present expansion of the potential silent carrier the beaver (*Castor fiber*) in Sweden, a species that is also expanding southwards.

RABBIT HAEMORRHAGIC DISEASE IN WILD EUROPEAN RABBIT (*ORYCTOLAGUS CUNICULUS*) POPULATIONS IN HUNGARY

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The wild rabbit population in Hungary is very small and fragmented. It is considered to be a remnant of a breeding stock introduced in the XVIII century. Scattered colonies were present in the hilly Transdanubia region while larger populations were typical of the central lowlands region on the left bank of the Danube. There is an age long debate about the legal status of the species as it is generally considered a non-indigenous and pest species despite its very limited distribution. On the other hand it is a suitable prey for the larger endangered raptor species, it has certain value for hunters and it had a local conservation role, since a „unique” juniper forest ecosystem was preserved and maintained by grazing of wild rabbits in the Bugac region.

Declines of major rabbit populations at the Kiskunság region began in 1991 and by 1995 the whole rabbit population of the Bugac region went extinct. The fragmentation, isolation and high predation pressure made these focal extinctions permanent. Wild rabbit populations were only preserved in areas where hunters managed and repopulated colonies after outbreaks and managed to maintain low predator (red fox) densities at the same time. The population declines and extinctions were attributed to Myxomatosis outbreaks but laboratory or indeed pathological investigations of these die offs of the past decade were never initiated and no measures were taken to preserve the country's wild rabbit populations. In February 2004 we diagnosed an RHD outbreak in a wild rabbit population near Paks, in a region along the Danube, where isolated wild rabbit colonies are being maintained by hunters. Suspected Winter-Spring epidemics of RHD are observed in the area at 2-3 year intervals. The presence of the virus was detected by an antigen ELISA and a PCR assay amplifying a 626 bp sequence of the VP60 gene. The PCR product was sequenced and a phylogenetic analysis was performed by parsimony, neighbour joining and UPGMA techniques. All these methods placed our wild rabbit isolate into a distinct, recently diverged cluster of RHD viruses (RHDVa subtype). The first members of this group emerged from Italy and Germany in 1996 while the so far only Hungarian strain in this group, from a Spring 2003 outbreak in a domestic rabbit stock.

These results demonstrate that there is a marked risk of RHDV transmission between domestic and wild rabbit populations. Coupled with any combination of other limiting or mortality factors this makes attempts to preserve or restore the few remaining wild European rabbit populations in Hungary a tough exercise.

NEOSPORA CANINUM IN WILD ANIMALS: A REVIEW.

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Neospora caninum is a recognised protozoan parasite of dog and livestock, but infection has been also reported in free-ranging wild ruminants, i.e. black and white tailed deer (*Odocoileus hemionus* and *O. virginianus*) (Woods *et al.*, 1994, J Vet Diagn Invest, 6: 508-510; Dubey *et al.*, 1999, Int. J. Parasitol, 29: 1709-1711), chamois (*Rupicapra rupicapra*), roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) (Ferroglio and Rossi, 2001, Vet Rec, 148:754-755), Alpine ibex (*Capra ibex*) (Ferroglio *et al.*, 2001, Z Jagdwiss, 47: 226-228) white rhinoceros calf (*Ceratotherium simum*) (William *et al.*, 2002, J S Afr Vet Assoc, 73: 38-43), Brown Hare (Ferroglio and Trisciuoglio, 2003, Vet Parasitol, 115: 75-78), zebra (*Equus burchelli*), eland (*Taurotragus oryx*), African buffalo (*Syncerus caffer*), Thompson gazelle (*Gazella thompsoni*), impala (*Aepyceros melampus*), warthog (*Phacochoerus aethiopicus*) (Ferroglio *et al.*, 2003, Vet Parasitol, 118: 43-49), and carnivores, i.e. coyote (*Canis latrans*) (Lindsay *et al.*, 1996, J Parasitol, 82: 657-659), red fox (*Vulpes vulpes*) (Buxton *et al.*, 1997, Vet Rec 141: 308-309), gray fox (*Urocyon cinereoargenteus*) (Lindsay *et al.*, 2001, Vet Parasitol 97: 159-164), lion (*Panthera leo*) and in a captive cheetah (*Acinonyx jubatus*) (Cheadle *et al.*, 1999, J Zoo Wild Med, 30: 248-251), spotted hyena (*Crocuta crocuta*) and in free-ranging cheetah (*Acinonyx jubatus*) (Ferroglio *et al.*, 2003). Infection has been recently revealed also in marine mammals (Dubey *et al.*, 2003, Vet Parasitol, 116: 275-296).

The majority of the above mentioned studies relies on serological test, mainly *Neospora caninum* Agglutination Test (NAT), which does not require a species-specific secondary antibody (Romand *et al.*, 1998, Parasitol Res, 84: 50-53). Unfortunately the specificity, sensitivity and cut-off value of serological tests have not been evaluated in wild species and moreover they indicate only exposure to the parasite. Considering that available data mainly refer to seropositivity and only few authors reports stillborn or neurological disorders, the pathological effect of *N.caninum* infection in wild animals health is largely unknown. Vertical transmission has been demonstrated in both herbivores (Peters *et al.*, 2001, Vet Parasitol, 97: 153-

157) and carnivores (Schaes *et al*, 2001, Int. J. Parasitol, 31: 418-423), but the importance and the incidence of vertical transmission in maintaining infection in wild species remain unrecognized.

The role of wild carnivore as a possible *N. caninum* definitive host is largely unknown. While an experimental trial in coyote can not demonstrate the shedding of oocysts in infected coyotes (Lindsay *et al*, 1996), further testing using more sensitive assays, such as PCR (McAllister *et al*, 2004, Int. J. Parasitol. 34:159-161) showed that coyote is a definitive *N. caninum* host.

Ferroglio *et al* (2003) report that in herbivores diggers and pure grazer species showed a higher seroprevalence than browser ones, and they suggest that this can be due to a great exposure to *N. caninum* oocysts shed on pastures by definitive hosts. The same authors suggest that in Kenya wild carnivore species could act as *N. caninum* definitive host considering the absence of infection in feral and rural dogs in the area. However, considering that to date the status of *N. caninum* definitive host has never been proved for species others than dog and coyote, this hypothesis can be only speculative. The demonstration of a *N. caninum* sylvatic cycle could be of great value not only to understand its epidemiology in wildlife, but also to clarify the epidemiology of the infection in domestic species and improve control measures. In fact the prevalence of infection in wild species in an area could reflect a high risk of acquiring infection also for domestic animals. Moreover the capability of wild canids to act as a *N. caninum* definitive host, and the related risk due to the transmission of the parasite from them to domestic animals, could not be rejected considering that the hypothesis of a transmission between wild canids and cattle is epidemiologically consistent (Barling *et al*, 2000, JAVMA, 217: 1361-1365).

Saturday 11 September

Oral Presentation 29

SHAGGY LAME FOX SYNDROME IN PRIBILOF ARCTIC FOXES (*ALOPEX LAGOPUS PRIBILOFENSIS*), ALASKA.

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A previously unrecognized condition characterized by failure to shed the winter pelage and lameness is described in arctic foxes (*Alopex lagopus pribilofensis*) from the Pribilof Islands of Alaska, USA. This condition is called Shaggy Lamé Fox Syndrome denoting the primary clinical signs. Prominent gross lesions included severe polyarthritis, small irregular and pitted kidneys, and over grown nails of the fore limbs. Histological lesions (26 foxes) included a generalized vasculitis, severe polyarthritis with lymphoplasmocytic synovitis, tendosynovitis, bursitis, and periosteal proliferation, renal cortical infarctions, myocarditis with myocardial infarctions, and meningoencephalitis with cerebral infarctions. The etiology and pathogenesis of SLFS is not known at the present time. The gross and histological lesions combined with the microbiological results suggest that SLFS may be an immune-mediated bacterial disease. *Streptococcus bovis* was isolated from the swollen stifle joint from one fox that appeared to be an extremely early case.

Oral Presentation 30

PATHOLOGY OF NECROTIZING ENCEPHALITIS IN SWEDISH ARCTIC FOXES (*ALOPEX LAGOPUS*)

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The arctic fox (*Alopex lagopus*) is an endangered species in Scandinavia. A few wild individuals were caught and have been kept at a breeding center for endangered species. These foxes and their offspring developed encephalitis, with new cases occurring over a period of nine years. Cases in adults appeared sporadically since 1994. Whole litters of puppies showed similar disease. Affected foxes showed successively aggravating clinical signs such as apparent loss of smell and sight, changes in behaviour, fearfulness, unstable gait, loss of balance, walking in circles, biting objects, depression, apathy, and somnolence. Clinical chemistry conducted in some of the foxes showed leukopenia and lymphopenia in whole blood, and increased protein, glucose, and leucocytes in cerebrospinal fluid. Most of the affected foxes died or had to be euthanized at up to one month after the onset of signs.

Pathology showed severe nonsuppurative meningoencephalitis with areas of necrosis. Inflammatory changes included multiple foci of gliosis and lymphocytic infiltration in the grey matter substance, prominent perivascular cuffs composed of lymphocytes, plasma cells and macrophages. Similar type of infiltrate was present in the leptomeninges. Rarified areas contained debris mixed with macrophages, gitter cells, plasma cells, lymphocytes, numerous gemistocytes, and binucleated

or multinucleated reactive astrocytes. The changes were more severe in the olfactory bulbs and frontal lobes of the cerebrum, but also involved other areas of the cerebrum and brainstem, and to less extent the spinal cord. Degenerative changes and intracytoplasmic inclusions in the choroid plexus epithelium were observed in one of the foxes.

The cause of the disease has not been determined by the laboratory testing done so far. Some of the known causes of encephalitis in foxes, such as *Toxoplasma gondii*, *Neospora caninum*, *Listeria monocytogenes*, canine distemper, rabies, adenovirus (fox encephalitis) and Borna virus tested negative. Further investigations, including virus culture and various PCR tests are ongoing.

Oral Presentation 31

DETECTION OF HERPESVIRUS BY PCR IN ARCTIC FOXES (*ALOPEX LAGOPUS*) WITH ENCEPHALITIS

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The arctic (*Alopex lagopus*) is an endangered species in Scandinavia, some of the individuals left in the wild were caught and kept at a breeding center for endangered species. Wild captured arctic foxes and their offspring developed encephalitis. The cause of the disease could not be determined by the laboratory testing done. Some of the known causes of encephalitis in foxes, such as *Toxoplasma gondii*, *Neospora caninum*, *Listeria monocytogenes*, canine distemper, rabies, adenovirus (fox encephalitis) and Borna virus tested negative. Further investigations, including virus culture and various PCR tests were conducted.

Cerebrospinal fluid, brain and lung specimens from affected foxes were tested by panherpes PCR targeting the DNA polymerase gene, and gB PCR targeting the glycoprotein B gene. DNA fragments amplified by these assays were sequenced and compared with sequences in gene bank. The amplified fragments showed a high degree of similarity to herpesvirus sequences. The finding suggests that herpesvirus may be a cause of the encephalitis in the arctic foxes.

Oral Presentation 32

HELMINTHS OF THE PREDATOR-PREY SYSTEM RED FOX – WILD RODENT AND EFFECTS OF URBANIZATION.

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Increasing fox populations in cities are reported throughout Europe. Red foxes (*Vulpes vulpes*) adapt to urban habitats by modifying their behavioural habits, such as social organization and diet. These variations in fox-behaviour as well as direct effects of urbanized environments on intermediate host populations might influence the epidemiology of the helminths of the red fox-wild rodent predator-prey system. In order to investigate the impact of urbanization on the epidemiology of monoxenous and heteroxenous fox parasites, we collected 267 red foxes from rural, residential and urban parts of the canton of Geneva, Switzerland. We underlined different response patterns of the dominant recovered intestinal helminths to the level of urbanization of the habitat. Whereas the monoxenous nematode *Uncinaria stenocephala* presented stable infestation rates (prevalence and abundance) throughout the study area, we recorded a significant decrease in prevalence of the non-strictly monoxenous nematode *Toxocara canis*, and of the dioxenous cestodes *Taenia* spp. and *Echinococcus multilocularis* with the increasing degree of urbanization. Additionally, a decrease in abundance was found for *E. multilocularis*. These findings suggest that rodent paratenic or intermediate hosts play a major role in the epidemiology of these species and highlight the significance of predator-prey relationships on the epidemiology of rodent-dependent non-monoxenous parasites of foxes. Urbanization-related variations in predation rates of foxes on small mammals, in small mammal population sizes, and in small mammal infestation rates may represent distinct or combine processes that could drive the recorded decrease in infestation rates of adult helminths in foxes. We are therefore currently investigating 664 rodents (4 genera), and initial results provide insights into the role the intermediate host plays in the epidemiology of infection with these parasites.

COURTSHIP AND MORTALITY IN FOXES

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A theme of the 6th meeting of the EWDA in Uppsala 2004, is the disease interactions between predators and prey animals. The present paper investigates the possibility that a predator, the fox (*Vulpes vulpes*), may attack and bite other foxes, particularly during courtship, causing injury and fatal bacterial infections. This could be considered as a variation on the conference theme, in that predators in close contact with one another, have the potential for efficient and direct con-specific transmission of pathogens by means of biting.

The study is based on a small series of published necropsy results (Duff 1995), where deaths in foxes were attributed to the results of bite wounds from other foxes during the vulpine rut. It was not possible to prove that foxes were responsible for the lesions, however circumstantial evidence to support this was given. The conclusions therefore were also speculative, including the primary one that death was usually due to bacteria inoculated by the bite. No further cases came to light, until 2004, in a completely different region of England. This recent one differed in some ways from the previous series, but appeared to support the hypothesis that foxes may deliver severe, infected bites to each other during the rut. This behaviour may be relevant for the following reasons: -

- For strategic timing of fox rabies vaccination, before the rut.
- Some foxes may be particularly susceptible to streptococcal infections.
- The pathology, in terms of severity, number and location of bites may give an insight into fox behaviour and interactions during courtship.
- The condition may be under-recorded. Many foxes with streptococcal septicaemia are jaundiced and may be diagnosed as cases of leptospirosis or hepatitis.
- 'There is some poison with all bites' (Celsus, 1st Century A.D), these cases support this old saying. In terms of evolution, particularly in the reptile phyla, and development of poisonous bites, this may be one possible origin of oral toxins, from commensal bacteria found in the mouths of predators.

The disease interaction between predator and prey may also be reflected in the disease interaction between predator and con-specific predator. These reactions in the cases of fox rabies, and badger bTB are significant but other examples may also be relevant. Predators have the potential to inoculate pathogens during a biting attack and this can provide facultative and opportunist pathogens with a means for direct, efficient, high dose-inoculum transmission.

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Oral Presentation 34

A NOVEL *HEPATOZOON* SP. CAUSING MYOCARDITIS IN PINE MARTENS (*MARTES MARTES*) IN SCOTLAND

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Although pine martens are considered endangered in many European countries, and are very rare in England and Wales, they are quite common in western Scotland. However, there is very little published data about the diseases or causes of mortality of pine martens in Britain. Post mortem examinations on five specimens killed in road accidents in Scotland revealed focal, granulomatous lesions in heart and skeletal muscles of four of them. Immunoperoxidase staining tentatively identified suspected protozoa in the lesions as *Hepatozoon* sp and this was confirmed by a polymerase chain reaction-based assay. *H. griseisciuri* infections have been observed in introduced grey squirrels (*Sciurus carolinensis*) in Britain and *H. erhardovae* in bank voles (*Clethrionomys glareolus*) but *Hepatozoon* infections do not appear to have been reported in carnivores in this country. Partial characterisation of the nucleotide base sequence of PCR products indicate that the organism in the pine martens is probably a new species that is closely related to, but distinct from, *Hepatozoon canis*. The

origin of the infection is not known but the muscle stages of the parasite closely resembled those of *Hepatozoon* sp. in Japanese martens (*Martes melampus*) and stone martens (*Martes foina*) in Germany (Yanai and others 1995, Geisel and others 1979). *Hepatozoon* spp. infections are typically acquired by the ingestion of infected arthropods, such as ixodid ticks, but the tick species present in Scotland are not recognised vectors of these parasites.

Oral Presentation 35

DISEASE RISK ANALYSIS FOR A REINTRODUCTION PROGRAMME: COMMON DORMICE (*MUSCARDINUS AVELLANARIUS*)

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An important facet of the common dormouse (*Muscardinus avellanarius*) conservation initiative in the UK has been reintroduction. Wildlife translocations, such as this, present more serious disease risks than are generally appreciated. The greatest danger is the accidental introduction of serious infectious disease into recipient free-living populations which can have deleterious effects on the abundance and dynamics of these populations. Consequently the IUCN recommends health monitoring of all animals involved in translocations. Translocations can also have negative effects on the health of animals through the stresses of shipment, social conflict and competition for resources.

Disease risk analysis and health surveillance of common dormice involved in the reintroduction programme started in 1999 as a part of the Zoological Society of London's partnership with English Nature to provide surveillance for the Species Recovery Programme. Over a five year period, over 300 common dormice have been screened for alien parasites, and their health monitored prior to release. From this pool of dormice, animals have been made available for release at 9 different sites. We have also increased knowledge of the infectious agents and diseases of free-living dormice. The aim of this work has been to ensure that new host-parasite encounters which occur as a consequence of reintroduction do not cause deleterious effects to the reintroduced populations of dormice or to other mammals at the release site, but also that the released animals are in good health to succeed in their new environment.

One potential alien cestode parasite has been eliminated from dormice prior to their release and the levels of another parasite are being closely monitored to ensure dormice are as fit as possible. Dormice with abnormalities which could affect post-release welfare have been held in captivity. Improvements continue to be made to the screening and monitoring process and analysis of the disease risks of release continues.

Oral Presentation 36

RABIES AND RACCOONS – BIOLOGY AND BEHAVIOUR

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There are important geographical variations in the main reservoir host species associated with rabies. In Europe the red fox *Vulpes vulpes* is most important. In the USA different species including fox species, raccoons, skunks and bats are important in different areas. Historically rabies in raccoons was endemic in the central coastal area of Florida, with spread occurring to reach southeastern parts of Alabama and Georgia. Translocation of raccoons into southwest Virginia between 1977 and 1981 is considered to have been the source of the raccoon rabies epizootic which has spread northwards and westwards in the mid-Atlantic states. This epizootic involves a recognised raccoon-adapted strain of rabies, although skunks are an important secondary species infected with this variant in some areas.

Understanding the biology and behaviour of raccoons is relevant to understanding spread of rabies in this species, e.g. rate of spread of an epidemic and periodicity of epidemic waves, and spread from raccoons to other species.

Both normal behaviour including social and sexual interactions as well as territoriality and dispersal, and abnormal behaviour associate with the effects of rabies on the CNS of infected individuals may be important in transmission within and between species.

Factors which may affect population density and therefore disease transmission include habitat type and food availability.

The ability of raccoons to adapt and utilise urban and suburban habitats and food sources means that raccoons may be found at high densities in close proximity to humans – for example in urban parks and suburban back yards. This increased the likelihood of human and domestic pet exposure to rabid raccoons.

Good information on biological and behavioural aspects of raccoons are vital if meaningful models of rabies spread, and of the effects of management strategies, are to be developed.

Oral Presentation 37

THE ROLE OF VETERINARY MEDICINE IN THE IBERIAN LYNX CONSERVATION PROGRAM.

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The Iberian lynx (*Lynx pardinus*) is considered the world's most endangered felid species, with an estimated total population of less than 200 animals distributed within two isolated metapopulations, Doñana and Sierra Morena. Its critical status is due to habitat loss and fragmentation, rabbit population decline from myxomatosis and viric hemorrhagic pneumonia, and non-natural death causes (road kills, illegal trapping).

Taking into account recommendations from the *Action Plan for the Iberian Lynx in Europe*, the *Iberian Lynx Population and Habitat Viability Analysis (PHVA)*, and the *National Strategy for Iberian Lynx Conservation*, the Spanish Ministry of Environment (MIMAM), promoted the establishment of an *Iberian Lynx Captive Breeding Action Plan (MIMAM, 2001)*. Besides the goal of establishing a healthy captive population, the Plan is designed to support *in-situ* conservation actions and to help increase knowledge about lynx conservation needs. The *ex-situ* conservation program is comprised by an interdisciplinary Captive Breeding Committee with a direction that coordinates several aspects: animal husbandry, genetics & demographics, health, reproduction, Biological Resource Bank, and ethology. In addition, there are several international ad-hoc advisors from the IUCN-CSG, the IUCN-CBSG and the EAZA Felid TAG. Each of these aspects includes specific goals, objectives, and actions, with an associated time calendar.

The Veterinary Aspects of the Captive Breeding Action Plan include four main objectives: to maintain the captivity population in an optimal health status, to investigate the health risks associated to the overall conservation program, to avoid disease transmission between the wild and captive populations, and to help control potential health problems in the wild. The specific actions to help achieve the proposed objectives include: to constitute a Veterinary Advisory Group (VAG), to develop and implement protocols for disease prevention and management, to elaborate a veterinary plan for Iberian Lynx Husbandry Manual, to determine disease threats to lynxes (both inter- and intra-specific), and to study and evaluate a safe vaccine protocol for the species. The Veterinary Advisory Group is formed by veterinarians with a background in wildlife disease research, zoo medicine, small animal practice, and wildlife rehabilitation. The Group has designed an Iberian Lynx Veterinary Protocol, which includes an *anesthesia and examination* form and *postmortem* form. The Group works in coordination with all other groups that constitute the Captive Breeding Committee.

Despite the Iberian lynx critical endangered status, few studies have been conducted on diseases of free ranging and captive Iberian lynx. Infectious diseases documented includes mycobacteriosis and piroplasmosis. A thorough health screening, together with appropriate quarantine measures, are necessary to reduce risks when incorporating wild animals to the breeding program, during animal movements, and in future translocations and reintroductions. A multi-institutional, multi-disciplinary, co-operative approach between biologists, veterinarians, and other conservation specialists will be necessary to fulfill the ultimate goal to re-establish Iberian Lynx in the wild.

Oral Presentation 38

DETECTION OF PREY MTDNA IN CARNIVORE SCAT BY POLYMERASE CHAIN REACTION (PCR).

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Identification of prey in wild carnivore feces provides useful data on dietary habits and predator-prey interactions that may influence risk exposures to certain infectious diseases. Traditional prey detection methods in feces rely on gross or microscopic inspection for distinctive hair, feather, or bone which has limited sensitivity and specificity [1,5]. Molecular techniques have successfully identified fecal origins in the field [2-4,6-10] presumably by amplifying DNA from sloughed colonic cells of the host species. Expansion of these techniques could potentially provide a highly sensitive and specific

means of identifying consumed prey in carnivore scat [11] as well as a means for evaluating disease risk in a region using noninvasive techniques. In this investigation, species-specific primers targeting various-sized portions of the cytochrome-b gene encoded on mitochondrial DNA (mtDNA) were tested on a carnivore model, the red wolf (*Canis rufus*), fed a variety of common mammalian prey items including white-tailed deer (*Odocoileus virginianus*), raccoon (*Procyon lotor*), and small rodents (*Rattus norvegicus* and *Mus musculus*). PCR was able to detect consumed prey in fresh wolf scat from 17 hours up to 4 days following a single feeding event. Positive results were obtained in the absence of gross prey evidence in the feces. Additional work evaluates the effects of scat age and storage method on prey assay sensitivity for field application. Molecular prey detection in feces has the potential for broad application in many species and may prove to be a useful adjunct for studying disease movement in wildlife communities.

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Oral Presentation 39

THE EPIDEMIOLOGY OF WEST NILE VIRUS WITHIN WILDLIFE POPULATIONS AND THE POTENTIAL RISKS OF INTRODUCTION AND SPREAD TO THE UK

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West Nile Virus (WNV) is a mosquito-transmitted arbovirus of the family Flaviviridae indigenous to Africa, West Asia and the Middle East but which has undergone recent and rapid expansion, particularly in North America. Migratory birds have been incriminated in the probable introduction of WNV into previously non-endemic areas and are potentially capable of spreading the disease both locally, and over larger distances through migration. The virus is maintained within bird populations by ornithophilic (bird-feeding) mosquitoes and its introduction to non-immune bird populations has resulted in large-scale mortalities. The virus is transmitted to mammals by "bridge vector" mosquito species that feed both from birds and mammals. The virus is capable of infecting a wide range of mammals as well as birds and has been responsible for a number of human deaths. As part of a project to identify the risk factors favouring the introduction and establishment of the virus in the UK, information has been gathered on the distribution of potential mosquito vectors together with migration routes of bird species, known to carry the virus and which migrate from WNV endemic areas, and risk maps produced using GIS. The findings and conclusion from this study are presented.

Oral Presentation 40

USUTU VIRUS SPREAD IN AUSTRIA MONITORED BY DEAD BIRD SURVEILLANCE.

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H-1143 Budapest; BERNHARD SEIDEL, A-3680 Persenbeug; NORBERT NOWOTNY, Clinical Department of Diagnostics, University of Veterinary Medicine, Veterinärplatz 1, A-1210 Vienna, Austria, and Department of Medical Microbiology, Faculty of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates.

Usutu virus (USUV), a member of the mosquito-borne clade within the genus Flavivirus, has been responsible for avian mortality in Austria since 2001. USUV activity has now been recognized during 3 consecutive years. As with West Nile virus, dead bird surveillance is an excellent tool for monitoring USUV activity. In total, USUV has been found in 132 birds with at least two independent methods, usually RT-PCR and immunohistochemistry. The affected birds belong to 8 different species, but more than 90 % of all positives were Eurasian blackbirds. In 2001, a few local episodes of bird mortality were recognized in Vienna and adjacent villages. In 2002 and 2003 the areas with USUV-associated bird die-offs gradually extended to the South and East and to a lesser degree to the North and West. Currently the distribution area of USUV comprises the city of Vienna, 11 (out of 25) districts of Lower Austria and 3 (out of 9) districts of Burgenland and covers approximately 3500 square kilometers in the East of Austria. So far USUV cases have only been noticed within the months July through September, a time period which we currently define as USUV season. In addition, USUV has been identified in several mosquito species. These data demonstrate that USUV has established an efficient bird-mosquito transmission cycle in Eastern Austria. We expect further extension of the affected areas and an involvement of neighboring countries, like Slovakia and Hungary soon. Although USUV belongs to the Japanese Encephalitis group of mosquito-borne flaviviruses, which includes several members with considerable pathogenicity for humans, USUV has not been associated with human diseases in Austria, so far.

Oral Presentation 41

PATHOLOGY AND VIRAL DISTRIBUTION IN USUTU VIRUS INFECTIONS OF BIRDS.

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In the summer of 2001, Usutu virus (USUV) was isolated for the first time in Europe during an episode of mortality among Eurasian blackbirds (*Turdus merula*) and great grey owls (*Strix nebulosa*) in the Vienna area. In the consecutive summers of 2002 and 2003 again numerous birds, most of which were blackbirds, succumbed to USUV infection. Following necropsy, the diagnosis had been confirmed by four methods: histopathology, immunohistochemistry (IHC), in-situ hybridization (ISH) and reverse-transcriptase polymerase chain reaction (RT-PCR). The major macroscopical finding was hepatosplenomegaly; histologically, neuronal necrosis, myocardial lesions and coagulative necrosis of the liver and spleen were observed.

IHC and ISH detected viral signals predominantly in brain neurons, myocardial fibres, cells of the splenic capsule, renal glomeruli, tunica muscularis of intestines and proventricular glands. Organs from USUV-positive birds from the summer 2003 showed a changed viral distribution predominantly characterized by considerably less signals in the brain neurons. These changes could be interpreted as a sign of beginning immunological response within the affected bird population.

Sunday 12 September

Oral Presentation 42

A VIROLOGICAL SURVEY IN MIGRATING WADERS AND WATERFOWL IN GERMANY

THOMAS MÜLLER, ORTRUD WERNER, ANJA GLOBIG, ELKE STARIK, HORST SCHIRRMEIER, FRANZ J. CONRATHS, Federal Research Centre for Virus Diseases of Animals, Germany; Ralf-Udo Mühle, Dieter Wallschläger, Institute for Biochemistry and Biology, Potsdam University, D-14469 Potsdam, Germany; SONJA LINKE, GEORG PAULI, MATTHIAS NIEDRIG, HEINZ ELLERBROK, Robert-Koch-Institute, D-13353 Berlin, Germany; Andreas Hlinak, State Veterinary Laboratory Brandenburg, D-15236 Frankfurt, Germany; Helmut Kruckenberg, Axel Degen, Melanie Buss, Workgroup of Geese Ecology, University of Osnabrück, D-49069 Osnabrück, Germany; Johann J. Seeger, NABU-Regional Association Westhavelland, D-14703 Rathenow, Germany

Migratory waterfowl is both a potential reservoir and/or a carrier of viral diseases and hence, may play a role in the epidemiology of economically important and zoonotic diseases. The high mobility of these birds across long distances between breeding and wintering grounds is considered to contribute to the spread of various avian pathogens. In 2001 and

2002, we conducted a survey with special emphasis on virus isolation in migrating waders and waterfowl. In a capture-mark-re-sighting study, throat and cloaca swabs respectively blood samples were collected from 945 bird specimen comprising 30 species.

In total 56 avian viruses could be isolated from the species captured. Among the virus isolates 48 could be identified as paramyxoviruses (PMV). The majority of them were PMV-1 known to cause Newcastle disease in domestic poultry and were isolated from teal (*Anas crecca*), dunlin (*Calidris alpina*), black-headed gull (*Larus ridibundus*), common sandpiper (*Actitis hypoleucos*), canada goose (*Branta canadensis*) and mute swan (*Cygnus olor*). However, molecular characterization showed the PMV-1 isolates to be rather less pathogenic variants. Whereas PMV-4 was found in white-fronted goose (*Anser albifrons*) and teal only, PMV-6 was detected in a broader variety of species comprising teal, dunlin, little stint (*Calidris minuta*), ruff (*Philomachus pugnax*) and meadow pipit (*Anthus pratensis*). Four avian influenza viruses (AIV) could be isolated from wild duck species (*Anas crecca*, *Anas querquedula*) and characterized. Furthermore, 4 reo-like viruses were isolated from dunlins. The majority of virus-positive birds were less than 1 year old and did not show any clinical symptoms. There was no evidence for the presence of West-Nile virus in these species.

Whereas the role of wild waterfowl in the epidemiology of avian viruses has been demonstrated earlier, this study for the first time also includes waders. The results confirmed previous findings and clearly indicate that next to wild waterfowl also waders represent a reservoir for certain viral pathogens. Though their breeding grounds are geographically separated from each other they gather in high abundances in the same resting sites. Hence, the restricted resting sites available in Western Europe could be important locations for the intra – and interspecific transmission of avian viruses.

Oral Presentation 43

Oral presentation number 43 was cancelled.

Oral Presentation 44

T. GALLINAE AND TRICHOMONIASIS IN WINTERING WOODPIGEONS (COLUMBA PALOMBUS) IN SOUTH-CENTRAL SPAIN: SUBLETHAL EFFECTS AND IMPLICATIONS FOR ENDANGERED BIRDS OF PREY

DIEGO VILLANÚA, URSULA HÖFLE, CHRISTIAN GORTAZAR

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Avian Trichomoniasis, a disease caused by the flagellated protozoan *T. gallinae* affects several species of birds including psittacines, gallinaceous birds and birds of prey, but the natural host appear to be columbiforms. In the latter infection is usually subclinical although pathogenicity with severe, lethal outbreaks occur. Prevalences among free-living and captive columbiforms are considered to be very high although few data exists, and the pigeon is considered the source of infection for more susceptible birds such as birds of prey. In order to study true prevalence of infection with the parasite among free-living woodpigeons and sublethal effects of the parasite on fitness and immunity of the birds we examined 60 hunter-harvested, apparently healthy woodpigeons. Presence of infection in each bird was investigated using culture, direct wet mount examination and inspection for characteristic lesions. In addition birds were weighed, sexed and underwent detailed necropsies including collection of biometric data, organ weights, data on body condition, parasite burdens and any pathologic lesions.

Culture proved most sensitive for the detection of infection, and the detected prevalence of infection with *T. Gallinae* was as high as 43.5% while only 3,28 % of the birds suffered from macroscopic lesions. Infection was independent from sex but was found to be significantly higher in adult birds. Infected animals had a larger bursa and were in poorer body condition than healthy birds even if no visible lesions were present.

The results suggests that infection with *T. Gallinae* is widespread among wood pigeons in Southern and Central Spain, and that sublethal effects of the parasite do exist. Parasitised birds are probably more susceptible to predation and thus constitute a source of infection for birds of prey. However it is yet unclear if the parasite may have a direct regulatory effect on woodpigeon populations.

AVIAN TRICHOMONIASIS IN BIRDS OF PREY: EMERGING DISEASE, EMERGING RESERVOIR OR BIOINDICATOR?

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Avian trichomoniasis is a well known disease of the upper digestive tract of pigeons caused by the protozoan parasite *Trichomonas gallinae*. The disease is historically known in wild and captive raptors that consume avian prey such as hawks and falcons. Recent emergence of trichomoniasis as an important disease and mortality factor in nestlings and fledglings of different endangered species of birds of prey has been attributed to a change in diet with columbiforms as substitute for disappearing traditional prey species such as. Typical forms with fibronecrotic lesions in the oropharynx, oesophagus and crop as well as atypical forms of the disease with sinusitis, hypema and conjunctivitis have been observed in the affected species that include the endangered Iberian Imperial eagle (*Aquila adalberti*), Bonelli's eagle (*Hieraetus fasciatus*), marsh (*Circus aeruginosus*) and hen (*Circus pygargus*) harriers. Also, severe outbreaks of trichomoniasis among wild columbiforms have been described recently in Spain, UK and USA. However, recent results of our studies suggest, that subclinical infections with *T. gallinae* exist in raptors as well as in columbiforms, either related to infection with less pathogenic strains or enhanced host resistance, thus trichomoniasis could be an indicator of impaired immunity rather than an emerging disease. Morphologic and molecular studies in order to compare trichomonas strains from different avian hosts and between diseased and healthy animals are under way, in order to elucidate the importance of trichomoniasis for endangered species.

Oral Presentation 46

HISTOLOGICAL LESIONS PROVOKED BY HYDROCARBONS TO OILED SEABIRDS

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The toxic effects of hydrocarbons can lead to lesions on various organs: digestive tract, lungs, kidneys, livers. On the other hand, hydrocarbons are often quoted as responsible for immunosuppression of oiled birds, in relation with an impairment of the Bursa of Fabricius. The treatment of the oiled birds has to take into account the potential risk of these various lesions, due to the ingestion and/or the inhalation of various hydrocarbons, to starvation of birds (due to oiling) and to secondary infection (due to captivity). To improve the knowledge on these lesions, all the oiled birds dead during winters 1999-2004 (included Erika and Prestige oil spills) in the wildlife centre of Nantes (France) were collected for the digestive tract, the Bursa of Fabricius, kidneys, lungs, livers for histological analysis. In parallel, the present type of hydrocarbon on the feathers of birds was analysed. The results are presented and interpreted, in order to distinguish between the lesions due to hydrocarbons, starvation and secondary infection. This will allow to better adapting the management of the oiled birds. The main lesions due to secondary infections are aspergillus's lesions, of lungs and livers. Concerning gut, the main lesions are lesion of autolysis and necrosis. We suggested that these lesions were provoked by starvation because of the poor condition of the birds arriving in the wildlife centres. Concerning kidneys, the main lesions were lesions of metaplasia but the origin of these lesions were unclear.

Oral Presentation 47

BACTERIAL AND FUNGAL CONTAMINANTS IN RAPTOR EJACULATES AND THEIR SURVIVAL TO SPERM CRYOPRESERVATION PROTOCOLS.

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Artificial insemination has successfully served captive breeding programs for endangered avian species, including raptors. Many are based on captive wild individuals (non-imprints) and therefore have to rely on the massage technique for semen collection. However, obtaining good quality semen is still a challenge mostly due to the presence of contaminants which render samples unusable. As an example, percentages of urine contaminated samples in raptors range from 28,7 to 48,2. Semen may also get contaminated with bacteria present either in the male reproductive tract or in the papilla and surrounding area during the collection process. If used for artificial insemination, it may cause systemic disease and infertility; i.e. ureaplasma in turkey semen has been associated to abnormal spermatozoa and infertility. The goal of this preliminary study was to determine the species, incidence, and degree of bacterial contaminants in ejaculates from eagles and falcons using massage and their resistance to the freezing-thawing process. A total of 45 semen samples, 25 from Peregrine falcons (n=6) and 20 from Imperial eagles (n= 7) were examined for urine and bacterial contamination. Bacterial contamination was demonstrated in 57,7% of the samples examined while fungi was cultured from only one sample (2,2%). From the 47 different strains isolated, 78,8% were *E. coli* spp and 2,1% *Proteus* spp., while the rest belonged to Gram positive strains including *Streptococcus*, *Micrococcus* and *Staphylococcus* spp. Number of colonies cultured per sample were not consistently related neither to the degree of urine contamination nor to a decrease in pH. Only in 6% of the samples, bacteria were killed by the freezing process. After thawing, the number of colonies were significantly reduced in 21,1% of the samples, while 24,2 % experienced a significant increase. In 42,4% of the samples freezing-thawing contributed to bacterial overgrowth. In conclusion, when obtained using massage, semen from captive wild eagles and falcons is frequently contaminated. Contamination is mainly by *E. Coli*, possibly because it forms part of the cloacal flora present at the site of the papilla in captive raptors. Steps and diluents needed for cryopreservation appear to promote bacterial growth in many cases. Artificial insemination with this material has the potential to transmit systemic diseases and/or affect the female reproductive tract leading to irreversible infertility. Further studies are in progress to determine strain pathogenicity, risk of transmission and develop methods to both minimize contamination and to exclude these and other potential pathogens from avian semen.

Oral Presentation 48

CHRONIC HYPERPLASIC GASTROPATHY WITH CYST FORMATION IN 13 GREEN IGUANAS (*IGUANA IGUANA*): A PRELIMINARY STUDY

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Gastric erosions, ulcerations and gastritis have been reported in reptiles several times, mainly associated with gastrointestinal parasites, foreign bodies, bacterial and mycotic agents. Predisposing causes to these pathologies include iguanas habits of ingesting foreign objects and multiple and additive stress factors that result from captivity.

A retrospective study was carried out in the Pathology Department, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica, including 56 necropsies done on adults iguanas (> 1 year old), between 1991-1994 and 1996. In total 13 green iguanas were diagnosed on histopathological examination with chronic hyperplasic gastropathy and glandular cyst formation.

Clinical findings: distribution included 7 females and 6 males. Five were reported as adults. In the remaining 8, age ranged from 3 ½ to 11 years (mean, 6 years). The main clinical complaint record was stomatitis (8 cases), weight loss with cachexia (4 cases), and dehydration (3 cases).

Pathological findings: Grossly, the lesions consist of proliferative mucosal changes with formation of polyps. Microscopically, 12 iguanas had chronic hyperplasic gastropathy and in 6 of these iguanas also had a cystic mucoid distention of gastric glands; one case had only a submucosal cyst. Classically in domestic animals, especially dogs, two forms of chronic gastritis with mucosal hyperplasia have been described. Hypertrophy gastritis and giant hypertrophic gastropathy both share some similarities with the present cases here. The main difference, however, is the absence of smooth muscle hypertrophy.

Oral Presentation 49

ACUTE DEATH DUE TO INFECTIOUS DISEASES IN FIVE MONKEYS

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Several infectious agents have been described as a cause of death in nonhuman primates, however only a few are responsible for sudden death. Five monkeys that died acutely (less than 24 hours) are presented in this report. Macroscopic and histopathological examination was done in four cases and only histopathology in one.

Case no 1: A male, *Saimiri oerstedii* died suddenly without clinical signs. Pathological and bacteriological diagnosis confirmed a systemic **Shigellosis**, affecting the intestine, lung, liver, and brain.

Case no. 2: A 16 month old male, *Alouatta palliata* died within 6 hours after showing clinical signs that included an initial episode of seizures, followed by depression and hypothermia. Seizure activity progressed despite treatment with diazepam and supportive measurements, and died. Final diagnosis was **Leptomeningoencephalitis** by *Listeria monocytogenes* which was cultured from brain, liver, spleen and heart samples.

Case no. 3: A free-range female *Saimiri oerstedii*, of approximately one year old, found dead on the ground in the National Park of Manuel Antonio. Liver and lung samples were collected by the referring veterinarian at the time of the finding. Histopathologically, an acute multifocal hepatitis due to *Toxoplasma gondii*, confirmed by immunohistochemistry was found.

Case no. 4 and 5: Two females *Alouatta palliata*, 1 and 2 years of age, respectively, from the same household. Clinical signs, the afternoon before sadness, next day found both death in the morning. Histopathological findings were consistent with a necrotizing acute hepatitis, enteritis and encephalitis caused by *Toxoplasma gondii*, positive by immunohistochemistry.

Conclusion: during this presentation, the importance of a complete histopathological investigation in cases of acute death will be emphasized. Routine examination of the brain is critical as a new world primates are highly susceptible to toxoplasmosis.

Oral Presentation 50

DISEASES FOUND IN FRENCH CARNIVORES ACCORDING TO SAGIR DATABASE – GLOBAL ANALYSIS

MARIE-EVE TERRIER¹, JEAN-ROCH GAILLET², FLORENCE CLIQUET¹, JACQUES BARRAT¹

¹AFSSA LERRPAS, BP 9, 54 220 MALZEVILLE, FRANCE. ²ONCFS, BP 20, 78 612 LE PERRAY EN YVELINES Cedex

Created in 1986 by the "Office National de la Chasse et de la Faune Sauvage" (ONCFS), the governmental agency in charge of hunting and wildlife surveillance, SAGIR is a national surveillance system of wildlife diseases.

SAGIR is organized as a network composed of the ONCFS, the French Food Safety Agency (AFSSA) in Nancy, the toxicology laboratory of the National Veterinary School in Lyon (ENVL) and other specialized laboratories, the "Departemental" Veterinary Laboratories (LVD) and the "Departemental" Federations of Hunters (FDC). The last two organisms form the basic unit of the whole system. A specificity of SAGIR is that the FDC pays for the different routine analyses. One of the advantages of SAGIR is the national coverage of the network. The drawback is the sampling method : the necropsy of animals found ill or dead is carried out according to the interest of the hunters for each case.

The data base integrates also cases external to the SAGIR network, such as data from the Lynx network, data from the National Parks, ...All the sources can be recovered.

Since 1986, more than 1900 carnivores were necropsied by SAGIR : over 1500 red foxes, more than 100 badgers, and a lot of lynx, stone martens, pine martens, polecats, otters, raccoon dogs, ...

After a short reminder on the SAGIR network, the presentation gives, for the red foxes on the one hand, for all the other carnivores on the other hand, the geographical origin, the evolution of the analyses year after year, the different causes of death and the principal agents found (bacteria, parasites, virus, toxics, radio elements).

The most interesting facts are:

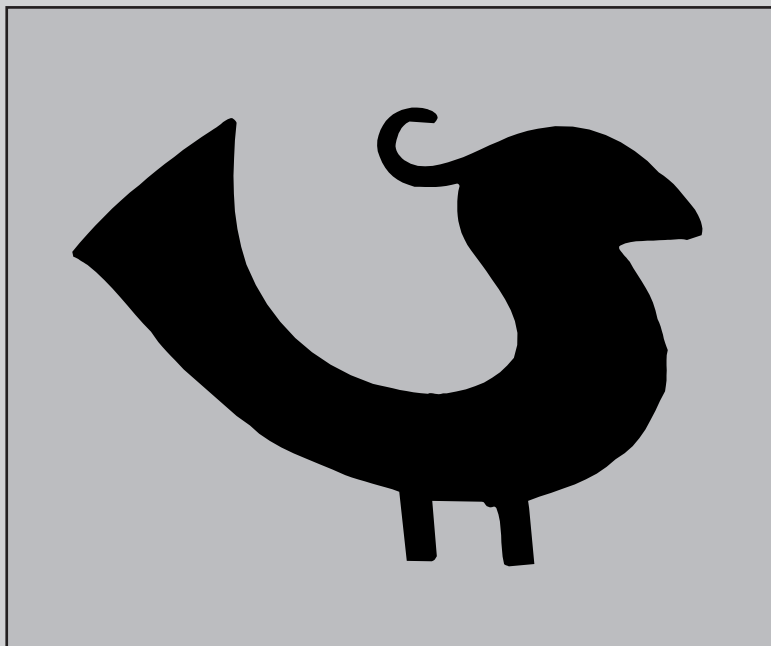
- the major cause of death of the foxes is linked with human activities. The microbial agents are responsible of only a quarter of deaths of foxes recorded by SAGIR, i.e. less in the whole wild population.
- the campaign against voles is responsible of a lot of deaths due to bromadiolone. The intoxications with cholinesterase inhibitors products as carbofuran, organophosphorus, ... concern the stone marten, the badger and the wild cat.
- the epidemiological analysis of the data about sarcoptic mange and echinococcosis is given.

This presentation is completed by a poster "Inventory of diseases found on French wild carnivores by SAGIR network", that gives the list of all microbial agents found in the laboratories.

Abstracts

Poster Presentations

September 9th and 11th



Poster Presentation 1

A CASE OF SIMULTANEOUS CEREBRAL SETARIOSIS AND NODULAR CUTANEOUS ONCHOCERCOSIS IN RED DEER (*CERVUS ELAPHUS HIPPELAPHUS*).

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We present a case of simultaneous cerebral setariosis and nodular cutaneous onchocercosis in a 5-year-old male red deer (*Cervus elaphus hippelaphus*) from upper Styria, Austria. The individual was observed for several days in early summer exhibiting clinical neurological signs like walking in circles, staggering and uncoordinated movements, as well as multiple subcutaneous nodules at the hind limbs. The deer was shot by a local hunter and a post mortem examination was performed on the head and the skin.

Pathological findings comprised severe hydrocephalus internus of the right ventricle filled with large amounts of reddish-brown fluid, inflammation of the meninges, and malacia of the right hemisphere. In total, nine adult nematodes were detected in the subdural space and identified as *Setaria cervi*. Skin nodules showed purulent inflammation and large numbers of filarioid nematodes of the genus *Onchocerca*.

Since both *Setaria cervi* and *Onchocerca* spp. are transmitted by arthropods, we speculate that wetlands near the place where the deer was found and humid weather conditions favouring a local boom of arthropod populations may act as ecological risk factors for the occurrence of these diseases.

Poster Presentation 2

COMPARISON OF THE EFFICACY OF TWO METHODS OF CAPTURING SPANISH IBEX (*CAPRA PYRENAICA*): DRIVE-NET AND BOX-TRAP

ENCARNA CASAS-DÍAZ, JORGE R. LÓPEZ-OLVERA, GREGORIO MENTABERRE, IGNASI MARCO, and SANTIAGO LAVÍN, Servei d'Ecopatologia de Fauna Salvatge. Facultat de Veterinària. Universitat Autònoma de Barcelona, 08193 – Bellaterra, Spain.

Different capture methods, like snares, corral-traps and box-traps have been used to capture Spanish ibex (*Capra pyrenaica*). The first experience to capture Spanish ibex with drive-nets started in 2002 in the National Game Reserve of Ports de Tortosa i Besòit (Spain). This experience was combined with capture by means of box-traps in the same Reserve.

During years 2002 to 2004, 10 capture operations by means of drive-nets were carried out, with a total of 31 individuals captured. Besides, up to 10 box traps were activated for 31 days during years 2003 and 2004, capturing a total of 26 animals. Efficacy of both methods, according to the parameters established by Berduco (1993), was estimated from the data obtained.

Drive net has a greater performance (0.64 days per animal) than box trap (1.19 days per animal), although it needs more personnel/day to capture an animal (1.15 compared to 0.37). Drive-net also allows the capture of related groups at the same time. During drive-net operations females were more frequently captured than males, whereas box-trap showed a higher percentage of males captured. No animal casualties related to capture method were registered in none of both methods, and risk for people was low and similar in drive-net and box-traps.

Poster Presentation 3

MYCOPLASMAS CARRIED BY SPANISH IBEX (*CAPRA PYRENAICA*) IN SOUTHERN SPAIN: FREQUENCY AND RISK FACTORS ASSOCIATED.

MÓNICA GONZÁLEZ CANDELA, GISELE VERBISCK BUCKER, PABLO MARTÍN ATANCE, MARIA JOSÉ CUBERO PABLO and LUIS LEÓN VIZCAÍNO. Infectious Diseases, Department of Animal Health, Faculty of Veterinary Medicine, University of Murcia, Murcia, 30100, Spain. e-mail: lleonvi@um.es

The populations of Spanish ibex (*Capra pyrenaica hispanica*) in southern Spain were investigated in order to identify which

potential pathogens were carried by these animals in the upper respiratory tract, reproductive tract and in ocular and ear canal mucous. This knowledge is essential to provide background information on the incidence and distribution of potentially pathogenic organisms in this wildlife species. Nasal, vaginal, ear and ocular swabs were collected from 321 Spanish ibex (186 males, 134 females, from which 116 were young). 271 animals were apparently healthy and 50 were naturally infected with *Sarcoptes scabiei*. About mycoplasmas, *Mycoplasma agalactiae* (14.3%) and *Mycoplasma arginini* (5.9%) were isolated most frequently; both were isolated with greater frequency (70% and 80% respectively) from ear canal swabs. The frequency of mycoplasmas without identifying was of 5.6%. Age was a risk factor in *Mycoplasma agalactiae*, *M. arginini*, and *Mycoplasma* spp. infection, most frequently isolated from young animals.

Poster Presentation 4

EFFECT OF ACEPROMAZINE ON DRIVE-NET CAPTURE STRESS IN SOUTHERN CHAMOIS (*RUPICAPRA PYRENAICA*).

JORGE R. LÓPEZ-OLVERA, IGNASI MARCO, JORDI MONTANÉ, ENCARNA CASAS-DÍAZ and SANTIAGO LAVÍN, Servei d'Ecopatologia de Fauna Salvatge, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193-Bellaterra, Spain.

Physical capture is one of the most stressful events that may happen in the life of wild ungulates. Chamois (*Rupicapra* spp.) have been captured for more than 100 years with scientific, translocation or restocking purposes. However, little information about their stress response is available in literature.

40 free-ranging Southern chamois (*Rupicapra pyrenaica*) were captured by means of drive-nets and underwent a three-hour restraint period after the capture. 19 randomly selected animals received 0.1 ± 0.02 mg/kg of acepromazine maleate intramuscularly at the moment of capture, whereas the remaining 21 animals received 0.5 mL of saline, acting as controls. Clinical parameters (heart rate and temperature) were registered throughout the study period. Haematological and serum biochemical parameters were analyzed from blood samples obtained at capture and one hour thereafter until the end of the study period. Treatment effects on the parameters analysed and their evolution over time were studied, as well as the combined effects of sex and treatment.

Heart rate decreased over time in both groups, but it stabilised earlier in treated animals than in control animals. It stabilised later in control females than in males (either treated or control) or treated females. Heart rate was higher in control females than in control males from minute 135 to minute 175. Body temperature also decreased over time in both groups, but it was statistically higher in control animals from the beginning of the monitoring to minute 135. Control males and females showed higher temperature than treated males and females from the start of temperature recording to minutes 55 and 125, respectively. Body temperature was lower in treated females than in treated males from minute 15 to minute 30 and from minute 80 to minute 125.

Treated animals showed statistically ($p < 0.05$) lower values for RBC, haemoglobin concentration, haematocrit and CK and higher values for monocytes, band neutrophils and glucose than control animals. Sex differences in the evolution over time were found for RBC, haemoglobin, MCV, MCHC, MCH, total protein, band neutrophils, glucose, cholesterol, triglycerides, total bilirubin, creatinine, urea, ALP, ALT, AST, CK, LDH, chloride, sodium and potassium.

Acepromazine modulates stress response in Southern chamois and could help to avoid the development of pathologies related to stress. Stress indicators suggest a stronger response to capture and physical restraint in females than in males.

Poster Presentation 5

TIME TRENDS OF METALS IN ORGANS OF MOOSE (*ALCES ALCES*) FROM SWEDEN

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The long-term monitoring of persistent and bio-accumulating chemicals in the Swedish environment is part of the Swedish National Environmental Monitoring Programme funded by the Swedish Environmental Protection Agency. It is based on chemical analyses of tissues and organs from species collected in selected reference areas of the Swedish mainland, lakes and coastal areas.

As part of the terrestrial contaminant monitoring programme, specimens of muscle, liver and kidney of moose (*Alces alces*) have been collected since 1980 from Grimsö, and a coherent hunting district in the Örebro county (T) in south-

central Sweden. In 1996, the monitoring was extended by collection and chemical analysis of organs of moose from six further counties and districts in Sweden. These districts are situated in the Norrbotten county (BD), Jämtland county (Z), Västmanland county (U), Älvsborg county (P), Jönköping county (F) and Kronoberg county (G).

Moose, with a diet dominated by twigs and leaves of trees and shrubs, was chosen in the monitoring programme as a representative of biota in the Swedish forest areas. Since the moose is distributed almost all over the country, it was considered as an ideal material also for studies of spatial distribution of environmental pollution and bio-accumulation, which is the reason for the extended collection of samples in 1996 onwards.

This report presents levels and time trends of Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, V and Zn in liver and kidney and Hg and Se in liver and muscle.

Poster Presentation 6

A CASE OF SWINE POX IN A WILD BOAR (*SUS SCROFA*) IN AUSTRIA.

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A male wild boar, less than 1 year old and shot because of abnormal behaviour, showed multiple skin lesions up to cherry size. Papules, pustules and scabs could be found all over the body. Light microscopic examination revealed ballooning degeneration of keratinocytes but no intracytoplasmic inclusion bodies. In the skin suspension virus particles could be demonstrated by negativ contrast electron microscopy. They were considered as orthopox viruses which cannot be distinguished morphologically from suipoxvirus. However by means of virus-specific PCR and subsequent sequencing of the amplification product suipoxvirus was unequivocally identified. Numerous lice (*Haematopinus suis*) parasitized on the wild boar which probably promoted the infection.

Poster Presentation 7

ANTIBIOTIC RESISTANCE IN WILDLIFE.

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Faecal samples were collected, as part of the National Health Surveillance Program for Cervids (HOP) in Norway, from wild red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), moose (*Alces alces*) and reindeer (*Rangifer tarandus tarandus*) during hunting seasons. A total of 50 samples were cultivated from each cervid species in order to isolate the indicator bacterial species *E. coli* for antibiotic resistance pattern studies. Antibiotic resistance was found in 13 (7.3%) of the 179 *E. coli* isolates tested (streptomycin resistance: ten isolates, tetracycline resistance: five isolates, sulphonamide resistance: six isolates, trimethoprim resistance: one isolate). The proportion of resistant *E. coli* isolates was higher in wild reindeer (24%) than in the other cervids (2.2%).

Twenty-eight other faecal samples, from bear (*Ursus arctos*), reindeer and moose, were plated out on a growth medium selecting for coliforms resistant to streptomycin or tetracycline (MaConkey agar with 30 µg/ml streptomycin or 30 µg/ml tetracycline added). Two resistant *E. coli* isolates were found; one from a bear and one from a moose, both were streptomycin resistant.

Genetic analysis of the resistant *E. coli* from the wild animals revealed the presence of known resistance genes commonly found in pathogens from humans and domestic animals. Conjugation experiments showed that it was possible to transfer the resistance determinants to susceptible *E. coli* (*E. coli* DH5a) from 11 of the 15 resistant *E. coli* strains isolated from the wildlife.

SEARCHING FOR TSE DISEASES IN REINDEER IN NORWAY AND ICELAND

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Introduction:

Deaths of semi-domesticated reindeer (*Rangifer tarandus tarandus*) are common in North of Norway specially in the winter months. In other times of the year deaths and abnormally poor condition on single animals may be seen both in the north and south area of the Sami's "reindeer-farming". The causes of death seem often to be poor condition only, or killing by predator animals. Scrapie or CWD cannot be excluded without testing. In Iceland suspect has risen of scrapie in some wild reindeer that were in close connection with heavy infected scrapie-flocks of sheep. In the year 2003 and 2004 the author has been working on reindeer diseases at slaughterhouses in Lappland and Røros Norway. Aims of the study:

- To look for changes indicating TSE-diseases (CWD, Scrapie, Mad-cow or BSE) in the brain semi-domesticated reindeer in Norway - Take samples of blood for genotyping reindeer from different areas in order to evaluate if possible the resistance against TSE-diseases (e.g. scrapie).

Material and Methods:

An investigation was made in march 2003, in november and december 2003 and in february and march 2004 at the reindeer-slaughterhouse in Karasjok, Kautokeino, Sussjäarvi Finnmark and Røros. Around 600 brainsamples were taken. In september 2004 there are plans of taking brain-samples of 350-400 in addition in middle-Norway(Gudbrandsdalur) and south-Norway(Setesdal) for testing for TSE-changes.

Findings:

Until now histological signs of TSE-diseases have not been found in brain of animals tested. Majority of the samples will also be tested with the Bio-Rad technique. Inflammatory changes were found in meninges of many of the reindeer indicating infestation with the brain-worm (*Elaphostrongylus rangiferi*). In a few animals, foci of inflammations of similar type were also found in the brain tissue. In the meninges of some of the reindeer were found creatures that remind of amoebae up to 30 in single animals. Such a parasites have been described in human, not earlier in reindeer after the knowledge of the author. Other interesting pathological changes have been found like gastric ulcers possibly not been described earlier. The investigation will be brought to an end in November-December 2004 or early in March 2005. There are plans of taking similar samples Finland and Sweden, and also from wild-reindeer in Setesdal, Svalbard and Iceland.

Poster number Presentation 9

RAPID HISTOLOGICAL EXAM APPLIED TO THE DIAGNOSIS OF WILD ANIMAL DISEASES: CASES REPORT.

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By means of the presentation of some characteristic cases, we propose to point out the contribution of rapid histological exam to the diagnostic check on wild animals. Rapid histological exam is based on freezed tissue dissection by cryostat and Hematoxylin-Eosin fast stain of specimens. The advantages of this analysis are:

- Brief time of execution (about 1 hour) and cheapness
- In many cases, closing of the diagnosis at the same time of the necropsy
- Diagnostic protocol management in relation to microscopic lesions
- The iconography of explained cases is subdivided in this way (Guarda F. and Mandelli G.):
- Parasitic lesions: trichinellosis in wild boar, sarcosporidiosis in roe deer, strongiloidosis in hare
- Infectious lesions linked to bacteriological exam (Carter G.R. and Cole J.R., Jr): chronic hepatitis for *Aerococcus viridans* in roe deer, purulent meningitis from haemolytic *Escherichia coli* in roe deer, purulent myocarditis from *Corynebacterium pseudotuberculosis* in chamois
- Neoplastic lesions: cutaneous squamous carcinoma in deer, renal cystic adenoma in beech-marten

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WILDLIFE DISEASE SURVEILLANCE IN ASTURIAS (NORTHERN SPAIN)

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Wildlife disease surveillance in the autonomic region of Asturias in Northern Spain is based on a collaborative effort between the regional government (Principado de Asturias), local hunters associations, the regional veterinary laboratory (SERIDA) and the National Institute for Investigation on Game Research (IREC). The network is being built up since 2002.

Surveillance includes analysis of dead or diseased wildlife submitted by the public, rehabilitation centres, wildlife officials, environmental patrols of the police and hunters (passive surveillance) as well as random and stratified sampling of hunter harvested game species, mainly ungulates (active surveillance). In addition, the scheme also includes the control of game-rearing facilities and wildlife translocations. Chest freezers and centrifuges have been distributed among the different game preserves and collaborating local hunter associations, although sampling in the field is mostly undertaken by personnel of the joint project. Species from all classes (mammal, avian, fish) are included in the surveillance.

Hunter harvested and submitted animals undergo detailed post mortem examination and routine sampling for diagnostics and for a backup storage of tissue and serum samples. Special attention is paid to bovine tuberculosis, paratuberculosis and brucellosis among other diseases in ungulates, and to rabbit haemorrhagic disease (RHD)/European brown hare syndrome (EBHS), tularemia and brucellosis in lagomorphs. Serologic analysis is carried out routinely for paratuberculosis, brucellosis, lyme disease and RHD/EBHS, and serum samples are stored for any additional analysis considered of interest. In birds and fish detailed necropsies are carried out as well as sampling for analysis of parasitic and infectious diseases as well as ecotoxicology. Ticks and tick-transmitted diseases are also surveyed within this scheme.

This surveillance permitted to diagnose different disease processes such as paratuberculosis among overabundant fallow deer (*Dama dama*) in one of the game preserves of the region. Low seroprevalence of Brucellosis was detected among red deer of a hunting reserve in 2003, while all ungulates examined to date were negative for bovine tuberculosis (absence of TB compatible lesions and culture negative). Sporadic cases include actinomycosis in wild boar (*Sus scrofa*), capture miopathy in a capercaillie (*Tetrao urogallus*) or septicemia from *Streptococcus sp.* in a chamois (*Rupicapra rupicapra*).

Poster Presentation 11

MYCOPLASMA AGALACTIAE INFECTION IN SPANISH IBEX (*CAPRA PYRENAICA*) POPULATIONS FROM ANDALUSIA, SPAIN

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Contagious agalactia syndrome (CAS) is a serious disease of small ruminants characterized by mastitis, arthritis and keratoconjunctivitis. CAS has been reported worldwide and is endemic in most mediterranean countries. In Spain, the species responsible for the majority of cases is *Mycoplasma agalactiae* (*Ma*), the main causal agent of CAS. In wild Caprinae species there are no descriptions of infection by *Ma* neither in Spain nor in the world. This paper reports the *Ma* infection in the spanish ibex populations studied from different provinces of Andalusia, in southern Spain. Conjunctival and ear canal swabs were collected from 500 animals to investigate the frequency of *Ma* infection, since October 1996 until May 2003. The frequency of *Ma* carriers was 10.2%; Almeria and Granada were the andalusian provinces with higher frequency of *Ma* isolates and Granada was the province with greater relative risk to present carrying animals, with 54.9% of infected ibex. The another provinces did not present significant statistical differences ($P > 0.05$). Sex and age were a risk factor in *Ma* infection within the spanish ibex population studied, with higher frequency of *Ma* isolation in females and at ages of 1 and 2 years. The infection by *Ma* represents a serious risk for demography and general sanitary state of these wild populations, once it generates severe consequences like blindness, weakness by starvation and poliartitis, affecting specially young animals. The obtained results mainly suggest the infection stays between wild animals and the domestic flocks by the contact when they graze and share habitat sometimes of the year during transhumance. Meanwhile, to affirm the infectious agent comes from domestic flocks and that wild Caprinae species play like reservoir, or if the isolated strains are common to the wild, deeper studies will be made.

SANITARY SURVEY OF THE IBERIAN LYNX (*LYNX PARDINUS*) AND ASSOCIATE SPECIES

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Endangered populations are markedly sensitive to diseases due to their low genetic diversity. Other more abundant wild or domestic species that share habitat with them can act as reservoir of diseases and would promote epizooties, that are one of the causes that can lead an endangered species to extinction. Recent evidences of these facts, as observed in carnivore species such as the cheetah (*Acinonyx jubatus*) or the black-footed ferret (*Mustela nigripes*), make necessary a sanitary survey in the areas where the Iberian lynx (*Lynx pardinus*) is still present. In spite of Iberian lynx being the more endangered felid species in the world, very few is known about its pathology, and only some case reports and helminthological surveys are available. There is also few information about the diseases of wild carnivores in the Iberian peninsula. However, surveys performed in wild ungulates in areas surrounding lynx populations reported high prevalences of infectious diseases such as bovine tuberculosis or pseudorabies.

The aim of the present study is to carry out a two-years survey of the diseases present in the lynx and species sharing habitat with it. Based on some previous information and on the scientific literature, the diseases of main concern are the above cited together with felid viral diseases, canine distemper, piroplasmosis, hookworms, or mange. The target species other than the lynx are feral cats, dogs, foxes and other wild carnivores, wild boar and red and fallow deer, among others.

Lynx samples will be obtained from stored sera collected for ecological studies, carcasses, from lynxes captured for the captive breeding program, and from faeces picked up in the field. Other species will be sampled by trapping (carnivores), after shooting drives (foxes and ungulates), and recovering carcasses of animals killed by cars or found dead by gamekeepers. The study will also include a survey of ticks in order to detect tick-borne diseases.

This study may help to detect endemic diseases that in case of epidemics would affect lynx populations, to determine the suitability of an area for future reintroduction, and to maintain the captive stock free from diseases.

Poster Presentation 13

DISTRIBUTION OF ALLELIC FORMS AT THE PORCINE NATURAL RESISTANCE-ASSOCIATED MACROPHAGE LOCUS (*NRAMP1*) AMONG WEST EUROPEAN POPULATIONS OF WILD AND DOMESTIC PIGS

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Some proteins are known to confer an innate resistance against salmonellas. NRAMP1 confers to macrophages resistance to a variety of bacterial pathogens such as *Salmonella* spp. Furthermore, in poultry, allelic polymorphisms at the *Nramp1* locus affect the probability of survival after experimental salmonellosis (Kramer *et al.*, 2003 ; Liu *et al.*, 2003), which raises the possibility that identification of a NRAMP1 isoform in pigs might allow selection programmes aimed at improving their innate resistance. The present study aimed at investigating the distribution of allelic forms among european wild boar, Landrace, Large White, Duroc, Berkshire and Pietrain pig populations. Different primers were designed to amplify several regions of the porcine *Nramp1* gene. Amplified products were sequenced and compared to identify allelic forms. The absence vs. wide distribution of the “multiple allelic forms” in wild and domestic pigs, respectively, provides a basis to investigate the relationship between the *Nramp1* genotype and susceptibility to salmonellas in the porcine population.

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LIU W., KAISER M.G., LAMONT S.J. Natural resistance-associated macrophage protein 1 gene polymorphisms and response to vaccine against or challenge with *Salmonella enteritidis* in young chicks. *Poultry Science*, 2003, **82**, 259-266

Poster Presentation 14

ANALYSIS OF PRION PROTEIN GENE (PRNP) SEQUENCE VARIATION IN ITALIAN WILD RUMINANTS.

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Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) occurring in North American cervids since more than 30 years. Until now, there is no evidence that TSE (BSE, CWD or scrapie) exists in cervids and other wild ruminants in Italy or any other European country. Prion protein gene (PRNP) alleles associated with relative susceptibility to TSE have been identified in sheep and humans. Sequence analysis of the PrP gene from Rocky Mountain elk showed only one amino acid change (Met to Leu at cervid codon 132) and homozygosity for PrP codon 132 Met were over-represented in both free-ranging and farm-raised CWD-affected elk when compared to unaffected control groups (O'Rourke et al. 1999). In addition, PRNP alleles encoding amino acid substitutions were identified in white tailed deer, with substitutions at residues 95 (Q→H), 96 (G→S) or 116 (A→G), each with serine (S) at residue 138 (N→S) (O'Rourke et al. 2004). Comparable data have not been derived for wild ruminants in Europe and characterization of PRNP single nucleotide polymorphisms (SNPs) in those species is still incomplete. In this study we describe nucleotide sequence variation in the PRNP locus of Italian red deer (*Cervus elaphus elaphus*) (n=30), chamois (*Rupicapra rupicapra*) (n=30) and roe deer (*Capreolus capreolus*) (n=30). DNA segments from blood and tissues samples, corresponding to the complete prion coding sequence, were amplified and sequenced by automated DNA sequencing on both strands. The investigation of PRNP polymorphisms in these wild ungulates and the comparison with North American cervids and domestic small ruminants PRNP polymorphisms can give preliminary data on the susceptibility of Italian wild ruminants to CWD and other TSEs. This research was supported by Ministry of Health grant IZSPLV008/2003.

Poster Presentation 15

DIAGNOSIS OF TSE STRAINS USING THE PRION SPECIFIC ANTIBODY 15B3

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Although scrapie has been known for more than 250 years the recent emergence of atypical scrapie cases has generated new interest into research on the detection, characterisation and transmissible nature of prion strains. Atypical prion strains are characterised by the glycosylation pattern of PrP^{Sc}, the distribution of PrP^{Sc} in the brain and in some cases by a reduced protease resistance of PrP^{Sc}. We have used the conformation specific antibody 15B3, which exclusively recognizes the pathological isoform of the prion protein, PrP^{Sc} from sheep, cow, deer and humans, for the diagnosis of TSEs. We have optimised the immunoprecipitation protocol and show that 15B3 is able to capture PrP^{Sc} in dilutions of brain homogenates up to 20'000 fold. We also performed immunoprecipitation experiments with 15B3 followed by Western blotting using the 6H4 antibody and we were able to detect PrP^{Sc} in various brain regions of atypical scrapie cases. If PrP^{Sc} in the atypical scrapie cases is associated with infectivity remains to be established.

Poster Presentation 16

NOVEL RAPID TSE DIAGNOSTIC TESTS

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Current testing for BSE is performed by rapid diagnostic tests which are based on Western blot or ELISA technologies. Some of the EU approved ELISA tests involve laborious sample preparation steps such as centrifugation or precipitation and require sophisticated laboratory equipment. The Prionics®-Check PrioSTRIP is a new rapid diagnostic test for transmissible spongiform encephalopathies (TSEs). In contrast to currently used rapid post mortem tests, the PrioSTRIP test is a dip-stick based immuno-chromatographic assay, similar to lateral flow devices. It is based on the antibody-mediated detection of the pathological form of the prion protein PrP^{Sc}. The design of the PrioSTRIP forces the analyte and the antibodies into close proximity thereby accelerating the reaction kinetics and thus minimizing incubation times. The lateral flow based format uniquely combines high testing speed with simple handling, resulting in very low total operation costs, while maintaining high reliability. The diagnostic performance of the PrioSTRIP was evaluated under field conditions for screening of BSE in cattle and compared to officially validated and accepted reference methods. The result of this study demonstrates that the PrioSTRIP performs as well as the reference methods, i.e. it shows 100% sensitivity and 100% specificity. We conclude that the PrioSTRIP opens new avenues to cost-effective, simple and accurate prion diagnostics.

ANALYSIS OF THE PRION PROTEIN OF BIG GAME CERVIDS IN SWEDEN INDICATE SUSCEPTIBILITY TO CHRONIC WASTING DISEASE

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The occurrence of CWD is a large problem in several free-ranging deer species in USA. CWD has also recently been diagnosed in ranches rocky mountain elk in South Korea and Canada (Salman, 2003; Sohn et al., 2002); discoveries that have heightened international awareness and concern regarding the spread of CWD in cervid populations outside USA. The best known susceptibility factor for TSEs is the amino acid sequence of the prion protein and some amino acid variants of PrP can modulate the susceptibility to disease (Goldmann et al., 1996; Prusiner et al., 1990; Scott et al., 1989; Westaway et al., 1987). To investigate if Swedish cervids have a PrP amino acid sequence similar to the cervids in North America that are affected by CWD, we deduced the PrP sequence in three important Swedish game animals, European moose (*Alces alces alces*), roe deer (*Capreolus capreolus*) and fallow deer (*Cervus dama*) as well as the semi-domestic reindeer (*Rangifer tarandus tarandus*).

It has been hypothesised that the level of proteolytic processing of PrP^C could affect susceptibility to prion infection (Vincent et al., 2001; Yadavalli et al., 2004) and in order to assist further investigations in these species, we analysed the cellular prion protein by immunoblotting before and after deglycosylation.

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WESTERN BLOT CHARACTERISATION OF A SWEDISH CASE OF NOR98

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Nor98 is an atypical form of sheep Scrapie, which recently has been identified in 5 cases in Sweden. Nor98 was first described in Norway in 1998 and differs from classical Scrapie in several features, among others, its epidemiology and proteolytic profile of the pathological prion protein.

We have extracted Nor98 prion from brain suspension of an affected sheep and result will be presented on the titration of proteinase K sensitivity and mapping of the fast migrating protein band by immunoblotting using various anti-prion antibodies.

SHEDDING OF THE BOVINE PRION PROTEIN

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The proteolytic processing and shedding of the bovine cellular PrP was studied by pulse-chase labelling in a cell culture system. The majority of labelled PrP was shed into the cell culture medium.

Analysis of the proteolytic processing showed that PrP^C is proteolytically cleaved at the cell-membrane into the so called C1-fragment. The N-terminal part can be found in the cell medium. After around 20 min at the cell membrane the C1 fragment is shed into the medium probably via an extreme C1 terminal proteolytic cleavage. A kinetic analysis of the proteolytic processing of bovine PrP will be presented.

Similarly sized processed PrP^C fragments are found in the bovine brain.

The significance of these processing events in relation to prion disease in animals and humans will be discussed.

Poster Presentation 20

VANADIUM CONTENT OF OILED BIRDS: IS IT A GOOD MARKER FOR OIL EXPOSITION?

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Vanadium is a trace element, contaminating oil products. It has also a specific toxicity (cellular and DNA toxicity). In this poster, we present a study about the vanadium content of organ of oiled bird since Erika oil spill in France. The aim is to study of there are differences between species and between the type of oil (with different vanadium value).

Vanadium was dosed by atomic absorption spectrophotometry in liver and kidney of several oiled seabird species, stranded on French atlantic and channels coasts and died in wildlife care Centers, during winter 1999-2003. The concentrations were not higher in oiled birds than in dead birds found on the beaches without visible traces of petroleum. However, we showed that there were differences between species, probably in relation with their diet. Moreover, it seems to exist differences between origins of birds (Atlantic *vs* Channel) but not between type of oil. We suggested that vanadium may be not a good marker of acute oiling but may be better to mark a chronic toxicity.

Poster Presentation 21

RISK ASSESSMENT OF LEAD POISONING IN WHITE-TAILED SEA EAGLES FROM GERMANY

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During necropsy of free-ranging white-tailed sea eagles (WSE) livers and kidneys were collected for analysis of environmental contaminants. Extraordinary high levels of lead, indicating acute lead poisoning (> 5 mg/kg ww), were detected in 27 % of 215 immature and adult WSE from Germany. Lead poisoning in predatory and scavenging birds, like WSE, results exclusively from alimentary ingestion of lead ammunition. Lead shot and fragments of lead bullets are ingested by preying on shot crippled (and therefore handicapped) waterfowl and game, or feeding on carcasses and gut piles with embedded lead ammunition. No other source for high lead concentrations in body tissue of wild birds has ever been detected other than for lead ammunition or lead fishing weights. Nearly all lead poisoned WSE were found dead or moribund in the fields from October to March, during and after the main hunting season. Lead levels during spring and summer were generally low and represent background levels for birds of prey. Regional pattern of lead exposure indicate a higher prevalence of lead poisoning with increasing distance from the Baltic coast. Whereas only one lead poisoned WSE was collected at the Baltic coast, we found most lead poisoned WSE in regions known for high game density and hunting activities. These results may suggest that foraging on carcasses and gutpiles of game animals which were contaminated with fragments of lead ammunition or lead shot were the main source for lead poisoning in WSE, which was supported by radiographs and analysis of gizzard contents. The only possibility to prevent lead poisoning in raptorial birds, and also in waterfowl and other bird species, is a legal ban of lead shot and other lead ammunition for hunting with substitution by non-toxic shot. In Europe, the countries Denmark and The Netherlands have prohibited the use of lead shot nationwide, whereas some other European countries banned lead shot for hunting waterfowl in wetlands or only in areas of conservation concern. Despite international recommendations and agreement of wildlife conservation there is no nationwide ban of lead shot in Germany, however some federal states of Germany have banned lead shot for hunting waterfowl.

Poster Presentation 22

DETECTION OF ORGANOCHLORINES IN MARINE TURTLES STRANDED IN THE CANARY ISLANDS

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Polychlorinated biphenyl compounds and organochlorine pesticides in general are well known as environmental contaminants due to three main facts: their stability, their strong bioaccumulative capacity and their global presence in different ecosystems. There for, it is essential to know these compound levels in the biological tissues, aiming at determining the potential impact that these accumulated substances have on the affected organisms. This is the main aim of our study on marine turtles. The chemical analysis was made using adipose and hepatic tissues from marine turtles stranded in the Canary Islands. In most of the cases the samples are from the species *Caretta caretta*. The chosen method of analysis is the one described by Tanabe et al. (1984). The application of such method in our work has been successfully tested by the certified reference material, CARP-2 (National Research Council Canada). The confirmed results refer to the congeners shown in the table below ($\mu\text{g}/\text{Kg}$ wet wt).

| PCB | Results | CARP-2 |
|-----|------------------|----------------|
| 52 | 119.7 \pm 13.9 | 138 \pm 43 |
| 153 | 116 \pm 16.6 | 105 \pm 22 |
| 180 | 59.2 \pm 10.7 | 53.3 \pm 13 |
| 28 | 27.3 \pm 1.5 | 34.0 \pm 7.2 |

The mean of the obtained results corresponding to the marine turtle adipose tissues are ($\mu\text{g}/\text{Kg}$ wet wt): PCB52= 61.875 \pm 10.130; PCB153= 34.541 \pm 1.235; PCB180= 44.914 \pm 1.861; PCB28= 26.617 \pm 1.069.

Poster Presentation 23

AN OUTBREAK OF CUTANEOUS POXVIRUS INFECTION IN STONE-CURLEWS (*BURHINUS OEDICNEMUS*) IN GRAN CANARIA ISLAND, SPAIN.

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The cutaneous form of avian pox infection has been reported in many wild bird species. However there are no confirmed reports of pox infection in Stone-curlew (*Burhinus oedicnemus*). During 2003 thirty-five stone-curlews were submitted to the Tafira Wildlife Rehabilitation Center (TWRC). Five birds were submitted with large cutaneous nodules on the legs and toes. In addition six birds submitted without initial lesions of poxvirus infection, developed cutaneous lesions some weeks later. Because the severity of the lesions some of them were humanely euthanized. Histologically, the stratified squamous epithelium of the cutaneous nodules was hyperplastic. Individual epithelial cells were swollen, rounded, and separated from each other. Their cytoplasm contained large, eosinophilic, finely granular, globoid inclusion bodies, characteristics of avian pox. Examination of formalin-fixed tissue from the cutaneous nodules with electron microscopy revealed numerous typical pox virions particles (150 x 300 nm). Pox virions were ovoid and contained a dumbbell-shaped nucleoid. Poxvirus particles are very persistent in the environment, and cutaneous infection can occur by direct contact with infected individuals or by mechanical transmission of the virus to broken skin.

Poster Presentation 24

IMPACT OF THE HUMAN ACTIVITIES ON THE MORTALITY OF SEA TURTLES STRANDED IN THE CANARY ISLANDS, SPAIN (2003-2004).

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Between January 2003 and March 2004, 29 loggerhead turtles (*Caretta caretta*) and 1 green turtle (*Chelonia mydas*) that stranded on the coasts of the Canary Islands were submitted for necropsy to the Veterinary Faculty (ULPGC). Some of them had been previously submitted to the TWRC for health evaluation, medical management, and possible rehabilitation. Four turtles (13.3 %) died from spontaneous diseases including different types of pneumonia, hepatitis, septicemic processes and salt gland adenitis. However, 26 turtles (86.6 %) died from lesions associated with human activities such as boat strike injuries (20 %), entanglement in fishing nets (30 %), and ingestion of hooks and monofilament lines (36.6 %). Turtles with boat strike traumatic injuries had severe fractures of the carapace/plastron, and severe lesions mainly penetrating into the lungs and kidneys. Entanglement in fishing nets resulted in severe ulcerative dermatitis, necrotizing myositis, amputation of flippers, and septicemic processes. A comparison of our survey and the data provided by the sea turtle stranding registry of the TWRC indicates that turtles with lesions induced by entanglement usually have a better prognosis for rehabilitation than those with traumatic lesions in the carapace or plastron. Affection of vital organs such as lungs and kidneys, because the anatomical location, dorsally attached to the carapace, explain the generally poor prognosis for turtles with severe traumatic injuries in the carapace. Ingestion of hooks and monofilament lines resulted in ulcerative and fibrinous esophagitis, fibrinonecrotic gastritis, fibrinous and necrotizing enteritis, intestinal intussusception, severe celomitis, and septicemia.

DETECTION OF METALS IN TISSUES OF LOGGERHEAD TURTLES (*CARETTA CARETTA*) STRANDED IN THE CANARY ISLANDS, SPAIN.

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Tissue distributions of metals (aluminium, arsenic, cadmium, copper, iron, mercury, nickel, lead, zinc) were determined by inductively coupled plasma atomic emission spectrophotometry in 78 specimens of juvenile and subadult loggerhead turtles (*Caretta caretta*) stranded in the Canary Islands between January 1998 and December 2001. The liver accumulated the highest concentrations for arsenic, copper, iron, and lead. For these metals the lowest mean concentration was detected in the skeletal muscle. The highest concentrations for nickel and cadmium were detected in the kidneys. The highest mean concentrations for aluminium and zinc were detected in the bone. Aluminium, nickel and lead levels found in loggerhead turtles stranded in the Canary Islands were higher than those found in sea turtles from different geographical areas. Mercury levels were lower than those reported in similar studies. Histological lesions were detected in the liver and kidney of the specimens with the highest levels of arsenic and lead respectively.

Poster Presentation 26

ZINC PHOSPHIDE – A RISK FOR GRANIVOROUS BIRDS

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The application of rodenticide treated cereal grains against voles and rats is a common practice in agriculture. Obviously, these products represent a high risk for several wildlife species esp. granivorous birds. One of the rodenticides used is zinc phosphide. Zinc phosphide hydrolyses in the tissues to the highly toxic gas phosphine, PH_3 . Death due to respiratory failure occurs rapidly.

Nonspecific necropsy findings include hyperemia of all internal organs and pulmonary oedema. First hints during necropsy are the presence of grains in the alimentary tract and a garlic like odour (from PH_3). Exact diagnosis needs chemical analysis of stomach and/or crop contents. As PH_3 is very volatile one must be very carefully when opening the digestive tract otherwise chemical analysis may give false negative results.

We proved cases of poisoning by phosphine in pheasants (*Phasianus colchicus*), ducks, rooks (*Corvus frugilegus*) and cranes (*Grus grus*).

Although we cannot exclude misuse or deliberate abuse of zincphosphide a legal ban of this highly toxic rodenticide preparation should be considered as it had already been in force for several years in Austria.

Poster Presentation 27

LEUCOCYTOZOON ZIEMANNI INFECTION IN A WILD POPULATION OF LITTLE OWL *ATHENE NOCTUA* IN SOUTHERN PORTUGAL

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Hemoprotozoa are generally considered only slightly pathogenic to wild birds. However, some evidence exist that they can cause subclinical/debilitating effects and depress breeding rates, and thus their influence on the dynamics of natural bird populations should not be neglected. Most studies on hemoprotozoa of wild birds only investigate prevalence and intensity of infection. The objective of our study was to investigate the relationships between host and environmental factors and the prevalence of hemoprotozoa in a wild population of Little Owls (*Athene noctua*).

Between February and August 1999, we captured 39 Little Owls in two areas with different Mediterranean habitats in southern Portugal. Blood was collected from the brachial vein and used to obtain smears, which were air-dried, fixed with methanol and stained with Giemsa solution. We searched for hemoprotozoa by scanning each slide for 30 minutes, constantly changing fields.

Sixteen (41.0%) of the owls were infected with *Leucocytozoon ziemanni*. Age was the only factor found to influence

prevalence in a multivariate logistic regression comparing infected and non-infected owls (Wald $^2 = 15.47$, $df = 1$, $p < 0.001$). Prevalence was much higher in adults (82.4%; $n = 17$) than amongst juveniles (9.1%; $n = 22$). When only adults were considered, none of the measured variables had a significant effect on prevalence. Nonetheless, adult females seemed to have higher prevalence (100.0%; $n=9$) than adult males (62.5%, $n=8$). Likewise, there was a trend for territories where infected owls were captured ($n = 14$) to be closer to water bodies (mean \pm SD: 332.14 ± 270.95 m) or streams (403.57 ± 290.98 m) than territories with uninfected owls ($n = 3$) (respectively 575.00 ± 253.72 m and 983.38 ± 774.73 m). Discussion of this results must take into account the relatively small samples involved. Age-related differences in prevalence, with adults more infected than juveniles, are commonly reported in studies of hemoprotozoa of wild birds. Although a trend towards higher prevalences of parasites in male than in female birds is generally recognized, there exists some data on hemoprotozoa of wild birds indicating the contrary. In this study area, where wetlands offering suitable habitat for potential vectors (*Simulium sp.* and *Culicoides sp.*) are scarce and often temporary, differences found in prevalence according to distance to water could reflect exposure to these vectors.

Poster Presentation 28

WILD CARNIVORES AS BIOINDICATORS OF *SALMONELLA* SP. INFECTIONS (AOSTA VALLEY, NW ITALY)

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Salmonellosis is a zoonosis of great economic importance in humans and animals. The rod-shaped bacteria are worldwide spread and potentially pathogenic for a variety of susceptible hosts.

In mammals, *Salmonella* sp has been implicated in a spectrum of diseases, including enteric or typhoid fever, bacteremia, focal infections, and enterocolitis.

Although uncommon, extraintestinal complications of salmonellosis caused by seeding of bacteria to other organs can include endocarditis and arterial infections, cholecystitis, hepatic and splenic abscesses, urinary tract infections, abortion, pneumonia or empyema, meningitis, septic arthritis, and osteomyelitis.

Salmonellae are common in the gastrointestinal tracts of mammals, reptiles, birds, and insects and many sylvatic species may be involved in its life-cycle as vectors and/or reservoirs for the disease in humans (Clarke & Gyles, 1986).

Among them, some wild carnivores, such as the red fox (*Vulpes vulpes*), the stone marten (*Martes foina*), which are typically generalist and sinanthropic predators, and the badger (*Meles meles*), could be also considered as "sentinels" of environmental sources of infection.

In the Aosta Valley the incidence of salmonellosis in this three species has been monitored since 2000 in specimens, mainly killed by cars, carried to Ce.R.M.A.S. laboratories for necroscopic exams by provincial foresters.

The isolation of *Salmonella heidelberg* in the urban area of Aosta town in two patients, three foxes and one water sample (Orusa *et al.*, 2003) suggested to analyse the spatial and environmental distribution of the records and to compare it with that of the human cases collected by the local medical service.

Furthermore, in order to obtain an accurate picture of the incidence of the disease in the study area, foxes' faeces were collected along transects spread in the more representative habitats.

Preliminary results about the potential role of carnivores as bioindicators of environmental contamination with regard to the risk for human health are discussed.

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Poster Presentation 29

TRICHINELLA SPP. IN LARGE PREDATORS IN FINLAND.

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Trichinella spp. are common zoonotic parasites in wild carnivores in Finland. All four species of large predators in Finland – brown bear *Ursus arctos*, wolf *Canis lupus*, lynx *Lynx lynx* and wolverine *Gulo gulo* – serve as hosts for *Trichinella* spp.

National Veterinary and Food Research Institute (EELA) conducts national wildlife trichinella surveillance. Material for surveillance consists mostly of hunted animals, but partly of animals found dead. Finnish Game and Fisheries Research Institute provides most samples from wolves and lynx. During 1999-2004, a total of 70 brown bears, 78 wolves, 282 lynx and 2 wolverines were examined for trichinellosis in EELA using the digestion method. *Trichinella* species from 55 lynx and 18 wolves were identified by multiplex polymerase chain reaction (PCR) in University of Helsinki. *Trichinella* spp. were most prevalent in lynx (44.9%) and wolf (35.9%). Prevalence of bears was 11.4%. One of the examined wolverines was infected, while the other one was not. Mean larval counts were low: lynx 6.1 lpg, wolf 5.1 lpg, bear 1.4 lpg and the single infected wolverine 0.2 lpg. The most common species in lynx and wolves was *T. nativa*, the etiological agent of sylvatic trichinellosis in arctic regions (72.7% of samples in lynx, 94.4% in wolf). In lynx, *T. britovi* was relatively common (16.4%), although it is typically a species of temperate climate. Both lynx and wolf had also a few infections of *T. spiralis* (7.3% and 5.6%, respectively), and in lynx there were also two cases of *T. pseudospiralis*. Large predators are all rare species in Finland, although populations of lynx and bear were slightly increasing in the 1990's. In 2003, it was estimated that there were about 920 lynx, 800 bears, 150 wolves and 125 wolverines living in Finland. Populations are strongest in the easternmost part of the country and elsewhere large predators are very rare. Lynx, however, are spread more evenly across the southern half of Finland. In lynx, *Trichinella* spp. were most prevalent in south-eastern Finland (70.1%). Red fox *Vulpes vulpes* and raccoon dog *Nyctereutes procyonoides*, common small carnivores, are considered to be the main hosts of sylvatic trichinellosis in Finland, and their populations are densest in southern and south-eastern Finland.

Poster Presentation 30

MIXED HEMOPROTOZOA INFECTION CAUSING DISEASE IN AN INJURED, WILD IBERIAN WOLF *CANIS LUPUS SIGNATUS*

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A juvenile (10 months old) wild Iberian Wolf *Canis lupus signatus* was run-over by a car and brought to a wildlife rehabilitation center in Parque Nacional da Peneda-Gerês, northern Portugal. It had a fracture in the L1 vertebra, causing hind leg paralysis.

On admission, routine hematological and biochemical analysis were performed, showing high AST (572 U/L) and ALT (696 U/L) values. The animal had ticks too, which were removed 6 days post-admission after topical Fipronil administration. As part of the treatment, the animal was submitted to a course of corticosteroids (Dexametasone 2 mg/kg, bid for 7 days).

On day 8 post-admission, another biochemical analysis was performed, showing again the high values of AST (208 U/L) and ALT (1876 U/L).

On day 11 post-admission, the wolf became anorectic, and the next day presented icterus and hemoglobinuria. Examination of blood smears revealed a mixed infection with *Babesia canis*, *Babesia gibsoni* and *Haemobartonella canis*. On the same day we administered Imidocarb 14,5 mg/kg and the wolf eventually made a full recovery from this infection.

We hypothesize that this animal presented a sub-clinic/chronic silent infection with those agents upon admission, which was exacerbated, probably due to the immunosuppression caused by the high-dose, long-term treatment with corticosteroids. According to Kreeger (2003), there is only one previous report of *Babesia sp* infection in wild wolves, involving *Babesia gibsoni* in India (Howe, 1971).

Our data show that besides being more widespread than thought before, this kind of parasitic diseases in wolves, specially in mixed infections, can be of major importance for the recovery of these kind of carnivores in rehabilitation centers.

Besides, as this kind of parasitic disease is common in dogs, its diagnosis and control is of major importance in domestic and stray animals that may live nearby some important nucleus of wild wolves.

WILD BIRDS AND WEST NILE VIRUS IN CAMARGUE, SOUTHERN FRANCE

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West Nile fever is a re-emerging mosquito-borne disease in Europe. Birds are known to be the principal hosts of this virus and to have a major role in its transmission cycle. The recent outbreaks in the South of France (Camargue in 2000, Var in 2003) emphasized the need of further investigation on the role of wild birds in the virus ecology in this area.

Birds might be involved in various aspects of West Nile virus epidemiological cycle, namely, introduction (for instance from Africa), amplification, spreading and transmission of the virus to a susceptible horse or man via an infected mosquito.

We present an ongoing study to test several hypotheses in Camargue, using both ornithological and serological approaches. Our aim is to identify birds potentially participating in the different epidemiological steps in Camargue and in the vicinity of infected horse farms in particular.

We undertook a serological survey in various migratory passerine bird species at their arrival in Camargue after their crossing of the Mediterranean Sea. Blood samples have also been collected in Cattle Egrets (*Bubulcus ibis*) and House Sparrows (*Passer domesticus*) to test the role of these species respectively in the amplification and the spreading of the virus.

This study is a part of a multidisciplinary project and skills in ornithology, entomology and virology will have to be shared to fully understand West Nile disease ecology in Camargue. The results of these preliminary investigations will help understand the role(s) of wild birds in the epidemiological cycle of this disease.

Poster Presentation 32

LEAD INTOXICATION BY INGESTION OF LEAD SHOT IN RACING PIGEONS (COLUMBA LIVIA)

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Introduction

Lead intoxication in birds by ingestion of lead shot has been recognised in many countries since Bellrose (1959) first described the long-term devastating effects on wild waterfowl populations. Secondary intoxication occurs in raptors preying on waterfowl (Müller et al., 2001). A wide variety of other bird species have been found susceptible to lead intoxication by mistaking lead shot for grit or food particles (Scheuhammer and Norris, 1995).

Only few descriptions of clinical cases of lead intoxication in pigeons are available. We comment a case of lead intoxication by ingestion of lead shot in a loft of racing pigeons. Similar casualties in domestic birds might reflect a more widespread intoxication problem in wildliving birds in Belgium.

Case report

Two pigeons of about 8 months old were presented in August 2004 with complaints of a distended crop and regurgitation of liquids. They belonged to a loft of 20 young pigeons, which during their daily flights alighted and fed on a nearby meadow where frequent shooting activity was noticed during and beyond the hunting season. The owner reported having seen similar cases in former years.

On clinical examination the two pigeons were thin and anaemic and displayed an unusual behaviour characterised by lethargy and a backwards stretching of the neck. Simultaneously lateral to and fro rolling movements of the distended crop could be seen under the skin. Deep palpation of the crops induced regurgitation of foul smelling liquid crop contents. In one pigeon ptosis was obvious. The urate fraction of the excreta was watery in one pigeon and a soft consistency of the faeces was noticed in both.

In the differential diagnosis we retained crop infection by trichomoniasis or candidiasis, adenovirus infection, salmonellosis and heavy metal intoxications including lead and zinc. Newcastle disease (paramyxovirus 1) was not considered because all the pigeons on the loft had been vaccinated *secundum artem*.

Microscopical examination of a wet crop swab (magnification x100) was negative for *Trichomonas gallinae*. A second crop swab was rolled on a glass slide, stained with Hemacolor® (Merck, Darmstadt, D.) and examined under immersion microscopy (x1000). No trichomonads nor *Candida albicans* could be detected but numerous lactobacilli were present on the smear. A

whole body radiograph of one pigeon confirmed the wide distention of the crop, which contained grit and food particles. In the gizzard one round radiopaque pellet, probably lead shot, could be easily distinguished from the grit particles. A heparinised blood sample was taken from the V. basilica in the same pigeon. Haematological examination of a blood smear stained with Hemacolor® showed numerous erythroblasts, polychromatic erythrocytes and reticulocytes, indicating a severe regenerative anaemia. Atomic absorption spectrophotometry performed in a commercial laboratory showed a blood lead concentration exceeding 7 ppm (7000 µg/l).

The two pigeons were treated by I.M. injection of 40 mg/kg calcium disodium EDTA twice daily in intermittent 5 day courses. No grit was offered because the grinding action of grit favours the fragmentation and ionisation of lead in the gizzard. Mineral oil was administered orally instead of attempting surgery to eliminate the lead shot. At the time of writing, treatment was still going on and no clinical improvement was obtained.

Discussion

The cytotoxic nature of lead and its capacity of inhibiting important enzymatic processes are responsible for the wide variety of symptoms observed in cases of lead intoxication. The most typical clinical picture is a combination of anaemia with neurologic, most often paralytic signs. Anaemia is partly regenerative, due to the destruction of red blood cells, and partly non-regenerative because of interference of lead with heme-synthesis through inhibition of δ -aminolevulinic-acid-dehydratase (ALAD) and of heme-synthetase. In waterfowl and raptors, blood lead levels above 0.4 ppm reflect abnormal exposure to lead and levels above 1 ppm are indicative of acute clinical poisoning (Pain et al., 1993).

The use of lead shot for hunting waterfowl has been banned and replaced by the use of steel shot in a number of countries. In the Netherlands and in Denmark the use for any kind of hunting, as well as the possession of lead shot, is forbidden. In Belgium, though lead shot is still used for hunting, no information is available on the impact of lead intoxication on wild or domestic birds. Due to the high density of building in this country, hunting is often taking place in the close proximity of backyards in inhabited areas. Lead poisoning was diagnosed in 16.6 % living (n = 30) and in 4 % dead (n=99) domestic anseriformes presented at the Bird Clinic of the Veterinary Faculty of Ghent University. These percentages were considered as minimal estimates (Tavernier et al., 2004).

Conclusion

Casualties of lead intoxication by ingestion of lead shot in domestic birds might indicate similar effects on wildliving birds. A systemic approach is needed to evaluate the impact of the use of lead shot on wild bird populations in Belgium.

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Poster Presentation 33

DISEASES FOUND IN FRENCH CARNIVORES ACCORDING TO SAGIR DATABASE – INTERESTING CASES

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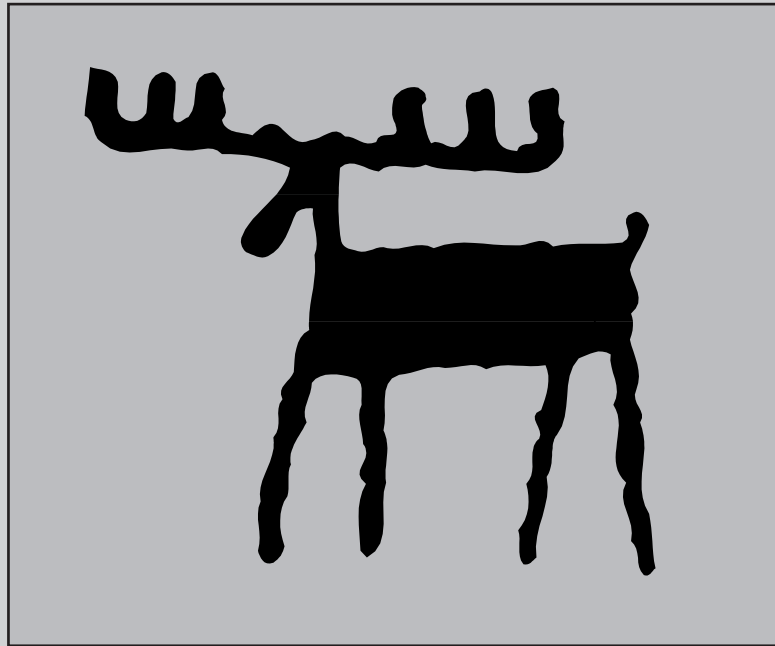
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This poster details some causes of death and pathological findings recorded in the SAGIR database.

These data are complementary to the global presentation given on “Diseases found in French carnivores according to SAGIR database – global analysis”. Epidemiologically interesting cases are selected.

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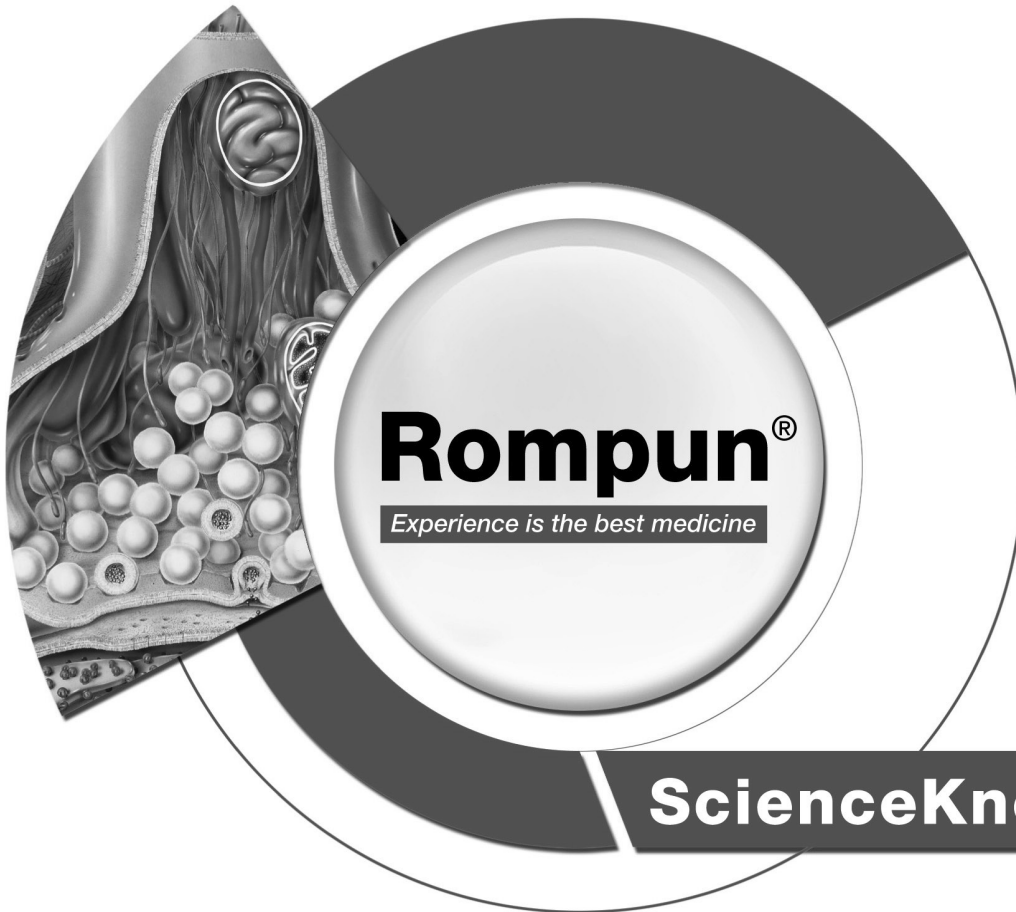
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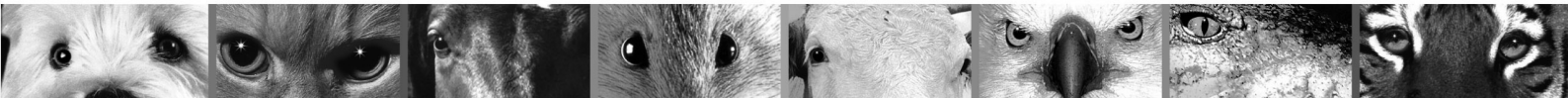
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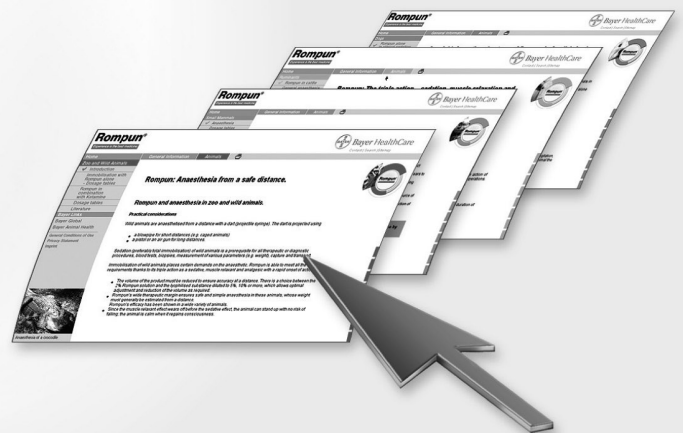
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