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Domodossola
11-13 Ottobre 2017

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IV CONGRESSO NAZIONALE DI ECOPATOLOGIA DELLA FAUNA

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11-13 Ottobre 2017

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Roberto Viganò, Nicola Ferrari, Carlo Citterio, Laura Stancampiano, Elisa Armaroli, Vittorio Guberti, Mauro Ferri, Andrea Cottini, Paolo Lanfranchi

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Sommario

Sessioni ed elenco delle comunicazioni

Il Congresso è organizzato dalla SIEF in collaborazione con l'Associazione Teriologica Italiana (ATIt), il Centro Italiano Studi Ornitologici (CISO) e la Società Italiana di Patologia Ittica (SIPI), la Provincia Verbano Cusio Ossola, il Comune di Domodossola, l'Associazione per lo sviluppo della cultura, degli Studi Universitari e della ricerca nel Verbano Cusio Ossola (ARS.UNI.VCO), il Dipartimento di Medicina Veterinaria dell'Università di Milano (DiMeVet).

Si tratta del principale appuntamento a livello nazionale nel campo dell'ecopatologia della fauna, che ha lo scopo di promuovere il confronto tra ricercatori, operatori della sanità pubblica ed animale, tecnici faunistici, esperti di gestione, appassionati e istituzioni sugli aspetti ecopatologici della gestione e conservazione della fauna, sulle implicazioni di sanità pubblica ed animale legate alla presenza e dinamica di popolazioni selvatiche, sui rapporti tra gli animali selvatici e le attività umane e, infine, sulle ricerche più avanzate in ecopatologia.

Il Congresso è articolato in 4 sessioni tematiche non contemporanee ed in uno Workshop di approfondimento. Si affrontano diversi temi, dalla conservazione e gestione alla ricerca, tutti in chiave ecopatologica.

Sessione 1
L'APPROCCIO ECOPATOLOGICO IN SANITÀ PUBBLICA VETERINARIA

Coordinatori: Vittorio Guberti & Andrea Maroni Ponti

Il tema della sessione è il ruolo dell'analisi epidemiologica quale strumento per coloro che nell'ambito della sanità pubblica veterinaria assumono decisioni in materia di gestione delle malattie che interessano animali selvatici, domestici nonché l'uomo.

A tale riguardo gli aspetti ecopatologici che caratterizzano le diverse fasi della malattia trasmissibile (early detection, controllo/eradicazione, sorveglianza e monitoraggio post azioni di controllo) rappresentano i principali argomenti di interesse della sessione.

Alla base di tali attività vi è sempre la raccolta di dati provenienti da piani di sorveglianza/monitoraggio il cui disegno varia in funzione delle fasi di malattia che si intende intercettare/analizzare e le cui modalità di attuazione non possono non tenere conto conto delle particolarità epidemiologiche della malattia considerata.

L'attività di sorveglianza e lo studio epidemiologico dei dati prodotti può consentire di attuare efficaci interventi di prevenzione e contenimento della diffusione di malattie zoonosiche o di malattie tramesse dai selvatici ai domestici attraverso l'applicazione di misure di biosicurezza negli allevamenti, restrizione alle movimentazioni, abbattimenti preventivi.

Per quanto riguarda gli animali selvatici non essendo possibile intervenire direttamente sui singoli animali infetti e mancando lo strumento vaccinale (disponibile esclusivamente per Rabbia e Peste suina classica) il controllo/eradicazione delle malattie in ambito selvatico sono possibili esclusivamente modificando i parametri demografici della popolazione ospite infetta.

Per quest'ultimo aspetto l'approccio ecopatologico si fonda sull'individuazione dei fattori di rischio intrinseci/estrinseci alla popolazione ospite che sono rilevanti nell'evoluzione della malattia e – nella pratica – nel gestirli con l'obiettivo di rendere la popolazione infetta ecopatologicamente inadatta all'infezione.

Guberti V. – Peste suina africana: 10 anni di lotta. *Relazione d'apertura*

Mignone W. *et al.* – Health surveillance of tuberculosis in wild boar (*Sus scrofa*) of the western Liguria region

Besozzi M. *et al.* – Monitoring of pseudotuberculosis in an Italian population of Alpine chamois (*Rupicapra r. rupicapra*): preliminary results

Martelli W. *et al.* – Hepatitis E virus in wild ungulates: serological evidence in chamois and red deer in the Alps and genetic assessment of viral variants in Europe

Cappai N. *et al.* – Salmonella detection in raccoons (*Procyon lotor*) of Foreste Casentinesi National Park

Formenti N. *et al.* – *Toxoplasma gondii*: spread, infection dynamics and effects on host behaviour in naturally infected red deer (*Cervus elaphus*)

Leopardi S. *et al.* – Disease dynamics of EBLV1 in colonies of *Myotis myotis* in northern Italy

Trogu T. *et al.* – Prevalence of zoonotic pathogens and epidemiological role of hunted Alpine wild ruminants

Obber F. ***et al.*** – Developing harmonized protocols for sanitary surveillance in the game control centres in the eastern Italian Alps

Bregoli M. ***et al.*** – Building networks for wildlife surveillance: from forestry workers and hunters to veterinarians

Sessione 2
ECOPATOLOGIA NELLA GESTIONE E CONSERVAZIONE

Sottosessione 2A
ECOPATOLOGIA DEI Mammiferi

(IN COLLABORAZIONE CON ATIt)
Coordinatori: Carlo Citterio & Luc Wauters

I temi ecopatologici che coinvolgono i mammiferi selvatici sono, anche nel nostro paese, numerosi e molto variegati. Tra questi si osservano, talvolta in concomitanza, problemi più tipicamente ecologici come l'impatto dei patogeni sulla conservazione; questioni che potremmo quasi definire zootecniche, come la gestione faunistico-venatoria; la sorveglianza e il controllo su malattie di importanza zoonosica e/o zooeconomica; il problema dei contaminanti ambientali; i problemi legati alla sovrabbondanza di alcune popolazioni e al loro impatto sull'ecosistema e sull'agricoltura; la questione dell'impatto e della gestione di specie alloctone. Il tutto, naturalmente, senza dimenticare la ricerca di base.
La grande diffusione, la plasticità ecologica di alcune specie e la loro condivisione del territorio con animali da reddito e attività umane rendono i mammiferi la classe forse più problematica dal punto di vista delle interazioni sanitarie tra animali domestici e selvatici e dei conflitti tra fauna e zootecnia. Il fatto che su di essa si giochi la maggior parte della gestione faunistico-venatoria moltiplica inoltre i portatori di interesse e, di conseguenza, le questioni ecopatologiche e sanitarie. In questa situazione, pur senza perdere la specificità delle varie competenze, la multidisciplinarietà appare ormai indispensabile per affrontare qualunque problema.
La sessione di ecopatologia dei mammiferi intende quindi privilegiare contributi che, proponendo nuovi approcci e metodi o verificando ipotesi di ricerca, riescano a collegarsi efficacemente ad una visione di insieme.

Stancampiano L., Ciarlo R. – Helminth of *Lepus europaeus*: parasite ecology in a declining population

Mazzamuto M.V. ***et al.*** – Squirrel invasions and their parasites: macroparasites of Pallas's squirrel in Italy

Santicchia F. ***et al.*** – The price of being bold: personality affects endoparasite infection in introduced eastern grey squirrels

Ebani V.V. ***et al.*** – Arthropod-borne pathogens in roe deer (*Capreolus capreolus*) in Tuscany. A preliminary report

Savio E. ***et al.*** – Health status of hunted wild ungulates population living in Belluno district

Salvadori C. ***et al.*** – Trombiculosis in northern chamois (*Rupicapra r. rupicapra*) from western Italian Alps: prevalence and pathological findings

Cavallero S. ***et al.*** – Alpine chamois population with impaired health status living in isolated habitat: evidences from pathology and molecular genetics

Sottosessione 2B
ECOPATOLOGIA DEGLI UCCELLI

(IN COLLABORAZIONE CON CISO)
Coordinatori: Guido Grilli & Gabriella Vaschetti

Gli uccelli rappresentano una delle principali componenti faunistiche per abbondanza e diversità; al contempo, gli allevamenti avicoli rappresentano un'importante componente della zootecnia.
La peculiare biologia caratterizzata dall'elevata mobilità, espressa nelle migrazioni e nella capacità di dispersione, determinano in questo gruppo di animali delle caratteristiche ecopatologiche peculiari, esponendoli da una parte a una pluralità di infezioni e agenti patogeni e dall'altra facilitando la trasmissione su vasta scala delle stesse infezioni.
Queste caratteristiche possono essere all'origine di alcuni problemi di tipo prettamente conservazionistico e implicare potenziali conseguenze per la salute umana e degli animali allevati. Scopo della presente sessione è affrontare le tematiche ecopatologiche legate agli uccelli sia dal punto di vista della conservazione sia per quanto riguarda la salute pubblica.

Franzoi A. *et al.* – Analisi di isotopi stabili nello studio dell'ecologia degli uccelli. *Relazione d'apertura*

Bertelloni F. *et al.* – Molecular detection of shiga toxins (*Stx1*, *Stx2*), *eaea* and *hlya* genes of *Escherichia coli* in wild birds

Cerutti F. *et al.* – The microbiota of hematophagous ectoparasites collected from migratory birds in Italy

Giammarino M. *et al.* – Heavy metals presence in hooded crow (*Corvus cornix*) as indicator of enviromental contamination

Iemmi T. *et al.* – Spread of *Toxoplasma gondii* in the Eurasian magpie and Eurasian kestrel from the Emilian territory

Viganò R. *et al.* – Alpine galliformes, domestic birds and introductions for hunting: sanitary and management risk factors

Sottosessione 2C
ECOPATOLOGIA DELLE SPECIE ACQUATICHE

(IN COLLABORAZIONE CON SIPI)
Coordinatori: Paola Beraldo & Andrea Gustinelli

Le molteplici attività umane hanno progressivamente mutato in modo diretto e indiretto l'equilibrio della natura, alterando l'integrità degli ecosistemi e spesso compromettendone la biodiversità. Negli ecosistemi acquatici, lo stato di salute delle specie animali, se correttamente interpretato, caratterizza gli elementi dell'ambiente in cui esse vivono (habitat, disponibilità di alimento, competizione), così come una deviazione da questa condizione, ad esempio un evento patologico, può indicare il deterioramento dell'ambiente stesso. Pertanto possiamo considerare le specie acquatiche come sentinelle fondamentali dello stato di salute dell'ambiente. In uno scenario globale contraddistinto anche da evidenti cambiamenti climatici, le attività antropogeniche possono interferire nei processi biologici degli organismi acquatici tramite la contaminazione dei corpi idrici con sostanze tossiche, diffondere malattie dalle specie ittiche allevate a quelle selvatiche e viceversa, alterare la dinamica delle popolazioni attraverso l'introduzione di specie alloctone e, così, pregiudicare lo stato di salute e la densità di popolazione della fauna acquatica. La sessione vede convergere per la prima volta la Società Italiana di Ecopatologia della Fauna e la Società Italiana di Patologia Ittica su queste tematiche comuni e di estrema attualità che saranno il filo conduttore delle comunicazioni selezionate.

Gustinelli A. – Fish-borne parasitic zoonoses in Italy. *Relazione d'apertura*

Pastorino P. *et al.* – Alien fish species in Italy: an overview about status and health risks to aquatic biodiversity

Toffan A. – Viral Encephalopathy and Retinopathy: the major viral threat for Mediterranean fish

Manfrin A. – Crayfish plague caused by *Aphanomyces astaci*

Dörr A.J.M *et al.* – Physiological response of red swamp crayfish to water salinity

Centelleghe C. *et al.* – The possible use of unmanned aerial system (drone) to collect information on wild cetaceans health status

Corazzola G. *et al.*– Anatomo-pathological findings on encephalic tissue of stranded cetaceans affected by *Toxoplasma gondii*

Mignone W. *et al.* – Marine mammal stranding in Italy: management and monitoring of the causes of death by the national reference center (C.Re.Di.Ma.)

Sessione 3

METODI E MODELLI IN ECOPATOLOGIA: DALLA DIAGNOSTICA ALL'ANALISI DEI DATI

Coordinatori: Nicola Ferrari & Antonio Lavazza

Le indagini sull'ecopatologia della fauna richiedono approcci metodologici adeguati nell'applicazione di protocolli di laboratorio specifici rispetto alla specie d'interesse e/o alle patologie considerate, anche al fine di ricomprendere le complessità intrinseche al contesto ecologico.
In particolare, se da una parte è necessario sviluppare, adattare e quindi validare i metodi diagnostici di laboratorio nelle singole specie, dall'altra è necessario sviluppare nuove metodiche per la valutazione di indici indiretti quali ed esempio *body condition*, *status* riproduttivo e tecniche non invasive *intra vitam* da applicare in campo sugli animali a vita libera.
Inoltre, l'analisi dei dati ecopatologici richiede metodiche epidemiologiche opportune al fine di fronteggiare la frequente incompletezza di informazioni disponibili. Per far ciò sono necessari approcci analitici e modellistici che siano in grado di includere le diverse componenti ambientali al fine di chiarire i processi epidemiologici in atto e sviluppare predizioni sui futuri trend.
Questa sessione si pone l'obbiettivo di affrontare queste tematiche al fine di mettere a confronto tra loro le diverse esperienze nello sviluppo di approcci metodologici inerenti l'ecopatologia della fauna selvatica.

Lavazza A. – Lagovirus e lagomorfi, un rapporto in continua evoluzione: una sfida per la loro identificazione? *Relazione d'apertura*

Formenti N. *et al.* – Applicability of blood sampling through intracavernous venipuncture: the example of *Toxoplasma gondii* in naturally infected red deer (*Cervus elaphus*)

Giammarino M. *et al.* – Morphological features of *Anas platyrhynchos* on postnatal growth based on FDA of c-r data: preliminary data

Cassini R. *et al.* – Species distribution models as part of a cost-effective surveillance approach for tick-borne diseases: case study in a natural park

Crovato S. *et al.* – A prioritization model to address infectious diseases surveillance in wild ungulates

Romeo C., Ferrari N. – When zero is a result: how to demonstrate the absence of an infection

Sessione 4
VARIE ED EVENTUALI IN TEMA DI ECOPATOLOGIA:
Sessione Libera

Coordinatori: Elisa Armaroli & Luca Rossi

Da sempre, l'interpretazione a vari livelli delle dinamiche ecopatologiche fornisce elementi necessari alla pianificazione di strategie sanitarie e di conservazione e gestione della fauna. In parallelo, anche la trattazione di temi non strettamente ecopatologici, quanto meno nell'accezione tradizionale del termine, ci aiuta ad ampliare lo sguardo e a cogliere le interazioni quanto mai dinamiche dell'ecopatologia con altre discipline e campi di interesse solo apparentemente distanti. Qualche esempio? La ricerca applicata alla gestione delle specie cacciabili, la filiera delle carni di selvaggina, il contenimento farmacologico e, più in generale, i metodi di cattura e manipolazione della fauna, la presenza di contaminanti ambientali, lo studio delle interazioni tra specie selvatiche, pratiche agricole e territorio, la diagnosi di predazione…ed altro ancora. Quindi libero spazio a tutti i contributi ecopatologicamente compatibili!

Demartini E. – Carni di grandi ungulati selvatici: qualità, valore e posizionamento di mercato.
Relazione d'apertura

Viganò R. *et al.* –
Characterisation of wild game meats: from animal welfare to nutritional, organoleptic and hygienic quality

Riccardi F. *et al.* – Evaluation of pH levels in wild game meat in relation to correct management and good hygienic practices

Cazzola P. L. *et al.* – Screening methods for control of radionuclides entry into the food chain

Ballocchi E. *et al.* – Presence of Cs–137 in wild ungulates of Ossola Valley (VB)

Mignone W. *et al.* – Cadmium exposure in wild boars in Italy: case report

Bandino E. *et al.* – Investigation on *Trichinella britovi* spreading in wildlife of Sardinia

– Workshop –

DATABASE ECOPATOLOGICI: UNA CHIMERA? VERSO L'INTEGRAZIONE DELLE COMPONENTI ANIMALI E SANITARIE

Moderatori: Carlo Citterio & Mauro Ferri

Dire che i dati di distribuzione e demografici delle popolazioni selvatiche, e le informazioni sulla presenza delle infezioni a cui esse sono suscettibili, sono basilari per la gestione e conservazione faunistica e per qualunque modello di sorveglianza e controllo delle malattie, sembrerebbe un'affermazione ovvia.

Quando però ci si trova ad affrontare concretamente la questione, presto ci si accorge di come molti suoi aspetti non siano affatto scontati. Ad esempio: quali sono le fonti dei dati e i metodi di raccolta? Quali i dati più pertinenti per i diversi fini? Quali le modalità più efficienti e sicure per conservarli? Quali i termini per condividerli? E quali le forme più efficaci di condivisione?

E' naturale che ciascuno possa raccogliere ed archiviare dati nelle forme più congeniali ai propri mezzi e alle proprie finalità, ma potrebbero essere trovate delle basi comuni che permettano, tutelando la proprietà del dato, un utilizzo e riutilizzo più ampio delle informazioni, così da aumentare l'efficienza complessiva del sistema ed evitare ripetizioni dello stesso lavoro?

Ancora, quali sono le popolazioni selvatiche per le quali tale raccolta sarebbe prioritaria ai fini sanitari, gestionali o conservazionistici? Quali esperienze, a livello nazionale e internazionale, si stanno dimostrando utili in questo senso?

E infine, che ruolo hanno gli enti pubblici e privati in questo contesto? Cosa li accomuna o li differenzia? Esistono basi normative che possono supportare questo sforzo?

A partire da casi concreti e da lavori in corso, il nostro workshop intende aprire una discussione su questi temi nell'ambito della comunità ecopatologica.

Paula Cáceres Soto, Organisation for Animal Health OIE (World Animal Health Information and Analysis Department) – *Wildlife health management and the implementation of databases: the WAHIS Interface.*

Roberto Lardelli, Ornitho.it (Comitato di Direzione) – *Un database per lo studio e la conservazione delle specie si apre all'ecopatologia: come sfruttare questa opportunità?*

Marco Farioli, Regione Lombardia (Direzione Generale Welfare Veterinaria) – *Gestione e pubblicazione di dati sanitari di animali selvatici: l'esempio del piano di monitoraggio e controllo sanitario della fauna selvatica della Regione Lombardia*

Nicola Ferrè, IZS delle Venezie (Ufficio Sistemi Informativi Geografici – Epidemiologia Veterinaria) – *Implementazione della Direttiva INSPIRE: armonizzazione delle informazioni geografiche secondo una prospettiva Europea.*

Andrea Maroni Ponti, Ministero della Salute (Epidemiosorveglianza e Anagrafi degli Animali, Direzione generale della Sanità Animale) – *Utilizzo e condivisione dei dati sanitari e di popolazione della fauna selvatica: il punto di vista del Ministero della Salute.*

Abstract

Sessione 1

L'APPROCCIO ECOPATOLOGICO IN SANITÀ PUBBLICA VETERINARIA

Coordinatori: Vittorio Guberti & Andrea Maroni Ponti

COMUNICAZIONI

HEALTH SURVEILLANCE OF TUBERCULOSIS IN WILD BOAR (*SUS SCROFA*) OF THE WESTERN LIGURIA REGION

MIGNONE W.[1], BONA M.C.[1], ZOPPI S.[1], BALLARDINI M.[1], MIGNONE G.[1], DONDO A.[1], GORIA M.[1], RU G. [1]

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The first case of tuberculosis in a wild boar (*Sus scrofa*) in the Western Liguria Region, Italy, was observed in October 1989; the diagnosis was made by observing pathological anatomical lesions, isolation of mycobacteria, identification with classical methods, inoculation of experimental animals and histological examinations. Laboratory examinations were carried out in collaboration between Istituto Zooprofilattico Sperimentale, Università di Torino and Università di Bologna. The territory of the Argentina Valley (Imperia province) and Mount Beigua (Savona province) was monitored from 1990 to 1994. In this period, a total of 278 samples were collected: 12 (4.3%) showed classical tuberculosis lesions, 44 (15.8%) had tuberculosis-like lesions, while 222 (79.9%) showed no lesions. Five *Mycobacterium bovis* strains and four *Mycobacterium tubercolosis* strains were isolated from classical tuberculosis lesions, using biological methods. From 1993 to 1995, 23 mycobacteria strains obtained from wild boars and 15 from cattle were isolated in the provinces of Imperia and Savona. After comparison by spoligotyping, the hypothesis of the disease transmission from one species to the other was confirmed. To further clarify the situation, we compared the geographical distribution of bovine tuberculosis outbreaks and the number of cases of wild boars with tuberculosis-related lesions in the territoriy of various municipalities. The majority of affected wild boars were caught in the territories located nearby infected livestock or breeding grounds where cattle from infected livestock had been bred. These findings clearly indicated an oral pathway of infection in wild boars, compatible with digging in wet fields contaminated with stools from infected bovines, while lesions observed in bovines indicated an aerial pathway of infection. We concluded that cattle were the reservoirs of the disease, while wild boars just played the role of bioindicators. Since 1995, a collaboration with Ambito Territoriale Caccia and Comprensorio Alpino of the Imperia province was established. An active surveillance was extended to the whole provincial territory and to all wild boars caught during the hunting season. Since then, more than 60,000 head (backward, sub-mandibular) samples have been examined. Concurrently with the improvement and intensification of the prophylaxis of bovine tuberculosis by the local veterinary service, a clear decrease in the number of samples with injuries and the number of *M. Bovis* isolates was observed. In 2014, the province of Imperia obtained the qualification of officially bovine tuberculosis-free (EU decision 1685, February 2016) and *Mycobacterium bovis* isolates have been rescinded.

MONITORING OF PSEUDOTUBERCULOSIS IN AN ITALIAN POPULATION OF ALPINE CHAMOIS (*Rupicapra r. rupicapra*): PRELIMINARY RESULTS

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Caseous lymphadenitis (CLA) of sheep and goats is a chronic and often sub-clinical disease, with high prevalence in different parts of the world, which can caused significant economic losses for farmers. The causative agent is *Corynebacterium pseudotuberculosis*, a gram-positive facultatively anaerobic rod resembling a coccus; primarily infects domestic small ruminants, but it has been isolated also in wildlife such as pronghorns (*Antilocapra americana*) and elk (*Cervus elaphus canadensis*). Furthermore, a recent research has demonstrated a maintenance of the infection on an endemic level in a Spanish ibex (*Capra pyrenaica hispanica*) population after an outbreak with high morbidity and mortality. The typical clinical manifestation is lymph node abscess that may fistulise and discharge pus contaminating the environment where can survive for months in faeces, fomites, and the soil; then the infection occurs through oral, nasal and ocular mucosa or skin wounds. Infected subjects with the sub-clinical form can also shed bacteria through respiratory tract and mechanical vectors such as flies. Human infection is a rare event and most of the reported cases have been related to occupational exposure and ingestion of raw meat and milk. Wildlife-livestock interfaces are dynamic and bidirectional and pathogens could be transmitted freely within and between the two species. Focusing attention on the Alps, the environment is probably one of the most valuable ecosystem, with a tricky balance.

The study area is the Alpine hunting district located in Vercelli province (formerly named C.A. VC1), with an extent of 77.668 ha, of which 51.182 ha are used for hunting. There are stable populations of chamois, roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), mouflon (*Ovis musimon*) and ibex (*Capra i. ibex*). Wild boar (*Sus scrofa*) is widespread, in particular in pre-alpine territories, and several flocks are present, in fact the province is famous for wool production. Bovids and cervids hunting is based on the selective cull method which provides the assignment of determined species, age class and sex to each hunter based on previous census, hunters can shot their heads in about two and half months. The study was carried out during 2016 hunting season, between September and December and performed on hunted chamois. A macroscopic examination of each carcass has been conducted at the Control Centre, where each hunter had to bring the chamois after culling for the control by a veterinarian. Hunters collected post-mortem-blood samples via jugular or heart clot from their own bag and leaded up to the Control Centre where serum is obtained by centrifugation and stored at -20°C until further processing. A commercial enzyme-linked immunosorbent assay (ELISA) kit (ELITEST CLA, HYPHEN BioMed, France) has been used to detect antibodies (IgG) anti-PLD (Corynebacterium pseudotuberculosis phospholipase D). Results have been expressed as OD after reading the plates at 450 nm in an ELISA plate reader. The aim of this monitoring is to confirm the circulation of *C. pseudotuberculosis* in the chamois population as suspected on the basis of the finding of several chamois with characteristic lesions during previous hunting seasons and to identify risk factors for the infection and the development of clinical signs of disease.

196 chamois have been hunted in 2016 hunting season, a total of 34 sera have been processed (1 kid, 7 yearlings, 17 adult males, 9 adult females) showing a seroprevalence of 8.82% (c.i. 95% 0.0-18.26). One yearling female and two adult females resulted seropositive; at the macroscopic examination the two adults had showed typical lymph node abscesses in abdominal region and the oldest was underweight and presented typical dermatophylosis lesions. Furthermore, a 4-year-old male showed a grey zone OD. The four chamois have been hunted in different area of the C.A.

The results of this first year survey on the territory are to be considered as preliminary, the starting point for a data collection that can become a historical series that could give useful management guidance in the near future. Sampling, in fact, is not sufficient to draw conclusions of any kind, either as sampling volumes or from the point of view of time. In spite of this, it can be safely stated that the first results obtained confirm the circulation of *C. pseudotuberculosis*, together with the pathological and bacteriological diagnoses of previous years. The fact that a yearling seropositive has been found without any lesions found is evidence of the current circulation of the pathogen as this subject has come into contact with the infection during the previous year.

We have to consider these preliminary results as the first step in the creation of a serological database of this population, for this reason the importance of continuing monitoring is evident in order to determine the actual impact on the population, the possible risk factors involved in the onset of clinical symptomatology and the level of endemic disease. Considering the role of goats and sheep in the transmission of the disease, the next step would be the planning of monitoring also in flocks sharing pastures with wildlife to verify whether or not there is a continuous spill-over of new strains from domestics and to understand the possible impact of the wildlife-livestock interface.

HEPATITIS E VIRUS IN WILD UNGULATES: SEROLOGICAL EVIDENCE IN CHAMOIS AND RED DEER IN THE ALPS AND GENETIC ASSESSMENT OF VIRAL VARIANTS IN EUROPE

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In recent years there has been an increasing interest in hepatitis E virus (HEV) because of its zoonotic nature.

The different ecological interactions among its numerous host species contribute to make HEV epidemiology unclear and the actual reservoirs and spill-over hosts have to be still defined. Domestic pig and wild boar are considered as the main reservoirs of zoonotic HEV in Europe, while wild ruminants are supposed as spill-over hosts.

This study aims to serologically investigate HEV infection in chamois and red deer in order to define their HEV seroprevalence and to clarify the epidemiology of this infection in alpine free-living wild ruminants. Moreover a genetic assessment of European HEV wild ungulates sequences retrieved from Genbank has been performed to identify the frequency of genotypes and subtypes, while European HEV sequences isolated from humans, domestic and wild animals have been checked to identify viral mutations associated with treatment failure and virus replication fitness in human.

Sampling of sera was carried out from 2013 to 2015 during autumnal hunting seasons and depopulation plans, from three study areas: i) 173 red deer from Stelvio National Park (Valfurva, Sondrio Province, Central Italian Alps), ii) 44 chamois from Orobic Alps, iii) 128 chamois, 81 red deer and 32 roe deer from Lepontine Alps (North-West Italian Alps).Sera were tested by a species-independent immune-enzymatic assay (HEV ELISA 4.0v, MP Diagnostics Biomedicals, Singapore).

All publicly available European HEV sequences from ruminant species (n=7) have been aligned with HEV reference and field strains, phylogeny was estimated by the maximum likelihood method and the percentage of nucleotide similarity was calculated. The presence of nucleotide variations have been checked in ORF1 available sequences of ungulates (n=18), humans (n=11) and other species (n=3).

Chamois and red deer showed a HEVseroprevalence of 1.16% (2/172; CI 95%:1.03-1.28) and 0.79% (2/254; CI 95%:0-1.87), respectively. Roe deer resulted HEV seronegative.

A high level of nucleotide identity (≥97%) has been observed among ruminants' genotype 3 sequences (ORF1 or ORF2) and HEV reservoirs or human ones isolated in the same country: i) a goat and red deer with a wild boar and swine respectively in Italy; ii) two red deer with a swine and human in Spain; iii) a roe deer and reed deer with swine and wild boar and also a roe deer with human in Hungary.

As regard to nucleotide variations, G1634 codon showed substitutions in 16 out of 32 available sequences (6 humans, 6 swine, 2 rabbits, 1 elk and 1 polecat) and V1479 codon in 23/32 sequences (6 humans, 12 swine, 2 rabbits, 1 wild boar, 1 elk and 1 polecat). In 5 humans, 7 swine, 2 rabbits, 1 elk and 1 polecat that mutations occurred together.

Sporadic HEV infection emerged in chamois, firstly highlighting the involvement of this species in virus transmission, and red deer from North-Western and Central Alps. These results, together with the increasing density and overlapping distribution of wild ungulates in the Alps, have to be considered in relation to virus spread in free-ranging animals. As this regards, although wild boar is known to be reservoir of HEV, this species shows a wide range of seroprevalences in Italy, ranging from 0% to 49% with the lowest observed in Alpine areas confirming the sporadic infection of wild ruminants. On the contrary, red deer from central Apennines, where this species shares its habitat with abundant wild boar populations, show the highest prevalence of 13.9 %. Therefore, data emerged support the hypothesis of a relation between HEV spread and the abundance of wild boar populations. Phylogenetic analysis showed significant clusters including ruminant and wild boar, swine or human sequences obtained in the same country, supporting ruminant as spill-over hosts but also highlighting the wide host range of zoonotic HEV. Moreover different mutations, supposed to increase virus replication and associated with treatment failure in human, have been identified in HEV sequences isolated not only from humans, but also from domestic and wild animals, such as polecat, elk and wild boar. These results and the increasing density and overlapping distribution of wild ungulates on the Alps have to be considered for the risk of virus spread among animals and infections in humans.

SALMONELLA DETECTION IN RACCOONS (*PROCYON LOTOR*) OF FORESTE CASENTINESI NATIONAL PARK

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Raccoon (Procyon lotor) is a medium- size mammal, native of North America. Actually European Union put this animal in the list of Alien Invasive Species (Reg. 1143/2014). The presence of raccoon in Foreste Casentinesi National Park is recorded from 2013. An eradication plan is in place since the beginning of 2016.

Raccoons (*Procyon lotor*) occupy agricultural, forested, and suburban areas; they prefer to make their dens in wooded areas near rivers or other water sources. The presence of raccoons in suburban areas is a source of concern because of the increased potential for wildlife-human contact. Raccoons can serve as reservoirs for a variety of known zoonotic agents, including rabies virus, *Baylisascaris procyonis, Toxoplasma gondii*, leptospires, and salmonellae.

Blood and fecal samples of all raccoons captured (N=6) were collected and analyzed at Istituto Zooprofilattico laboratory.

Blood samples were analyzed for: *Toxoplasma gondii* (SL); Cimurro (SN); Leptospira spp. (*L. Bratislava, L. Canicola, L. Copenhageni, L. Icterohaemorrhagiae, L. Saxkoebing*) (MAT); *Anaplasma Phagocytophilum* (PCR) ; *Ehrlichia Canis* (PCR) ; Leishmania spp. (PCR); Rickettsia spp.(GLTA) (PCR); Leishmania spp. (PCR).

Fecal samples were analyzed for: enteroparasites research (fecal flotation); bronchopulmonary nematodes (Baermann); pathogenic bacteria (culture method); Salmonella spp (culture method).

Salmonella was isolated from the faeces of 2 raccoons, and the serotypes isolated were *S. Napoli* and *S. Coeln.*

S. Napoli appears to be distributed in Europe, specifically in France, Italy, and Switzerland. Is an emerging serovar in Italy. It accounts for 2-4% of all serovars isolated from human infections. It was reported in rucola and in wild birds from Italy. Although it is a nontyphodial *Salmonella* strain some if its symptoms, including a prolonged incubation period (7–14 days), high fever, bacteremia, and elevated rates of hospitalizations, are similar to those caused by typhoidal *Salmonella*.

S. Coeln has been isolated in high frequency (21.8%) in wild boars in Italy. This serovar is very rare and scarcely associated with human cases.

This foundings could have public health implications, particularly in locations that are shared by humans and wildlife.

TOXOPLASMA GONDII: SPREAD, INFECTION DYNAMICS AND EFFECTS ON HOST BEHAVIOUR IN NATURALLY INFECTED RED DEER (*CERVUS ELAPHUS*)

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The complex life cycle of *Toxoplasma gondii* and its broad spectrum of host species rise zoonotic, economic, and conservation issues. Moreover, recent studies supposed the parasite's activity to manipulate intermediate host behaviour leading to neurological symptoms/alterations ("host behaviour manipulation" or "manipulation hypothesis") that promote a longer parasite survival, necessary to complete its life cycle and thus to increase its spread. While in humans, loss of psychomotor performance and concentration, schizophrenia and other mental disorders were related to Toxoplasmosis, in animals several "risky behaviours" that could increase *T. gondii* transmission are reported. Indeed, this parasite is trophically transmitted and behavioural host manipulation may favour its transmission from intermediate to other/s or to definitive hosts.

Among *T. gondii* intermediate hosts, wild ungulates can be source of human infection and thus the risk should be evaluated in relation to game meat consumption. Moreover, until now, the infection dynamics and effects of *T. gondii* in these species has received little attention. Focusing on red deer (*Cervus elaphus*) since its intense culling and the increasing consumption of meat, the need is to investigate *T. gondii* in this host species and to evaluate its epidemiology in wild ecosystem. Moreover, no assessments are reported about *T. gondii* associated-behavioural modifications in naturally infected red deer. Therefore, we carried out a serological study composed by two parts. In the first one we investigated (1) the epidemiology, spread and dynamics, of *T. gondii* infection while in the second part we analysed (2) if the parasite may induce behavioural modifications leading to an increase in the culling probability.

Overall 376 sera of free-ranging red deer were collected during three culling management plans (2014-2016) from three areas in Stelvio National park (Italian Central Alps) for epidemiological analysis while the behavioural analysis was performed on 53 adult females hunted in 2016. Samples were tested by a commercial ELISA kit (IDVET, Montpellier, France) and results were analysed through Generalized Linear Models. In particular, we used a Binomial and a Gaussian GLMs to investigate factors influencing the probability of being seropositive and the dynamics of the infection, respectively. Moreover, the behavioural hypothesis was tested by a Gaussian GLM that modelled the "days from the beginning of hunting" to evaluate the potential increase in the culling probability induced by *T. gondii*.

An overall prevalence (p) of 26% emerged. The seroprevalence increased with age: adults (p=41.5%) were more infected than yearlings (p=19%) and calves (p=5%). Subjects of low anthropised area (p=13%) were less infected than those of the high (p=36.3%) and moderate (p=22.8%) anthropised ones. Subjects of 2014 (p=16%) were less infected than those of 2015 (p=27.8%) and 2016 (p=35.7%). Focusing on seropositive deer (n=98), red deer of 2014 showed serological titres significantly lower than those of both 2015 and 2016. As regard *T. gondii* activity to induce risky behaviour, seropositive adult females (36/53) had a significant higher culling probability than the seronegative ones.

The effect of age class and anthropization on the spread of *T. gondii* supports horizontal transmission as the main route. Considering the sporadic presence of lynx (*Lynx lynx*) in Italian Alps, feral and semi-domestic cats are the only definitive hosts responsible for its spread. The difference in prevalence and serological titres between study years leads to a boost event of *T. gondii* spread during 2015 and then a maintenance of the infection during 2016, as the stable prevalence and serological titres between 2015 and 2016 showed. In adult females, the infection seems to favour the increase of the probability to be culled sooner than seronegative individuals. *T. gondii* appears therefore to induce behavioural alterations even in red deer making them more "at risk" to be hunted. The supposed emerged mechanism leads to a *T. gondii* manipulation in this host species that could indeed increase its spread and transmission.

Data arisen give evidence to the presence of *T. gondii* in the study area with horizontal transmission as the main route of infection. In this sense, a widespread exposure to infection is supposed and should be considered in relation to the parasite zoonotic potential and, besides, the supposed cyclic introduction-re introduction of the parasite in the study area. Although the emerged relation between the infection and the increase in the culling probability of adult females could support *T. gondii* host behaviour manipulation, further analyses should be carried out to extend this analysis to other classes and to preyed/found dead/roadkill animals for a wide assessment of the potential alterations induced by *T. gondii*.

DISEASE DYNAMICS OF EBLV1 IN COLONIES OF *MYOTIS MYOTIS* IN NORTHERN ITALY

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The interest for bats as possible reservoir for EIDs has been steadily increasing since the concomitant discovery in 2005 of Ebolavirus and SARS-like viruses in these animals, which has led to the discovery of a great number of novel viruses whose zoonotic potential has not yet been fully assessed. Among these, rabies-related lyssaviruses (RRLVs) are the only proven zoonotic pathogens found in European bats, as they determine in mammals an acute and always fatal encephalomyelitis indistinguishable from that caused by rabies virus. While rabies virus (RABV) in Europe is still mostly associated with non-flying mammals, at least five different LYSVs are reported in bats. European bat Lyssaviruses 1 and 2 (EBLV-1 and EBLV-2) are the most widely distributed and have been associated with spillover events to humans and animals. To date in Europe four lethal cases have been associated with bat RRLVs. In Italy, passive and active surveillance measures have been implemented since 2008, providing serological evidence for bat LYSV circulation in different species but leading to no confirmed cases.

Anthropogenic activities altering the ecological landscape are considered as a major driver of the increasing emergence of zoonotic pathogens from wildlife. The progressive restriction of wild areas coupled with the increasing human presence in natural environments for scientific and entertaining purposes are changing the way in which wild animals, including bats, interact with us and our domestic animals. Different bat species are indeed assuming synanthropic habits and active colonies are frequently found in buildings and public areas, creating new opportunities for the spillover of bat pathogens to the human population.

The present study is included in the framework of the Young Researchers Project GR-2011-02350591, funded by the Italian Ministry of Health and implemented to better evaluate the risk associated with bats in Italy in terms of circulation of viruses with zoonotic potential. In particular, the main aim of this study is to investigate disease dynamics of LYSV infection in myotis bats, which is crucial to evaluate their potential to maintain and eventually transmit the pathogen.

We investigated 5 maternity colonies of *Myotis myotis* and *Myotis blythii* between 2015 and 2017, covering almost the whole Bolzano province to the border with Austria. These colonies were selected due to their relevance in public health, being located in roofs of officiant churches. All colonies were sampled at least twice in May and September, with 7 sampling campaigns performed in 2017 for two colonies. At least 30 individuals were live sampled for blood in each colony at each time point, allowing for the estimation of the sero-prevalence with 95% confidence level. Blood samples were analyzed for the presence of antibodies against EBLV-1 using a modified Rapid Fluorescent Focus Inhibition Test (RFFIT), in which EBLV-1b was used as challenge virus.

Antibodies against EBLV1 were detected in almost all the sampling campaigns, which suggests that the virus is maintained within each colony and across the whole area. This is particularly relevant as it confirms that *Myotis* bats do unexpectedly survive LYSV infection. Furthermore, we were able to identify a seasonal pattern, characterized by the lowest sero-prevalence immediately after the birth pulse, followed by a peak at the end of the season, when colonies mainly host young individuals born during the summer. Similar patterns would suggest increased virus circulation during late pregnancy and lactation, which is consistent with the report of increased shedding of gastrointestinal viruses such as *Coronaviruses* in the same time-span. This finding is likely associated to the increase of susceptible individuals during this period, which is particularly relevant is *Myotis myotis* due to the synchronization of births within colonies.

In conclusion, we identify the maintenance of LYSV within colonies of *Myotis myotis* in Alto Adige, with particular risks for human exposure during the birthing season. Thus, intervention measures should be intended to minimize bat-human encroachment during this period. Importantly, we predict that a similar strategy would be critical to protect humans as well as bat populations.

PREVALENCE OF ZOONOTIC PATHOGENS AND EPIDEMIOLOGICAL ROLE OF HUNTED ALPINE WILD RUMINANTS

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The importance of monitoring diseases in wildlife lies in the zoonotic, zooeconomic and ecological value. Indeed these aspects have to be considered in relation to the strong interactions with humans, livestock and wild populations. This study focuses on zoonotic pathogens that are emerging and usually not monitored in wild ruminants, in particular Hepatitis E virus (HEV), *Toxoplasma gondii*, *Cryptosporidium* spp. and *Giardia duodenalis*. The aim is to investigate the susceptibility and the prevalence of these pathogens in chamois, red deer and roe deer in order to define the zoonotic risk for humans, in particular concerning the hunting activity.

The study area lies in Lepontine Alps, within the hunting district of Verbano-Cusio-Ossola (VCO2). From 2013 to 2015 128, 81 and 49 sera, and contextually 193, 106 and 113 faecal samples have been collected from chamois, red deer and roe deer respectively. Sera samples were analysed by immune enzymatic test through commercial kit for the detection of antibodies against HEV (HEV ELISA 4.0v, MP Diagnostics Biomedicals, Singapore) and *T.gondii* (ID Screen Toxoplasmosis Indirect ELISA, IDVET, Montpellier, France). Faecal samples were analysed by immune enzymatic test for the detection of copro-antigen of *Cryptosporidium* spp. and *G. duodenalis* (RIDASCREEN® *Cryptosporidium* – RIDASCREEN® *Giardia)*. Positive faecal samples have been subsequently analysed through PCR in order to identify *Cryptosporidium* species and *Giardia* assemblages.

Chamois and red deer showed a HEV seroprevalence of 1.56% (2/128; CI: 1.37-1.75) and 1.23% (1/81; CI: 0.96-1.5) respectively, whereas no roe deer (0/32) resulted positive to HEV ELISA. As regard to *T. gondii* seroprevalences stood around 2.35% (2/85; CI: 2.00-2.70), 17.74% (11/62; CI: 16.64-18.84) and 24.48% (12/49; CI: 22.99-25.97) in chamois, red deer and roe deer respectively. On faecal samples, we detected *Cryptosporidium* antigens in one chamois out of 193 (p=0.51%; CI: 0.44-0.58), one red deer out of 106 (p=0.94%; CI: 0.76-1.12) and 4 roe deer out of 113 (p=3.53%; CI: 3,22-3,84). The same samples analysed for *G. duodenalis* showed a prevalence of 5.69% (11/193; CI: 5,46-5,92), 2.83% (3/106; CI: 2.53-3.13) and 8.84% (10/113; CI: 8,37-9,31) in chamois, red deer and roe deer respectively. Isolates from two roe deer clustered within the zoonotic genotype *Cryptosporidium ubiquitum.* As regard to *G. duodenalis*, PCR confirmed positivity in 8 chamois, 2 red deer and 3 roe deer; the zoonotic assemblage A in chamois and red deer and assemblage E in chamois were identified. Sequencing for roe deer is still ongoing.

On the whole roe deer, whose habitat overlaps with more anthropized areas, showed the higher prevalence for *T. gondii*, *Cryptosporidium* and *Giardia,* underlining the higher risk of contamination. Conversely, roe deer resulted negative for HEV, although this may have been caused by a limited sampling (n=32). As regard to foodborne zoonoses, prevalence recorded suggests that the zoonotic risk associated with manipulation and consumption of raw meat is higher for *T. gondii* than for HEV, in particular from cervids. Previous studies carried out on Alps demonstrated that the low prevalence of HEV are in fact likely consistent with the low frequency observed in alpine wild boar, while *T. gondii* is more affected by anthropization and environmental contamination. This is further highlighted by the low parasite prevalences recorded in chamois, explained by the remote habitat-use of this species. On the other hand, despite the low prevalence recorded for both intestinal protozoans, their strong environmental survival and the detection of zoonotic species and assemblages highlight the role of wild ruminants as a potential source of *Cryptosporidium* and *Giardia* infection for livestock and humans. The zoonotic risk associated with wild ruminants can not be ruled-out, above all considering the large number of culled animals during every hunting season, and the strong intensification of outdoor activities that increases the chances of getting in contact with these pathogens.

DEVELOPING HARMONIZED PROTOCOLS FOR SANITARY SURVEILLANCE IN THE GAME CONTROL CENTRES IN THE EASTERN ITALIAN ALPS

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Game control centres could represent an important infrastructure for surveillance on wildlife diseases and, due to the increasing interest in game meat, the distribution of these facilities should become more and more widespread in North-Eastern Italy. At the moment, however, their distribution is still very patchy, being present in some areas, while absent in others. Therefore, consolidated traditions in data collection and surveys from these centres are still not present.

In such a situation, and considering the probable increase of these facilities, it appears worthwhile to start the developing of shared protocols, so that data collected at their level could be more easily compared and analyzed among different administrative units. This need is all the more reason evident, when considering the effort in progress to develop and harmonize the surveillance on wildlife diseases in Europe, regarding diagnostics, population dynamics and distribution and epidemiological data.

To this aim, the project herein described, funded by the Italian Ministry of Health, was focused collecting and organizing information concerning game control centres at a medium-wide geographical range (North-Eastern Italian Alps).

Main objectives of this project were:

- to draw protocols for sanitary surveillance at the game control centres, for the purpose of passive surveillance and early detection of prioritary pathogens and possible active surveillance programmes;
- to develop methods for population and sanitary data collection and management at the game control centres;
- to share protocols and methods between stakeholders and Authorities in charge for disease surveillance and meat hygiene and safety.

Main results and outputs of the project were:

- a map of the presence and distribution of game control centres in the study area;
- the detection and description of the basic required metadata from a centre to acquire relevance in wildlife disease surveillance, useful to develop present facilities and to plan new ones;
- a basic protocol for passive surveillance on prioritary diseases at the game control centres;
- a training programme for hunters, gamekeepers and veterinarians focused on wildlife disease surveillance in the field and in the game control centres;
- an Information Technology model, integrated within the wider georeferenced infrastructure of the wildlife surveillance network, taking into account the distribution and characteristics of the game control centres, also for possible active surveillance programmes.

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BUILDING NETWORKS FOR WILDLIFE SURVEILLANCE: FROM FORESTRY WORKERS AND HUNTERS TO VETERINARIANS

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During the last decade wildlife surveillance in the Friuli Venezia Giulia Region was mainly focused on compulsory national/regional programmes such as for rabies, trichinellosis, avian influenza, West Nile disease and Aujeszky's disease; only locally in Pordenone Province a general surveillance was planned. This endorsed, for instance, the prompt detection and management of *Salmonella* Choleraesuis outbreaks in wild boar (*Sus scrofa*) populations. Regional Hunting law (LR 6/2008) includes health monitoring; nevertheless general surveillance was more based on personal initiative rather than on a structured basis, although sampling for research purposes has been performed. In this context, since 2013 the main efforts have been addressed to training of forestry workers and hunters in order to build networks of collaborations and thus increase the early detection capacity of the system in the framework of passive surveillance. In this frame local legislation was improved implementing European Regulation (853/2004/EC) with the release of a regional decree (Decreto 820SEVE dd3.9.14) instituting trained hunters. Since no other teaching on wildlife diseases was regularly addressed to hunters previously, these courses include not only hygiene issues but also general knowledge on diseases, epidemiology, surveillance, zoonosis, vector borne diseases, domestic livestock-wildlife interface and conservation. Moreover, much energy was dedicated to practical training including activities in captive red deer (*Cervus elaphus*) facilities and necropsies in the laboratory. In particular these undertakings enhanced the diagnosis of diseases such as European brown hare syndrome (EBHS), toxoplasmosis and yersiniosis in brown hare (*Lepus europaeus*), *Neospora caninum* in red deer, and pestivirus in roe deer (*Capreolus capreolus*). Furthermore, the involvement of practitioners allowed the detection of suspects of canine distemper virus (CDV) in badgers (*Meles meles*) and leptospirosis in red fox (*Vulpes vulpes*).

The pathway is just at the beginning and more actors shall be involved within a multidisciplinary approach, including protected areas, universities and conservation biologists and strengthening the involvement of public and practitioner veterinarians.

POSTER

WEST NILE VIRUS CIRCULATION IN PIEDMONT REGION IN 2016: THE ROLE OF SYNANTHROPIC BIRDS AS EARLY DETECTION SENTINELS AND PUBLIC HEALTH IMPLICATIONS

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West Nile virus (WNV) is a worldwide-distributed mosquito-borne flavivirus, causing growing concern in Europe and Mediterranean countries because of its ability to induce neuroinvasive disease (West Nile Neuroinvasive Disease - WNND) at least in horses and humans. The virus is maintained in the environment in an enzootic cycle involving birds, both migrating and residential species, and mosquitoes, mainly *Culex pipiens*, as vectors.

Birds are considered amplifying hosts, whereas mammal species including humans are considered dead-end hosts, because they may become infected and display a wide range of symptoms, but are unable to develop a sufficient viremia to infect mosquitoes.

In addition to the risk of mosquito-borne infection, there is a concern related to the risk of WNV transmission via blood transfusion and organ transplantation.

As suggested by the CDC Guidelines for West Nile disease "Surveillance, Prevention and Control", entomological and ornithological surveillance can play a key role in order to maximize sensitivity and early detect WNV appearance in risk areas, long before the onset of clinical symptoms in dead-end hosts. Whereas human cases occurred later in the epidemic season, mosquitoes and birds were the first indicators of WNV circulation, and detection of virological positivity can lead to prompt application of systematic screening of blood donations by Nucleic Acid Test (NAT) analysis.

According to national legislation, since 2016 an integrated surveillance system has been implemented throughout Northern Italy Regions with the aim to prevent the spread of WNV infections in humans.

As a part of this network in Piedmont region, together with clinical surveillance in humans or horses, mosquitoes and synanthropic birds are actively catched and tested in order to early detect WNV circulation. When virological positivity is detected in a province by surveillance activities, the screening for WNV in donors is systematically applied in order to ensure safety in blood transfusion.

In 2016, the surveillance was carried out throughout the regional territory from the beginning of July to the end of October.

Entomological surveillance: mosquitoes were sampled in 49 fixed geo-referenced sites with fortnightly samplings by CO_2-baited traps. Collected mosquitoes were counted, identified at the species level and pooled according to date, location, and species. Virological analysis were performed by Real Time RT-PCR.

Ornithological surveillance: synanthropic birds were retrieved in the framework of the regional control program enforced to protect cultivated land. Pest birds mainly belonging to magpies (*Pica pica*) and hooded crows (*Corvus cornix*), were weekly captured by Larsen traps and then killed, or directly shooted by trained hunters. Then they were necropsied and from each bird two samples consisting of brain and of a pool composed by heart, spleen and kidney, were processed by the same Real Time RT-PCR method applied for mosquitoes testing.

An amount of 973 mosquito pools and 556 synanthropic birds were analyzed during the surveillance period. Over the regional territory, WNV was detected in one *Culex pipiens* mosquito pool collected in Alessandria province at the beginning of September (0.001%; CI 95% 0.0004-0.0058).

Seven synanthropic birds tested RT-PCR positive (0.013%; CI 95% 0.006-0.026): two hooded crows killed in Vercelli province at the end of August; two hooded crows killed in Alessandria province in mid-September and three subjects killed in Torino province: one hooded crow at the end of September, one magpie at the end of October and one rock pigeon at the end of December.

Following any WNV confirmation, NAT testing was timely introduced in the interested provincial areas. More than 35.000 blood units were screened. Fortunately no positive donors were identified in 2016.

The timeline of events in 2016 underline that synanthropic birds testing revealed early positivity for each province involved in WNV circulation. In Alessandria province vectors became positive almost simultaneously as birds.

In areas where population control program against synanthropic birds are carried out, they are easily available may acting a good sentinel role for WNV surveillance together with mosquitoes screening.

Early detection of WNV at the enzootic level can provide useful data to Public Health Authorities in order to promptly apply prevention or control measures against WNV spread.

Sottosessione 2A
ECOPATOLOGIA DEI MAMMIFERI

Coordinatori: Carlo Citterio & Luc Wauters

COMUNICAZIONI

HELMINTH OF *LEPUS EUROPAEUS*: PARASITE ECOLOGY IN A DECLINING POPULATION

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Since the Seventies, a reduction of the European brown hare population (*Lepus europaeus*) has been observed in most European Countries, including Italy. This reduction suddenly accelerated, at least in some italian areas areas, from 2008 and is still continuing.

The studied hares come from the eastern hunting-management district of the Province of Bologna (Emilia-Romagna Region) known as ATCBO2. In ATCBO2, both the hares hunted in the hunting areas and the hares captured in the protected areas severely dropped from 2008-09. For example, about six thousands hares were hunted in 2009-10 while about four thousands were hunted in 2015-16; consistently, captured hares were more than 2 thousands in 2008-09 and about seven hundred in 2015-16. However, the same data suggest a reduced speed of hare decline from 2013-14, when the hare population seems to stabilize at low densities.

54 and 61 hares legally hunted in 2013-14 and 2015-16 hunting season, respectively, were examinded for helminth parasites. Sex and full weight was recordered by hunters; age was estimated observing Stroh's tubercle.

No significant difference in the sampled hare population was found between the two hunting seasons: sex-ratio, age-ratio and full weigh was similar in both years.

In both hunting seasons, the intestinal nematode *Trichostrongylus retortaeformis* and the visceral larval form of the tapeworm *Taenia pisiformis* (cysticercus) were collected; moreover, lesions ascribed to lung nematode infection were observed in 3+3 hares collected in each hunting season. In the second hunting season only, the nematode *Trichuris sp*. and the filarial nematode *Micipsella numidica* were also collected from the intestine and the sierosae, respectively. Parasite biodiversity was, therefore, particularly low in both seasons, but improved in the second one.

Significant differences between the two hunting seasons were observed for both *T. pisiform*is and *T. retortaeformis*. *T. pisiformis infection* was less frequent in 2015-16 (3.28%) than in 2013-14 (14.81%); on the contrary, *T. retortaeformis* abundance was significantly higher in the second hunting season.

While *T. pisiformis* abundance appeared to be negatively related with the weight of the hares in the first hunting season, *T. retortaeformis* abundance was higher in heavier hosts in both seasons. Both parasites are typical of adult hosts: *T. retortaeformis* was significantly more abundant in adults and *T. pisiformis* cysticerci were never isolated in animals younger than 7 month.

From 2007-08 hunters (and mass-media) reported a sudden increase of hares infected by *T.pisiformis* cysticerci, seldom or even never found before in the same area. Previous reports about hare parasites showed quite composite helminth communities, with different helmith species parasitising the gastro-intestinal tract of the hares. The results of the present study reveal an atypical parasite situation, with the strong dominance of one gastro-intestinal helminth species only and the appearance of heavy infections due to cysticerci of *T. pisiformis*.

The difference in parasite infections suggest a modification of the host-parasite relationship over time. The hares examined in the first hunting season show a very critical parasite situation, with *T. pisiformis* possibly affecting host condition and a strongly depauperated intestinal helminth community dominated by *T. retortaeformis* alone. The reduction of *T. pisiformis* infection two years later and the appearance of other helminth species suggest improved host-parasite relationship and could indicate that the period of hare population decline is going to end up.

It is impossible to know if parasites played any role in promoting hare population decline, but it is very difficult that *T. pisiformis* alone could have induced such a dramatic host decline. Hosts and parasites appears, rather, to be tied by a common ecological destiny.

SQUIRREL INVASIONS AND THEIR PARASITES: MACROPARASITES OF PALLAS'S SQUIRREL IN ITALY

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Biological invasions can affect native species through several ecological processes such as competition, predation and hybridization. The outcome of the invasion may also be affected by parasites, as has been reported for several introduced species, and infection transmission can become a threat to native species and humans.

The aim of the present study was to investigate the macroparasite fauna of Pallas's squirrel, *Callosciurus erythraeus*, a tree squirrel native to South-East Asia, co-occurring with the native Eurasian red squirrel *Sciurus vulgaris* in Lombardy. Pallas's squirrel is known to be invasive and has been introduced to other countries like Japan, Argentina, Belgium, France, the Netherlands and, in the last decade, introduced to Italy. A threat to native wildlife, and in particular to the native Eurasian squirrel, may be processes of parasite spillover, spillback or parasite release. We examined a total of 74 squirrels (females = 34, males = 40) for gastrointestinal parasites, and identified five nematode species all with prevalence below 10%. In particular, the helminth with the highest prevalence was the Oxyurid nematode *Rodentoxyuris (Trypanoxyuris) sciuri*, species specific to the native Eurasian red squirrel. All parasites, except *S. callosciureus*, were native to Europe and were acquired in the invaded range. We also found two main species of ectoparasites collected from the fur of 135 Pallas's squirrels (females= 61, males = 74): the flea *Ceratophyllus (M.) sciurorum sciurorum* (prevalence 50%, mean intensity 1.0 ± 0.1) and the tick *Ixodes ricinus* (prevalence 47%, mean intensity 3.0 ± 0.7). While the hard tick *I. (I.) ricinus* infests a wide variety of terrestrial vertebrate hosts in many Italian habitats, *C. sciurorum* is acquired from red squirrels.

The poor parasite community of the Pallas's squirrel in Italy and the loss of native parasites might have contributed to their successful invasion. The acquisition of local parasites, and the introduction of the Asiatic nematode *S. callosciureus* may potentially lead to spill-back and spill-over processes towards red squirrels. The low prevalence of *T. (R.) sciuri* seems however to preclude, at least at present, any premises for parasite spill-back. On the other hand, further studies are needed to better understand the potential consequences of *C. (M.) s. sciurorum* spill-back and *S. callosciureus* spill-over to red squirrels and the role of the ongoing parasite colonisation process on the invasion success of *C. erythraeus*. Moreover, special attention should be paid to vector-borne diseases, especially pathogens transmitted by *I. (I.) ricinus* in Europe, since the high nymph-to-larvae ratio might indicate that Pallas's squirrels actually play a significant role in the circulation and transmission of such pathogens in the vertebrate community, humans included.

THE PRICE OF BEING BOLD: PERSONALITY AFFECTS ENDOPARASITE INFECTION IN INTRODUCED EASTERN GREY SQUIRRELS

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Animal personality has been defined as consistent between-individual differences in behaviour maintained over time and/or across ecological contexts. Differences in individual personality are measured through traits such as reaction to potential risks or handling novelty, aggressiveness and sociability. Among vertebrates, individual variation in personality has been demonstrated to affect variation in fitness among animals within a population. One of the effects of personality is its potential role in the transmission of parasites and pathogens: risk-taking behavior, exploration tendency, activity and sociality are all traits that may affect hosts' exposure to parasites by altering contact rates among individuals and/or increasing chances of encountering infective stages in the environment.

The aim of the present study was to investigate the relationship between a personality score and the abundance of macroparasites of Eastern grey squirrels (*Sciurus carolinensis*) as invasive alien species (IAS) introduced in N. Italy. Invasiveness may be enhanced through the process of enemy-release, that is the absence of important predators and/or parasites in the new range compared to the native range. In fact, grey squirrels from populations in Italy have a reduced macroparasite fauna compared to populations in the native range in North America and, as far as endoparasites are concerned, only one intestinal helminth species occurs with high prevalence and intensity in grey squirrels in Italy: *Strongyloides robustus*. Here we test the hypothesis that bolder squirrels with a high tendency to explore have a higher abundance of *S. robustus* than more shy, less exploring individuals. We used capture-mark-recapture data gathered over three trapping sessions in 5 high-quality mixed deciduous woods and park in Piedmont to estimate trappability and trap diversity indices (n = 207). Both indices were strongly correlated: therefore a Principal Component Analysis (PCA) was used to derive a new variable (score of the first component, PC1) as single best estimator of tendency of being bold. Next we used PC1 as boldness score in generalized linear models with abundance of *S. robustus* as dependent variable.

We examined 95 grey squirrel carcasses: 61 out of 95 were infected by *S. robustus* (prevalence 60 ± 4%). Abundance varied from 0 to 86 helminths per host (mean ± SE = 6.68 ± 1.36) and mean intensity (mI) of *S. robustus* was 10.4 ± 2.0 helminths per host. Only 15 grey squirrels harboured also other helminth species: *Trichostrongylus spp.* was found in 13 squirrels (13.7%, mean Intensity ± SE: 3.5 ± 1.0) and in two hosts we found 6 oxyurid nematodes and 1 specimen of cestode, respectively, but these parasites were not included in the analyses. The first, general model on *S. robustus* infection showed that endoparasite abundance in grey squirrels increased with both the host's PC1 score and body mass. However, further models showed that PC1 score only affects squirrels' infection status, with bolder, more explorative individuals having a higher probability of being infected by *S. robustus*. Conversely, intensity of infection in infected animals did not vary with PC1 score, but was positively affected by host body mass. There was no significant difference between males and females in either of the infection parameters. Hence, our results supported the first, general prediction that bolder, more explorative animals are more heavily infected by *S. robustus* than shy grey squirrels. However, we also found that host personality mainly influences the probability of acquiring *S. robustus,* whereas it has no effect on parasite intensity in already infected hosts, which is instead determined by host body mass.

ARTHROPOD-BORNE PATHOGENS IN ROE DEER (*CAPREOLUS CAPREOLUS*) IN TUSCANY. A PRELIMINARY REPORT.

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The European roe deer (*Capreolus capreolus*) is an important and common part of the indigenous fauna in Europe, as well as an important host for *Ixodes ricinus* ticks, the vector of several arthropod-borne agents, most of them zoonotic. *Anaplasma phagocytophilum*, *Coxiella burnetii* and *Francisella tularensis* are well known zoonotic pathogens, able to infect both wild and domestic animals. Piroplasms may infect wild animals mostly causing asymptomatic forms; some species, as *Babesia venatorum* and *Babesia divergens*, may infect humans too.

The aim of the present study was to evaluate the occurrence of *A. phagocytophilum*, *C. burnetii*, *F. tularensis* and piroplasms in roe deer and ticks recovered from them in Tuscany.

During the regular hunting season 2016-2017 in some provinces of Tuscany, spleen samples were collected from 22 roe deer. Sixteen animals were male: 12 of them were > 24 months old, 3 were 12-24 months old, 1 animal was < 12 months old. Six roe deer were female: 1 female was > 24 months old, 3 were 12-24 months old and 2 were < 12 months old.

A total of 69 ticks were collected from 13 roe deer, then morphologically identified at genus level. Twenty-three pool with 2-4 ticks belonging to the same genus and collected from the same animal were analyzed.

DNA extraction was performed on the spleen samples and the ticks' pool using a commercial kit. All DNA samples were submitted to different Polymerase Chain Reaction (PCR) assays to detect *Babesia* sp./*Theileria* sp., *A. phagocytophilum*, *C. burnetii*, and *F. tularensis*, respectively.

None of the tested roe deer resulted positive for *C. burnetii* and *F. tularensis.* Negative response for the agent of Tularemia is in agreement with previous studies carried in Central Italy that did not find positive results testing wild rodents, red deer (*Cervus elaphus*) and red foxes (*Vulpes vulpes*). Past investigations showed that *C. burnetii* is present in Central Italy, even though its prevalence is not high among wildlife. However, both pathogens are causative agents of severe diseases in humans, thus they should be regularly monitored in animal populations.

A. phagocytophilum infection was detected in 5 (22.73%) roe deer. Wild ruminants are well known as possible reservoirs of this agent, as demonstrated by studies worldwide. Moreover, the present results confirm the presence of *A. phagocytophilum* in central Italy, as showed by previous investigation carried out in wild and domestic animals.

Three (13.64%) roe deer resulted PCR positive for *Babesia* sp. /*Theileria* sp. Sequencing of the PCR products allowed to identify 2 samples as *B. capreoli,* whereas the third sample is still under investigation. One animal resulted co-infected by *A. phagocytophilum* and *B. capreoli*.

Twelve out of 23 ticks' pool resulted positive for one or more pathogens. In detail, 9 (39.13%) pool were positive for *Babesia* spp. (5 *B. venatorum*, 3 *B. capreoli*, 1 *B. capreoli* and *B. divergens*), 8 (34.8%) for *A. phagocytophilum* and one (4.35%) for *C. burnetii.* *A. phagocytophilum* and *Babesia* sp. were found in 5 pool, while *A. phagocytophilum* and *C. burnetii* were co-present in one pool.

A. phagocytophilum, *C. burnetii* and *B. capreoli* were detected in pool of *Ixodes* sp. ticks, whereas *B. venatorum* in ticks of the genus *Ixodes* and *Rhipicephalus*. Molecular analysis for species identification of the positive ticks are in progress.

For what concerns piroplasms occurrence, *Theileria* sp. was not detected in the examined samples. The only available data about the presence of *Babesia* species among roe deer in Italy are referred to Alps region and are in agreement with our preliminary findings.

Even if the present work was performed on a limited number of both roe deer and ticks, the obtained results would appear as promising and further research in this sense would be advisable.

HEALTH STATUS OF HUNTED WILD UNGULATES POPULATION LIVING IN BELLUNO DISTRICT

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74 wild ungulates from Belluno district (Veneto, Italy), a mountainous area with altitudes ranging from less than 500 metres to over 3000 metres above the sea level, culled during the hunting season 2016/2017 were examined for the prevalence for lesions in liver and heart. Investigated population belongs to different species, such as 11 chamois (*Rupicapra rupicapra*), 17 moufflon (*Ovis musimon pallas*), 17 roes (*Capreolus capreolus*), and 29 red deer (*Cervus elaphus*) from various age classes and hunting areas.

Macroscopically and microscopically examination were performed with the aim of describing major detected lesions both in cardiac and hepatic tissues, which are the most important edible viscera.

The presence of parasitic cysts in the myocardium were detected in most of the animals examined (65% of the total ungulates), most likely related to *Sarcocystis spp*.; this finding was not associated to any macroscopic evidence and/or histologic lesion. Multifocal, mild, chronic myocarditis, usually associated with necrotic debris, was also detected (10% of the total samples).

Liver lesions included steatosis (3% of the total animals), lymphoid aggregates (Kisselev nodules, 3,5%), mild focal chronic hepatitis (43.8%), congestive hepatopathy (24,6%), and parasitic lesions consisting of cholangitis, mineralization and necrosis (14%). Detected parasites belonged to Trematoda class and only in few cases parasitic elements were evident at the macroscopic analysis.

The most relevant findings in the liver and heart were parasitic and degenerative lesions; the interpretation of the results obtained require further in-depth analysis to understand the role of each single damage.

In ungulates, most pathological investigations have involved farm-raised animals, and there are only a few reports on disease in the wild. Therefore, continuous surveillance for parasitic, bacterial and viral diseases potentially responsible for epizootics in wild animals is needed to establish conservation programs and is important also to establish the sanitary status of consumed hunted meat with the aim of informing hunters about the risks of consuming it.

TROMBICULOSIS IN NORTHERN CHAMOIS (*Rupicapra r. rupicapra*) FROM WESTERN ITALIAN ALPS: PREVALENCE AND PATHOLOGICAL FINDINGS

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The *Trombiculidae* family includes more than three thousand species with a worldwide distribution. Of the seven stages in the life cycle of *Trombiculidae*, only larvae (chiggers) may infest a wide range of terrestrial vertebrates including mammals, birds and reptiles. In wild ruminants, trombiculosis has been recently reported in white-tailed and sika deer and the Alpine or Northern chamois. Aims of this study were to further investigate the epidemiology of trombiculosis in the Alpine chamois in Western Italian Alps and to describe the related histopathological changes.

Superficial scraping and skin samples were obtained from 191 alpine chamois during the hunting season 2015 (September-December). Sixty chamois originated from Lombardy Region (Lecco province) and 131 from Piedmont Region (Verbano-Cusio-Ossola province). Superficial skin scraping was performed on lesions bearing areas, or election site of infection in asymptomatic subjects. Clarification with 10% potassium hydrate solution was followed by morphological identification of mites at 10X magnification. Skin biopsies were also collected from affected and un-affected areas. Specimens were fixed into 10% neutral buffered formalin and routinely processed for histology. Five-micrometer thick sections were stained with hematoxylin and eosin (HE), Toluidine Blue (TB), PAS (periodic Acid-Schiff) and Goldner trichrome staining. Immunohistochemistry labelling on skin sections was performed and number of eosinophils, mast cells, CD3, CD79α, CD68-positive cells were counted on eight 10,000 μm^2 random fields of each biopsy using a semiautomatic analysis system (LASV 4.3, Leica, Germany). Eosinophils were counted on HE sections. Mast cells were counted on TB stained slides.

Forty-one (41/191; 21.5%) chamois scored skin scraping positive for presence of *Trombiculidae* larvae. Of them, 15 were from Lombardy and 25 from Piedmont. Histologically, trombiculid mites were localized on the surface of the epidermis, over the keratin layer. Generally, a low number of mites (1-2 mites at 10X HPF) were detected, except for a pinnae skin biopsy where mites were more numerous (4-6 mites at 10X HPF). Focally extensive or diffuse slight to moderate parakeratotic hyperkeratosis was observed. Rarely slight crusting was also detected. Multifocal erosions were observed only in the fore-mentioned pinnae skin specimen. No alopecia was evident in histological sections. In the superficial dermis, an eosinophilic stylostome with a diaphanous not stained center was associated with mites. Stylostome was surrounded by inflammatory infiltrates and necrotic debris and associated with erosion/necrosis of epidermis and slight exocytosis of degenerated neutrophils. Inflammatory infiltrates were composed mainly by macrophages and T-lymphocytes. Degenerated neutrophils, eosinophils and B-lymphocytes were also present. Mast cells were rarely observed. Inflammatory cells were also moderately diffused in the surrounding superficial dermis.

Based on data, trombiculosis is frequent in chamois in the Western Italian Alps, with similar prevalence in Lombardy and Piedmont regions (19% and 25%, respectively). Macroscopical lesions were moderate with involvement of body regions in contact with ground, especially muzzle (pinnae, and areas around eyes and mouth) and limbs, characterized by thin skin where stylostome is easily formed. Infested chamois, showed a normal body condition, except for a 2-year-old female whose body mass was lower than in peers. Histologically, trombiculosis in alpine chamois causes a focal moderate to severe dermatitis with epidermal necrosis, crusts and hyperkeratosis. Lesions were like those described in white-tailed deer or other wildlife. Inflammatory infiltrates are suggestive of granulomatous reaction facing on stylostome, formed to mite saliva and necrotic debris of host epidermal and dermal tissue. However, a wide difference of immune response was detected in affected chamois that could reflect the development of a hypersensitivity reaction to the mites, as described in cats. Finally, chiggers may have a potential zoonotic role as suggested by frequent co-occurrence of infection in humans and animals and their vector role for *Rickettsia* spp., *Bartonella* spp., and other pathogens. In chamois, the role of trombiculosis as vector of other diseases is unknown and further investigations are warranted.

ALPINE CHAMOIS POPULATION WITH IMPAIRED HEALTH STATUS LIVING IN ISOLATED HABITAT: EVIDENCES FROM PATHOLOGY AND MOLECULAR GENETICS

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The Alpine chamois *Rupicapra rupicapra rupicapra* is the most abundant subspecies of the genus, considered at "least concern" by IUCN. Despite their large areal, some chamois population may suffer because of areal restriction, fragmentation and loss of habitat. For instance, population of Mount Generoso (Prealpi Luganesi) inhabiting an isolated mountain surrounded by barriers as lake or highways, showed impaired health status. In fact, after a period of strong population growth (600-700 animals), a reduction of birth and other signs of diseases were reported during veterinary surveillance of animals.

In the framework of a collaboration between ISPRA and the Hunting Office of Como Province, chamois were collected, inspected at necropsy, preliminary viral-bacteriological and parasitological investigations were performed together with genetic analyses at neutral (microsatellites) and adaptive (DRB1 gene of the MHC Class II) molecular markers in order to evaluate the amount of genetic variation of populations with an indirect estimation of adaptive potential.

Fifty animals were collected and examined for sign of diseases and subsamples of fifteen specimens were analysed using genetics. Almost all animals (98%) showed severe alteration of the dental arch; 100% of them showed an abnormal growth of the hoof, the front limbs, with deformation of the soles and most of adults (between 16-19kg) the detected body weight was significantly lower than the average. In thirty animals, *Sarcocystis* spp. in myocardium and mitral valve degeneration were reported.

Fifteen specimens were genetically characterized at exon 2 of the DRB1gene (MHC class II) by sequences analysis, using in-phase haplotypic composition, haplotype frequencies and variability indices. Six haplotypes were observed, five previously reported and one newly described. Among the five haplotypes already described, four were observed in Alpine chamois and one in Pyrenean chamois. Haplotype diversity was 0,71 with an observed heterozygosis of 47%.

The aminoacidic alignment of molecules confirms that most of the genetic variation is on the Peptide Binding Region coding residues, involved in antigen presentation and immune response triggering. Despite the level of haplotype diversity is in agreement with results obtained by previous studies on genetic variability at DRB1 of *R. rupicapra* and *R. pyrenaica*, the heterozygosity and the number of observed allele is lower, similar to those observed in individuals belonging to subspecies living in small areals and considered as endangered, such as *R. r. tatrica* and *R. p. ornata*. Such preliminary results highlight possible decreased adaptive potential of the population.

POSTER

OCCURRENCE OF A NEW PARASITE OF THE UNGULATES IN ITALY: *LIPOPTENA FORTISETOSA* (DIPTERA: HIPPOBOSCIDAE)

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In the last decades the presence of wild ungulates in Italy has increased its levels causing management issues. It is assessed that between 1980 and 2010 *Cervus elaphus* has increased its presence of more than 700% and *Capreolus capreolus* of 350%. The other species' increments range from the 120% of *Rupicapra rupicapra* to the 300% of *Ovis musimon*. Tuscany, in particular, is the region with the highest presence of roe deer, wild board, fallow deer and red deer. In 2012 it was estimated the presence in Tuscany of 200˙000 roe deers, 200˙000 wild boars, 8˙000 fallow deers and 4˙000 red deers.

The massive presence of ungulates leads not only to several problems related to wildlife management and its social and environmental implications, but also to a consequent increase of their parasites. Among these, the Diptera hippoboscid ectoparasites are relevant under a sanitary point of view; they live at the expenses of various species of ungulates and can accidentally infest other species including humans. This flies damage the hosting animals causing sickness and stress and maintaining the transmission of pathogens and zoonoses (Borreliosis, Anaplasmosis, Tripanosomiasis).

In addition to the problems related to native ungulates, it is important to consider the ones related to allochtonous ones. The presence of the latter in nature is the consequence of intentional or accidental immissions, a common phenomena motivated by leisure interests, such as hunting or the creation of wildlife parks. Especially cervids were involved in these operations representing the major species naturalized in Europe and worldwide. For example, the Sika deer (*Cervus nippon*) was recently discovered in Italian territory in the provinces of Trento and Modena.

Hence, the increase of cervids, the recent confirmed presence of the *C. nippon* in our territory and the climate changes, as the rising of temperature that favours the spread of parasites, are some of the reasons to intensify the monitoring of wildlife, considering also the parasites control.

To this purpose a research was made in order to determine the presence of the hippoboscid *Lipoptena cervi* and to have a deeper morphological, biological and ecological knowledge of this species that has not been recently studied and has not been properly described yet.

In the preliminary step five animals were analysed: three specimens of *C. elaphus*, a male fawn, a female fawn and a yearling male, each shot in the province of Prato in 2017, on January 29th, February 11th, and March 11th respectively; and two specimens of *Capreolus capreolus*: a male fawn shot in the province of Grosseto (2017 August 26th) and an adult female living at the experimental farm of DISPAA in Florence.

The examined subjects hosted a great number of ectoparasites: among these the presence of the hippoboscid *Lipoptena fortisetosa* was detected; this species, already reported in other European countries, had never been found in Italy yet.

From the five samples we collected 630 parasites of *L. cervi* and 150 of *L. fortisetosa.*

A morphological study allowed to identify the peculiar characteristics that facilitate the distinction between the two species. The biggest difference is the dimension, *L. fortisetosa* is smaller and less hairy than *L. cervi*. A deeper observation showed that the abdomen of *L. fortisetosa* is less sclerotized and of a lighter colour, and the abdomen in males is less developed compared to *L. cervi*. The head of *L. fortisetosa* has a characteristic rhomboidal shape, whereas in *L. cervi* it is ovoid. The thorax also differs for the distribution of bristles and for other structures and sutures.

The analyzed ungulates were from different habitats and areas far from each other, thus the presence of *L. fortisetosa* in the territory of the Northern Apennines could be considered neither sporadic nor accidental.

This study is the first reporting of *L. fortisetosa* in Italy; at the present it is not possible to confirm if the presence is due to *C. nippon*, its principal host; the Sika deer could had spread in Europe by bringing its ectoparasites, that could have adapted later infesting other cervids and carrying further potentially pathogenic agents. This hypothesis would need an increment of controls in order to determine the degree of the spread of this parasite, either geographically or related to the host species, its present degree of adaptation and moreover to prove that *C. nippon* is responsible for its presence.

IDENTIFICATION AND DISTRIBUTION OF *HIPPOBOSCIDAE* ECTOPARASITES ON WILD UNGULATES IN SONDRIO PROVINCE

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We examined specimens of Hippoboscidae collected on wild ungulates given to the "Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna, Sondrio Section". The study of the specimens allowed to detect the presence of three species: Lipoptena cervi (Linnaeus, 1758), Melophagus rupicaprinus Rondani, 1879, Melophagus ovinus (Linnaeus, 1758).
A total of 388 *L. cervi*, 65 *M. rupicaprinus* and 10 *M. ovinus* were collected and classified.
The largest number of specimens belong to the species L. cervi, which was found on red deer (n=10) and roe deer (n=50) but also in chamois (n=7). This shows the great adaptability of L. cervi in terms of host species, and its ability to spread and colonize rapidly different areas, exploiting the populations of wild ungulates present in the province of Sondrio.
We, then, examined the different distribution of *L. cervi* on his type hosts (red deer) and the most represented host on this study (roe deer).
Statistical analisys showed that roe deer have a lower intensity of infestation (ANOVA, n=60, F=4.088, p=0.048) but no effect of age, pathological findings and cause of death on intensity of infestation.
M. ovinus was detected in one chamois and *M. rupicaprinus* from six chamois and two alpine ibex.
We didn't find any other *Hippoboscidae* of the Genus *Lipoptena* previously described in the same host species collected in our study.
Our observations confirm the low specificity of these parasites described by some authors. Low specificity demonstrated from both genus *Lipoptena* (red deer vs roe deer and chamois) and *Melophagus* (chamois vs alpine ibex).
As a matter of fact, we found a complete absence of the roe deer type parasites *L. capreoli* (Rondani, 1878).
These last result could demonstrate a possible replacement in roe deer from his type parasite. The lower intensity could be due to the residual specificity of the parasite or simpler correlated to the dimension of the host.
The knowledge about the distribution of these species are very important because they could reflect relationship and population dynamics of their hosts and because they are potential vectors of transmission of zoonotic bacteria of the genus Bartonella.

SURVEY OF SOME TICK-BORNE ZOONOSES IN UNGULATES OF THE COLLI EUGANEI REGIONAL PARK

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A survey of zoonotic bacterial infections was carried out during the period March-November 2014, in wild boars (*Sus scrofa*) and fallow deer (*Dama dama*) of the Colli Euganei Regional Park. This is a tourist and recreational area, characterized by a wide botanical and faunal biodiversity and by complex relationships between wild animals, livestock and humans.

Wild boar and fallow deer are recognized as important hosts for maintaining *Ixodes* ticks and several pathogens transmitted by ticks to other animals and to humans.

Wild boar was illegally introduced in the park in the nineties and since then the population has been progressively growing up despite of the increasing number of animal culled in the framework of the population control programme, established by the park administration (about 500 animals culled in 2007 and more than 1000 in 2016).

Fallow deer represents another allochthonous species of Euganean hills submitted to population control. The population of fallow deer is estimated to be few hundred individuals.

Blood samples were collected by park operators from 211 wild boars and from 61 fallow deer. DNA was extracted from 200 µl of blood using a commercial kit and the presence of vector-transmitted zoonotic bacteria (*Borrelia burgdorferi sensu lato*, *Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp. and *Bartonella* spp.) was evaluated by biomolecular analysis. *B. burgdorferi s.l.* and *Rickettsia* spp. detection was performed by real-time PCR assays targeting *23S rRNA* and *gltA* genes respectively. The presence of the other microorganisms was assessed by PCR amplifying a portion of the *16S rRNA* (*Anaplasma* spp. and *Ehrlichia* spp.) and *ribC* (*Bartonella* spp.) genes. Nucleotide sequences obtained from positive samples were compared to homologous fragments in GenBank by using BLAST. *A. phagocytophilum* was further confirmed applying a specific real-time PCR targeting *msp2* gene.

Only one out of the 211 wild boars sampled resulted positive to *B. burgdorferi s.l.* (prevalence: 0,4%; 95% c. l.: 0%-1,4%). No wild boar was positive to the other zoonotic bacteria.

Out of the 61fallow deer, 18 (29,5%; 95% c. l.: 19.6%-39.5%) were positive for *A. phagocytophilum* (nucleotide similarity between 99% and 100%) and 1 (1,6%; 95% c. l.: 0%-4,4%) for *Ehrlichia* spp. (nucleotide similarity between 97% and 99%).

Bartonella spp. was detected in 37 blood samples (60,7%; c. l. 50,0%-71,3%); 18 amplicons were sequenced and were 98-99% similar to *Bartonella capreoli* homologous nucleotide sequences. None of the tested fallow deer were positive for *B. burgdorferi* and *Rickettsia* spp.

These results demonstrate the presence of zoonotic tick-borne pathogens (*A. phagocytophilum, B. burgdorferi s. l., Ehrlichia* spp.) in the Colli Euganei Regional Park. Furthermore, the presence of wild boar and fallow deer populations promotes the maintenance of ticks in the environment. The risk of livestock and of human exposure to infections during the outdoor recreational or professional activities or manipulation of hunted animals should therefore not be disregarded.

FIRST REPORT OF FELINE LEUKEMIA VIRUS (FELV) IN EUROPEAN WILD CAT (*FELIS SILVESTRI SILVESTRIS*) IN NORTHEAST ITALY

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Feline leukemia virus (FeLV), like other viral and bacterial aetiological agents, described in domestic cats, is considered a potential threat for isolated populations of European wild felines: European lynx (*Lynx lynx*), Iberian lynx (*Lynx pardinus*) and European wildcat (*Felis silvestris*). The most important data concerning the FeLV in the wild environment was obtained from studies of free living populations of European wildcat in France, Great Britain, Scotland, Switzerland, Germany, Slovenja, Spain and Portugal. In these areas the FeLV antigenemia was detected from 0 to 49%. Despite this, the epidemiological data on this viral infection among this specie appear very scarce and fragmented if compared to the range of wildcat in Europe, in particular of the eastern area. Although in Italy there are two subspecies of this carnivore: *Felis silvestris libica* in Sardinia and *Felis silvestris silvestris* in Sicily and in peninsular regions, until now FeLV had never been described. Lately (April 2017) one male wildcat (*Felis silvestris silvestris*) was accidentally trapped in a rural environment of Carnia, an eastern alpine area of Friuli Venezia Giulia. This subject was captured with a single entrance box trap inside a hen-house and gradually anesthetized using a combination of ketamine (Imalgene®) and medetomidine (Domosedan®). During the clinical examination, the cat showed a good hair quality, but appeared slightly emaciated with icteric gums mucous. After the blood sampling, the animal was measured, weighed, and equipped with a radiocollar. Before the releasing, atipamezol (Antisedan®) was used to reverse the effects of medetomidine and facilitate a faster recovery. The hematological and biochemical evaluations of blood samples showed an increasing of WBC, ALT, AST, ALP, GGT values, probably correlated with an infection and hepatic damages. A European wildcat blood aliquot was tested by the commercial rapid immunoassay for the simultaneous detection of FeLV antigen and FIV antibody (Feline Immunodeficiency Virus) (SNAP Combo FeLV Ag/FIV Antibody Test®, IDEXX), which was positive to FeLV. The clinical, hematological, hematochemical and serological data allowed us to describe the first FeLV) presence in European wildcat in Friuli Venezia Giulia, and probably in Italy. This report reaffirms the necessity to increase the knowledge on health statusof Italian populations of European wildcat, a mammal specie with high conservation value.

SURVEY ON THE PRESENCE OF TICKS AND RELATED TICK-BORNE PATHOGENS IN OSSOLA VALLEY, PIEMONTE REGION, NORTH-WESTERN ITALY

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In Italy, the availability of eco-epidemiological data on the distribution of different tick species and associated tick-borne pathogen (TBPs) is highly variable. Such information constitutes a useful guideline for the medical healthcare system which in general has a proper awareness on the epidemiology of these vector borne diseases. However, the incidence of human tick-borne diseases (TBDs) in non-endemic Italian areas is probably underestimated, perhaps due to limited surveillance, asymptomatic cases, improper diagnosis (e.g. false negative in serological tests) and lack of follow up studies.

Despite a recent study has provided data on *Borrelia burgdorferi* sensu latu prevalence in *Ixodes ricinus* ticks collected in Piemonte, responding the increase in the number of reported Lyme disease cases in the area, this region remain one of those territories rarely investigated for the presence of ticks and TBPs.

In our survey, a molecular screening for four important agents of zoonoses (*Rickettsia* spp., *Borrelia burgdorferi* sensu lato complex, *Francisella tularensis* and *Coxiella burnetii*) was performed on both engorged ticks collected on wild and domestic animals, and questing ticks collected by dragging on vegetation in different forested areas in Ossola valley.

Two different species of rickettsiae (*R. helvetica* and *R. monacensis*), known to cause human illnesses and five different *Borrelia* species, proved (*B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*) or suspected (*B. valaisiana* and *B. lusitaniae*) to cause clinical manifestations of Lyme disease in humans, were found in both questing and engorged *Ixodes ricinus* ticks. Moreover, PCR positivity for *Francisella tularensis* was obtained in engorged ticks collected on two different wild ungulate species (*Capreolus capreolus* and *Cervus elaphus*). This work provided further data and broadened our knowledge on bacterial pathogens present in ticks in North-Western Italy.

PREVALENCE OF *SALMONELLA ENTERICA* SUBSP. *ENTERICA* IN HEDGEHOG (*ERINACEUS EUROPAEUS*) IN FERRARA PROVINCE (EMILIA ROMAGNA, ITALY)

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Hedgehog (*Erinaceus europaeus*) is a small mammal, largely distributed in Europe, adapted to a nocturnal way of life. Hedgehog is omnivorous, and its diet includes invertebrate animals (e.g. worms, snails, spiders, centipede), vertebrate animals (e.g. frogs, toads, snakes, bird eggs), carrion, and vegetables (e.g. mushrooms, berries, melons, acorns). Hedgehogs have a fairly wide range of action, and in a night, they can walk 1-3 km, move in hunting areas that can range up to 30-100 hectares. Generally, specimens living in open environments move more than those who settle in wooded or sheltered areas. During the summer, they could change nest 0-30 times.

Hedgehogs are host to a wide variety of bacterial and protozoan pathogens, of which a number have become a matter of concern to public health. In addition to vector-borne agents, hedgehogs are a potential reservoir for enteric bacteria (such as *Salmonella* and *Campylobacter*), and protozoan parasites (*Giardia* and *Cryptosporidium*), which may cause enteritis in humans, livestock, and pets. *Salmonella enterica* subsp. *enterica* is a microorganism largely distributed all over the world, able to survive for long time in the environment, and, it can cause disease in humans and animals. The primary transmission route to humans is believed to be food-borne (via the fecal-oral route through the ingestion of contaminated water or food), however, (indirect) contact with an animal reservoir can be an alternative source of infection. According to EFSA (European Food Safety Authority) *Salmonella* is the second most common cause of human gastroenteritis in Europe. The role of wildlife as *Salmonella* spp. carriers has been highlighted in several studies on a variety of species, included hedgehogs. Wild animals are relevant to the epidemiology of salmonellosis because of their role as healthy carriers of a broad range of *Salmonella* serotypes as observed by in the United States and in Norway. Additionally, similar strains have been isolated from both humans and wildlife, suggesting that wildlife species could be a reservoirs for *Salmonella* spp.

From 2012 to date, 166 hedgehog carcasses have been tested for the presence of *Salmonella* spp.. All the hedgehogs have been collected in the Ferrara province and delivered to a local wildlife rehabilitation center. The animals that died in this center were stored frozen and, about once a week, delivered to the laboratory where a necropsy and the research of *Salmonella* spp. were carried out. In most cases the cause of death was due to traumatic injuries.

The research of *Salmonella* spp. has been carried out from the intestine of the animals, according to ISO method 6579:2002/Amd 1:2007 using the selective medium MSRV (Modified Semisolid Rappaport Vassiliadis). The suspected colonies were confirmed biochemically with API 20 E system and the serotyping of *Salmonella* strains was performed using commercial antisera; polyvalent *Salmonella* O and H antisera were used to obtain a presumptive diagnosis but the definitive antigenic designation was assigned using monovalent antisera following the White-Kauffman-Le Minor serotyping scheme.

Salmonella enterica subsp. *enterica* was isolated in thirty-five samples out of the 166 tested (21,1%). Twenty-one strains out of 35 belonged to the serovar *S.* Enteritidis (60%). In the current study, hedgehogs in Ferrara areas harbor a not negligible number of zoonotic agents, and therefore may contribute to the spread and transmission of zoonotic diseases. Although the majority of *Salmonella* spp. infections are foodborne, other sources should not be ignored. Wildlife could play a notable role in maintaining *Salmonella* spp. in the environment, even if, to what extent can hedgehogs maintain the enteric zoonotic agents in natural cycles, and the role of (spill-back from) humans or other bred species, mostly avian species, remains to be investigated.

REPORT ON TRAUMATIC MYASIS IN CRESTED PORCUPINES *HYSTRIX CRISTATA* IN ITALY

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The Crested porcupine *Hystrix cristata* is distributed in Northern Africa and in sub-Saharian Africa. Italian peninsula it is the only European distribution area but its population increasing and enlarging towards North.

In the mainframe of a research on the ecological and parasitological role of the species in the Italian ecosystems, in cooperation with the Istituto Zooprofilattico sperimentale della Lombardia ed Emilia Romagna, Forlì Section (IZLER), we examined *H. cristata* specimens recovered for accident trauma. In total, three cases of traumatic myasis were found on 68 specimens examined.

Myiasis is an infestation of vertebrate animals with dipterous larvae which feed on the host's dead or living tissue. These parasitosis can be caused by many species of Diptera, but larvae of Calliphoridae, Sarcophagidae and Muscidae are the main agents causing traumatic myiasis in humans and animals.

The first case was a young female of around 4,5 kg that suffer of a car impact in Lido di Classe (RA) at the end of December 2016. The animal was brought to a local wildlife recovery center but died the following morning. The animal had a fracture to pelvic girdle and decaying wound in the left side with large number of maggots feeding on the dermal and muscle necrotic tissues. Sample of the collected larvae were killed by immersion for 1.5 minutes in boiling water and later fixed in 95° ethyl alcohol, while other specimens were reared in the laboratory to the adult stage. Both larvae and adults were identified as *Calliphora vicina* (Diptera: Calliphoridae).

Other cases of traumatic myiasis involved porcupines were found on two females sent to the IZLER in May 2017 for the trauma caused by a road accidents. The first female was found in Ravenna with a thoracic wound on right hip. The lesion was found infested by many dipteran maggots. The second gravid female was found in Rimini with a wound on the back, also infested by many dipteran larvae. In this last case *Escherichia coli* was found infecting all organs. For both cases it was not possible to collect larvae.

In order to increase the knowledge on the Diptera that cause myiasis in wild animals it is need an accurate data collection of larvae and then their taxonomic identification. These data together with other investigations on the ecology and health of wild *Hystrix cristata* could be very useful also for the future management and welfare of this specie.

GIARDIA AND *CRYPTOSPORIDIUM* IN PORCUPINES *HYSTRIX CRISTATA* AND BADGER *MELES MELES* IN ROMAGNA REGION

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Giardia duodenalis is one of the most common intestinal parasitic infection among humans and animals in both developed and developing countries, and its zoonotic potential and public health relevance have been assessed by several studies. Among assemblages of *G.duodenalis*, the A and B group have the broadest host choice. In particular assemblage B outbreaks in humans have been broadly reported. *Cryptosporidium spp*. is also causative agent of zoonotic infections worldwide. Together with *Giardia spp*. is considered to be amongst the most common waterborne diseases, since oocyst of both the parasites are able to contaminate the environment, particularly water sources and streams for long periods of time.

In this study 24 samples collected from *Hystrix cristata* and 38 from *Meles meles* have been tested for presence of *G. duodenalis* by qualitative RT-PCR. 16 out of the 24 *Hystrix* specimens resulted positive for infection, while all *M.meles* samples were negatives. 18 of the samples collected from porcupines were also tested to assess the presence of *Cryptosporidium spp*.. Diagnosis was established by microscopical observation of concentrated fecal matter smears colored with the modified Zihel-Neelsen method. *Cryptosporidium spp*. was detected in 11 samples, 7 of which were also positive for *G. duodenalis* too, showing a tendency to mixed infection. The presence of these parasites in wild animals fuels the open dialogue about the importance that this protozoan parasites can have in contaminating environment, especially sources of drinking and potable water for both animals and humans and the possible role of *Hystrix* as a reservoir or amplifier host in the maintenance of zoonotic pathogens' cycles.

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SPINE ARE NOT ENOUGH: TRAUMAS IN CRESTED PORCUPINES FROM TRAFFIC CASUALTIES

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Wildlife is at risk for traffic around the world and is important also to increase the knowled in how the different species are affected by. This study focuses on the anatomo-pathologic information of 65 *Hystrix cristata* specimens delivered to the Forlì lab of the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna between March 2014 and August 2017. Each of the 65 specimens was first examined and necropsy was carried out in all cases. Among the *H. cristata* of this study the causative agent of death was found to have been trauma due to impact with motor vehicles in 93.85% (n.61) of the cases, and only 6.15% (n.4) deaths were ascribable to other causes. A record of the most frequently reported fractures and traumas was kept. Fractures were found to be frequent in skull (n.23), rib cage (n.7), spinal column (n.14), pelvis (n.7), pelvic limb (n.4) and sacrum (n.1). Dislocation of the femoral head and acetabulum was also frequent (n.4). Traumas were reported to the abdomen (n.7) and thorax (n.5) accompanied by internal injuries such as evisceration (n.8), organ rupture (n.4) and diaphragmatic hernia (n.3). In order to study the dynamics of the impacts and to evaluate the exact structure of fractures, several skulls and pelvic bones were gathered for further investigation. It was nevertheless noted that many of the injuries reported occurred in animals which externally showed little or no signs of trauma. This study aims both to highlight the most frequent traumatic areas and the features of fractures present in the porcupine, a very stout and robust animal capable of enduring significant impacts when involved in vehicle accidents, and to gather important information which could be of use to veterinarians in charge of the recovery of wounded animals.

INVESTIGATION OF *GIARDIA DUODENALIS* IN WILD RUMINANTS IN THE HUNTING DISTRICT C.A. VCO1

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Giardia duodenalis is a cosmopolitan flagellated parasite of humans and many species of animals, and is considered as the most common cause of protozoan diarrhea worldwide. For many years, the zoonotic potential of *Giardia duodenalis* has been a controversial problem in *Giardia* research. This species is genetically heterogeneous, and eight major assemblages of genotypes (A-H) have been recognized. While the role of pets and livestock in *Giardia* transmission has been studied intensively, the role of wild animals, particulary ruminants, has recently been taken into consideration. Wild ruminants might play a significant role in contamination of the environment with *Giardia* cysts because large populations occur worldwide. They excrete a relatively large volume of faeces, and their feeding ranges usually overlap with cattle pastures. Thus, hey might be potential reservoirs of *Giardia* infection both for humans and livestock, and they might represent a public and veterinary health interest..

The diagnosis of Giardiasis is frequently based on detection of trophozoites or cysts by microscopic examination of stool sample. However, microscopic examination of a single stool specimen has a low sensitivity due to intermittent shedding of the cysts.

An alternative method to diagnose Giardiasis infections it could be the enzyme-linked immunosorbent assay (ELISA) , that can be integrated into the microscope test, because it increases the sensitivity of the analysis, and because it provides confirmation data in samples with a low number of parasites, but above all because it also diagnose Giardiasis even in faecal samples where parasites are absent.

The aims of this study was to obtain information about the presence of Giardia infection in wildlife in the hunting district of Piemonte region, located in Verbania Province (formed named Comprensorio Alpino Verbano-Cusio- Ossola 1 (C.A.V.C.O.1).

In this study we have examined a total of 39 faecal samples collected from wild ruminants and 5 faecal samples from other animals. All the samples were collected in 2016, from march to september , by ASL VCO staff in the C.A. VCO1. Wild ruminants (11 *Cervus elaphus* deer, 22 *Capreolus capreolus* roe deer, 2 *Rupicapra rupicapra* chamois, 2 *Ovis aries musimon* mouflon, 2 *Dama dama* fallow deer) and other species (2 *Vulpes vulpes* red fox, 1 *Marmota* woodchuck, 2 *Martes foina* beech marten, 1 *Sus scrofa* wild boar) were tested with fecal flotation using 33% $ZnSO_4$ solution with centrifugation and Lugol iodine and with the RIDASCREEN Giardia-an ELISA test.

The Giardia ELISA is an enzyme immunoassay based on the detection of antigens of *Giardia duodenalis* cysts and trophozoites in stool specimen. Here, Giardia-specific antibody is coated on the surface of the well of microtitre plate. Then stool sample is added followed by addition of conjugate; if *Giardia* is present in the specimen, a sandwich complex forms which is made up of the immobilized antibodies, the *Giardia duodenalis* antigens and the conjugates antibodies. Unattached enzyme-labelled antibodies are removed during the washing phase. In positive test, on addition of the substrate, there is a change in the color of the well of the microtitre plate from colorless to blue. On adding the stop reagent, the color changes from blue to yellow. The absorbance of controls and animal samples was read at 450 nm using an ELISA micro-titre plate reader.

Although the samples tested were all negative, we must not overlook the epidemiological roles that these wild ruminants play in the environmental contamination (including watercourses and watersheds) and transmission to other animals.

WILD ANIMALS DISEASES: 2013-2016 RESULTS FROM THE ITALIAN WILDLIFE SURVEILLANCE NETWORK.

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One Health recognizes that the health of people is connected to the health of animals and the environment. The diseases shared with wildlife have a significant impact on human and domestic animals health, economy, ecology but also on effective wildlife management or conservation. Surveillance and monitoring programs are the first steps to provide an appropriate level of understanding of wildlife health, in particular to detect diseases and monitor their temporal trends and spatial distribution.

Aim of our study is to show the main results of the recent four-year period activity of the Italian wildlife surveillance network .

Annually, on the basis of the reporting activity of a team of *ad hoc* referents, the Italian Reference Centre for Wild Animal Diseases (CeRMAS), on behalf of the Ministry of Health, collects and analyses monitoring data of wildlife diseases. The referents are based in each of the ten Istituti Zooprofilattici Sperimentali that make up the network of Italian official laboratories for prophylaxis and diagnosis of animal diseases. Samples of any wild species are examined in each local lab; animal data and diagnoses are entered by the referents in a shared spreadsheet file and periodically submitted to CERMAS. National data are collated to fill the "OIE Wild animal diseases questionnaire", a national dataset of qualitative and quantitative information. Then the overall Italian questionnaire is submitted to the OIE World Animal Health Information System. Here data produced over the 2013-2016 period have been considered.

Several diseases have been diagnosed every year (e.g. African swine fever, Trichinellosis, Echinococcosis /hydatidosis),while others are detected only periodically or sporadically in small subpopulations (e.g. Transmissible gastroenteritis, Infection with Plasmodium spp.). Highly pathogenic avian influenza in birds has been reported just in the last year. Finally, Toxoplasmosis has been reported in many animal species.

Our work shows the success of the surveillance network targeting wildlife diseases: it allows an overall, national description of the epidemiological situation. However, the latter may be in part biased due to some targeted activities specifically focussing on: 1. diseases notifiable to the OIE (listed diseases affecting wild animals, non-listed wildlife diseases and non-infectious diseases); 2. diseases object of specific scientific projects aimed at prevalence estimation or at disease detection; 3. diseases detected as single case report in the frame of the routinary diagnostic activity. Disentangling the sources of the collected data helps in interpreting the health status of the Italian wildlife.

The Italian surveillance network, born thanks to an OIE stimulus, provides fundamental insights to effectively manage problems regarding zoonotic and emerging diseases (e.g. Tick borne encephalitis), the consequences of livestock and wildlife interaction (e.g. Brucellosis) and relevant issues related to conservation (e.g Infection with Sarcoptes scabiei or Pestiviruses).

MAN-WOLF INTERACTION: A POSSIBLE SCENARIO

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More than an animal, the wolf is an archetype, a symbol of fear and death; it has always stimulated the human imagination and for this it appears in the culture and in the traditions of a lot of civilizations, evoking fantastic legends and tales.
The relationship between wolf and man has been very variable over the centuries; the wolf has raised too many reactions through phases of coexistence and phases of conflict.
Bibliographic data indicate the beginning of this complex relationship from the Paleolithic, 1-2 million years ago, when the man was hunting in areas populated by abundant wildlife, especially wild ungulates; at that stage the wolf and the man used to live together sharing that abundance without conflict.
The coexistence lasted until the man became a shepherd: at that stage, the wolf began to exploit the opportunity to surrender domestic livestock, much easier to capture than wildlife, thus becoming a predator considered wicked and cruel. More or less, such a negative role of the wolf has remained until our times.
A quite recent review of wolf attacks on humans from Europe, Asia and America affirms that "the risks of wolf attacks under present circumstances are very, very low throughout Europe and North America".
Despite this, the recent reappearance of wolves in territories where they seemed to have disappeared, has generated a wide and controversial discussion.
The strong impact due to the conflict and the hostility to them was the reason for a request for collaboration from a Veterinary field unit to undertake a study on the assessment of the risk of wolf attack on humans.
Risk assessment, as defined by CODEX and OIE, is the process of estimating the likelihood that a health effect will occur after an individual has been exposed to a specified amount of a hazard.
The exercise consists of four phases, and the first one is the identification of the possible hazards. In this case the factors related to the human-wolf interaction. We have considered: human factors (age, gender, awareness, type of activity, knowledge), wolf factors (illness, density of the population, social characteristics, availability of alimentary resources) and external factors such as weather conditions, season, presence of domestic livestock, vegetation, degree of anthropization. All these factors have been used to build up a couple of scenarios i.e a worst-case and one adapted to current situations.

CANINE DISTEMPER VIRUS & WILD CARNIVORES: AN IMAGE IN NORTH-WESTERN ITALY

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Canine Distemper Virus (CDV) is an enveloped, single-stranded, negative-sense RNA virus belonging to *Morbillivirus* genus, *Paramyxoviridae* family, causative agent of Canine Distemper (CD), a severe systemic disease affecting carnivores. CD occurs with gastrointestinal, respiratory and nervous symptoms often complicated by secondary bacterial infections; severity of disease depends on strain pathogenicity and immune status of the host. Domestic dogs are the main reservoir, but a recent spatial phylogenetic reconstruction showed a widening of the host range: for this reason any new CDV outbreak could represent an important threat especially for endangered species as wolf (*Canis lupus*), due to its potential role on population decline. Nonetheless, how wildlife are exposed to CDV is still an open question, especially when there is an evidence of viral circulation in wildlife even with low infection levels in domestic animals. Consequently, it has been supposed a circulation of CDV in wildlife within complex reservoir systems, in which some interconnected wildlife populations may act as a single reservoir. Currently, in Italy the circulation of three CDV lineages is reported: EU1/SA1, EU2 and EU3. Only the last two have been isolated from wildlife until, 2006, when it was identified a distinct subgroup belonging to EU1, called "Europe Wildlife 2006-2009", responsible of several outbreaks in the Eastern Alps with abnormal mortality rate among wild carnivores. Phylogenetic analysis revealed that all identified CDV strains were related and part of a single transnational European CDV epidemic characterized by a widening of the host range and an increased virulence. This epidemic seems to move from East to West along the Alps. Our research project (funded by the Ministry of Health) aimed to fill the gap about the circulation of CDV in North-Western Italy (NWI; Piedmont, Liguria and Aosta Valley regions). From 2013 to 2015, 548 wild carnivores found dead, hunted or euthanized because of irreversible pathological conditions were collected and analyzed. Each animal was necropsied, observing suggestive CD lesions, especially on lung and eyes. For 543 animals CDV screening tests, as Fluorescent Antibody Test (FAT) and/or RT-PCR on step were performed on lung and nervous tissue. Data from animals with at least one positive screening test were analysed with S.A.S. software (version 9.4). At age level, statistical analysis showed a raw prevalence of 39.67% (32.61%, 46.74%; IC 95%) in young animals and of 31.32% (26.45%, 36.19%; IC 95%) in adults (χ^2 3.730; p-value 0.0534). Animals found dead had a raw prevalence of 42.07% (36.39%, 47.75%; IC 95%), while symptomatic animals 62.50% (51.32%, 73.68%; IC 95%) and hunted animals 9.83% (5.39%, 14.26%; IC 95%). Differences were statistically significant (χ^2 79.052; p-value < 0.0001). The diagnostic protocol was integrated with neuropathology and histopathology evaluations on selected animals with anamnesis or gross lesions suggestive of CD. Microscopic findings were in agreement with those described in previous scientific literature except for the frequent detection of intracytoplasmic or intranuclear inclusions in the transitional epithelium of urinary bladder. It is interesting to point out how in the samples examined, even in the absence neuropathological lesions, positivity to the immunohistochemical examination has been observed. This could be connect with death at the earliest stage of infection. Also a serological test on blood samples was performed by an ELISA kit (Biopronix-Agrolabo). Although the protective antibody titre in the wildlife is not known, the obtained seropositivity (33%) confirmed a CDV circulation. Whereas the samples panel was constituted for half by animals found dead, their conservation status prevented efficient viral isolation and affected the number of isolates that could by characterized by sequencing. Nevertheless, CDV was isolated in 6 foxes and 3 badgers using Vero Dog SLAM cell line. Regarding phylogenetic analysis, all sequences that could be analyzed (n= 31) were carriers of amino-acid mutations at position 266 (L → F), 590 (S / T → I) and 597 (R → H) typical of the Wildlife Europe 2006-2009 cluster. Therefore, molecular characterization showed that the CDV circulating in NWI was closely related to the emergent CDV strain identified in the Alpine regions and Central Europe. Moreover, the detection of the same strain in different species and the presence of the amino acid mutations Y →H at position 549, suggestive to be responsible for a widening of the host range confirms the hypothesis of a multi-host epizootic spread. The lack of enough knowledge of the disease epidemiology in our territory hampered to define a proper sampling plan to determine accurate prevalence and incidence values of CDV. On the bases, this research project was structured with the aim of acquiring of information on CD ecology, as well as of developing diagnostic workup procedure also applicable to other wildlife diseases.

CASE REPORT OF DEMODECTIC MANGE IN BADGER (*MELES MELES)* IN ALTO ADIGE

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Wild mammals are affected by a wide number of parasitic species, coping with these by means of their fitness related to individual factors (as age, sex, physiological and nutrition status, genetics) and population/environmental conditions (as population distribution and density and resource availability). Concerning carnivores, malnutrition represents an important factor influencing susceptibility to parasitic infection. Demodectic mange, due to *Demodex* mites, is a parasitic disease transmitted through direct contact. Mites are elongates, 200-400 μm length, inhabiting the hair follicle from where they provoke a complex hypersensitivity reaction of the skin.

Here we describe a case of a demodectic mange in a badger *Meles meles* in an Alpine valley of Bolzano province. The badger was a one year old male that was sent to Bolzano lab of the Istituto Zooprofilattico Sperimentale delle Venezie the 15th of May 2016. The specimens was found at Campo Tures, 46°57'5''N and 12°3'38''E, at 1500 m asl.

The specimens presented large cutaneous scabby inflammatory lesions with severe dermal thickening mostly present at neck and on the back, less invading the head. Also some myasis was present on the back wounds.

The skin was examined and samples were prepared by scraping the skn lesions and later with clearance with KOH and mounted on glasses for microscopic observations. In all the samples an important presence of *Demodex* sp. was evidenced.

The case is the first report of a *Demodex* infestation in a badger in Italy and the identification of the species of the parasite is undergoing, considering the morphology and genetic of this particularly complicated taxon.

Sottosessione 2B
ECOPATOLOGIA DEGLI UCCELLI

Coordinatori: Guido Grilli & Gabriella Vaschetti

COMUNICAZIONI

MOLECULAR DETECTION OF SHIGA TOXINS (*STX1*, *STX2*), *EAEA* AND *HLYA* GENES OF *ESCHERICHIA COLI* IN WILD BIRDS

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Shiga toxin-producing *Escherichia coli* (STEC) are important human pathogens. These bacteria could be the cause of serious illness in man, such as haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS), and sometime death can occur. In Europe STEC represented the fourth cause of zoonosis. In addition to the classical STEC serotype O157:H7, other non-O157 serogroups *E. coli* have been frequently involved in human infections in recent years (O26, O76, O91, O103, O111, O128, O145, O146). The main virulence factors responsible of STEC-associated disease are Shiga toxins 1 and 2 (Stx1, Stx2), that have the same mode of action even if they are immunologically distinct. The Stxs (also known as Vero toxins) are a group of bacterial protein toxins that inhibit the protein synthesis in sensitive eukaryotic cells. Furthermore, most strains associated with human illness possess genes responsible for the 'attaching and effacing' histopathological lesion in the intestinal epithelium. In particular, *eae* gene encodes the outer membrane protein intimin, which mediates the attachment of *E. coli* to the epithelial lining of intestine and effacement of microvilli. Another virulence factor is enterohaemolysin that promotes the intestinal invasion. In general, STEC are not pathogen for animals that represent asymptomatic carriers. Bovine is considered the main reservoir host, even if other animal species can harbor these bacteria at intestinal level. Some authors have suggested that also avian species may be involved in STEC spreading, even though data available in literature about this topic are scant.

The aim of the present study was to evaluate the spreading of STEC in wild birds. From January 2015 to December 2016, 100 gut samples were collected from wild birds during routinely investigations. In particular, 57 samples were collected from hunted animals: 22 common teals (*Anas crecca*), 15 mallard ducks (*Anas platyrhynchos*), 11 Eurasian wigeons (*Anas penelope*), 3 common shelducks (*Tadorna tadorna*), 3 northern shovelers (*Anas clypeata*), 1 pintail (*Anas acuta*), 1 Eurasian coot (*Fulica atra*) and 1 common wood pigeon (*Columba palumbus*). Furthermore, 43 samples were collected from birds dead at a recovery center: 35 seagulls (*Larus michahellis*), 3 common kestrels (*Falco tinnunculus*), 2 grey herons (*Ardea cinerea*), 1 little owl (*Athene noctua*), 1 peregrine falcon (*Falco peregrinus*) and 1 Eurasian sparrowhawk (*Accipiter nisus*). After DNA extraction, the following genes were investigated with different PCR assays, respectively: *stx1* encoding for Shiga toxin 1, *stx2* encoding for Shiga toxin 2, *eaeA* encoding for intimin, and *hlyA* encoding for haemolysin.

Overall, 16 (16%) samples resulted positive for at least one of the investigated genes. In detail, 6 (6%) samples were positive only for *stx1*, 5 (5%) only for *eaeA*, 1 (1%) for *hylA*, 2 (2%) for *eaeA*+*hlyA*, 1 (1%) for *eaeA*+ *stx1*, 1 (1%) for *stx1*+*stx2*+*eaeA*+*hlyA*. In conclusion, 8 (8%) birds could be considered STEC positive: 3 seagulls, 1 little owl, 1 grey heron, 1 common teal, 1 Eurasian wigeon and 1 pintail each. Only 1 specimen, collected from a little owl, had all the searched genes.

These results suggest that wild birds can be carrier hosts for different types of pathogenic *E. coli*. In particular, some of the examined animals harbored Shiga-toxins producing *E. coli*, other birds had enteropathogenic *E. coli* strains, other enterohaemorrhagic *E. coli* strains. All but one of the positive samples were from water birds, suggesting a possible correlation with habitat and/or diet. However, the only samples positive to all genes was collected from a bird of prey placing emphasis on the involvement of these animal in STEC epidemiology. Three samples positive to *stx1* were collected from migratory hunted birds (common teal, Eurasian wigeon and pintail): this finding highlights the role that these animals cold have in the spreading of bacterial pathogens in the environment, even for long distances, and the possible risk for humans during carcasses manipulation.

THE MICROBIOTA OF HEMATOPHAGOUS ECTOPARASITES COLLECTED FROM MIGRATORY BIRDS IN ITALY

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Arthropod vectors are responsible for the transmission of a large number of human pathogens worldwide, including viruses (e.g. West Nile virus, Zika virus), bacteria (e.g. Borrelia, Rickettsia) and parasites (e.g. plasmodia, trypanosomes). A number of these arthropods are ectoparasites of birds. To our knowledge, no study has been carried out to characterize the microbiota of ectoparasites collected directly from birds. Arthropod symbionts play a pivotal role within the microbial community, interacting with pathogens. Here, we sampled ectoparasites feeding on migratory birds during ringing sessions, and then we performed 16S rRNA gene metabarcoding to characterize their microbial community, with a special regard to pathogens.

A total of 116 pools of ectoparasites were processed for metabarcoding and classified into three groups: a) Hyppoboscidae diptera; b) ticks; c) other ectoparasites. Arthopods were collected from resident, short- and long-distance migratory birds. The most represented genera were endosymbionts: Wolbachia for Hyppoboscidae diptera, Candidatus Midichloria for ticks, Wolbachia and Arsenophonus for the other ectoparasites. The metabarcoding identified the following potential pathogens: Rickettsia, Ehrlichia, Borrelia, Coxiella, Francisella, Bartonella, Anaplasma. Molecular tests confirmed the presence of Rickettsia spp. (40.5% prevalence), B. valaisiana (8.6%) and A. phagocitophilum (1.7%). Ehrlichia was not confirmed, but further analyses showed that these reads were misclassified as E. ovina, and belonged mainly to Candidatus Midichloria, Anaplasma and Wolbachia. Also, co-infections were observed: Borrelia-Rickettsia (n=9), two of which occurred in individual samples, and Anaplasma-Rickettsia (n=2).

Our study showed that NGS applied to the 16S rRNA gene metabarcoding allowed a global overview on the bacteria present in/on the ectoparasites collected alive from migratory birds in Italy, including symbionts and pathogens. Targeted PCR tests confirmed part of the detected pathogens, and the not confirmed were probably symbionts not detected by the diagnostic PCRs. The high presence of symbionts in all the samples may be of interest if we consider that a pathogen like C. burnetii evolved from a tick-borne symbiont.

This approach was a valuable method to detect multiple potential pathogens in one shot using universal primers. The targeted investigation confirmed the presence of the pathogenic species only on a reduced sample number.

HEAVY METALS PRESENCE IN HOODED CROW (*CORVUS CORNIX*) AS INDICATOR OF ENVIROMENTAL CONTAMINATION

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Heavy metals that are present in fertilizers remain in the ground and are absorbed by organic components, thus contaminating surface water. These elements are absorbed by plants, especially in acidified soils, and are then consumed by the animals, including humans. Once absorbed, cadmium and lead bioaccumulate in high concentrations in liver and kidneys. This cycle may cause the decline of populations of some avian species in intensively farmed areas. This may also directly affect humans that consume contaminated water and plants and animals, and secondary contamination of lead and cadmium in edible parts of animals is also of concern. Monitoring the concentrations of contaminants within the tissues is an useful way of assessing the potential damage of chemicals to organisms and their predators or scavengers. Liver and kidneys are the most important organs for detoxification processes. Lead poisoning manifests itself as a chronic or acute disease in wild birds. Signs usually include progressive weakness, green-stained feces and vent, deposition of eggs with thin shells, disorders of the central nervous system, weight loss and emaciation, as well as making no attempt to escape in the presence of humans. Lesions consist of severe wasting of breast muscles, impaction of esophagus or proventriculus, enlarged gallbladder containing dark green bile, and hemorrhagic enteritis. High levels of lead exposure decreases resistance to infectious disease and can result in nestling and adult death. Cadmium is an extremely toxic element; it is able to cause both acute and chronic tissue lesions and has been found to have teratogenic, mutagenic and carcinogenic effects. The aim of this study is to determine the prevalence of lead and cadmium levels in kidney and liver of hooded crowns (*Corvus cornix*) collected from province of Cuneo (Piedmont, Italy) and to compare the levels recorded in hooded crows that reside in intensely cultivated territory where chemical fertilizers are used, with those of hooded crows that reside in regions used for pasture where no chemical fertilizers are used. Hooded crows are quite sedentary, never venturing far from their nests and can therefore be considered as residing in this contained area. In addition, their propensity to scavenge, their trophic level, and the fact that they are abundant, makes them candidates for bioindicators of environmental contamination by lead and cadmium. The crow may be contaminated either by consuming water and forage directly or by eating animal carcasses with the pollutants. We found that lead is stored in a non-significantly different proportion in the two tissues (W = 7,803, p = 0.987), while cadmium tends to accumulate more in kidneys than in the liver (W = 5,206.5, p = 0.000). Both in kidneys and livers, the differences of lead and cadmium levels between the three age categories (young, subadult, adult) are highly significant ($p < 0.001$), with the exception of the case of lead for categories subadult and adult (in liver: W = 196.5, p = 0.925; in kidney: W = 174.5, p = 0.0289). Lead and cadmium levels recorded from category adult are significantly higher than those from category young: for lead in liver W = 66.5, p = 0.0009; for lead in kidney W = 190.5, p = 0.0000; for cadmium in liver W = 82, p = 0.0000; for cadmium in kidney W = 29, p = 0.0000. Cadmium levels recorded from category adult are significantly higher than those from category subadult: in liver W = 120.5, p = 0.0014; in kidney W = 69.5, p = 0.0000. We also compared levels obtained in the municipality of Caramagna Piemonte with those of the other municipalities considered in this study, and the observed differences turned out to be highly significant ($p < 0.0001$): median for lead in liver: 0.00 versus 0.08 mg kg^{-1}; median for cadmium in liver: 0.04 versus 0.19 mg kg^{-1}; median for lead in kidneys: 0.00 versus 0.11 mg kg^{-1}; median for cadmium in kidneys: 0.09 versus 0.42 mg kg^{-1}. Hooded crows, when subjected to population control, could be used as valid bioindicators of environmental contamination by heavy metals due to their large geographical distribution, feeding habits and easy sample collection. The high tolerance of the hooded crow to heavy metal allowed us however to determine exceptional extreme values of contamination. In particular, we highlight the value of 9.42 of cadmium in the kidneys of an adult male from Murello and to the value of 2.93 of lead in the liver of an adult female, also from Murello. These are extreme values, previously unreported in passerine birds similar to those of carnivorous birds, perhaps due to their propensity to scavenge. While lead content is similar in both liver and kidneys, cadmium tends to accumulate in kidneys, rather than in the liver, as already observed in other species. The trend to bioaccumulate in different tissues is evidenced by the statistically significant differences of the median values, considering the three age categories: young, sub-adult and adult. Adults and sub-adults have, on average, higher levels of contamination. The principal difference between the two habitats compared in this study is in the practices of agricultural production: in the municipality of Caramagna Piemonte there are pastures and meadows dedicated to the production of hay. The others municipalities are devoted to intensive cultivation for which inorganic fertilizers are essential to obtain high productivity. Among the inorganic fertilizers, phosphorus fertilizers in the Province of Cuneo are still widely used: 1.6 kg per hectare of area in 2004. However, they contain heavy metals that can contaminate the soil and threat the health of animals and humans and European Commission is aware of the need to take action in accordance with its risk reduction strategy. The main source of lead and cadmium contamination, in this study, could be identified as being phosphate fertilizers.

SPREAD OF *TOXOPLASMA GONDII* IN THE EURASIAN MAGPIE AND EURASIAN KESTREL FROM THE EMILIAN TERRITORY

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The present study is about the investigation of the *Toxoplasma gondii* diffusion in two wild ornithic species, very common in the Italian Northern Regions, the Magpie (*Pica pica*) and the Eurasian (or common) Kestrel (*Falco tinnunculus*), in particular taking in exam healthy birds trapped in the context of a mitigation project of the birdstrike hazard in the pertinences of the "Giuseppe Verdi" airport of Parma . The mitigation hazard project foresaw the capture, the birds marking with numerated rings and the following release in a station located at 18 km from the airport; it was observed a certain homing phenomenon with 22 recapture case on 345 total captured and transferred kestrels, for that birds had been possible to have second blood sample .

For both species, the diagnostic research was conducted through serology employing the micro agglutination technique (MAT) considering two dilution level 1:5 (cut-off) and 1:25. Muscular and myocardial sample of magpie (species subject to culling management plan) were molecularly analyzed in RT-PCR (target sequence 529 bp). For the magpie, the research in MAT and RT-PCR were not performed on the same birds group.

In the Magpies was observed a prevalence of 43% (n° tested birds=21) with the RT-PCR and 47% (n°=16) of which the 29% with high title 1:25 performing the MAT; four Magpies RT PCR positive were genotyped with the PCR-RFLT highlighting the circulation of intermediate types II / III (1 sample) and III (3 samples).

For Kestrels the study was made for a part of the subjects captured, considering the age class, highlighting a prevalence of 87% (n°=31) for subjects over 1 years of age (17 adults and 14 sub-adult,

at least first molting), of 31% (n°=55) for young subjects included in the age class between 45 to 75 days of life and of 45% (n°=55) for young birds with an age between 120 to 190 days of life.

An interesting data comes from the observation of the antibody titles, for the adults the 50% of positive was with low title 1:5 , in the sub-adults (at the first molt) the 28% and in the young 15 %, in addition it was observed two cases of seroconversion in two young kestrels recaptured after 30 days from the first capture and sampling, both in the second sampling with title 1: 5, based on the observations, we could hypothesis that low titles 1: 5 is indicating newly acquired infections or chronic forms .

Data showed the presence of *T.gondii* in the Magpie and Kestrel populations of the study area and in consideration of remark made the two species could to contribute to the maintenance of the parasite in the environment, acting as a concentrator through the predatory activity on micro mammals (and through the consumption of carcass by the magpies) making the parasite available again to their death for saprophytic homeothermic animals (including rodents), moreover, in view of the above, we could take in consideration sedentary carnivorous birds, as Magpie and Kestrel, as sentinel species for the estimation of *T.gondii* oocysts environmental charge and the infection hazard for a specific environment of study.

ALPINE GALLIFORMES, DOMESTIC BIRDS AND INTRODUCTIONS FOR HUNTING: SANITARY AND MANAGEMENT RISK FACTORS

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Biodiversity in natural ecosystems is the essential condition for their existence. This concept has to be related to at least three interdependent contexts of diversity: natural ecosystem diversity (regarding environments in which life is present), specific diversity (defined "species richness" in a specific area) and genetic diversity (variety of genes of a species). Since each ecosystem is the result of the interaction of various species, both plants and animals that live in, it is sufficient that one of these species is missing to compromise the dynamic balance of the whole ecosystem.

In the Alps, the modification of natural habitats due to the abandon of traditional activities and to the development of tourist infrastructure, above all those for winter sports; the extinction of native species; the introduction of foreign species; the appearance of new parasite strains and, finally, climate changes are source of extreme danger to biodiversity conservation. Conservation and management of threatened species have an essential role in the biodiversity maintenance; for this reason, during last years, veterinary medicine has enlarged its knowledge beyond traditional clinical and zootechnical fields.

Because of their high biological and conservational value, alpine Galliformes are considered as the target species of this concept. In the Italian Alps, both hunted species, such as alpine rock partridge (*Alectoris graeca saxatilis*) and white ptarmigan (*Lagopus muta*); and protected species, such as capercaillie (*Tetrao urogallus*), are in the red list of IUCN and considered vulnerable; while black grouse (*Lyrurus tetrix*) and hazel hen (*Tetrastes bonasia*), although not threatened, need precautionary measures of management. Notwithstanding, shared guidelines for the management of hunting activity, agroforestry and tourism are still missing. Moreover, attention is seldom paid to the health status of these populations, including possible interaction with rural poultry and or farmed wild birds that are released for hunting purposes.

Genetic interactions have been largely demonstrated between rock partridge and red-legged partridge (*Alectoris rufa*), not only in Maritime Alps where the two species are innately present, but also where red-legged partridge is not native (Western Alps), as evidence of illegal introductions for hunting. Moreover, other studies and photographic evidences have documented the introduction of some chukar partridge (*Alectoris chukar*), very similar to rock partridge, but very unsuitable for the alpine territory.

Furthermore, releases of grey partridge (*Perdix perdix*), quail (*Coturnix coturnix*), and pheasant (*Phasianus colchinus*) for hunting dogs training are still common not only in low-altitude areas less suitable for alpine Galliformes but also in their typical environments. In addition, the presence of domestic avifauna in the alpine pastures should be considered, that could contaminate the soil with gastrointestinal parasites, such as *Heterakis spp* and *Raillietina spp*, found in rock partridge and black grouse population. It is proven that alpine Galliformes evolved together with some gut parasites, such as *Ascaridia compar*, *Aonchotheca caudinflata* and some Coccidia, reported in alpine, Scandinavian, Icelandic and Scottish populations; but it is necessary to pay attention to new helminth strains and to the interaction between host and parasite. In this respect, it should be noticed as an example the completely lack of gut parasites in some meta-populations of white ptarmigan in the Alps, in particular Western Alps. This imbalance, probably linked to critical events influencing population dynamic, underlines white ptarmigan vulnerability. Climate changes on Alps had caused temperate and bit snowy winters and hot and wet summers, negatively affecting alpine Galliformes survival and the evolution of their environment, also determining higher survival of infectious parasitic stages in the soil. In fact, UV rays are less able to penetrate the expanding forest cover, thus losing their role in limiting the vitality of helminth eggs; while the lower thermal excursion and the fewer number of days with under 0 °C temperature promote eggs survival also during winter season. This situation may therefore determine an increase of gut protozoa and helminths in relation to the habitat of the species and to the climate conditions.

Stress is finally another important factor able to influence alpine Galliformes health status. Winter and summer tourism in suitable area for the species is demonstrated to have a negative effect on these populations in relation to the use of their habitat and to the health and immune status of hosts.

For all the reasons above, cooperation between wildlife managers, wildlife conservation experts and veterinarians is needed, to develop a risk profiling model and draw situation based guidelines for both rural poultry raising and famed game birds release in alpine areas important for the conservation of Alpine Galliformes and for biodiversity in general.

POSTER

DETECTION OF ZOONOTIC AGENTS FROM WILD BIRDS OF FRIULI VENEZIA GIULIA REGION

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Wild birds, particularly migratory species, may carry zoonotic pathogens that can be directly or indirectly transmitted. In the Friuli Venezia Giulia region (located in northeastern Italy), wild birds are under sanitary surveillance for Avian Influenza (AI) and West Nile Disease. Passive surveillance includes the monitoring of mortality events. The aim of this work is to focus on the data obtained from general and scanning surveillance activities implemented in the last five years. A total of 381 carcasses were delivered to the laboratory during the study period: 190 Passeriformes, 132 Columbiformes, 26 Anseriformes, 13 Galliformes, 12 Pelicaniformes, 10 Ciconiiformes, 7 Falconiformes, 2 Strigiformes and 1 Charadriiformes. Necropsies, parasitological and bacteriological examinations were performed according to the clinical or epidemiological suspect and following standard protocols. Search for avian influenza viruses was generally performed all year round, whereas analyses for West Nile virus were conducted only during the risk-period (July-October).

Causes of mortality included an outbreak of avian botulism type C in mallard ducks (*Anas platyrhynchos*) and frequent avian paramyxovirus-1 (pigeon paramyxovirus 1) cases in Columbidae (*Columba livia* and *Streptopela decaocto*).

Zoonotic bacterial pathogens were detected sporadically and only at the occurrence of mortality outbreaks. They included isolation of *Salmonella* spp. (*S.* Typhimurium and *S.* Houtenae) from 1 Northern bald ibis (*Geronticus eremita*), 6 sparrows (*Passer -domesticus- italiae*) and 1 grey heron (*Ardea cinerea*). *Vibrio cholerae* was detected from 2 mute swans (*Cygnus olor*); these animals had been recovered in a single hotspot. The only parasitological evidence was a case of sarcocystosis in a mallard duck. Avian Influenza viruses emerged only last winter (2016-2017), with 4 cases of HPAI in migratory birds: 1 case of H5N5 in a widgeon (*Anas penelope*) and a gadwall (*Anas strepera*) and 1 case of H5N8 in a widgeon and a mute swan.

Salmonella species occurred as pathogenic bacteria in wild birds in the focal area, although mortality events had already been recorded in passerines, involving in particular green finches (*Carduelis chloris*). A great concentration of animals, as in the case of feeding stations, may favour the transmission of this pathogen. Although not frequently, these episodes are considered as possible source of transmission to humans and domestic animals.

V. cholerae has already been reported in waterfowls in different countries, making us assume that these species may be carriers of the bacterium, while it is still unclear whether it plays a crucial role in swans mortality.

The emergence of highly pathogenic AI viruses highlights the need for a continuous detection of mortality events in free-living birds, aquatic species in particular, in the frame of routine surveillance in order to promptly detect cases of infection and disease.

HEALTH SURVEY OF NORTHERN BALD IBIS (GERONTICUS EREMITA) ON CAPTIVE COLONY IN ITALY

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A survey was conducted at the “Oasi dei Quadris” of Fagagna (Udine, Italy) on a captive colony of Northern Bald Ibis (*Geronticus eremita*, Linnaeus, 1758) with the aim to investigate the health condition of the colony. The Northern Bald Ibis is a large bird in the ibis family Threskiornithidae, and it is classified as Critically Endangered by the IUCN. In Europe, the specie is present only in captive colonies, which represent an important resource for projects aiming to increase the knowledge on its biology and ecology. The studied colony, composed of 80 birds of different ages, was situated in a large aviary with a natural grass ground, a small artificial stream and wooden shelters. The birds of the colony were fed with a mix diet composed by beef hearth, mice, chicks and mealworms. Inside the aviary, there was also a small group of white stork (*Ciconia ciconia*, Linnaeus, 1758). During 10 months between 2009 and 2010, 40 ibises were sampled for faeces (twice in the study period for coprological analysis) and blood, and external examination for ectoparasites was also performed on 20 subjects. The birds were habituated to the close presence of humans, and each bird was marked with an individual combination of coded colour rings. Individual droppings were collected immediately after defecation on clean plastic sheets placed under the perches and kept in plastic tubes inside a portable fridge until the arrival at the laboratory, where the faecal smear and flotation techniques have been performed. Blood samples were collected from the ulnar vein and stored in plastic tube with EDTA; at the laboratory, the thick and the thin blood film tests were performed. Faecal and blood subsamples were sent to an external laboratory for serological and microbiological analysis. During the sampling period, in the aviary 3 birds have been found dead which were necropsied and gastro-intestinal contents were analysed by total worm count technique.

Serological and faecal microbiological analysis did not show any relevant pathogen. Copromicroscopy by flotation detected few parasitic elements (prevalence 21.2%), consisting of eggs and larvae of nematode (probably strongyles). No parasites were observed in the blood. The 75% examined ibis for ectoparasites were infested with phthirapteran specie, *Ardeicola geronticorum* (Brinck, 1955). This finding represents the first record of this specie in Italy and could be confirm the Moroccan origin of the captive colony. The necropsies showed no presence of parasites, and attributed the causes of death to non-infective agents.

TICKS AND TICK-BORNE PATHOGENS: AVIAN ROUTE OVER DENSELY POPULATED AREA IN NORTHERN ITALY

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Migratory birds can transport ticks and related tick-borne pathogens (TBPs) of humans and animal importance over long geographic distances. In addition, many avian species are also considered reservoirs of several TBPs. Over the last decades, climate and environmental changes have contributed to drive the range expansion of some tick species to northern latitudes and to higher altitudes. Similarly, ticks and related TBPs are rapidly expanding from wild and natural areas to urban and peri-urban areas increasing human health risks.

In our survey, migratory birds were intercepted and captured by nets in the ringing station Fondazione Europea il Nibbio–FEIN (Arosio, Como, Italy 45°43'54.87'' N, 9°12' 40.10''E, 353 m a.s.l.) during their back migration from central Europe. The ringing station is located in a woodland area at the border of a densely populated and infrastructure-rich area in northern Italy. The aim of this study was to investigate the presence of four important zoonoses-causing bacterial agents (*Rickettsia* spp., *Borrelia burgdorferi* sensu lato complex, *Francisella tularensis* and *Coxiella burnetii*) in ticks collected from trapped birds. All the collected ticks, larvae and nymph, were identified as *Ixodes ricinus* and subsequently PCR-screened for the presence of TBPs. Four species of *Borrelia* (*B. afzelii*, *B. garinii*, *B. lusitaniae* and *B. valaisiana*), proved or suspected to cause clinical manifestations of Lyme disease in humans and three species of *Rickettsia* (*R. helvetica*, *R. monacensis* and *R. mendelii*) were detected respectively in 74 (29.5%) and 25 (10%) out of a total of 251 examined ticks. Co-infection with both pathogens was encountered in 7 out of 99 (7.1%) infected ticks. These results confirm the role of birds in tick dispersal and provide an explanation to the observed spreading of ticks and ticks-borne pathogen outside their own elective environments.

RETROSPECTIVE ANALYSIS OF DATA OBTAINED FROM POSTMORTEM INVESTIGATIONS CARRIED OUT ON TRAUMATIZED BIRDS FROM 1996 TO 2016, SÃO PAULO, BRAZIL

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Until now, no retrospective studies on mortality and morbidity of birds in Brasil have been carried out, even if the avifauna is rich, lives in anthropogenic contexts and a large number of its species are in risk of extinction. Several studies reveal that trauma is the most frequently cause of entry and mortality in the rehabilitation centers: collisions between wildlife and human-structures (vehicles and buildings) are a common cause of injury or death especially in birds. Interspecific and intraspecific interactions can result in traumatic lesions to both free-living and captive animals but territorial defense, self-mutilation and aggressions are described especially in captive birds. The aim of this study was to evaluate biological, clinical, environmental data and anatomopathological findings of traumatized birds submitted to the Laboratory of Wildlife Comparative Pathology, Department of Pathology, School of Veterinary Medicine and Animal Sciences, University of Sao Paulo, between 1996 and 2016. A total of 327 necropsy reports were selected and birds were divided in 22 orders and 113 species. Cases were classified according to order, species, date of death, date of necropsy, year, season, and month of reception, age-class (adult, young, immature), gender (female, male), body condition (good, regular, bad), weight, provenance, housing condition (captive or free-ranging), terms of death (euthanasia or spontaneous), clinical suspect (where present), history (where present). Gross finding were divided in traumatic and non-traumatic lesions: most of the birds presented both of them (218; 67%), suggesting a predisposition to trauma in debilitated animals. The primary systems affected by traumatic findings were skeletal muscle (232; 70.9%) and integumentary one (151; 46.2%), while non-traumatic findings were found mainly in the alimentary (219; 63.9%) and respiratory (143; 43.7%) systems. We first analyzed the necropsy findings focusing on causes of traumatic injuries: besides the "not reported" data were the majority (243; 72,4%), we grouped all the causes in "human-associated" (34; 10.4%) and "animal associated" (56; 17.1%) traumas. They comprehend, respectively, building collisions (7, 20,5%), vehicles collisions (8; 23,5%), wires trapping (12, 35,3%), gunshot (5; 14,7%) and traps (2; 5,9%) associated with human activity, while inter and intraspecific aggressions (26; 46,4%), predations (22, 39,3%), not confirmed aggression (6; 10,7%) and falling from the nest (2; 3,5%) were grouped as animal-associated traumas. In all these categories recurrent necropsy findings were described, such as the head localization of the lesion after building collision (71,4%) and this could provide an interpretation key to suppose a possible traumatic event as cause of hospitalization for birds received without history. Most representatives orders of birds were grouped in categories according to common biological and ecological features: birds of prey - Accipitriformes, Cathartiformes, Falconiformes and Strigiformes - residential avifauna (mainly captive birds) - Anseriformes, Galliformes, Psittaciformes and Piciformes - and "urban" birds - Apodiformes, Charadriformes, Columbiformes, Passeriformes and Pelecaniformes. Orders of each group are connected by similar percentage of pathological traumatic findings and a relation with the same causes of trauma previously described was found. Although in this study lack of information in necropsy reports was a limiting factor, a standardization of traumatic lesions that characterize single and similar orders, linked with physical, biological and ecological features could allow to describe single species' predisposition to incur death in determined contexts and eventually take action to avoid this occurrence.

Sottosessione 2C
ECOPATOLOGIA DELE SPECIE ACQUATICHE

Coordinatori: Paola Beraldo & Andrea Gustinelli

COMUNICAZIONI

ALIEN FISH SPECIES IN ITALY: AN OVERVIEW ABOUT STATUS AND HEALTH RISKS TO AQUATIC BIODIVERSITY

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Alien species are species introduced by humans (either intentionally or accidentally) outside its natural past or present distribution. Synonyms of "alien" that are widely used are: exotic, allochthonous, introduced, not native species (NNS) or not indigenous. Otherwise, a species occurring in its original area is defined as autochthonous or native or indigenous. In Italy, Zerunian (2004) reports the presence of 48 autochthonous freshwaters species (with 22 endemisms). Nocita and Zerunian (2007) reports 38 allochthonous species, 13 of them are naturalized. The last check list drafted by Italian Association of Freshwaters Ichthyologist (A.I.I.A.D.) in 2016 reported the presence of 72 allochthonous species. The increase is also due to the implementation of new genetics techniques and biogeographic knowledge. The most common negative effects that an alien species may cause are: 1) predation; 2) competition with autochthonous species; 3) changes of the habitat; 4) hybridization with indigenous species; 5) spreading of new pathogenic agents (parasites, bacteria, viruses, fungi) that may be more virulent to new hosts due to the lack of innate immunity in the autochthonous species. In Italy, the introduction of fish fauna from foreign countries, both for restocking or for aquaculture, has been going on for years. Problems related to international trade are not just an Italian issue. In fact, the presence of globalization and the paucity of rules and controls at frontiers has led to the introduction of alien species in the Member States and their spread throughout the territory. A classic example is the Anguillicolosis, a parasitic disease caused by *Anguillicoloides crassus* (Nematoda), introduced in Europe through the import of *Anguilla japonica* from Asia. It causes health problems (inflammation of the swim bladder and secondary bacterial infections) in aquaculture facilities and in eel wild populations. In addition, certain pathogens introduced with the import of live fish may potentially be dangerous for humans (zoonotic agents), such as some etheroxenic parasites present in freshwater fish. Health issues are not only related to the introduction of alien species from other countries, but are also extended to the handling of indigenous species from different geographic district within the national territory.

Thus, risks from the introduction of alien species are several: while the ecological risks have been repeatedly defined, and reaffirmed at national and international scientific meetings, the health risks are often marginally considered.

The aim of this presentation is to contribute to the assessment of alien freshwaters fish introduced in our territory and related health risks.

VIRAL ENCEPHALOPATHY AND RETINOPATHY: THE MAJOR VIRAL THREAT FOR MEDITERRANEAN FISH

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Viral Encephalopathy and Retinopathy (VER), otherwise known as Viral Nervous Necrosis (VNN) is caused by RNA viruses belonging to the *Nodaviridae* family, genus *Betanodavirus*. Their genome is constituted by two ssRNA molecules. The RNA1 gene encodes the viral replicase and gives rise to the sub-genomic transcript RNA3 translated into protein B2. The RNA2 segment encodes the capsid protein. Based on the phylogenetic analysis of the RNA2 molecule, betanodaviruses have been classified into four genotypes: RGNNV, SJNNV, BFNNV, TPNNV. Due to the segmented nature of their genome, fish nodaviruses can undergo reassortment events resulting in antigenic shift. In the Mediterranean basin, the occurrence of RGNNV, SJNNV and reassortants RGNNV/SJNNV and SJNNV/RGNNV have been documented.

Since its first description in Europe in the 1990s, Betanodaviruses have been considered a major problem for marine aquaculture, but they are also considered as an ecological hazard for wild fish. As a matter of fact VER virus has been detected in 160 fish species belonging to 79 families and 24 orders and this number is growing year by year. Recently an increasing number of outbreaks of VNN in freshwater fishes (farmed and wild) have been reported suggesting that salinity is not a limiting factor for the development of the disease. Furthermore the virus has been also detected in several marine invertebrates (shellfish, cephalopods, crustacean) and due to its extremely resistance to chemical and physical agents it can contaminate marine water for long time.

In the fish farms and in the hatcheries VER can cause severe diseases inducing up to 100% mortalities especially if associated to other pathogens (i.e. *Vibrio* spp., *A. ocellatum*) and due to the lack of properly effective prophylactic measures this disease represents a major constraint for fish farming sustainability. VER seems to be a considerable threat also for wild species, in particular for some species considered more susceptible than others: grouper (*Ephinephelus spp*.), flatfish (*Solea* spp.*, Scophtalmus maximus*), and drum (*Umbrina cirrosa, Argyrosomus regius*). Indeed, in the past years recurrent and severe VNN outbreaks have been reported in Mediterranean wild groupers (*Epinephelus* spp.).

From 2008 to 2013 a surveillance for VER prevalence in marine areas surrounding the Apulia region was performed with the collection of more than 500 wild fish samples. Fish were caught during scuba diving excursions and with experimental fishnets close to sea cages of a local sea bass/sea bream farm, in its surroundings as well as in some faraway areas. Surveillance was performed by real time RT-PCR and virus isolation. Several fish especially belonging to *Epinephelus* spp. and *Labridae* spp. were found to be positive with different prevalence according to the sampled species and the diagnostic method. The overall positivity for VNN was 24% suggesting a high prevalence in the area monitored.

In another survey, genetic and epidemiological data related to documented VNN episodes affecting wild groupers from Algeria, Greece, Italy and Tunisia between 2001 and 2014 were collected. Overall, 42 RNA1 and RNA2 partial sequences were obtained. The phylogenetic analyses indicated that all the strains detected in wild groupers were typed as RGNNV. The RNA1 topology showed that groupers betanodaviruses were distributed within six different genetic clusters, corresponding to specific geographic origins. On the contrary, the RNA2 phylogenetic tree highlighted that betanodaviruses from different locations were grouped within the same cluster, thus suggesting a low antigenic diversity among viral strains circulating in wild Mediterranean groupers. P-distance estimated among wild groupers betanodavirus sequences highlighted a higher genetic variability of the polymerase gene in comparison to the coat protein gene.

In both surveillance activities, the high similarities between some sequences from wild and farmed fish reared in close proximity of the observed outbreaks suggested inter-species viral exchange, although it was not clear which of the species was a source of infection for the others. The collection of genetic and epidemiological data, and the notification of new VER outbreaks in the farms and in the wild are crucial to deeply understand betanodavirus ecology and to develop strategies for preserving the Mediterranean wild fauna.

CRAYFISH PLAGUE CAUSED BY *APHANOMYCES ASTACI*

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The aetiological agent of crayfish plague, *Aphanomyces astaci*, firstly identified in 1903 in Germany, is an aquatic organism closely related to the Saprolegnia genus, which are responsible of skin fungal infections in freshwater fish. *A. astaci*, belonging to the Chromista phylum, Oomycota class, Saprolegniales order and Saprolegniaceae family, is the most infectious and lethal pathogen of freshwater crayfish all over Europe. The susceptibility to infection and the clinical course can vary considerably among the different species of crustacean, in particular the European species belonging to the Astacidae family, including the autochthonous *Austropotamobius pallipes*, *A. torrentium*, *Astacus astacus* and *A. leptodactylus*, which are extremely susceptible to plague. On the contrary, the North American species of the Cambaridae family, such as Louisiana's red shrimp *Procambarus clarkii*, *Pacifastacus leniusculus*, *Orconectes limosus*, are resistant to infection and act as biological carriers. The Australian family Parastacidae, which includes the genera *Euastacus*, *Cherax*, *Geocherax*, *Astacopsis*, are also susceptible, with the exception of the *Cherax* genus, which exhibits a certain degree of resistance. Due to the considerable pathogenicity of indigenous species, it is essential to know the symptoms of the disease, to ask the laboratories to perform correct sampling and diagnosis protocols and to avoid spreading pathogens by applying disinfection procedures to disinfect the equipment used during sampling. It is also a disease that shall be notified to O.I.E. (World Organization for Animal Health), so it must be reported to the local competent veterinary services and to the Ministry of Health.

PHYSIOLOGICAL RESPONSE OF RED SWAMP CRAYFISH TO WATER SALINITY

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Disturbance to ecosystems due to alien invasive species represents a well-known negative ecological phenomenon. The red swamp crayfish *Procambarus clarkii* (Girard, 1852) is one of the most threatening freshwater species widely introduced in the world, especially for its economic importance. This species is inserted in the Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014, on the prevention and management of the introduction and spread of invasive alien species.

P. clarkii has a large ecological valence, elevated adaptive capacity employing a r-strategy and acts as a vector for potential pathogens leading to a decline of biodiversity. Its high fertility, the plasticity of the reproductive cycle and the ability to withstand environmental extremes are making impossible its complete eradication from the colonized environments. The high tolerance to environmental factors, including salinity, indicates that this species could be able to invade wetlands, brackish water and lagoons causing ecological imbalances in transitional ecosystems and external waters.

Salinity, among the natural stressors, is an essential abiotic factor influencing several biological processes such as feeding, growth and reproduction, thus affecting survival and ecological success of animals in aquatic ecosystems. Imbalance of optimal salinity rate may also increase the production of reactive oxygen species (ROS) with changes in activities of antioxidant enzymes. Although the damages related to oxidative stress are not promptly identifiable, the effects can be extremely harmful leading to fitness reduction and even death. There are still scanty information concerning how red crayfish copes with large salinity changes in terms of the antioxidant mechanisms counteracting ROS production. Oxidative stress occurs when a system's antioxidant defenses (e.g., reduced glutathione [GSH], superoxide dismutase [SOD], catalase [CAT], and glutathione peroxidase [GPx]) are overcome by pro-oxidant forces, and ROS cannot be adequately removed, resulting in physiological impairment.

The aim of the present study was to determine if high salinity concentration may be a limiting factor for survival and dispersion of this invasive species. Furthermore, we investigated if long-term treatment with high salinity concentration may induce oxidative stress in *P. clarkii*. Specimens were collected from Lake Trasimeno (Central Italy), where the species established nearby 15 years ago. The crayfish population is very well acclimated in Trasimeno even if its water has a high conductivity value.

During the experiment both sexes were exposed to increased antimicrobic iodized salt concentration, up to 35‰, to simulate the natural transition from freshwater to marine water. Chemical and physical water quality parameters were monitored every day throughout the experiment in order to keep them constant and similar in all tanks. Several biochemical indicators, including SOD, CAT, GPx, glutathione S-transferase (GST), glutathione reductase (GR), and total glutathione (GSH + glutathione disulfide [2GSSG]), were measured in hepatopancreas and gills of both sexes of crayfish treated with increasing saline concentrations for 60 days.

Through the experiment, higher mortality was found for salinity-treated females than males. A tissue-linked change of antioxidant parameters was observed in the treatment groups and major variations occurred mainly in hepatopancreas, than in gills. Furthermore, a sex-linked change of oxidative stress biomarkers was recorded as major changes occurred in females, than in males. Although high mortality rate occurred in females under salinity pressure, the survived crayfish showed a strengthening of the markers levels of SOD, GPx, GR and GST. Survival of *P. clarkii* at very high salinity concentration as used in our experiment may allow us to understand if both sexes of red swamp crayfish are able to descend freshwater streams reaching brackish waters, lagoons and the shoreline of the Mediterranean Sea and, along the latter search for new water courses to ascend and so invade new environments favorable to its dispersion. Although our findings are the result of a laboratory experiment, they may help to improve predictive models inferring the invasiveness of alien species and for elaborating control strategies.

THE POSSIBLE USE OF UNMANNED AERIAL SYSTEM (DRONE) TO COLLECT INFORMATIONS ON WILD CETACEANS HEALTH STATUS

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In the last ten years, Unmanned Aerial Systems have been used extensively in various research studies on vertebrates because they can provide an economical and portable platform for aerial surveys of animals habitats and possible threats. Drones are an effective means of obtaining information performing low-disturbance surveys with minimum reactions in terms of behavioral and physiological responses. In recent years, their use has been incorporated in numerous studies on the field of different vertebrate species such as Sumatra orangutans (*Pongo abelii*), American alligators (*Alligator mississippiensis*), African elephants (*Loxodonta africana*), black bears (*Ursus americanus*) and other mammals species. However, the use of drones for studying and monitoring marine mammals species is still at an early stage. There are many publications regarding the use of marine mammals as sentinels of ecosystem health using blood samples, that provide the most accurate assessment of both individual and population health. However, obtaining blood samples from cetaceans is impractical without severe trauma or culling of the animal. Here we are presenting some preliminary results obtained during our research in order to assess a standard non-invasive method for sampling and analyzing blow samples of marine mammals collected using drones.

In order to set sampling and analytical methods on blows, a series of blow samples from a group of 6 under-human-care bottlenose dolphins (*Tursiops truncatus*) located at Genova Aquarium were collected; those dolphins belong to different sex and age classes. Blow samples were collected using both Petri dishes and 6-well plates, positioned at different heights over the animal's blowhole. Total RNA and DNA extraction from blow samples has been performed with two different methodologies: by pressure filtration method, using commercial kits following manufacturer's instructions and with TRIzol Reagent (Ambion, Thermo Scientific) following the supplier's protocol. The obtained nucleic acids were quantified using NanoDrop 1000 (Thermo Scientific), amplified employing PCR technique and sequenced.

Subsequently, the same feasible sampling method, assembled on a waterproof drone, was tested on a population of wild bottlenose dolphins living in the Ambracia Gulf, western Greece. Unfortunately, this kind of sampling, applied to wild small cetaceans, did not provide biological matrix for genetic and pathological biochemical studies. However, it was possible to identify the collection of microorganisms (microbiota) present in the dolphins' upper respiratory tract using 16S Illumina Amplicon Protocol, as previously isolated by other research groups from sterile swabs inserted into the blowhole during animal breath during bottlenose dolphins' capture and release programs.

Cetaceans could be considered crucial indicators of the health *status* of animals, and blowhole sampling using drones could be a truly technological advancement and a completely new remarkable approach to wild cetaceans health studies.

ANATOMO-PATHOLOGICAL FINDINGS ON ENCEPHALIC TISSUE OF STRANDED CETACEANS AFFECTED BY *TOXOPLASMA GONDII*

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Toxoplasma gondii is a parasitic protozoa, able to infect all warmblooded animals. Cats and other wild felids are the definitive hosts and can shed millions of oocysts into the environment. Anthropogenic factors could influence and alterate the ecology of this parasite. In this way, a massive amount of oocysts can reach and contaminate the marine ecosystem, where they can survive for several months. This turn toxoplasmosis into one of the most important emerging infectous disease for several cetaceans populations, representing a serious hazard to their health and conservation. Like other mammalian species, some cetaceans are characterized by a great susceptibility to *T. gondii* infection, due to their separated evolutionary history with this parassite. In these animals the main lesion observed is usually a severe nonsuppurative meningoencephalitis, characterized by multifocal perivascular mononuclear cell cuffing and areas of necrosis.

The aim of this study was to observe *T. gondii* lesions in different cetacean species living in two different marine condition, such as in shore and pelagic, and to classify them in order to define and compare the severity of the pathological processes.

In particular, on brain tissue slides of 10 striped (*Stenella coeruleoalba*) and 13 bottlenose dolphins (*Tursiops truncatus*), biomolecular positive for *T. gondii*, standard histological (hematoxylin & eosin) and immunohistological stains (anti-Glial Fibrillary Acidic Protein antibody and anti- Caspase 3 antibody) were performed. Microscopically examination of the slides were carried out and the differences of inflammatory and non-inflammatory patterns between the two species were detected and described. Striped dolphins, a pelagic species, showed more severe lesions than bottlenose dolphins, a coastal one that probably passed through a stronger host-parasite co-adaptation.

The results obtained in this study set up an anatomo-pathologic methodology finalized to analize and compare the differences between inflammatory and non-inflammatory pattern in encephalic tissue of different cetacean species, even if the outcome is not statistically significant due to the small sample size analyzed.

MARINE MAMMAL STRANDINGS IN ITALY: MANAGEMENT AND MONITORING OF THE CAUSES OF DEATH BY THE NATIONAL REFERENCE CENTRE (C.Re.Di.Ma.)

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The phenomenon of marine mammal stranding has always attracted the interest of the public opinion and the scientific community. As all cetaceans are protected by national laws and international agreements, monitoring their health is a priority not only for the non-profit organizations involved in their conservation, but also for public administrations. There are many factors that may interact to determine the death of these animals: infectious, non-infectious, anthropic. The first group comprehends agents such as viruses, bacteria, fungi and protozoa. Some of those, like Morbillivirus, may cause epizootic diseases that can affect cetaceans' population dynamics, while others have a zoonotic potential. Their detection helps in assessing the health status of coastal ecosystems and the potential risk of transmission to humans.

The second group includes degenerative and metabolic pathologies, neoplastic diseases, intoxications from algal biotoxins and injuries resulting from intra and inter-specific interactions.

The anthropic factors are related to human activity, in the form of accidental catches in fishing gear, collisions with boats, noise in the marine environment, low- and medium-frequency military sonar, environmental pollution and, in some cases, intentional killing. The causes of stranding are often considered multifactorial, within a general condition of immunosuppression; in this sense, non-infectious factors such as pollutants or other environmental factors can disrupt ecological balances in the marine habitat and contribute to promote the spread and expression of virulence of various infectious agents.

The examination of the stranded animals is a precious source of biological and diagnostic data, useful to assess the impact of human activity on the marine environment and the effect of pathogens on human and animal health.

In order to ensure harmonized diagnostic intervention at national level and a central point for the collection of diagnostic data, the Ministry of Health supported the research activity of Istituti Zooprofilattici Sperimentali (IIZZSS), creating a network of specialized laboratories established at each IZS, and the creation of a National Diagnostic Data Register (Re.Da.Ce.), managed by the IZS of Piemonte, Liguria and Valle d’Aosta, aiming at collecting the diagnostic results of all necropsies performed on marine mammals stranded along the Italian coasts.

Furthermore, thanks to its multi-year surveillance activity on cetacean strandings, the IZS of Piemonte, Liguria and Valle d’Aosta was recognized as the National Reference Centre for diagnostic activities on dead stranded cetaceans (C.Re.Di.Ma) by the Italian Ministry of Health (Decree of October 22, 2014), in order to ensure the availability of a reference technical-scientific instrument at national level for diagnostic intervention on stranded subjects and the determination of the causes of mortality, with particular reference to factors of infectious origin.

Summarizing, the objectives of C.Re.Di.Ma are the standardization of diagnostic protocols at national level (autopsy, sampling, laboratory surveys), with the aim of obtaining comparable diagnostic conclusions, the support to the activities of the IIZZSS network and the Ministry of Health, the collection and analysis of diagnostic results, focused to elaborate annual reports on the death’s causes, the promotion of research activity and the collaboration with other research institutions in Italy and abroad.

As for the field activity, in addition to guaranteeing the intervention on the animals stranded along the Ligurian coast, C.Re.Di.Ma., in collaboration with the Cetacean Emergency Response Team (C.E.R.T.), managed the unusual mortality event occurred in 2013 along the Tyrrhenian coasts, and supported the diagnostic activities performed on the occasion of an extraordinary stranding occurred in Vasto (CH) in 2014, involving 7 Physeter macrocephalus individuals.

Regarding the research activity, some projects are currently underway to implement the knowledge of Dolphin Morbillivirus (DMV), which is considered the pathogen of greatest concern for marine mammals (at least 9 morbilliviral epizooticss have occurred worldwide throughout the last 25–30 years). The activities are focused to implement diagnostic capabilities, to sequence the genome of DMV strains found in the Mediterranean Sea and to clarify some eco-pathological aspects of the disease, such as the influence of the animals' age and diet.

Considering the large diffusion of plastics in the marine ecosystem and the consequent risk for marine organisms, a new project is starting to investigate the presence of microplastics in odontocetes and mysticetes living in an area of the Mediterranean characterized by high anthropic impact, through the standardization of the analytical methods applicable at the level of the gastro-intestinal tract.

Finally, C.Re.Di.Ma is actively supporting the activities of the Ministry of Health and the Ministry of the Environment (MATTM), focused to create an efficient National Stranding Network, based on official guidelines, opportunely shared between all bodies involved in the management of a stranding event.

POSTER

GASTROINTESTINAL HELMINTH COMMUNITY OF *CARETTA CARETTA* IN THE NORTH WESTERN ADRIATIC SEA: PRELIMINARY RESULTS

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The Adriatic Sea is one of the most important neritic feeding grounds for *Caretta caretta* inside the Mediterranean basin. The diet of this species at neritic ontogenic stage includes a variety of benthic preys, as molluscan and crustacean species, that are often involved as intermediate host in the indirect life cycles of digenetic trematodes and nematodes. The aim of this survey was to describe gastrointestinal helminthic fauna of loggerheads feeding in northern Adriatic grounds and to compare it with surveys made in other Mediterranean areas.

In the period 2009-2016, 113 loggerhead turtles, found stranded dead along Italian north Adriatic coasts, were investigated for the presence of gastrointestinal (GI) parasites. Content of stomach and gut were examined by mean of a filtration-sedimentation process. The parasites were isolated at stereomicroscopy, counted and stored in 70% alcohol. Identification of parasites was achieved by observation of morphometric features at stereo and light microscopy and comparison with literature. Prevalence (P), mean intensity (MI), mean abundance (MA) and relative abundance (RA) were estimated for each parasite species.

Totally 62.8% (71/113) of the turtles were positive for the presence of GI parasites, with total burden ranging from 0 to 626 individuals (mean abundance: 32.4 helminth parasites per turtle). Eight species of two different taxa were found: *Sulcascaris sulcata* (P=23.9%; MI=29.7; MA=7.1; RA=21.9%) and *Kathlania leptura* (P=5.3%; MI=78.5; MA=4.2; RA=12.9%) among Nematoda. *Rhytidodes gelatinosus* (P=38.9%; MI=23.2; MA=9; RA=27.9%), *Pleurogonius trigonocephalus* (P=15.9%; MI=30.3; MA=4.8; RA=14.9%), *Pachypsolus irroratus* (P=22.1%; MI=11.0; MA=2.4; RA=7.5%), *Orchidasma amphiorchis* (P=19.5%; MI=11.8; MA=2.3; RA=7.1%), *Enodiotrema megachondrus* (P=15.9%; MI=15.8; MA=2.5; RA=4.2%) and *Calycodes anthos* (P=1.8%; MI=2.0; MA=0.0; RA=0.1%) among Trematoda.

All the species encountered in our study were already observed in other loggerhead populations. The number of isolated taxa (richness index: 8) is similar to that observed in precedent surveys in the Adriatic basin (Gračan et al. 2009). However, these values are lower than number of parasite species reported in turtle populations of north Atlantic neritic areas, where up to 16 different species of GI parasites were recorded in single turtles population (Greiner 2013).

R. gelatinosus and *S. sulcata* showed the highest values of prevalence and abundance. They were reported in all previous studies in the Adriatic Sea with similar prevalence. This finding copy with assumption that life cycles of these parasites involve benthic intermediate hosts, mostly present in the Eastern part of the Mediterranean basin and thus they are tipically present among the helminthofauna of neritic stage turtles. On the contrary, *E. megachondrus* and *C. anthos* life cycles is supposed to involve pelagic intermediate hosts, and their abundance and prevalence are usually higher in oceanic habitats. Nevertheless, these two species were encountered in the investigated population and *E. megachondrus* in particular showed an unexpected high prevalence value. The significance of this finding has to be further addressed.

In conclusion, the investigated population showed a moderate prevalence of infection. Light infection levels (<100 parasite specimens per host) were present in most cases (63/71). The influence of biological and ecological parameters on infection levels and species prevalence should be evaluated, taking into account age and sex of the host and season.

HISTEROMORPHA TRILOBA (DIGENEA: DIPLOSTOMIDAE) REPORTED IN CYPRINIDS OF LIGURIAN INLAND WATERS

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Hysteromorpha triloba (Digenea: Diplostomidae) is a parasite that affect fish muscle. This fluke is characterised by a complex life cycle, which include a snail (Genus *Gyraulus*) as first intermediate host, a fish (Ictalurids or Cyprinids) as second intermediate host and birds (Cormorants) as definitive hosts. It has been described in Europe and America; in Italy, the metacercariae stage has been found in wild chub (*Squalius cephalus*) caught in Emilia-Romagna watercourses and in catfish (*Ameiurus melas*) reared in a farm placed in Modena, while the adult stage of the parasite was found in Eurasian bittern (*Botaurus stellaris*). Thus, during spring 2015 a study has been carried out by the Liguria region in collaboration with the Fish Diseases Laboratory of the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta to better understand the native range of *H. triloba* and to establish the health status of fish settled in Ligurian inland waters. In total 93 Cyprinids were sampled, in particular 46 Italian riffle dace (*Teslestes muticellus*), 25 Italian barbel (*Barbus plebejus*) and 22 chubs (*Squalius cephalus*) caught in various Ligurian streams. All samples were submitted to internal and external parasitological exam, with particular focus on the muscle to detect digenean metacercariae. *H. triloba* metaceriae are clearly visible since they are characterized by a white colour and they measure 1-2 mm in diameter. Each found parasite was analysed with the microscope to define morphological characters and fixed in 70% alcohol. Metacercariae were found in 10 fish (10,7%), especially in only one Italian barbel (4%) and in 9 chubs (40,9%). Parasites were identified as *H. triloba* through morphology characterisation. All positive fish were found with numerous parasite in the muscle. Despite the fact that this parasite doesn't affect humans, it is a problem from a market point of view since they appear repugnant and impossible to sell. Nowadays dates about *H. trilobata* prevalence and distribution in Italy are limited, so the current study provides new information about how this fluke affect the Italian fish fauna. However more data are necessary, in particular in the other Italian region in order to better understand *H. triloba* real distribution.

A FORUNCOLOSIS EPISODE OCCURRED DURING A BROWN TROUT (*SALMO TRUTTA*) STOCKING ACTIVITY

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Fish stocking is the practise of releasing reared fish into the natural environment with the purpose of regenerating or building up fish population or for recreational fishermen entertainment. However, alive animals stocking come with the potential risk of pathogens introduction in the wild, that may cause diseases for both wild population and for aquaculture facilities nearby. The existing legislation (Council Directive 2006/88/EC), to prevent this risk, provides for notifiable diseases prevention and control measures. Instead, other diseases are not proper regulated, since the law only specifies that fish has to be clinically healthy before restocking and has to come from a hatchery where unexplained mortality rates are not occurred. Frequently, some aetiological agents in latent form are difficult to detect in fish which appear to be healthy and in these circumstances accidental pathogens releasing in the environment increased. At the end of July 2016, seventeen brown trout (*Salmo trutta*) were found dead in 3 pools of Carpasina stream (IM), placed in the municipalities of "I Carpi", "Mulino d'Arianna"and "Roccai". The unusual mortality occurred following a programmed fish stocking down the stream. Analyses were performed at the fish diseases laboratory of the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta. Numerous samples were not well-preserved, consequently only four fish were analysed. Fish did not show any macroscopic lesion on the skin or on the internal organs. Columbia blood Agar (CA) was inoculated with kidney samples from the 4 fish and incubated at 22°C for 72 h. Isolated culture were cloned and identified through the Analytical Profile Index (API, bioMérieux) tests and serological test (rapid agglutination test, Bionor). *Aeromonas salmonicida*, the aetiological agent of foruncolosis, were found in all four samples. Otherwise, no mortality events occurred neither in the wild population before the fish stocking activity nor in the farm where brown trout came from. It is impossible to exclude the pathogen presence in the environment, but the mortality event cannot be considered a natural episode since its rapid appearance. The most likely scenario is that the *A. salmonicida* was already presence in a latent form in the stocked fish or that it was present in a not diagnosed sub-acute form. That explains how the disease has occurred as a consequence of the stress caused to the fish by manipulation and the transport. This recent case define how diseases control and regular monitoring are essential to ensure wild and farmed fish health, in particular, during fish stocking activity or fish transport, that may cause significant losses. Moreover, fish intended for public waters restocking should be control for a period of time useful to establish the presence of a disease and accompanied by a certificate of the result. With all the proper measures will be possible to avoid the introduction of affected fish in the natural habitat and consequently avoid possible pathogens spreading that may affect the natural population.

PLACOBDELLA COSTATA (MULLER, 1846) (HIRUDINEA: GLOSSIPHONIIDAE) ON *EMYS ORBICULARIS* (LINNAEUS, 1754) (CHELONIA: EMYDIDAE) IN SALENTO PONDS

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Placobdella costata (Muller, 1846) (Hirudinea: Glossiphoniidae) is an ectoparasitic haematophagous leech that feeds eminently on the pond turtle *Emys orbicularis* (Linnaeus, 1754) (Chelonia: Emydidae). This leech in considered a Mediterranean taxa widespread in central-sothern Europe, northern Africa and Anatolia. In Italy was in the past reported in Mantova, Padova, Roma, Sassari and recently, for the southern part of the peninsula, in Lecce province with a unique free specimen.

E. orbicularis is an endangered Emididae pond turtle that actually is mainly represented by relict populations in Italy. For the first time here is reported the association between the pond turtle and the leech in the Regional Natural Reserve “Palude del Conte e Duna Costiera” at Porto Cesareo (LE). In the Reserve still a large population of *E. orbicularis* inhabits the reeds and small lakes in the area, sometime has taken place also in the drainages channels around the ponds. Thanks to a temporary permission the health status of 14 specimens was checked in 2009.

All of the turtles present some parasites with a mean number of 17.8 leeches, with a range between 6 and 48 specimens of different age and size.

Leeches were found moving free on the plastron and carapace or feeding on the neck, laterally to the mouth, within the axillary and inguinal cavities and near the tail. Juveniles and adults were found at the same time on the turtles.

Where the parasites feed, it was possible to observe hyperemic areas and ecchymosis but these injuries resolved spontaneously over a short period of time without causing the formation of skin lesions that could affect the health of the turtles, as evidenced by the lack of scars in the examined turtles. As this group of ectoparasites can transmit haemoparasites, the blood smears of the turtles are under study in order to check the presence of parasites owing to the haemogregarines complex.

ACUTE MORTALITY EVENT IN BROWN TROUT (*SALMO TRUTTA*) FROM MOGLIANA RIVER (BORGONOVO, GENOA)

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The Mogliana stream is a tributary of the river Sturla, flowing in Borgonovo (Genoa) and in part in Regional Natural Park of Alveto. This stream hosts a brown trout (*Salmo trutta*) hatchery, aimed at fish stocking of inland waters. During 2015, an acute mortality episode occurred, and all the farmed fish as well as some wild brown trout in upstream river were found dead.

In addition to water turbidity, a barrel labelled as flocculant-aluminium based was found in the water stream. Flocculation is in fact, an essential process in water treatment, used to remove suspended colloids. The most used substance is the aluminium sulphate (Alum), commonly utilised in water treatment plants to clarify drinking water and in lakes to reduce the amount of the nutrient phosphorus. After the contact with water, alum forms a fluffy aluminium hydroxide precipitate called floc that binds with phosphorus to form an aluminium phosphate insoluble compound.

Fingerlings and wild trout muscle have been tested to detect aluminium (Al) concentrations in order to confirm the cause of the death. Samples mineralization was performed using a microwave digestion lab station (Ethos 1, Milestone, Shelton, CT, USA), equipped with a 10 positions rotor for high pressures PTFE (polytetrafluoroethylene) digestion tubes. Fish samples (0.5-0.6 g) were directly weighed into PTFE digestion tubes. 7 mL of HNO3 (70% v/v) and 1.5 mL of H2O2 (30% v/v) were then added before the microwave digestion process. Al determination was performed by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS Xseries II, Thermo Scientific, Bremen, Germany) equipped with a multi-vial autosampler (ASX 520, CETAC Technologies, Omaha, NE, USA). The Limit of Quantification (LOQ) of the method is 0.010 mg/Kg.

Aluminium was recorded in all the samples at levels up to 41 mg/kg, suggesting a diversified contaminant distribution in the water stream. Some authors already pointed out that alum treatments involve environmental risk to fish and aquatic biota and that treatments should be carefully established to minimize these risks. In fact, due to its toxicity to many aquatic organisms such as fish, aluminium was not found to bio concentrate easily in the aquatic tropic web. In 1998, the US Environmental Protection Agency (EPA) already published an ambient water quality criteria document for the protection of aquatic organisms from the toxic effects of Al. Striped bass and brook trout were observed to be the two most sensitive North American species; the toxic effects of aluminium are increased mortality, changes in feeding habits, genetics, growth and gills malfunctioning. The level toxic to fish is above 0.2 mg/L, even if toxicity is even possible at 0.1 mg/L. The amounts of Al in the fish tissues here investigated were significantly higher than those reported in other studies. Moreover, in moderate acidity conditions (pH 5.5 to 7.0), fish and invertebrates may be stressed due to Al adsorption onto gill surfaces and subsequent asphyxiation.

We suggest that an excessive aluminium sulphate concentration in the considered water stream has caused the fish death for asphyxiation.

Sessione 3

METODI E MODELLI IN ECOPATOLOGIA: DALLA DIAGNOSTICA ALL'ANALISI DEI DATI

Coordinatori: Nicola Ferrari & Antonio Lavazza

COMUNICAZIONI

APPLICABILITY OF BLOOD SAMPLING THROUGH INTRACAVERNOUS VENIPUNCTURE: THE EXAMPLE OF *TOXOPLASMA GONDII* IN NATURALLY INFECTED RED DEER (*CERVUS ELAPHUS*)

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Health surveillance of wildlife faces several field and logistic issues. In particular, the difficulty of sampling is one of the most important limitations for monitoring programs in free-ranging animals, especially in case of blood, a very delicate matrix. As blood is frequently collected in the field directly from the major blood vessels by the hunters during the bleeding of carcasses, this sampling method may affect quality or quantity of sera samples. Indeed, sampling protocol has to be adapted to cope with the harsh field conditions to avoid sera alterations such as coagulation, contamination and haemolysis induced, for example, by the delay (several hours) between the death of the animal and the collection of the sample. As these alterations lead to a low sample size/lack of samples and/or to low volume/low quality/suitability of samples, the need is to define alternative sampling methods to solve these diagnostic concerns, mainly in case of zoonosis surveillance. Here, we chose *Toxoplasma gondii* in red deer (*Cervus elaphus*) as a representative host-infection model since in wildlife the protozoan detection is carried through serological tests. Therefore, we investigated the reliability of sera obtained from blood by intracavernous venipuncture (IV), a sampling method developed by Arenas-Montes et al. (2013, Eur. J. Wildl. Res. 59: 299-303), as an alternative to sera from blood of major vessels (MV) in *T. gondii* ELISA test.

Overall 142 sera were collected in Stelvio National park (Italian Central Alps) during two culling management plans (2015 and 2016) and obtained by both IV and MV techniques. Samples were tested by a commercial ELISA kit (IDVET, Montpellier, France) and the concordance between results was evaluated through the calculation of the Kappa (K) value. Prevalence of 31% and 26% emerged from MV and IV, respectively, and showed an "Excellent agreement" (K value=0.8450 (95% CI: 0.7478-0.9422)) between the two sampling techniques.

The concordance between results emerged from the two techniques supports the use of IV as a useful and valuable alternative for sera field sampling. Moreover, IV technique permits to sample high quality sera from individuals till 8 hours after the shooting and the bleeding of carcasses leading to an increase of the sample size with both a reduction of the time required for sampling and the potential risk of exposure to pathogens. This innovative technique can be thus a useful tool that could be further widely used in wild ungulates health surveillance, especially in case of programs for zoonosis control. Although in this study a valuable concordance between *T. gondii* antibody detection emerged between the two sampling techniques, further analysis should be carried out to investigate the comparison between sera from IV and MV in electrophoresis to evaluate the suitability of this new matrix even for hematologic and biochemical analyses.

MORPHOLOGICAL FEATURES OF *ANAS PLATYRHYNCHOS* ON POSTNATAL GROWTH BASED ON FDA OF C-R DATA: PRELIMINARY DATA.

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The descriptions of the pattern of growth in the wild mallard ducks (*Anas platyrhynchos*) are very poor available in literature. However intrinsic growth rate, defined as the relationship between size and age, is an important factor to consider in life history theory: it plays an important role in the evolution of age trajectories of fertility and mortality. In fact, for a species is an optimal life history strategy maximizing lifetime reproduction, which is determined by maximizing age survival and fecundity. But for most organism is the size rather than age that directly affects both survival probabilities and individual fecundity. We can apologize that organism grows as fast as possible to achieve mature size. Size can be increased through many strategies, for example by increasing initial size or time of growth. Juvenile birds grow as quickly as possible for reducing the time of vulnerability to predator, but no correlation between growth rate and predation among temperate passerine birds was founded. Food limitation, poor quality food, deseases and ability of parents to provision their offspring are invoked as factors to explain slow growth in birds. For the birds, rate of increase in body weight and in many morphological features are studied for many altricial species, but for the precocial species are poorly known. The altricial species was chosen for this kind of studies because its nests are abundant and/or because they are readily recaptured. The problems associated with repeatedly catching and measuring the offspring of precocial birds means that it is not easy to task and as a consequence studies, that quantity the growth of precocial species are under-represented in the literature. However, the rate of postnatal growth varies widely among species of birds: it seems that growth rates are negatively related to adult mass and that in altricial birds are generally more rapid than precocial species. Also the study of the avian growth curve and development is important for comparisons within and among species. The form of the avian growth curve varies considerably, and for this several mathematical functions have been used to describe growth: logistic, Gompertz, von Bartalanffy equations: these equations are inappropriate for precocial species, like wild mallard ducks, where are rarely available frequent measurements of known-age birds and growth data often results incomplete. Fitting these equations to incomplete data can bias estimates of growth rate and some models are difficult to fit due too many parameters for practical. This study examines development changes in the morphology of the wild mallards in the stage hatching-fledging. Young wild mallard ducks were ringed and measured at first capture, and, recaptured as often as possible thereafter, were measured again in a natural Park in Piedmont (Italy) during period of March-September for seven years. Rates and patterns of development of 4 morphological features (head-and-bill-length, bill-length, tarsus-length, and weight), were examined and compared with adult measurements. Offspring of wild mallards is capable of running and swimming immediately after the hatching: the ability to fly (fledging) is reached at 50-60 days of life. We hypothesize that the features which wild mallard ducks used immediately after hatching, bill for eating, tarsus for swimming and running, would reach mature size as fast as possible, when the adult weight is not achieved. Also, this study propose a first quantitative assessment of the growth of mallard duck chicks and also an original statistic approach: modeling the growth of wild mallard ducks, by the capture-recapture (C-R) method of young individuals and by Functional Data Analysis (FDA). The C-R method allows to measure the parameter of interest several times in a same individual and, therefore, to have information on the evolution of the growth of that individual. The FDA method allows to fit the growth curve to the measurements obtained, although they are few, and thus to get the plausible growth values. The trend of the average curves for the three parameters considered is the convex type. The curve relative to tarsus reaches the asymptote faster and precociously (some mallards already at 20 day of life). Indeed the individual growth curves relative to tarsus are steeper. The bill and, also, the BH grow still after fledging and reached adult values about at 80 days for bill, over 80 for BH. The tarsus at fledging (50-60 days of life) is 98,1% of asymptote (50-60 days of life), bill at fledging: 86,2% (50 days of life) and 91,8% (60 days of life), BH: 86,3% (50 days of life); 92,4% (60 days of life). Rapid tarsus development is not exclusive to mallards and may relate to efficient early locomotion in a variety of avian taxa. The fact that the tarsus may grow faster than the other parameters may be due to the need to move as fast as possible both on the ground, to escape predators, both in the water, for a more effective search of food at a time of life in which there is still the use of the wings. You can assume that the whole skeleton, along with the tarsus, reaches the final size after fledging: rising further weight after fledging could be due mainly to the muscular component that necessarily strengthens the flight itself. However it seems that the weight is reached later probably for the further growth of the muscles engaged on the fly. Here we propose a comparison with some other mathematical functions. FDA of capture-recapture data has been shown to be a valid approach and can be useful to represent the growth curves and the daily values, despite the fact that the observations are very few and the date of birth is unknown. This approach could be used for studying the growth of other animal species (in addition to the avian species), when standard methods are not applicable, as in case of precocial species or species hard to recapture before adult size.

SPECIES DISTRIBUTION MODELS AS PART OF A COST-EFFECTIVE SURVEILLANCE APPROACH FOR TICK-BORNE DISEASES: CASE STUDY IN A NATURAL PARK

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In Europe, ticks are among the most important vectors of viruses, bacteria and parasites, which are responsible for a high number of zoonotic diseases (e.g. Tick Borne Encephalitis, Lyme Borreliosis, Rickettsiosis, Human Babesiosis and Human Granulocytic Anaplasmosis). For many of these diseases, different types of wildlife species have a proved or suspected epidemiological role either as reservoir of the etiological agent, or in maintaining and amplifying tick population. In response to the observed general increase in the geographical spread and incidence of these zoonotic diseases, many ticks and tick-borne pathogens (TBPs) monitoring projects have been implemented. However, due to limited resources, more cost-effective surveillance systems need to be developed. The present study investigated the applicability of species distribution modelling based on remotely sensed environmental data in a natural Park of north-eastern Italy, where *Ixodes ricinus* is the dominant tick species.

The study area corresponds to the territory of the Colli Euganei Regional Park, a hilly area of volcanic origin located in the central part of Veneto Region. Data on tick occurrences from TBPs field monitoring activity conducted in 2009-2010 were used to develop a model of habitat suitability for *I. ricinus*, using the maximum entropy algorithm (MaxEnt). Initial explorative analysis was performed to exclude factors with low variability level and to select the most ecologically meaningful variables among highly correlated ones (rho ≥ 0.80). Normalized Difference Vegetation Index (NDVI) and day and night Land Surface Temperature (LSTday and LSTnight) were finally included in model development. Besides, the cost-effectiveness of different sampling strategies, based on sub-sets of the data (permanent sites vs. temporary sites; all-year sampling vs. seasonal sampling), hypothesized *a-posteriori*, were compared according to different criteria (sampling effort, number of ticks collected, pathogen detection, model accuracy).

The study showed that the MaxEnt modelling approach performed well (measured by the ”leave-one-out” validation approach), maintaining stable predictive performance, even with few occurrence sites as input. The model identified spring NDVI as a good predictor and summer high LSTday as the most important limiting factor for *I. ricinus*, with 32°C as an upper threshold of habitat suitability. This is consistent with previous preliminary ecological analysis, based only on field-collected environmental data.

Using only temporary sites (sites monitored only once) seems to be the best strategy for ensuring cost-effectiveness. Though permanent sites may help in a sound description of tick species seasonality, they represent an additional cost when the monitoring objectives are the development of a risk (suitability) map and the detection of TBPs. The seasonal monitoring in temporary sites in May-June (peak season of ticks presence) was the most cost-effective choice, in terms of detected occurrence at sites, number of ticks collected and detection of TBPs.

The model prediction provided a greater understanding of ecological requirements of this tick species in the study area, suggesting that the main limiting factor is represented by the hot and dry climate during late spring and summer, in place of the cold winter of pre-alpine environment. Notwithstanding the geographical area covered by the study is limited, the obtained results may serve to design further surveillance strategies, in order to optimize the resources employed, achieving an efficient and cost-effective tick and TBPs monitoring approach.

A PRIORITIZATION MODEL TO ADDRESS INFECTIOUS DISEASES SURVEILLANCE IN WILD UNGULATES

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In the eastern Italian Alps the amount of wildlife, and mainly of ungulates (as red deer, roe deer, chamois, ibex, mouflon and wild boar) is constantly increasing. As a consequence, contacts at the wild-domestic and animal-human interface have also been increasing, making the circulation of pathogens among the different components of the ecological interface more likely. In such a situation, early detection and the prompt response to the introduction or re-introduction of a pathogen are a pre-requisite of any human and animal health-risk management model.

Based on these assumptions, a research project was implemented with the aim of both defining a representative list of infectious diseases affecting wild ungulates or having chance of being introduced/reintroduced in these populations, and prioritizing them in order to focus the health surveillance and optimize available resources.

In line with the definition given by the World Organisation for Animal Health (OIE), the prioritization model was selected as a process aimed at providing decision-makers with a tool to help them select the disease-related threats that are worth being addressed by public policies.

A research team composed of scientific experts, sociologists and statisticians was involved in the project to implement the prioritization model which was divided into three phases: diseases selection; identification of criteria to prioritize the diseases; diseases ranking. In the first phase, the diseases were defined by the team of scientific experts, with particular focus on the eastern Italian Alps. 26 infectious diseases were identified, 12 of which were zoonoses. The Nominal Group Technique combined with Focus Group Technique was then implemented (second phase), in order to identify the relevant criteria for diseases prioritization. In this phase of the project, ten scientific experts were involved: 2 medical doctors, 4 veterinarians, 4 wildlife managers. 30 criteria were established and weighed by these experts. The Delphi method was then applied in the third phase of the project, in which fifteen scientific experts were involved independently through an online questionnaire: 8 veterinarians evaluated each of the 26 diseases through the identified criteria, assigning to each criterion a severity score from 1 to 5; while 7 medical doctors evaluated only the 12 zoonoses applying the same procedure. For each disease, the weighed score of each criterion (as defined in the second phase) was multiplied by the criterion severity score obtained. The total for each of the 30 criteria were added up to obtain the final score of each disease. In this way, two lists were produced: one related to non-zoonotic diseases, and the other to zoonoses. The results of this prioritization process will be useful for planning health surveillance activities in the territory of interest.

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WHEN ZERO IS A RESULT: HOW TO DEMONSTRATE THE ABSENCE OF AN INFECTION

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Demonstrating the absence of an infection from a population can be problematic since it would theoretically require the testing of all individuals with a test that had both 100% sensitivity and specificity. However, this is hardly realistic since every diagnostic test retains some inherent measure of uncertainty and disease surveys are typically carried out on a subset of individuals sampled from the target population.

This problem can be overcome by estimating the confidence of freedom from a disease, which represents the probability that an infection is truly absent given that a determinate number of individuals from that population are all negative to a specific diagnostic test. Confidence of freedom extends the concept of negative predictive value to the whole population by taking into account sample size, expected prevalence of the disease and population-level specificity and sensitivity of the diagnostic test of choice.

This approach can be most useful in the context of biological invasions, when there is a concrete risk of alien hosts introducing new pathogens that may threaten public health or native wildlife but, at the same time, there is no previous epidemiological history about either the host or the disease in the introduction area.

To illustrate this method, we will apply confidence of freedom on two case studies involving the potential introduction of a disease by alien hosts. Although in both surveys we did not find any evidence of the target pathogen, an accurate interpretation of negative results was crucial for either public health (case study I) or conservation (case study II) purposes.

Case study I – Raccoons (*Procyon lotor*) and *Baylisascaris procyonis*: we applied confidence of freedom to preliminary results obtained during a survey aimed at detecting whether introduced raccoons had carried along to northern Italy the zoonotic nematode *B. procyonis*

Case study II – Grey squirrels (*Sciurus carolinensis*) and squirrelpoxvirus: we applied confidence of freedom to data obtained through two different diagnostic methods to determine whether alien grey squirrels had introduced to Italy a poxvirus which is known to severely threaten native squirrels in the UK.

Through these examples, we will show how assuming different prevalences and choosing different diagnostic tests (i.e. with different specificity and sensitivity) and sample sizes will affect confidence of freedom estimation.

POSTER

AN ECO-EPIDEMIOLOGICAL MODEL FOR TICK-BORNE DISEASES: WHICH ARE THE KEY PARAMETERS AFFECTING TRANSMISSION?

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Currently, vector-borne diseases, especially those transmitted by ticks (e.g. Lyme disease, TBE), represent a very significant zoonotic risk. In particular, rodent species are associated with numerous zoonoses of public health interest, due to their ecology and transmission competence.

This eco-epidemiological modelling exercise employed a deterministic single-vector multi-host compartmental model, also including non-host species and ecological relationships such as competition and predation, to describe transmission of tick-borne diseases in woodland rodent communities.

The aim was to analyse how model structure and community composition affected model outputs such as number of ticks, or host and vector disease prevalence. Also, dilution effect was investigated by assembling a progressively complex community.

Key differences in model structure, consisting of a set of differential equations, concerned rodent species population dynamics, which was modelled with a seasonal or constant growth rate, and vector distribution on rodent individuals, which did or did not include a dispersion parameter estimated from empirical data. Sensitivity analysis was performed on parameters that were hypothesised to affect model outputs (e.g. efficiency in transmission/competence, host-vector encounter rate, feeding/moulting probability of success).

To add confidence to the model, where relevant, parameters where estimated from field data. Rodent live-trapping was conducted in Welsh woodlands across two seasons (spring and autumn) for three consecutive years.

Modelling results, in agreement with literature, confirmed that community composition was essential in determining disease prevalence, and significant parameters affecting the tick-borne disease system were: efficiency in transmission/competence; survival rate larvae to nymphs; nymph/competent host encounter rate.

Finally, models with different structures led to considerably different results, reinforcing the importance of model selection when making predictions of disease prevalence and human risk, and when employing modelling in decision-making to improve wildlife management and public health.

Sessione 4

VARIE ED EVENTUALI IN TEMA DI ECOPATOLOGIA: SESSIONE LIBERA

Coordinatori: Elisa Armaroli & Luca Rossi

COMUNICAZIONI

CHARACTERISATION OF WILD GAME MEATS: FROM ANIMAL WELFARE TO NUTRITIONAL, ORGANOLEPTIC AND HYGIENIC QUALITY

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The product characterisation is a key step in the identification of traceability guarantees and for the food safety of a product. These requirements are essential for the marketing of the product, which on the one hand must not damage the consumer and on the other hand must be characterized by a clear and certificated origin also as a guarantee of the production chain that it is meant to structure.

Wild game meat comes from animals that were born, lived and culled in wild condition, without any direct human contact, without force-feeding systems and without any pharmacological or vaccine treatment.

This makes the wild game meat definitely healthier and also more ethical than meat reared in farms where animals were born in captivity and are bred on an intensive feeding regime in order to increase production (mais, soybean, silage, food supplements etc.); where the wild game meat undergoes vaccine treatments against communicable diseases and pharmacological therapeutic and preventive treatments and it exposed to stress caused not only by the kind of breeding, but also by the phases of slaughtering (loading, transport, unloading, slaughter line, stunning and bleeding). Furthermore wild fauna has no environmental impact on the habitat considering soil consumption, production of manure and CO2 emission. The only negative issue about the presence of wild fauna on the territory is the eventual conflict with human activities generated by road crashes, crop damage and by the use of the domestic livestock same grazing areas.

In addition, wild fauna causes damages and alterations to forests regeneration and to the grass sub-strata over 2000 m.

It follows that, in order to reduce these conflicts, a proper management of wild fauna as hunting resource is essential, also trying to respect a proportionated number of wild fauna on the territory.

As far as it regards animal welfare, it is also necessary to compare farm animals slaughtering stages to selective culling stages deriving by hunting activities. Although the farm animal welfare requirements established by community regulations and european guidelines in slaughtering stages are extremely rigorous, it is inevitable that some stressing factors can impact not only on animal welfare, but even on the quality on the final product (movement, transport and entering in slaughterhouse).

However, although nowadays at a social level hunting is poorly considered by public opinion in all respects, the culling/slaughtering of the subject is such that it does not affect animal physiology; indeed the hunter must cull the animal by using precision rifle with scope. In this way, no stressing factors caused by manipulations, transport or other factors can affect the animal and the shot has to kill the subject immediately reaching it in vital areas (heart/lung).

It follows that the product quality, considering animal welfare, organoleptic and hygienic characteristic is directly proportional to hunter's ethical values. For this reason, as a part of the project "Filiera Eco-alimentare" funded by Cariplo Fundation, some training courses have been organized in order to improve hunters attitude and activities related to culling and carcass management stages. However, during the carcass management, any danger must be considered and monitored in order to contain risk levels and to guarantee food safety of the final product: physical and chemical dangers (presence of foreign bodies like bones and/or bullet sliver and their possible chemical dissolution in the matrix), biological dangers (incomplete bleeding; bacterial contamination caused by evisceration on the field and during the trasport; improper management of cold-chain during the transport). It follows that, considering the analysis led during the 2015/2016 hunting season on 177 chamois (*Rupicapra rupicapra rupicapra*), 79 roe deers (*Capreolus capreolus*) and 129 deers (*Cervus elaphus*), it was possible to consider the present management of the carcasses, and, consequently, the level of animal welfare, within the context of *ars venandi*.

Data show how the 70.6% of chamois, the 82.3% of roes and the 66.7% of deers were killed by one single lethal shot. Considering ammunition kind, 30.0% of hunters used lead-free bullet, and no difference emerged between Pb bullets and lead-free bullets in relation to lethality of the shot. The firing distances are averagely higher for the chamois (average 163 m – max 450 m) compared with roe (average 101 m – max 300m) and deer (average 129 m – max 356). It is remarkable that more than 33% of hunters who shot down at least one prey, are not member of any fire range.

Considering these data, which are currently submitted to an improvement process through product formation and certification, it is necessary to point out the organoleptic qualities of wild game meat, that shows a health-conscious/healthy relation between SFA, MUFA e PUF, with high values of Omega3 (chamois 53,48 mg/100g, roe 20,28 mg/100g, deer 94,70 mg/100g) well balanced with the Omega6, keeping in all the species Ω6/Ω3 ratio under 4. Also the content of Conjugated Linoleic Acid (CLA), given by the alimentation of green forage (values between 3.36 and 7.83 mg/100g), was found to be high.

Laboratory surveys on meat stock life have shown how vacuum conservation of the product at a temperature of 2-3 °C (wet-aging) since over 30 days is possible by using an anaerobic environment with pH of 5.2, which can potential bacteria that could lay on the meats because of an improper management.

EVALUATION OF pH LEVELS IN WILD GAME MEAT IN RELATION TO CORRECT MANAGEMENT AND GOOD HYGIENIC PRACTICES

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During the last 40 years, in Italy, wild ungulates populations, such as roe deer (*Capreolus capreolus*), alpine chamois (*Rupicapra r. rupicapra*), red deer (*Cervus elaphus*), and wild boar (*Sus scrofa*), considerably increased giving birth to the necessity of a specific hunting management. The opportunity for hunters to sell wild game meat, in accordance with the law (Reg. CE 853-854/2004), encourages competitive local economy systems in the restaurant industry. Starting from this notion, thanks to the funding of Cariplo Foundation and with the involvement of Ars.Uni.VCO. association located in Domodossola, a new project arose with the name “Filiera Eco-Alimentare – Progetto di valorizzazione delle carni di selvaggina attraverso il miglioramento della qualità igienico-sanitaria, la creazione di un marchio d’origine e la promozione alimentare locale e turistica dell’Alta Val d’Ossola (prov. VB)”.

The aim of this project is to increase the value of hunted game meat designing a guidance for the correct management of the carcass, the veterinary inspection and the subsequent food processing and selling. In this framework, wildlife could be considered an important factor for the biodiversity protection, the environment promotion and a high quality food processing.

To reach the aim, the study is based on the measurement of pH values as an important and useful means for the first quality and wholesomeness analysis of carcasses of wild ungulates hunted during 2015/16 and 2016/17 hunting seasons. Then a statistical analysis of pH values recorded at the control centre has been carried out; data have been divided into classes of species and sex. Then the analysis has considered the difference between age classes, time interval between shot and pH measurement, and management aspects related to the correct cull and bleeding.

After considering the errors committed by the hunter before and during the killing, data (ANOVA Test $p<0,005$) clearly demonstrate how the pH levels of the subjects killed by only one shot are definitely lower than those of the subjects killed by more than one shot. Furthermore, the more shots are exploded, even without reaching the animal, the highest the level of pH, generated by stressing factors, negatively affecting the meat maturing processes. Therefore the result of the shooting impacts on the fact that the animal is taken by a single shot immediately letal or after an agony phase due to the wounding.

Provided that the hunter, in order to be regarded as such and ethic in his behaviour, must shoot the animal with the almost entire certainty of killing it without sufferings, therefore exploding one single shot and aiming it to vital areas, the fact that many subjects suffered from hunting activities negatively impacts on the status of the hunter, on his role of overseer of the fauna and on the phases of welfare of the culled animal. Also in this event, it is significantly evident (ANOVA Test $p<0,05$) that the subjects killed without sufferings show pH levels lower than those died after a longer or shorter agony.

From the point of view of the management of the phases following the killing, it is pointed out how subjects showing values of pH higher than 5,8 are for the most part animals improperly bled. Indeed, it has to be considered that the blood within the muscle mass may retard the proper lowering of the pH due to the buffering effect of the blood itself and alter the health aspects of the meat through proliferation of bacteria.

Another aspect emerging from the analysis is the influence of external temperature: the subjects culled in the hottest hours of the day (from 10 am to 4 pm) tend to show levels of pH higher than those culled in the first hours of the day wich shows mostly levels lower than 5,8, if the correct management of the carcass is respected.

At the analytical level, in order to better highlight how stressing factors could affect hunted game meat’s quality, clearly emerges (ANOVA test $p<0,01$) how subjects killed in absence of stress show most appropriate values of pH for the meat maturing processes than those not killed by one fatal shot and/or by shooting twice or more.

The results obtained show how to get game meat with optimal pH values also from hunted animals in absence of errors during culling and, after that, through the correct management of the carcass. The study confirms that the measurement of pH in muscles is a useful tool to obtain predictive data about the maturation of meat. Therefore, pH could be considered as a quality index of wild game meat also for commercial purposes. The investigation has shown that it is possible to promote game meat in order to obtain a high quality, eco-friendly and “farm-to-table” product following the correct production specifications.

SCREENING METHODS FOR CONTROL OF RADIONUCLIDES ENTRY INTO THE FOOD CHAIN

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The presence of large contaminated areas by radionuclides of anthropogenic origin, such as Cesium-137, poses the question on how to quickly address the screening of a large number of samples within a reasonable time. The main reason is the development of we need a prompt health intervention, complementary to the traditional surveillance systems, aimed to detect and interrupt the transmission of nuclides into the food chain. The method should consist in a continuous monitoring with a methodology able to screen a significant number of samples and at the same time with a sensitivity able to identify the critical points.

We propose here a new preliminary screening method able to detect a human-driven radioactivity contamination in zootechnical milk, blood and muscles without necessarily having the knowledge of the original responsible radionuclides, so as to recognize the places where the latter is present and to identify the routes of possible spread to humans. Moreover, the method allows to collected samples both *intra vitam* (milk and blood) and *post-mortem* (muscle and heart clot).The choice of using the farm milk as a main indicator derives from ~~for~~ its ease in being sampled, from its analytical simplicity and from the sufficiently widespread of the dairy herds available (e.g. sheep, goat and cattle). The latter additionally allows for an indirect control of the territory in terms of veterinary surveillance of animal origin foods. On the other hand, the choice of screening muscles collected from wildlife, in particular wild ungulates, allows to obtain data regarding territory areas not reachable by domestic herds even during pasture season.

The method proposed is based on a liquid scintillation (LSC) readout, which ~~can~~ ensures the rapidity and sensitivity required. Furthermore, experimental campaigned conducted with spectrometric quantization techniques on those samples not detected by the aformentioned method have confirmed the reliability of a control system of the total radioactivity screening based on the LSC.

In particular, the use of LSC-based methods on milk at the milking stage enables us to discriminate and highlight those samples in which the human-driven radioactivity is present, which are therefore suitable for a prompt screening program directly on the territory to safeguard the food chain.

In our laboratory in Vercelli, we are also investigating the possibility of using automated equipments for the radioimmunological determinations, such as the Perkin Elmer COBRA, for the identification of the food contaminated with gamma-emitters at levels above the legal limits. Although showing a sensitivity considerably lower than that of other methods (with contamination levels certainly higher than 100 Bq/Kg), the method is however capable of over 90 determinations per day.

By comparing the results obtained with the two methods on wild boar muscle samples at different levels of contamination, we observe a Pearson Correlation Index value very close to 1, overlapping ~~of~~ the responses from the two methodologies regardelss the concentrations present and the validity of the system starting from 100 Bq/Kg of ^{137}Cs.

This method has also been successfully applied to determine this latter radionuclide in blood clots, where a concentration correlated to that present in the muscle has been demonstrated.

The ability to detect a radioactive contamination by simply testing small amounts of blood and to perform several tests per day gives the possibility of addressing important “in vivo” screening campaigns on animals and on human tissues, as well as the possibility of monitoring the biological elimination of ^{137}Cs out of the body.

PRESENCE OF Cs-137 IN WILD UNGULATES OF OSSOLA VALLEY (VB)

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The accident in the nuclear power plant of Chernobyl (Ukraine, 26th April 1986) had released a significant amount of radionuclides in the environment that have been deposited in the soil. Above all, after Chernobyl accident, Cesium (Cs-134 and Cs-137 isotopes) has been deposited in large areas of Northern and Central Europe, including Italy, in particular Alps, because of rainfall and atmospheric winds. So Cesium has been deposited in the surface layers of the land, creating a fragmented patchwork of contamination in relation to the rainfall of that period, to the vegetation and to the soil permeability. In this background, to date, the isotope Cs-137 is noteworthy, in particular because of its half-life of 29,5 years and its biological behaviour similar to potassium, in fact they are competitors for the same adsorption site in the organism.

In previous Studies soft fruit, mushrooms and farming products have been sampled and processed; the results had demonstrated the presence of high risk areas in some territories in Western Alps, such as Ossola Valley (Verbania Province), Sesia Valley (Vercelli Province) and Biella Province. In fact, in these samples Cs-137 has been resulted higher than other samples collected from agriculture and food of animal origin.

An investigation has been carried out to evaluate the presence of Cs-137 in the environment by means of a monitoring in wild hunted ungulates leaded up to the control centres in C.A. VCO2 and C.A. VCO3 during 2013, 2014, 2015 hunting seasons, from which samples of muscles (diaphragm and/or tongue) have been collected.

An overall of 246 chamois (*Rupicapra rupicapra rupicapra*), 206 roe deer (*Capreolus capreolus*), 309 red deer (*Cervus elaphus*), and 113 wild boar (*Sus scrofa*) have been collected.

The analysis have been performed at the laboratory of food radioactivity of the Istituto Zooprofilattico Sperimentale Piemonte, Liguria e Valle d'Aosta (IZS PLV) located in Vercelli. The test, carried out on muscle samples has been performed using "Packard COBRA" gamma counter mod. 5003 featuring a 3 inches NaI detector equipped with a read window for Cs-137 in the 580-754 KeV energy range and count time up to 120 minutes.

Results have shown a different distribution in the various species of wild ungulates. Wild boar could be considered the sentinel species in the research of *Caesium* probably because of the eating habits such as rooting and research for berries, tubers and mushrooms that may be accumulators of the radionuclide. In fact, during years, wild boar had shown the highest values among ungulates (35 subjects with values between 100 and 400 Bq/Kg, 3 between 400 and 600 Bq/Kg, 9 over 600 Bq/Kg; maximum value 2255 Bq/Kg). It is necessary to underline that the highest values in wild boar have been detected in subjects culled between March and July during control activities. Values higher than 600 Bq/Kg have been also detected in roe deer and chamois. Roe deer is known to be another sentinel species because of its habit of eating a radio-absorbent mushroom, named "deer truffle", during certain periods of the year; while so high values in chamois are surprising. 55 roe deer and 41 chamois have shown values between 100 and 400 Bq/Kg, 9 roe deer and 3 chamois values between 400 and 600 Bq/Kg, one chamois (603 Bq/Kg) and 2 roe deer (maximum value 986 Bq/Kg) have exceeded the cut-off point of 600 Bq/Kg. Regarding red deer, only in 22 subjects *Caesium* values are between 100 and 400 Bq/Kg, with a maximum value of 222 Bq/Kg.

Considering that the Cs137 remains in the body of ungulates between 30 and 45 days on average, data have shown that there were no particular risks in the consumption of game meat of ungulates hunted as provided by the shooting plans.

However, the presence of this radionuclide in several wild animals with different habitats and food habits, even if values are below the reference cut-off, underlines the importance of wildlife as sentinel species for environmental contaminants.

CADMIUM EXPOSURE IN WILD BOARS IN ITALY: CASE REPORT

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Cadmium (Cd) is a toxic and carcinogenic heavy metal widely distributed in the environments as a result of anthropogenic activities. Animals are exposed to environmental Cd through inhalation and ingestion but the primary route of exposure for wild animals is through the diet. Severe exposures to high doses of Cd lead to an acute disease with respiratory problem and renal failure. Chronic exposure at low concentrations can evolve to the development of a variety of pathologies including hepatic, pulmonary and renal dysfunctions. Many studies demonstrated that Cd2+ is a non-infectious stressors, that can modulate the activity of cellular enzymes, initiate oxidative stress, suppress mitochondrial functions, disrupt calcium homeostasis and modulate the immune response. In this study we reported 4 cases of debilitated wild boards unearthed in Ligurian area (Italy) from November 2015 to December 2016.

After complete necropsy, all animals showed evident cachexia conditions, smaller size respect the estimated age, rarefaction mantle with large alopecia areas. Moreover we observed anemic mucous membranes.

Over 70 analyses were runned on each animal (microbiological, toxicological, chemical, parasitological, virological, serological and histological). In particular we analysed macroscopic lung lesions and microscopic kidney, liver and lymphoid lesions. We detected cadmium (Cd) exposure in all animals with a mean value of 1.3±0.5 mg/Kg in liver and 15.9±3.2 mg/Kg in kidney. Kidneys histological examinations showed two types of changes: degenerative and proliferative changes.

Degenerative changes affecting the epithelial cells in some proximal tubules, and proliferative changes in the interstitium. The major histopathological changes were mainly in the epithelium of proximal tubules, these lesion are probably due to chronic Cd exposure. Furthermore 2 animals out of 4 were positive for *Salmonella choleraesuis;* this microorganism was isolated in many organs like lung, spleen and liver where we found typical injuries. In pigs, *Salmonella* infection is generally sustained by host-adapted serotypes of *S. enterica* (especially *S.* Typhimurium) and less frequently by *S.* Choleraesuis; however this microorganism can determines an acute or chronic typhoid form some time lethal. Moreover 3 animals out of 4 were Porcine circovirus (PCV2) positive in serum, lung and lymphonodes; and we observed typical lesion in these organs. PCV2 diagnosis was based on the presence of histopathological lesions (lymphadenomegaly, lymphocyte depletion and / or hystocyte infiltration, para-cortical hyperplasia) associated with the presence of viral antigen found on tissue, by immunohistochemical analysis or "in situ" hybridization and PCR assay to detect viral DNA. Pig circoviruses can play a role in postweaning multisystemic wasting syndrome (PMWS) whose etiopathogenesis is unreliable, but many authors describe the onset of symptomatology with a state of down regulation of the immune response attributed to both infectious and non-infectious factors. In this report we evidence the exposure to Cd in animals infected with PCV2 and *S.* Choleraesuis

The results of different studies conducted both in vitro on T cells and in vivo lymphocytes, aimed at delineating cadmium-induced interference with cellular immunity, showed strong depression with decreased phagocytic activity of peritoneal macrophages, reduced activity of natural killer cells and decreasing resistance to infections.

This study showed the presence of clinical sign of PCV2 or *Salmonella* infection in wild boars naturally expose to cadmium; this heavy metal could be cause of alteration of immune response, so pathogens that usually are not cause of disease in wild boars could determine dead of these animals.

INVESTIGATION ON *TRICHINELLA BRITOVI* SPREADING IN WILDLIFE OF SARDINIA

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Trichinellosis is a zoonotic disease caused by nematode worms of the genus *Trichinella* circulating in domestic and wild animals worldwide, except Antarctica. In humans, *Trichinella* spp. cause more than 2,800 infections per year. Worldwide, the main sources of infection for humans are swine meat. However, in the last decade, the importance of wild boar as sources of infection for *Trichinella* is increased in Europe. Humans acquire the disease solely by the consumption of raw meat, from wild or domestic animals infected by *Trichinella* larvae. In Sardinia, the first human outbreak of trichinellosis occurred in 2005. Human trichinellosis appeared in 19 persons who had consumed raw sausages made with pork from a free-ranging sow illegally reared in the Orgosolo municipality and slaughtered without veterinary control.

From 2005 to 2017, all *Trichinella* larvae isolated from animals of Sardinia have been identified as *Trichinella britovi*.

The aims of the study were to assess the current status and spread of *Trichinella* infections in wild and domestic animals, georeferencing the infected animals, and to assess the presence of *Trichinella* taxa in addition to *Trichinella britovi*.

The study area was the Nuoro province, the Ogliastra province and an area of the Sassari province, contiguous to that of the Nuoro province. The provinces were splitted in sub-areas for epidemiological analysis.

From 2014 to 2017, during the hunting season, we analyzed by the artificial digestion test, performed according to EC Regulation 2075/2005, replaced in 2015 by the EC Regulation 1375/2015, 7,679 wild boar muscle samples and 77 fox muscle samples.

Moreover, to detect anti-*Trichinella* IgG, a serological investigation was carried out on 2,848 wild boar blood samples from different macro areas of the study area. All samples were tested by ELISA and ELISA-positive samples were tested by Western blot as confirmatory test.

Blood samples from 184 wild boars were also collected from animals hunted in an area of southwestern Sardinia (Cagliari province) and used as control, since this area was considered *Trichinella*-free for the lack of reports. Results: three wild boars (0.04%) and three foxes (3.9%) hunted in the Orgosolo municipality (Nuoro province) were positive by artificial digestion. *Trichinella* larvae were identified as *T. britovi*.

A total of 298 blood samples were positive by ELISA and 93 of them tested positive by Western Blot (serological prevalence 3.2%).. Unexpectedly, also in the southwestern part of Sardinia, three wild boar samples tested positive by both ELISA and Western blot.

In conclusion, this study shows that parasitologically positive animals, i.e. animals with a high larval burden, are circulating only in the Orgosolo municipality. Instead, serological screening indicates that *Trichinella* is widespread in the study areas with different sub-area prevalence. *Trichinella* continues to be a public health concern in in the Nuoro province, the Ogliastra province and in the municipalities of the Sassari province, contiguous to the Nuoro province . However, the serologically positive wild boar detected in the Cagliari province suggest that *Trichinella* is widespread also in other areas of Sardinia island.

The different prevalence detected by digestion and by serology (0.04% vs. 3.2%) is related to the analytical sensitivity of the two tests:1 larva per gram (LPG) for the digestion and 0.01 LPG for serology. The different prevalence obtained by the direct and indirect tests is similar to the prevalence detected in wild boar of other Italian regions .

Free-ranging pigs, raised for self-consumption in the Orgosolo municipality, were found positive for *Trichinella* by the artificial digestion test and probably they play an important role in the maintenance of this zoonotic parasite in the area and in the increase of the larval burden among wildlife .

There is the need to implement *Trichinella* surveillance plans in pigs and wild boar of the Sardinia island and to organize educational programs for farmers, hunters and stakeholders to avoid human infections and to control the spread of the parasite in the island.

POSTER

POISONED BAITS AND WILD SPECIES: A STILL OPEN QUESTION ABOUT ACCIDENTAL AND DELIBERATE POISONING IN NORTH WESTERN ITALY

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The improper use of poisoned baits is still a widespread practice in many European countries, causing death of thousands animals and it is mostly related to an inappropriate or careless use of commercial legal and illegal pesticides formulations, urban or hunting interpersonal conflicts as well as the simple intolerance towards animals. Cats and dogs are the most involved domestic species in poisoning episodes as opposed to livestock probably due a lower risk of exposure or because the economic loss is insufficient to justify post-mortem investigations. Poisoning episodes in wild species are also reported and encountered among the most frequent causes of death with considerable implications especially for endangered species, avian predators and scavengers. The most common poisons involved are pesticides as insecticides (carbamate, organophosphate and organochlorine) and rodenticides, followed by herbicides, molluscicides and fungicides. The type of pesticides used is linked to several factors such as the agricultural context, awareness and wide availability in commercial formulations without any particular sell restriction. Moreover, despite the ban on the use of highly toxic pesticides, the cases of animal poisoning by aldicarb, carbofuran, diazinon, fenthion and endosulfan are still frequently reported. In Italy, the enforcement of the Ministerial Order 18/12/2008 with its extensions and the previous law n. 189/2004 have yielded illegal the use of poisons for purposes other than management of pests. In Piedmont, the ministerial protocol has been consistently applied by Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (IZSPLV). The aim of this work is to describe poisoning of wildlife through a descriptive statistical analysis of all data collected by IZSPLV from 2012 to 2016. During routinely diagnostic activities, toxicological examinations were conducted on all animals with a suspect of poisoning based on anamnesis or necropsy findings. All samples were processed applying a protocol issued by the Official Regional Antidoping Center and based on GC-MS and LC-MS / MS techniques. A total of 123 toxicological investigations were carried out on 50 birds of prey, 30 foxes, 18 wolves, 7 wild boars, 6 ungulates, 5 hares, 3 squirrels, 2 badgers and 2 hedgehogs. Among these, 35 were positive and distributed as follow: 20 foxes (66.6%), 10 birds of prey (20%), 3 wolves (16.6%), 1 hedgehog (50%) and 1 badgers (50%). In all cases it was detected a pesticide, i.e. 24 rodenticides anticoagulant, 6 insecticides and 5 mixtures of rodenticides with insecticides or molluscicides. Foxes and birds of prey birds was the most involved with 66.6% (CI 95%: 47.2-82.7) and 20% (CI 95%: 10.0-33.7) of positive sample respectively. Particularly, 16 foxes and 6 birds of prey were poisoned by anticoagulant rodenticides which are able to persist in the tissue leading them to a sub-lethal doses exposure that predisposes to different kinds of trauma or other pathologies also described as "indirect" fatality. This frequency could be related to the diet and ethology of these predatory and/or scavenger species, which make them more exposed to secondary poisoning through ingestion of poisoned animals by both legal or illegal manner. However, the causes of wildlife poisoning are also to be found in illegal use of toxic substances against several species (i.e. wolf) perceived as "undesirable", because of its potential competition with anthropic activities such as rearing and hunting. Since the use of poisoned baits is not a selective method, the involvement of non-target species should be considered a public health problem which can cause damage to ecology and economy spheres besides conservation problems for non-targeted species, in particular the wolf, for which it is known a spontaneous recolonization of Piedmont territories over the last twenty years. Also insecticides have been detected as cause of death of wildlife: in that case the substances were methamidophos, methomyl and endosulfan despite they are banned in Italy or even in some cases worldwide. Despite some possible underestimation in wildlife, mostly due to the difficulty of rescue of carcasses, the problem is present and persistent. So, wildlife is involved directly and indirectly by both legal and illegal poisoning. However, our observations are not sufficient to provide a defined framework to develop an appropriate risk assessment, that is essential to plan control and targeted communication strategies. In fact, the knowledge of the animal poisoning in our territory contextualized in depth is crucial to any action in order to reduce and control the phenomenon. Moreover, it is essential to follow up and share information produced with judicial authorities for management and reduction of the risks of accidental and deliberate poisoning and to enforce stricter control on the possession of any prohibited chemicals substances as endosulfan, whose recourse seems to be recurring and inveterate in restricted areas of Piedmont.

EVALUATION OF POST-MORTEM TEMPERATURES IN HUNTED GAME

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During inspection and forensic activities, the evaluation of the *post-mortem* temperature in carcasses of hunted game is difficult to assess objectively since the operations carried out during hunting activities related to the management of the culled animal can influence the trend of the temperature. On the basis of local rules, for a good hygienic practice minimizing the risk of meat contamination, hunters have to bleed the culled animal via jugular and eviscerate it soon after shooting, directly on the field. They can partially or completely eviscerate the carcass choosing not to remove heart, lungs and liver or to remove both abdominal and thoracic organs. Transport phases could also influence the temperature of the carcass, sometimes causing favorable situations for bacterial growth resulting in adverse effects on hygienic and sanitary quality of the product.

As part of the project "Filiera Eco-Alimentare" funded by Cariplo Foundation, during 2015 and 2016 hunting seasons, at the VCO2 Alpine Hunting Area - Ossola Nord (project partner) pH and temperature measurements have been collected from semimembranosus muscles of hunted animals by means of DeltaOHM® 21052.02 pHmeter in order to provide certification of game meat production. Measurements of pH are susceptible to the sample temperature, for this reason, they are always associated with the measurement of temperature in depth of the muscle. In the present study, data have been collected from 211 chamois (*Rupicapra rupicapra rupicapra*), 70 roe deer (*Capreolus capreolus*) and 155 red deer (*Cervus elaphus*) leaded up to the control centre. For each hunted animal information have been collected on culling date and location, altitude, sex and age classes, weight, time of shooting, arrival time at the control center, pH of meat, deep temperature of semimembranosus muscle, evisceration evaluation (complete or partial). During each hunting day, weather conditions and average outdoor temperatures have been recorded according to the hunting territory divided into two hunting districts: Antigorio/Formazza and Vigezzo/Isorno. The heads that have been stored in cold room in slaughterhouses and/or in in a locale with legal permissions (5 chamois, 2 roe deer and 3 red deer) have not been considered for further processing.

The analyses show that outdoor temperature, weather-climatic conditions and rate of sampling do not significantly influence the decreasing rate of internal temperatures of game meat, except for special conditions, as well as there are no significant differences between bled and unbled subjects, completely and partially eviscerated subjects, age and sex of classes. It should be underlined that the subjects with the highest muscle temperatures, even if the values are not significant, were generally those not dead on the spot, in which stress has compromised the decreasing rate of *post-mortem* temperature. In addition, it should be considered that, if the hunter does not proceed quickly with proper carcass management immediately after death, the internal meat temperature can rapidly increase up 40.0 °C because of the absence of homeostatic regulation and chemical-physical reaction within the muscle mass. In particular, it has been observed that some chamois and roe deer placed in backpacks or bags immediately after culling, that the temperature of the carcass has remained high even after several hours from the death (for example, the subject 472 shot at 9.00 a. m. and leaded up to the control center at 6.30 p.m. had muscle mass temperature of 31.7 °C).

Regarding those subjects with extremely low temperature in relation to the time interval between death and measurement, in two red deer the recorded temperatures was 17.5 °C and 20.5 °C respectively after 447 and 460 minutes from culling. The first subject was reported at the end of the hunting season by other hunters as culled during the night before the hunting day, however due to the late reporting it was not possible to proceed with its immediate requisition; while the second, reported immediately as a cull made during a period of time not allowed, has been seized by the Provincial Police confirming the time of shot (10.30 p.m.) during the night before. As a result for both animals, the correct range of time between death and the muscle temperature measurement is well over 16 hours.

Analyzing the data and without considering the subjects placed in the freezing cell and those illegally culled, it is possible to evaluate the downward trend of the temperature for each single species through the formula ($y = ax + b \pm c$); where y is the recorded temperature, x the minutes between the time of death and the measurement, a the angular coefficient, b the intercept and $\pm c$ the 95% Confidence Interval: Chamois: $y = -0.022x + 35.73 \pm 6.65$; Roe deer: $y = -0.028x + 36.90 \pm 5.64$; Red Deer: $y = -0.021x + 38.45 \pm 5.78$.

The application of these formulas, with corrections as appropriate, may provide useful guidance both in the context of an assessment of proper carcass management practice and investigations about the exact time of the culling of the animal, providing a useful tool to be combined with field investigations in case of controversy.

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