





ABSTRACT BOOK

CONSERVATION MEDICINE AND WILDLIFE HEALTH INTERNATIONAL SEMINAR

Thursday 16th and Friday 17th June, 2022

Teramo, Campus Aurelio Saliceti Polo Didattico Silvio Spaventa - Aula magna Benedetto Croce Via Renato Balzarini 1 - Località Colleparco.







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Opening address.

On behalf of the Organizing Committee, the University of Teramo and the Maiella National Park, who have worked on this educational and cultural exchange project, we would like to thank to all those people who kindly cooperated with us in preparing for the present Seminar.

It's our pleasure to welcome all students, researchers, lecturers and all those who have spent part or all of their professional life for knowledge of the health status of wild animal populations and in the experience of Conservation Medicine.

Special thanks to our three keynote speakers, which will be our point of reference, our headlights can guide us in the composition of the experiences that will be told here in these two days, all very interesting, but certainly, in many cases, expression of a certain heterogeneity and complexity that are, on the other hand, typical characteristics for those who deal with diseases in the complex scenario of ecosystems.

We thank very much Alonso Aguirre, Chair and Professor of the Department of Environmental Science and Policy, College of Science, George Mason University (USA). He heads an academic and research program using the One Health/Planetary Health approaches to understanding wildlife diseases and their links to human health, and he co-founded the emerging discipline of Conservation Medicine, that's the main topic for which we have gathered in this international seminar, developing practical, sustainable and effective solutions understanding local socio-economic factors and a solid grasp of complex national and regional health and environmental policies.

We thank very much for joining us Jorge Ramon Lopez Olvera, Professor of the Department of Animal Medicine and Surgery, Universitat Autònoma de Barcelona (Spain). He is a very long-standing experience wildlife veterinarian, focused inon the study of diseases, population management and capture and handling of wildlife. He already knows our mountains and our Apennine chamois and has already had collaborations with Maiella National Park in last years, and today he deals mainly with merging ecological and health approaches to produce a comprehensive understanding of the drivers of wildlife management issues.

And, last but not least, thanks a lot to James D. Forester, Associate Professor Institute on the Environment - Dept. Fisheries, Wildlife, and Conservation Biology, University of Minnesota (USA). The overall goal of his research is to develop an understanding of the spatio-temporal processes that affect the distribution and abundance of wild animals. He is developing a specific research line on how animals alter their habitat selection and movement paths in response to heterogeneity in resources and risk. This kind of research that is focused





primarily on how large, mammalian herbivores respond to changing landscapes, is one of the reasons why he collaborates with the Maiella National Park and will also help us at the University of Teramo to place in a complex ecological context the reading of wildlife health data.

Because, after all, this is the challenge that awaits us, not only increase our knowledge on wildlife diseases and health risks of wild populations, but above all know how to read the presence of diseases in the ecological contest in which they occur. This is a historic moment in which many people, not only the experts, are asking themselves what should be the orientation of relations between humans, environment and animals. And in the world there are different and deep changes in ecosystems, led by humans, that will require us to do more and more in-depth studies and adapted to local ecological conditions, before global.

We are hosting this debate, in these days, in an area of Central and Southern Europe where the depopulation of rural areas by humans, and the associated abandonment of cropland, had marked ecological consequences on wildlife: shrub and tree encroachment, and the expansion of mammalian ungulates and carnivores, are the major consequences of the rural exodus in this corner of the Earth. But we know that this is not the case everywhere and, indeed, there are areas in the world where the destruction of the forest and the advance of cities on the rural landscape causes human/wildlife interfaces very different and dangerous in other ways.

This is the meaning of this Seminar. Attempting to build a discussion, oriented by our high-skilled colleagues, that represent how the work of veterinarians in the coming years cannot be separated from solid competences in the ecological field and how the concept of the animal population to be managed has profoundly changed and needs new skills and new approaches.

Organizing Committee:

Prof. Cristina E. Di Francesco (Faculty of Veterinary Medicine, Teramo); Prof. Fulvio Marsilio (Faculty of Veterinary Medicine, Teramo) Dr. Simone Angelucci (Wildlife Research Center, Maiella National Park, Italy) Dr. Antonio Antonucci (Wildlife Research Center, Maiella National Park, Italy)

Advanced in wildlife health and conservation medicine

ASSESSING THE EFFECTIVENESS OF COMBINED VACCINATION AND IMMUNOCON-TRACEPTION STRATEGIES IN REDUCING CANINE DISTEMPER VIRUS PREVALENCE IN FREE-ROAMING AND AFRICAN WILD DOGS

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Canine distemper virus (CDV) is a multi-host viral pathogen that is endemic in free-roaming dog (Canis lupus familiaris) populations. Free-roaming dogs act as a maintenance host and source of CDV to the endangered African wild dog (Lycaon pictus), contributing to infrequent but highly fatal outbreaks. This study used a system dynamics Susceptible-Exposed-Infect-ed-Recovered (SEIR) model to assess the efficacy of vaccination and fertility control strategies in reducing CDV prevalence in the wild dog and free-roaming dog populations. We investigated combinations of vaccination in the wild dog population. Vaccinating African wild dogs, alone or with concurrent vaccination of free-roaming dogs, at a 50% coverage, was most effective at reducing CDV prevalence in wild dogs and maintaining the population above a viable threshold. Vaccinating free roaming dogs, with or without the addition of immunocontraception, only marginally reduced the impact of CDV in the African wild dog population and made no change to CDV prevalence in the free-roaming dog population. This study concluded that vaccination protocols primarily targeting African wild dogs are the best course of action for protecting this endangered speciesthe threat of CDV.

IMPORTANCE OF PASSIVE SURVEILLANCE AND EXISTENCE OF EXPERT NETWORK FOR A CORRECT ECOLOGICAL AND EPIDEMIOLOGICAL APPROACH IN WILDLIFE: THE EXAM-PLE OF GOLDEN JACKAL (CANIS AUREUS) IN PIACENZA PROVINCE

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- 3. Student Sciences of Nature and the Environment-University of Parma, Italy
- 4. Veterinary Freelance, Modena, Italy
- 5. Zoologist, Zoological Section of the Friulian Natural History Museum, Udine, Italy
- 6. Emilia Romagna Region, Piacenza, Italy
- 7. Local Health Authority of Piacenza Animal Health Operating Unit, Piacenza

Objectives

The present work describes the identification in Piacenza province (northern Italy) of a golden jackal (Canis aureus), a species not yet recorded in this area and reports the investigations carried out on its carcass, according to the regional wildlife control plan and to the recommendations of a group of experts.

Methods

The canid was found dead close to the state highway in Bobbio district (44°46′56.9″N, 9°24′14.6″E) in August 2021 by a group of volunteers of the Piacenza Wildlife Rescue. The carcass was delivered to IZSLER diagnostic section of Piacenza and submitted to necropsy. Several samples were collected for diagnostic investigations.

Results

The phenotypic features analyzed during necropsy matched up with those of golden jackal. It was a young male (aged about 18 months) and the main findings recorded were traumatic lesions, attributable to a collision with a vehicle, and a hemorrhagic aspect of internal organs. Laboratory analysis detected the presence of *Canine Parvovirus type 2* and gave negative results for rabies virus, canine distemper virus, *Leishmania infantum, Leptospira* spp., *Echinococcus granulosus* and *multilocularis, Tenia* spp., *Trichinella* spp. and mange mites. Furthermore, considering the hemorrhagic findings, we carried out toxicological tests for the detection of anticoagulants, revealing the presence of brodifacoum, bromadiolone and difethialone, three common rodenticides.

Conclusions

This first description of investigations carried out on a golden jackal in Piacenza Province, underlines the importance of wildlife surveillance, both from health and ecological points of view, and of the collaboration among different competences.



LISTERIA MONOCYTOGENES PASSIVE SURVEILLANCE AND STRAIN CHARACTERIZATION IN WOLVES

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Listeria monocytogenes (Lm) is an important zoonotic pathogen and wildlife plays an important role in its persistence and spread in environment, hence to humans. In *"One health"* approach the Istituto Zooprofilattico Sperimentale Abruzzo e Molise is involved in a surveillance plan for monitoring the diseases in wild. Deep genomic analysis of the detected strains allows monitoring virulence and antibiotic resistance genes to forecast possible hazards to livestock and human.

Between 2020 and 2022, 37 brain and 43 feces samples from dead wolves were collected mostly in Abruzzo and Molise Regions (Central Italy). *Listeria* spp. and *Lm* were detected according to ISO11290-1:2017. WGS was carried out using Illumina platform and sequences were used for Clonal Complexes (CCs) detection and strain clustering using Institut Pasteur's BIGSdb-Lm cgMLST scheme.

In the brain *Lm* (8), *L. innocua* (2), *L. ivanovii* (1) and *L. seeligeri* (1) were detected, while in fecal samples *Lm* (3), *L. innocua* (1) and *L. ivanovii* (1).

In silico analysis of NGS data for *Lm* showed eleven CCs, among them also CC1.

Among endangered species, wolves are protected animals in Italy and proximity to farms and livestock, canhappenduetomore frequent presence of herbivore wild animals in urbanized areas. Detection of zoonotic pathogen like *Lm*, above all belonging to hypervirulent CC, may represent a hazard to public health. In *"One health"* approach, passive surveillance and characterization of strains isolated in wildlife allow to assess the risk they may pose to human health.

CLINICAL PARAMETERS, BIOCHEMICAL ANALYSIS AND VENOUS BLOOD GAS OF APEN-NINECHAMOIS(RUPICAPRAPYRENAICAORNATA)CAPTUREDBYASSOCIATEDMECHAN-ICALANDCHEMICALIMMOBILIZATION IN CONSERVATION TRANSLOCATIONS ACTIVITIES.

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The Apennine chamois (Rupicapra pyrenaica ornata) is one of the rarest species in Italy, is listed in Annexes II and IV of the Habitats Directive and as a subspecies at risk of extinction in the IUCN Red List. The Maiella National Park (MNP) population has recently been defined as a source population for reintroduction to other parks. The aim of this study was to evaluate a new capture method that includes mechanical immobilization, handling methods and chemical immobilization on free-ranging chamois, to allow captures of groups of animals, considered suitable for reintroduction activities. This method foresees the associated use of the "Up-net" or box-trap and an acepromazine-medetomidine-ketamine administration. A standardized monitoring form was used to record the clinical findings. Out of 37 translocated chamois, 21 individuals were evaluated clinically and by comprehensive investigation (18 female - 1 yearling, 4 subadults and 13 adults - and 3 male -1 yearling and 2 adults -). The effects of this protocol on the captured animals was evaluated by means of hematological and chemical analysis. In addition, for the first time on this species was applied the venous blood gas analysis during the field activities, in order to assess the animal welfare during the capture sessions and prevent critical situations. The method and the chemical protocol applied in this study represent an innovative and useful approach for the free-ranging chamois captures, especially in conservation and management contexts, in which groups of animals should be captured and moved for reintroduction purposes.

SEROLOGICAL INVESTIGATION ABOUT TREPONEMA PARALUISLEPORIDARUM ECOVAR LEPUS IN NORTH ITALY

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Since the 1960s, Lepus europaeus populations have suffered a widespread density decline. In addition to habitat changes and inadequate hunting management, other causes influencing regional hare population decline are diseases. Our study aims to investigate Treponema paraluisleporidarum ecovar Lepus (TPeL) seroprevalence in North-Italy. The infection is usually asymptomatic, not included among the pathogens commonly investigated in passive surveillance. Current prevalence in hares is, therefore, likely underestimated. In 2018-2019, 248 and 82 hare sera were analyzedlive-trapped animals in North-Italy restocking areas andHungarian imported animals, respectively. We used a commercially available Treponema pallidum particle agglutination assay (TPPA) cross-reactive for anti-TPeL antibodies. Our results demonstrate a 36.9% seroprevalence and an even distribution of the infection across our study area. Further confirmation of the TPPA test results was achieved by additional testing of 39 positive and 20 negative sera using an immunofluorescence test (FTA-ABS). In our sample set, haresHungary were significantly less infected than animals that originatedItaly (p0.001), suggesting that the disease is likely endemic in Italy and a limited role of imports in diffusion, although this would need to be further explored. Whereasthe serologically tested hares were asymptomatic, the presence TPeL was confirmed with a specific PCR targeting polymerase I gene (poIA) on skin samples and oral/genital swabsother 5 hares presenting lesions referred to syphilis. TPeL epidemiological cycle is still to be studied in depth and presence and impact of other ecovariants, such as rabbit (TPeC), should also be investigated.



VETERINARY FORENSIC MEDICINE IN THE FIGHT AGAINST POACHING: THE CASE OF THE NORTHERN BALD IBIS IN ITALY

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The Centro di Referenza Nazionale per la Medicina Forense Veterinaria (CeMedForVet) at the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" ensures the contribution to justice of the Veterinary Medicine in the fight against poaching. Recently, Ce-MedForVet has been involved in a LIFE project for the conservation of the northern bald ibis (Geronticus eremita). The main goal was to increase the European population size to at least 120 individuals. Italy is the overwintering area of the ibisthe project. Here, however, the project was severely hampered by illegal killing. Poaching was the main cause of death, followed by disease, electrocution and predation. In 2017-2021, nineteen ibis were found dead and subjected to necropsy by CeMedForVet, where we diagnosed 9 cases of poaching (47%), 2 of disease (10%), 2 of starvation (10%), 2 of trauma (10%), 1 case of drowning (5%), 1 of electrocution (5%) and 1 of predation (5%). One case (5%) remained unsolved due to the high degradation of the carcass.ibisthe project were monitored by GPS trackers. This proved critical to the retrieval of dead birds. In two shot ibis GPS data helped to trace the point of origin of the shot. In poaching cases, forensic approach was necessary to provide evidence to the authorities. In our experience, good quality X-ray analysis, accurate necropsy and collaboration with other forensic scientists, such as ballistic experts, led to successfully solve caseworks.



UNUSUAL SEROTYPES OF SALMONELLA ENTERICA SUBSP. ENTERICA ISOLATEDTER-RESTRIAL TORTOISES IN THE EMILIA ROMAGNA REGION (ITALY)

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Objectives

Captive and wild reptiles are reservoirs of various Salmonella sp. and could be a direct source of infection for humans and animals. Usually zoonoses caused by cold-blooded animals are due to companion reptiles, but wild reptiles could also pose a risk of zoonoses. In the EU this is due to the increasing presence of reptiles as companion animals and their role as reservoirs of several zoonotic agents, including Salmonella. Reptiles are known to harbour Salmonella sp. in their intestines.

Methods

In this study, we examined the carcasses of 42 land tortoiseswildlife rescue centres (33 Testudo hermanni, 6 T. graeca, 3 T. marginata) to determine the cause of death. They were analysed for the presence of Salmonella, according to the ISO 6579-1:2017 method.

Results

Salmonella sp. was isolated24 animals (57.14%) out of 42.isolated strains were serotyped according to Method 6579-3:2014. The most frequently isolated serovar was S. Richmond (19.05%), followed by S. Halle (7.14%), S. Patience (7.14%) and S. Hermannswerder (4.76%). Of the serovars isolated, S. Veneziana was one of the least frequent but was the most frequent in humans in the region.

Conclusions

This study confirms the presence of unusual Salmonella serotypes in land tortoises. This should be considered and further investigated with regard to potential zoonotic risk, and information on potential health risks may be useful to minimise the risk of transmission to workers and volunteers involved in wildlife management and working in wildlife rescue centres.

VASECTOMY IN CAPTIVE DEER (CERVUS ELAPHUS) AS MANAGEMENTTOOL OF NON-RE-LEASABLE WILDLIFE

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The aim of the study was to describe the technique of vasectomy in deer (Cervus elphus) in the Majella National Park to reduce overcrowding conditions in enclonsures that host animals (with permanent disabilities or mal-imprinted animals that have lost fear of people and/or cannot recognize their own species, or habituated animals, for whom it's foreclosed successful rehahilitation, while keeping the hierarchical and hormonal conditions of the intact animals. Three 7-month-old deer were subjected to teleanesthesia using Dexmedetomidine, Tilet-amine and Zolazepam, Butorphanol and Ketamine. Before surgery, antibiotic coverage with Amoxicillin and Clavulanic Acid was performed.

At the level of the scrotal neck an anterior approach was done with a 3 cm incision of median raphe of skin and dartos, in the proximal-distal direction. Then the tunica vaginalis proper of each testis was incised and the ductus deferens was isolated, separating itthe deferential vessels. Two ligatures, approximately 1 cm apart, were made on the duct with Assufil USP 1 thread and the tract of the vas deferens between the two ligatures was removed. Subsequently, the skin was sutured with detached U-shaped stitches.

At the end of surgery, long-acting Cefovecin and Dexamethasone were administered. The surgical technique was quick and easy to perform. There were no complications either during surgery or during the post operative period. Amonth after the vasectomy, the same animals were anesthetized again to be transferred to another area of the park; at the same time the surgical dieresis was checked, and it was perfectly healed and did not present pathological processes. In conclusion, vasectomy in deer appears to be the minimally invasive surgery of choice for birth control without altering hierarchical status and hormone-dependent sexual characters.

Infectious and parasitic diseases, perspective in ecosystem health, environmental change and human-wildlife interface

CONTRIBUTION TO THE STUDY OF WILDLIFE'S PLACE IN WESTERN SOCIETIES: OVER-VIEW OF THE SITUATION AND ETHICAL ANALYSIS

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According to biological principles, the human species is only one species among others. Since over one million animal species have been fully identified, we represent a small fraction of animal kingdom. However, for more than 200 years now, human societies have impacted the environment they share with other animals, in an unprecedented way, leading to a major extinction crisis. The "defaunation" process expresses this critical situation which endangers not only wildlife but also humans.

In this context, western societies, which have for a long time defined the human species as distinct other animals, are behaving in an ambivalent way which doesn't make it possible to solve the problem. On the one hand, the western world is following a social and economic development model which consumes a lot of natural resources. Therefore, western countries seem deeply responsible for defaunation. However, on the other hand, they become aware of the problem and undertake significant efforts to counter the damage they are causing. Nonetheless, failure is persisting. This can be partially explained by the current difficulties encountered in the conservation field.

This bibliographic study investigates the relationship between western societies and wildlife. It highlights a complex situation which leads to this persistent biodiversity crisis. Provided they exist, solutions to this problem will certainly arisetransdisciplinarity, mainly biology, humanities and social sciences.

WILDLIFE POISONING IN ABRUZZI REGION DURING 2011-2020 PERIOD

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Objectives

Providing clustered data on toxicological investigations in wildlife carcasses recoveredAbruzzi region during 2011-2020.

Methods

A wildlife health archive of toxicological investigations was set up, where each case-record was defined as single or cluster of poisoning events, that is carcasses and/or poisoned baits having space-time and toxic connection.

Results

In the investigated period, overall 58 case-records of single or cluster of poisoning were reported, involving 139 wildlife and 98 baits. Red fox and wolf were the main target species reported in 48 case-records where they were or simultaneously involved with dogs, cats, other wildlife and baits. Among wild birds, 10 case-records were reported mainly involving the griffin. Carbamates (mostly aldicarb) were responsible for 24 case-records, followed by Organophosphates (mostly phorate) and Rodenticides (strychnine, anticoagulants) responsible for 20 case-records. The banned 2-Metil-4,6-Dinitro-Fenolo (DNOC) was responsible for 2 case-records involving 9 animals and 26 baits. L'Aquila was the province most troubled by the poisoning phenomenon, having reported 44 out of 58 (75,8%) events, mainly concerning the agro-forestry interfaces of Marsica's municipalities and the border areas between Abruzzi and Lazio regions.

Conclusions

The clustering methodology was more suitable to analyse the poisoning phenomenon than the simple enumeration of positive/negative reports. The main clusters of wildlife poisoning and baits were reported in the agro-forestry interface in Marsica's territory where fox and wolf are the target species and Carbamates the toxics mainly used, followed by Organophosphates and strychnine, still detected after its ban since 2006.

INFLUENZA VIRUS IN WILD DUCKS AND A WILD-LIVESTOCK ECOSYSTEM CHARACTER-IZATION

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The study of Influenza A Virus (IAV), its hosts, and their environment is crucial due to the emergence of highly pathogenic influenza outbreaks in poultry and wild waterfowl, its interspecies transmission, and zoonotic potential. However, studies based on this integrative perspective in southern North America, especially Mexico, are scarce. We aimed to identify IAV in waterfowl and determine the socio-environmental characteristics of a particular winter stop wetland. The study was conducted in a natural protected area and RAMSAR site, "Ciénegas de Lerma", located in the migratory Central Flyway. In winter 2019-2020, six hundred and sixty-nine cloacal samplesresident and migratory ducks were collected. Molecular detection by Real-Time PCR was performed. Water physicochemical parameters were measured. Nineteen IAV-positive Anatidae with different worldwide geographic distribution were identified: Anas acuta, Anas crecca, Spatula cyanoptera, Spatula clypeata, Spatula discors, and Anas diazi, the latter being a resident species. It means 2.84% IAV prevalence. The swamp is characterized by anthropogenic influences due to socio-environmental conflicts, loss of territory, pollution, land communication routes, agricultural lands, and backyard animals. The water parameters, such as temperature, pH, salinity, and ammonia, are conducive to the maintenance and dissemination of IAV. The birds identified as IAV-positive coincide with those reported in previous studies. Likewise, the characterization of the ecosystem indicates that it is favorable to IAV transmission between different species in a wild-domestic interface. Therefore, further studies are being conducted to identify the viral subtypes since Mexico is a hotspot of viral genome modifications for significant outbreaks in poultry.

FIRST DETECTION OF AVIAN METAPNEUMOVIRUS SUBTYPE C IN A EURASIAN WIGEON (MARECA PENELOPE) WINTERING IN NORTHEAST ITALY

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Avian metapneumovirus (aMPV) is a relevant poultry pathogen which affects turkeys and chickens causing respiratory and reproductive disorders. Repeated detections of aMPV in wild birds brought to hypothesize their potential role in the viral epidemiology. A molecular survey was therefore carried out on 23 species of the orders Anseriformes, Charadriiformes and Passeriformes sampled within the national Avian influenza surveillance plan, implemented2007 to 2010 in Northeast Italy. RNAs extracted492 oropharyngeal swabs were analyzed using a Real-Time RT-PCR able to detect and subtype aMPV. A single swab collectedan Eurasian wigeon (Mareca penelope), sampled in November 2007, was found positive to aMPV subtype C. The strain was named aMPV/C/IT/Wigeon/758/07. Matrix, fusion and attachment glycoprotein viral genes were amplified, sequenced, and phylogenetically compared with known aMPV homologous sequences. As a result, close genetic relationships were identified between the strain hereby reported and aMPV strains belonging to the subtype C, particularly with those isolated in the late 1990s in domestic ducks in France and belonging to the Eurasian genetic lineage. Considering that Eurasian wigeons are medium to long-range migrant dabbling ducks that move along the Black Sea/Mediterranean flyway, our finding might be related to migratory bridges between countries. To conclude, this study reports the first molecular detection of aMPV subtype C in a waterbird wintering in Italy and suggests the susceptibility of an additional wild species to aMPV. Additional research is needed to assess the role of wild birds in the aMPV-C epidemiology.

EPIDEMIOLOGICAL SURVEY OF TICK-BORNE ZOONOTIC PATHOGENS IN IXODES TICKS FROM MIGRATORY BIRDS FLYING THROUGH EASTERN ALPS, ITALY

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Migratory birds are recognized hosts of ticks and may also spread them, and associated pathogens, to distant geographical areas while migrating. The study aim was to evaluate the infestation burden and the presence of zoonotic pathogens in ticks collected from birds captured at the 'Malga Confin' ringing station (Julian Prealps Natural Park, Northeastern Italy) during post-reproductive migration along the Adriatic-Flyway, autumn 2019-2020. Data on sex, age, level of fat deposition and muscle development were recorded. Ringed birds (n=3414)wereinspectedforectoparasitesand88ofthem(blackbird,Europeanrobin,brambling, chaffinch, song thrush, redwing, water pipit, coal tit) harboured ticks. A total of 209 immature ticks were identified as Ixodes ricinus (n=207), Ixodes acuminatus (n=1) and Ixodes frontalis (n=1). DNA/RNA were co-extracted from ectoparasites and molecularly screened for Rickettsia spp., Anaplasma phagocytophilum, Borrelia burgdorferi s.l., Bartonella spp., Ehrlichia spp., and TBEV. Ticks tested positive for Rickettsia helvetica (16/209), Anaplasma phagocytophilum (9/209) and (3/209) Ehrlichia spp.. Of note, Borrelia burgdorferi s.l. was detected in 114/209 ticks and zoonotic species as Borrelia garinii, Borrelia afzelii, Borrelia valaisiana, and Borrelia miyamotoi were identified. A statistically significant correlation emerged between Borrelia burgdorferi positive ticks and birds with low fat and scarce muscle development. Borrelia burgdorferi s.l. positive ticks were also found coinfected with R. helvetica and Anaplasma phagocytophilum. All ticks tested were negative for Bartonella spp. and TBEV. Although migratory birds seem poorly infested with ticks, the remarkable prevalence of tick-related pathogens is noteworthy and highlights the importance of monitoring migrating bird populations.



ANTIBODIES AGAINST TICK-BORNE ENCEPHALITIS VIRUS IN WILD UNGULATES FROM NORTHEASTERN ITALY: PRELIMINARY STUDY

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Objectives

The tick-borne encephalitis virus (TBEV) is the causative agent of human tick-borne encephalitis. TBEV can infect several domestic and wild species other than humans. Some species, such as red deer, have been considered as sentinels of virus circulation. This study aimed to investigate TBEV antibody seroprevalence in wild ungulates from Friuli Venezia Giulia (FVG) and Veneto Alps.

Methods

A total of 110 blood samples, collected from wild ungulates culled during the 2018 and 2019 hunting seasons, were tested for TBEV antibodies by a commercial ELISA kit. In particular, a total of 64 and 46 samples were collected from FVG and Veneto regions, respectively.

Results

A total of 24 out of 110 samples (21,8%) tested positive and other 10 (9,1%) were borderline for antibody detection with the following breakdown: 10 positive samples were from FVG (10/64; 15,6%), and 14 from Veneto (14/46; 30,4%), while 7 borderline samples were from FVG (7/64; 10,1%) and 3 from Veneto (3/46; 6,5%).

Except mouflon, all surveyed animal species showed a positive or borderline antibody response.

Conclusions

This serological survey shows a high TBEV antibody seroprevalence in wild ungulates from northeastern Italian Alps, confirming the endemicity of the virus in the studied area. Direct detection of the virus in ticks and reservoir hosts rarely gives positive results, and the evaluation of seroprevalence in wild ungulates, applying a correct sampling approach, can be considered a useful tool to investigate the epidemiological trend of TBEV presence over time.



A ONE HEALTH APPROACH TO THE PATHOGENS TRANSMITTED BY TICKS IN THE ITALIAN CENTRAL ALPS IN THE PERIOD 2018-2021.

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Objectives

Ticks are the main vectors of pathogens in temperate climate zones. They are capable of transmitting pathogens of both human and veterinary interest. In recent decades, demographic and environmental changes have facilitated contact between humans and ticks with a consequent increase in infections caused by the pathogens they transmit. The World Health Organization promotes approach One Health with the aim of studying the interdependence between human, animal and environmental health in order to protect public health.

Methods

Between 2018 and 2021, 768 ticks were collected from humans and 934 collected in seven species of mammals. We identified by species and cycle stage vital, washed in 70% ethanol and homogenized in PBS using Tissue Lyser. Nucleic acids were extracted with the NucleoSpin® Tissue kit (Macherey-Nagel) and tested by amplification with endpoint PCR and Real-Time PCR for the following pathogens: Francisella spp, Borrelia, Coxiella burnetii, Rickettsig and for the ticks of humans TBEV.

Results

We detected in humans samples 58 ticks positive for Rickettsia, 86 positive for Borrelia and 2 for Francisella. Only 1 positive for TBEV (tick got in Germany) and 1 for Coxiella burnetii. In mammals we detected 106 positive for Rickettsia spp. and 10 positive for Borrelia spp. Conclusions

Our results confirm the existence of common epidemic cycles between wildlife and human populations frequenting the natural environment of the central Italian Alps. For some pathogens the risk of transmission appears higher. This epidemiological surveillance model will be extended to the entire Lombardy region.



EFFECTS OF CLIMATE CHANGE ON THE DISTRIBUTION OF A CONSERVATION INTEREST SPECIES IN TWO POSSIBLE FUTURE SCENARIOS: THE CASE OF MEADOW VIPER IN CEN-TRAL APENNINES

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Some recent ecological studies are about how habitats and organisms can response to ecosystems and biodiversity transformations. The aim of this work is show how a typical, endangered species of Central Apennines high altitude, Vipera ursinii, can be influenced by bioclimatic changes in two different future scenarios, also according to the recent COP 26. Distribution data of V. ursinii are based on Liberatore PhD thesis (2016). MaxEnt and GIS Softwares identify the main variables that influences potential distribution and its potential area in "good" scenario (SSP1-2.6: low level of emissions of greenhouse gases and CO2 emissions reach null value on about 2050) and in "bad" scenario (SSP5-8.5: very high emission rate during XXI Century). Scenarios are based on elaboration of 19 bioclimatic and elevation variables (CMIP6) extractedWorldClim website. This work demonstrates that highest suitability area of Vipera ursinii can decrease in medium period (2040-2060) if emissions of greenhouse gases continue rising both in "bad" scenario (-6,3%) and in "good" scenario (-3,8%) compared to actual distribution area, but the medium suitability area decreases only in "bad" scenario (-32,8%). Main variables that influence model are "Minimum Temperature of Coldest Month" and "Mean Temperature of Coldest Quarter". So, to know how species and their habitats are influenced by environmental changes can help scientist to stop biodiversity decline in future scenarios, considering socio-economic needs too, in particular studying temperature-related species,V. ursinii, which are indicators of global warming and ecosystem changes.



KLEBSIELLA SPP. ISOLATES FROM WILD ANIMALS IN CENTRAL ITALY: ANTIMICROBIAL RESISTANCE AT THE WILDLIFE-ENVIRONMENT-HUMAN INTERFACE

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Objectives

Nowadays wildlife is continuously exposed to anthropogenic activities due to the expansion of urbanized areas. The aim of this study was to evaluate the *Klebsiella* spp. occurrence in wild animals from Central Italy and the possible role of wildlife as reservoir of multidrug- resistant bacteria.

Methods

From 2019 to 2022 a total of 457 diagnostic samples including faeces (n = 316), intestine (n = 95) and brain (n = 46) were collected from carcasses of 420 wild animals (18 mammals' and 15 birds' species) in Central Italy. *Klebsiella* spp. was isolated using microbiological approach and confirmed by MALDI-TOF mass spectrometry. A subset of 16 *K. pneumoniae (Kp)* isolates was selected to perform antimicrobial susceptibility testing by broth microdilution and to check the correspondence of antimicrobial resistance with whole genome sequencing.

Results

A total of 106 *Klebsiella* spp. isolates were collected, including *Kp* (n = 85), *K. oxytoca* (n = 13), *K. variicola* (n = 6) and *K. aerogenes* (n = 2). *Kp* isolates displayed significant phenotypic resistance to tetracycline, beta-lactams and importantly to ertapenem, mainly used in human medicine. Moreover, three of 16 isolates (18.8%) were classified as multidrug resistant. Several antimicrobial genomic determinants were found in this dataset.

Conclusions

These preliminary data suggested the need of continuous surveillance on wildlife exposed to anthropogenic antibiotic resistance contamination through agricultural activities, livestock farming, waste disposal and wastewater management. The presence and persistence of multidrug resistant *Klebsiella* spp. strains in the environment could pose an increasing health risk for humans and animals.



EXPOSURE TO MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN ALPINE PAS-TURES: EVALUATION OF CATTLE AND RED DEER CONTRIBUTION THROUGH ENVIRON-MENTAL FAECAL SAMPLES.

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3. Stelvio National Park, Bormio, Italy

Objectives

Aim of the study was to assess the contribution of bovine and red deer in pasture contamination by Mycobacterium Avium Subsp. Paratuberculosis (MAP) in the Stelvio National Park (Lombardy area), where the prevalence of MAP in wild deer was previously estimated (2011-2015) to be around 20%.

Methods

Areas, either shared or not by deer and bovine, were selected during two successive grazing seasons. Environmental faecal samples (2020: N=757; 2021 N=2000) were analysed by qPCR for MAP detection.positive samples were cultured and MAP isolates were subtyped using MI-RU-VNTR and Short Sequence repeats. MAP shedding was quantified by digital PCR (dPCR).

Results

In 2020, 19 (2.5%) samples (16 deer; 3 bovine) were positive by qPCR. In 2021 only 3 deer samples tested positive. Digital PCR showed a low concentration of MAP in faeces (1000 copies/g). The same genotype was observed among the 6 obtained isolates.

Conclusions

Our results showed a wide but low contamination of Alpine pastures suggesting how MAP contamination on pasture does not likely represent a risk factor for transmission in this area. This low prevalence in both species compared to previous findings could be related to a selective deer population control and culling of clinical cattle over the years. Additional samplings are ongoing on culled animals to clarify if the low environmental contamination depends on prevalence reduction on hosts or because environmental faeces are not an appropriate matrix for achieving the desired sensitivity.



EMERGING RESISTANCE PROFILES IN BACTERIAL STRAINS FROM WILD AND DOMESTIC RUMINANTS IN CENTRAL ITALY

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The study was carried out to identify and characterize prevalence and antimicrobial resistance profiles of E. coli and Enterococcus spp. isolated from wildlife and livestock inhabiting the Maiella National Park, Italy. Ecological and georeferenced data were used to select wildlife and livestock sympatric or not. Bacterial isolates (n=88) from fecal samples collected between October and December 2019 were examined for species identification, antibiotic susceptibility testing and molecular detection of antibiotic resistance and virulence genes. Data were analyzed using Episheet and STATA software. Forty isolates were identified as E. coli and forty-eight as Enterococcus spp. Phenotypic antibiotic resistance to at least one antimicrobial agent was detected in 25/88 (28.41% CI95%: 19.3-39) isolates and multidrug resistance was detected in 5/88 isolates (5.68% CI95%: 1.87-12.76). The resistance genes were identified in 51/88 (57.95% CI95%: 46.95-68.40) and the virulence genes in 75/88 isolates (85.23% CI95%: 76.06-91.89). Indeed, the sympatric animals showed a prevalence of resistant isolates and potentially virulent isolates 2.75 and 2.88 times greater that animals that do not share the grazing lands, suggesting the potential transmission of drug resistance bacteria between animals cohabiting at wildlife-livestock interface. It emphasizes the need of further investigations focusing on environment-wildlife-human interactions, in order to improve an early detection of emerging AMR profiles and possible transmission routes.

POSTER SESSION

PREVALENCE OF DIFFERENT SALMONELLA ENTERICA SUBSPECIES AND SEROTYPES IN WILD CARNIVORES IN EMILIA-ROMAGNA REGION, ITALY

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Objectives

This study aims to investigate the presence of different Salmonella subspecies and serotypes in wild carnivores of the Italian region Emilia-Romagna.

Methods

Foxes (Vulpes vulpes), badgers (Meles meles) and wolves (Canis lupus) carcasses submitted between 2016-2021 were included for the present work. Isolation of Salmonella spp. was performed according to ISO 6579-1. Serogroup identification and serotypization were performed according to ISO/TR 6579-3:2014.

Results

almonella analysis was carried out in 142 badgers, 10 wolves and 591 foxes and positivity was observed in 18/142 (12%), 3/10 (30%) and 34/591 (5.7%) respectively. Isolated Salmonella enterica strains belonged to 4 different subspecies and 23 different serotypes. Salmonella typhimurium and S. veneziana were the most frequently isolated strains in foxes (4/34 each) and badgers (5/18, 3/18). The serotypes isolatedwolves, S. infantis (2/3) and S. stanleyville (1/3), were identified also in foxes (2/34, 1/34).

Discussion and Conclusion

The prevalence in both foxes and badgers was similar to other Italian surveys. Nevertheless, the prevalence of 12% found in badgers was higher than other European studies. Concerning wolves, even though few animals were analysed, a prevalence of 30% was assessed. Amongthe serotypes identified, many are relevant for human, livestock and pets' health. The present work highlighted the potential role as reservoirs or dead-end hosts of the investigated species. However, further analysis should be carried out to assess the actual role of predators: for instance, by relating strains isolated wild carnivores with thoseother wildlife species, livestock, pets and humans. Moreover, analysis of the antimicrobial resistance of the isolated strains could help to improve our knowledge under a One Health framework.

5

COMPARISON OF THE PREVALENCE OF DIFFERENT MICROORGANISMS (LEPTOSPIRA SPP., MYCOPLASMA SPP.) IN WILD AND CONFISCATED TESTUDO INDIVIDUALS.

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Objectives

Natural populations are the fundamental benchmark for any investigation aiming to assess the actual significance and disease ecology of any infectious organism considered to impact wildlife. Most studies on chelonians' diseases were carried out on captive individuals. Comparison between captive and free-ranging population allows to better highlight and discriminate the agents which could represent a threat for the investigated species. Following a multidisciplinary ministerial project aimed at appropriately relocating confiscated Testudo individuals, we carried out environmental, genetic, and clinical-diagnostic investigations on Testudo hermanni, T. graeca, T. marginata. A general study involving various disciplines was essential to protect natural populations, comparing them with confiscated animals. Our studies focused on the prevalence of some infections, including Leptospira spp. (L) and Mycoplasma spp. (M), and are discussed in our presentation.

Methods

A multidisciplinary team collected samplesfree-ranging animals and confiscated animals; direct test (PCR on cloacal samples for L and oral swabs for M) and indirect test (microag-glutination test for L) were performed.

Results

The detected prevalence by PCR was 20% and 0% for L, 34.3% and 2.2% for M, for confiscated and free-ranging animals, respectively; seroprevalence was 0.8% for L, with significant differences between free-ranging and confiscated animals.

Conclusions

Overall, the results show that the infectious agents L and M are more present in the confiscated animals than in free-ranging tortoises, limiting the possibility of relocation of the confiscated animals to contain the risk of spreading of these infections wild. A multidisciplinary approach is required before any relocation action is taken.



EVALUATION OF COASTAL AQUATIC ANIMAL BIODIVERSITY IN THE TRABOCCHI COAST (CH): THE IMPORTANCE OF ECOSYSTEM HEALTH AND WILDLIFE CONSERVATION WITH-IN THE CONTEXT OF GROWING HUMAN ACTIVITIES

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Objectives

This work is aimed to provide a qualitative evaluation of the fauna in a specific section of the Trabocchi Coast, with a focus on the fish living between the shoreline and the breakwater rocks. This is a renowned territory in the Italian mid-Adriatic portion, characterized by a growing tourism for its environmental uniqueness.

Methods

Semi-submerged breakwaters in the southern coast of Rocca San Giovanni (Chieti, Italy) were inspected through dynamic Underwater-Visual-Census (UVC)2017 to 2020. In each immersion (20/year), 300 m were examined and filmed through a GoPro7 and Fujifilm XP. The recorded media were post-produced in order to classify the animal species observed.

Results

We identified 2 Molluscae, 4 Crustacea, and 31 Osteichthyes, including Sparidae (10), Mullidae (1), Moronidae (1), Atherinidae (1), Serranidae (1), Labridae (3), Gobiidae (2), Sphyraenidae (1), Mugilidae (1), Carangidae (1), Sciaenidae (1), Blenniidae (5), Pomatomidae (1), Belonidae (1), Congridae (1).

Conclusions

Results showed one of the richest Italian underwater coastal fauna, consistent with Arbuatti's previous findings in 2015, in which the research was performed 2520 mt further north. The completion of the cycle path by 2022 will determine a greater anthropic impact, due to a growing tourism. The existing infrastructure and services might not be sufficient to face touristic needs. To support local economy and prevent possible outcomes on biodiversity and ecosystems, an increase of structural investments, as well as scientific research and education are required to improve locals and tourists' environmental consciousness and promote a real sustainable eco-tourism.



INTESTINAL PARASITES INFECTING SQUIRREL MONKEYS (SAIMIRI CASSIQU-IARENSIS) IN A HUMAN - NON HUMAN PRIMATE INTERFACE IN COLOMBIA

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Objectives

This study aimed to identify and molecularly characterize intestinal parasites of free-ranging squirrel monkeys living in three forest fragments in Colombia where non-human primates (NHP) are in close proximity to humans, and often subjected to food provisioning.

Methods

Ninety-seven fecal samplessquirrel monkeys (Saimiri cassiquiarensis) were collected immediately after defecation and stored in 96% ethanol and 10% formalin solution. Faecal smears and flotation were performed, and samples microscopically classified as positive for Ascarididae were processed for molecular characterization.

Results

98% of the samples were positive for intestinal parasites. Protozoans (Blastocystis sp., Dientamoeba sp., Entamoebidae, Giardia sp.), Nematodes (Ascarididae, Strongyloides sp., Trypanoxyuris sp.), Cestodes (Hymenolepis sp.), Trematodes (Controrchis sp.), and Acanthocephalans were identified based on morphology, while Ascaris lumbricoides was confirmed by molecular techniques.

Conclusions

The finding of intestinal parasites with zoonotic potential suggests epidemiological implications. We recommend conducting regular parasite surveys in NHP in order to monitor the potential zoonotic transmission risk. Additionally, educational activities with the exposed local communities should be encouraged in order to increase the awareness regarding the potential risk of zoonotic transmissions, and the importance of avoiding food provisioning and physical contact with NHP.



PERITONEAL LARVAL CESTODIASISMESOCESTOIDES SP. IN A RED FOX (VULPES VULPES) IN GRAN SASSO MONTI DELLA LAGA NATIONAL PARK (ITALY)

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Objectives

Discuss a case of peritoneal Cestodiasis in an adult female fox (Vulpes vulpes) found agonizing with uncontrolled limb movements near Gran Sasso Monti della Laga National Park (L'Aquila, Italy).

Methods and Results

At necropsy the fox presented tracheitis, large areas of pneumonia, catarrhal gastritis and enteritis. Abdominal cavity was invaded by hundreds of white bodies similar to grains of rice with a soft consistence, adhered to the omentum, and serosa of the intestine, liver, kid-ney and spleen. No adult intestinal parasites, including adult tapeworms, were found. Virological investigations on lung and brain revealed viral molecular signals of Canine Distemper Virus (CDV). Histopathological examination of liver showed multifocal parasitic cystic formations containing single or multiple larvae, surrounded by a thin fibrous capsule. The recovered immature worms were identified as tetrathyridia of Mesocestoides sp. (Vailant, 1863) (Cestoda, Cyclophyllidea, Mesocestoididae) by molecular analyses. Total DNA was extracted with DNeasy Blood & Tissue Kit after a lysis step with protein-ase K. After PCR amplification (ITS-D1 / D2 regions) and sanger sequencing, obtained sequences were analysed using BLAST (nucleotide collection nr/nt).

Conclusions

Although foxes can serve as both the second intermediate and definitive hosts for Mesocestoides spp., only larval stages were observed. To our knowledge, tetrathyridia have only been found in peritoneal cavity in two foxes in Spain and in one fox in Turkey and encysted in the hepatic parenchyma in one fox in Italy. This is the first report of proliferative peritoneal larval cestodiasisMesocestoides sp. in a fox in Italy.



TAENIID CESTODES IN APPENNINE WOLF (CANIS LUPUS ITALICUS) LIVING IN AN AN-THROPIC AREA: A ONE HEALTH PERSPECTIVE

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The wolf plays a key role in the maintenance and transmission of several taeniids species, some of which are relevanta one health perspective. The colonization of human-modified environments by the wolf leads to an increase in zoonotic infection transmission risk. In this study, the presence of taeniids in a wolf pack living in a highly anthropic area of Pisan hills characterized by an interaction between wildlife, livestock, and recreational human activities was evaluated. Thirty-eight wolf fecal samples were collected and microscopically analyzed. Fecal samples positive for taeniids eggs were molecularly analyzed to identify Taeniidae species. On fecal samples collected diet analysis was also performed. The frequency of taeniid eggs detection resulted 34.2% (13/38). At molecular analyses, only Echinococcus granulosus s.s (26.3%, 10/38) and Taenia hydatigena (10.5%, 4/38) were identified. The wolf pack diet in this highly anthropic area resulted mainly based on wild ungulates (78.79%, N=104) as happen also for wolf living in the natural landscape (80-90%), while the frequency of livestock occurrence resulted higher (28.79%, N=38) than those detected in wolf in wild environments (4-10%). The high frequency of E. granulosus should be ascribed to the highly anthropized environment with a high livestock density and proximity to farms with infected dogs and sheep. The low biodiversity of taeniids detected in wolves could be due to low parasite biodiversity in wild prey in this restricted anthropized area.



NO DETECTION OF OPHIDIOMYCES OPHIDIICOLA IN SNAKES FROM "RITO DEI SERPA-RI" OF PRETORO (ABRUZZI, ITALY) SHOWING GROSS SIGNS OF DISEASE

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Two ancient religious rites, involving snakes as part of San Domenico Abate worship, are celebrated annually in Abruzzi (Central Italy). The less famous celebration is the one of Pretoro (Chieti): born in the last part of the XIX century, it consists in a competition between ophidians captured by snake hunters called "serpari". Since 2015, thanks to a consortium, the ritual has been subjected to regulations aimed to protect the ophidians and to instruct snake hunters. In 2019, a preliminary screening for the fungus *Ophidiomyces ophidiicola* (Oo), an emergent pathogen for snakes, has been performed. All the snakes participating to the ritual underwent to clinical examination, and the ones showing signs consistent with ophidiomycosis have been dry swabbed along the skin and cutaneous lesions with a single sterile cotton-tipped applicator. The samples were stored at +4°C until DNA extraction. A broad-range panfungal PCR (D1-D2 region) and two conventional PCRs using specific sets of primers – targeting Oo ITS2 and IGS genes – have been performed and run on agarose gel for visualization.

Twelve out of 61 snakes (19,7%: 11/49 *Hierophis viridiflavus* [22,4%]; 1/8 *Zamenis longissumus* [12,5%]; 0/4 *Natrix helvetica* [0%]) showed signs consistent with Oo infection (dysecdysis, serocellular crusting, swellings) and have been swabbed. No PCRs showed product amplification.

So far, the presence of Oo in sampled snakes cannot be excluded, and improvement of diagnostics methods is in progress. Oo screenings will be further carried out to scientifically valorize events like Pretoro celebration and its centenary dimension linking religion, human and wildlife.

MOLECULAR DETECTION AND PHYLOGENETIC ANALYSIS OF CANINE DISTEMPER VI-RUS IN MARSICAN BROWN BEAR (URSUS ARCTOS MARSICANUS)

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In 2021, a live-trapped Marsican brown bear resulted positive for Canine distemper virus RNA by means of diagnostic RT-PCR carried out in accordance with the monitoring activities of National Conservation Plans. The sequences analysis revealed the specificity of amplicons for the Europe Wildlife lineage of CDV, the same viral strain recovered from three foxes and two unvaccinated dogs coming from the same territories where the positive bear was captured. These results confirm the receptivity of Marsican brown bear for CDV, apparently without any pathological consequences for the positive animal, and suggest the presence in the area under study of a unique wild host-adapted lineage of the virus, able to spreading in domestic animals, too. The small size of population along with the numerous threats that can further affect the conservation of this species make it necessary specific surveillance programs, focusing the early identification of pathogens potentially able to influence the health status of animals. The presence of CDV-infected domestic dogs inhabiting with Marsican brown bears once again confirms that more effective vaccination program in dog populations is crucial, with particular emphasis for the protected areas of Central Italy.

ECOCARDIOGRAPHIC AND ELECTROCARDIOGRAPHIC STUDY OF TWO ADULT BEAR MALES (URSUS ARCTOS ARCTOS) UPON AWAKENING FROM HIBERNATION

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The population of European brown bear (Ursus arctos) present in the Alps, counts just over one hundred individuals. The reintroduced subjects belong to the Slovenian population and are of the same subspecies as the native one (Ursus arctos arctos). The subjects reintroduced in the territory of the Adamello Brenta Natural Park between 1999 and 2001 were 10 (3 males and 7 females). Given the important size and athletic performance of the species, the evaluation of cardiac function, for the first time, in bears of the Trentino population has been posted or as the purpose of this study. The study in question is carried out on wild adult specimens of the Alps, housed in a provincial structure waiting for reintegration into the wild. The bears, at the time of the examination, upon awakening from hibernation, had, the first 5 years and a weight of 220 Kg and the second 3 years and a weight of 160 Kg. Subjects underwent telenarcosis prior to each investigation. A portable ultrasound with phased-array multifrequency probes and a portable ECG device were used . For echocardiography, the acoustic window of the right axillary region of both specimens was used, after trichotomy, with the subjects in sternal decubitus on a special natural straw bed. For both, no anomalies of electrical conduction emerged. Echocardiographic examinations have shown values of cardiac function in the reference ranges compared to previous studies. Unlike previous studies, mild aortic insufficiency with a low-velocity retrograde flow was appreciated; no evidence of other abnormalities either in the valve systems or in the working myocardium level. In conclusion, it can be said that, in the two bears under study, no significant cardiac alterations have emerged that could compromise their reintegration into nature.

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