



S.I.E.F.
Società Italiana
Ecopatologia
della Fauna

ATTI DEL

**V CONGRESSO NAZIONALE DI
ECOPATOLOGIA DELLA FAUNA**

Udine
14-17 settembre 2022

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- Università degli Studi di Udine

In collaborazione con la Società Italiana di Medicina dei Viaggi e delle Migrazioni (SIMVIM), con l'Associazione Teriologica Italiana (ATIIt) e con la Società Italiana Medici Veterinari degli Animali Selvatici e da Zoo (SIVASZOO).

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V CONGRESSO NAZIONALE DI ECOPATOLOGIA DELLA FAUNA

14-17 settembre 2022

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Con il patrocinio di



E con il patrocinio della Regione Autonoma Friuli Venezia Giulia

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Razionale del Congresso

Il Congresso Nazionale di Ecopatologia della Fauna è il principale appuntamento a livello nazionale nel campo dell'ecopatologia della fauna. Lo scopo del Congresso è quello di promuovere il confronto sugli aspetti ecopatologici della gestione e della conservazione della fauna, sulle implicazioni di sanità pubblica e sanità animale legate alla presenza e alle dinamiche delle popolazioni selvatiche, sui rapporti tra gli animali selvatici e le attività umane e, infine, sulle ricerche più avanzate in ecopatologia. Il Congresso si rivolge a ricercatori, operatori della sanità pubblica ed animale, tecnici faunistici, esperti di gestione, appassionati e più in generale a tutte le istituzioni coinvolte nelle tematiche affrontate.

Il Congresso è articolato in quattro sessioni tematiche non contemporanee e in un workshop, che abbracceranno diversi temi, dalla conservazione e gestione alla ricerca, sempre in chiave ecopatologica. Nell'ottica del confronto e della trasversalità, alcune sessioni tematiche saranno tenute in collaborazione con altre Società Scientifiche specificamente attive sugli argomenti in questione.

Atti del V Congresso Nazionale di Ecopatologia della Fauna

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Abstract – Comunicazioni

Sessione 1
INFEZIONI TRASMESSE DA VETTORI

In collaborazione con SIMVIM

Moderatori: Marco Bregoli - Rudi Cassini - Andrea Rossanese

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ECOLOGY AND ETOLOGY OF *IXODES RICINUS* TICK, IN RELATION TO THE MAIN TRANSMITTED PATHOGENS

DEL FABBRO S.¹, ANNOSCIA D.¹, BORTOLOMEAZZI R.¹, D'AGARO P.², FRIZZERA D.¹, GOLLINO S.³,
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Introduction

The hard tick *Ixodes ricinus* L. is the vector of pathogens of medical and veterinary importance; including the tick-borne encephalitis virus (TBEV) and the spirochete *Borrelia burgdorferi*, etiologic agent of Lyme disease, which are both endemic in some areas of the Friuli Venezia Giulia region (Italy). Rainfall is the most important environmental factor promoting the spread of *I. ricinus* in the region followed by habitat availability, altitudes and solar radiation. The infection's risk for humans is mainly influenced by ticks' abundance and the prevalence of pathogens that they transmit. In addition, the incidence of tick-borne diseases can depend on the behavioural manipulation capacity of the pathogen. This can promote ticks' host-seeking behaviour (questing) and maximise the transmission to the final host.

Materials and Methods

In order to investigate if *B. burgdorferi* is able to manipulate the questing activity of *I. ricinus* to maximize its opportunity of host-to-host transmission, a laboratory assay has been conducted to observe if the questing activity and behaviour vary in accordance with the spirochete infection.

Secondly, since the importance of the cuticle in water transpiration, we investigated the effects of the pathogen infection on the cuticle conformation. Finally, ongoing analysis will reveal if *Borrelia* infection is able to enhance ticks' survival in stressful humidity conditions.

Results and Discussion

Our results indicate that infected ticks are more mobile than the uninfected ones and showed a greater propensity to station at stressful humidity altitudes. In general, because of the disadvantageous surface area/volume ratio, the ability of an arthropod to tolerate low relative humidity depends on the permeability of the cuticular layer. Infected ticks highlighted a reduced percentage of unsaturated cuticular fatty acids if and a higher percentage of saturated fatty acids compared to the uninfected ones. This may limit the fluidity of the cuticle and make the infected ticks more resistant to dehydration. The higher mobility of the infected ticks and the modification of the cuticular lipids suggest that the pathogen has the potential to influence the vector activity of *I. ricinus*, maximizing the chances of transmission to the final host. Future analyses are necessary to confirm this aspect. Vector's behavioural manipulation by the pathogen is another important factor to be considered in studies about the incidence of tick-borne diseases.

VECTOR-BORNE DISEASES, AN EMERGING RISK TO HUMAN HEALTH

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Introduction

Vector-borne diseases, such as ticks and mosquitoes, will represent an emerging public health challenge over the coming decades. The underlying reason is related to the modification of the ecosystems, caused, to a large extent, by climate change, which contributes to higher proliferation rates of vectors, longer transmission seasons, changes in the ecological balance of wildlife, reservoir of various pathogens.

Lyme disease and TBE

Lyme borreliosis is the main vector-borne disease in continents with a temperate climate, caused by the spirochaete *Borrelia burgdorferi* s.l., which is transmitted by hard ticks, generally of the Ixodes complex (*I. ricinus* or *I. persulcatus*).

Lyme borreliosis is a systemic disease with protean manifestations, including dermatologic, rheumatologic, neurologic and cardiac abnormalities. The most common clinical marker for the disease is erythema migrans, the initial skin lesion that occurs in 60-80% of patients.

It is not always easy to diagnose and is treated with common antibiotics, which, in most cases, lead to healing. A percentage of patients between 10 and 20% have symptoms persisting even after treatment.

The same type of tick can transmit a flavivirus responsible for TBE (Tick-borne encephalitis). The infection can remain asymptomatic or present with fever, it can regress in a few days or become complicated with the involvement of the central nervous system in the form of meningitis, meningo-encephalitis or meningo-encephalo-mielitis, thus requiring a long convalescence and sometimes causing permanent damages. In a limited percentage of cases in Western Europe (1-2%) it could be fatal, this percentage exceeds 20% in Eurasian countries. TBE is the only tick bite-borne disease that can be preventable by vaccine.

West Nile fever

The proliferation of mosquitoes (*Culex*), in particular during 2022, caused the spread of West Nile virus (WNV).

It is an important zoonotic flavivirus responsible for mild fever to severe, lethal neuroinvasive disease in humans, horses, birds, and other wildlife species. Since its discovery, WNV has caused multiple human and animal disease outbreaks in all continents, except Antarctica. Moreover, globalisation and climate change are also important drivers of the emergence and re-emergence of the virus and disease.

Conclusions

Climate change has a great impact on the vector-borne diseases, by affecting the interactions of the vector–host–pathogen triangle. The need for epidemiological surveillance is considered to be of major importance since these diseases, do not only constitute a future threat, but at the same time affect the present.

ENDEMIC AND EMERGING VECTOR-BORNE ZONOSIS IN NORTH-EASTERN ITALY: EPIDEMIOLOGY AND RISK FACTORS FOR HUMANS

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Introduction

Zoonoses are animal diseases that occasionally affect humans. In recent years we have witnessed a widespread increase in cases of zoonosis and this trend has not spared even Italy. In particular, in the north-eastern part of the country, cases of Lyme borreliosis (LB), tick-borne encephalitis (TBE) and West Nile fever (WNF) have significantly increased.

Epidemiology

LB is classified as a rare disease and for this reason clear epidemiological data are not available: experts estimate that about 500 new cases of LB occur in Italy every year, about 90% of which in the north-eastern corner. According to the latest regional bulletin on arboviroses (09/09/2022), in Veneto during this year there were 31 confirmed cases of TBE (23 regional autochthonous and 8 autochthonous from another region) without deaths; 138 confirmed cases of WNF (134 autochthonous) and 107 confirmed cases of WNND (106 autochthonous) with 15 deaths (80% males, mean age 83.1 years). In the autonomous province of Trento about 25 cases/year of TBE (particularly from the Valleys of Non, Cembra and of Lakes) are notified, whereas WNV infections are less frequent. Data from Friuli-Venezia Giulia say that in the last year there have been 14 autochthonous and 4 imported TBE cases, 10 cases of WNF and only 1 case of WNND.

Risk factors

The ticks involved in the transmission of the most widespread zoonoses in our regions are mainly *Ixodes ricinus* and *I. persulcatus*, but occasionally also *Dermacentor reticulatus* can be involved. The Ixodidae are the most common hard ticks in Europe, they feed mainly on small rodents and ungulates. *Dermacentor* feeds mainly on dogs. Rare above 1300 meters, they live in wooded environments rich in bushes, humid (up to about 80%) and shaded, with low vegetation and beds of dry leaves. These are mainly the border areas between meadow and forest. The causative agent of LB is a borrelia that lives in the stomach of the tick: at the end of the meal, at the moment of detaching, the mite regurgitates the last part of what it was sucking, thus inoculating the bacterium in the bloodstream of his host. For this reason, quick tick removal can help prevent LB effectively. It should be emphasized that the most common borrelias in Italy (*B. afzelii* and *B. garinii*) are those that give the typical manifestation of erythema migrans and have a more marked tendency to invade the nervous system, while arthritis is characteristic of the infection from *B. burgdorferi s.s.*, rare in Europe and vice versa prevalent in the United States. However, when we talk about the tick-borne encephalitis virus (TBEV), the story is different: it lives in the salivary glands of the tick and, therefore, its passage to the host's bloodstream is contextual to the beginning of the blood meal. In this case, early tick removal will have a much lower chance of preventing infection. As with most viral infections, also in this case the TBEV infection occurs mostly asymptomatic (about 70% of cases) or paucisymptomatic (20-25% of cases), while the severe neurological forms fortunately do not concern more than 5-10% of the total number of infected. While we have effective antibiotics (tetracyclines and beta-lactamines) available for the treatment of LB, there are no effective antivirals for the treatment of TBE, for which one must act in advance with the specific vaccination. The main risk factor for contracting a tick bite disease is obviously represented by the frequentation of environments infested with these mites. It will always be worth wearing suitable (covering) clothes, preferably pre-treated with pyrethroids, and using repellents to support. In the case of TBE vaccination prevention, in addition to laboratory personnel exposed to the virus and people slaughtering potentially infected animals, the vaccine is indicated for all those living in highly endemic areas (≥ 5 cases of TBE / 100,000 inhabitants / year) and for visitors of "outdoor" environments who plan to engage in risky activities (eg camping, hiking, jogging, mountain biking, orienteering, hunting or picking flowers / mushrooms / berries). WNF is caused by the West Nile virus (WNV), transmitted to humans by mosquitoes of the genus *Culex*. Since these are common domestic mosquitoes, the actual prevention of this infection is difficult, since it is impossible to think of being able to completely eliminate the vector. As already seen for TBE, the virus responsible for the disease is inoculated with the insect's saliva at the time of the bite. At least 75-80% of WNV infections are asymptomatic or paucisymptomatic, no more than 20% are WNF and only 1% of infections involve the nervous system with the neuroinvasive form (WNND), which can, although rarely, be fatal or leave heavy neurological relics. The prevalent asymptomatic nature of the infection makes it difficult to know the real prevalence of the disease, which is often attempted to detect after some forms of WNND have been identified. The portion of the most complex framing of the problem is represented by WNFs, which should be 150 times more frequent than WNNDs and which, on the other hand, are very often of an equal or slightly higher number, if not even lower. Since there are no valid treatments or vaccines available for this zoonosis, prevention is mainly based on personal protection from mosquito bites, avoiding unnecessary outdoor exposure, especially at dusk, using appropriate mosquito nets, skin repellents and insecticides.

Conclusions

Zoonoses are animal diseases that are increasingly affecting humans. A special attention must be given to prevention of mosquitoes and tick bites with every single personal protection measure and device available. Having effective vaccines available would be of great help against diseases like LB and WNF.

RAPID IDENTIFICATION AND GENOTYPING OF *BORRELIA* SPP. IN AN ENDEMIC AREA: PRELIMINARY RESULTS

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Introduction

Ticks transmit a great variety of zoonoses, and those transmitted by the genus *Ixodes* are frequently occurring throughout North-Eastern Italy. In particular, Lyme disease is a widespread severe zoonosis whose etiological agent, *Borrelia burgdorferi* (s.l.), has a number of different genotypes (i.e. *B. burgdorferi sensu stricto*, *B. garinii*, *B. afzelii*, *B. bavarensis* and *B. spielmanii*) causing the typical form of Lyme-disease. Moreover, the genotype *B. miyamotoi* induces relapsing fevers and a variety of significantly different clinical symptoms, while for genotypes such as *B. valaisiana* the zoonotic potential is still debated. The core area of the research is the Belluno Province, recognized as endemic for Lyme disease, where different genotypes of *Borrelia* have been detected in past surveys. This study focuses on the ecology and epidemiology of *Borrelia* spp., aiming to create a collection of *Borrelia* strains for genotypic characterization and antimicrobial-sensitivity tests, as well as to build libraries of protein spectra for species identification by mass spectrometry for both *Borrelia* and tick species.

Material and Methods

Ticks were collected in 2021-2022 as follows: i) weekly, from March to August, by dragging/flagging in 7 different sites of the province; ii) throughout the two years, from wild animals retrieved by passive surveillance; iii) throughout the two years, from people accessing the Emergency Room of the Belluno Hospital. Ticks were morphologically identified and grouped according to their developmental stage. Single adults ticks, pooled larvae and nymphs (maximum 10 specimens per pool) were washed in serial dilutions of ethanol, rinsed with distilled water and then dried. Ticks were homogenized using the TissueLyser II in 400 µl of Barbour-Stonner-Kelly H (BSK-H) medium; 200 µl of homogenate were used for nucleic acid extraction for molecular detection of *Borrelia* using a Real time PCR and 200 µl were added in a 1 ml of BSK-H and then incubated at 30°C for cultivation. Broth cultures of *Borrelia* spp. positive ticks were examined every 15 days for the presence of spirochetes using dark-field microscopy. Multilocus sequence typing (MLST) based on eight housekeeping genes was performed for the positive *Borrelia* samples. Different reference strains of *Borrelia* and ticks collected in the field and laboratory breeds were used to create the reference spectra database for matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS).

Results and Discussion

About 4000 ticks were collected from the field and all of them belonging to *Ixodes ricinus* species. A total of 545 DNA/RNA extracts were obtained: 200 from adults, 110 from pools of larvae (n=1043) and 235 from pools of nymphae (n=2218). The 19% (n=103) of samples were positive to *Borrelia* spp. and the most frequently genospecies was *B. afzelii* (44.7%; n=46), followed by *B. garinii* (24.3%; n=25), *B. miyamotoi* (11.7%; n=12) and *B. burgdorferi* s.s. (6.8%; n=7); 12.6% (n=13) of samples has not been genotyped yet. Overall, 89 *I. ricinus* ticks (33 female, 50 nymphae and 6 larvae) were collected from humans, of which 15.7% (n=14) tested positive for *Borrelia* spp. The most frequently detected genospecies was *B. afzelii* (42.9%; n=6) followed by *B. garinii* (14.3%; n=2) and *B. burgdorferi* s.s. (7.1%; n=1); coinfections with 2 genospecies (*B. afzelii* and *B. burgdorferi* s.s.) was also found (7.1%; n=1). In 28.6% (n=4) of *Borrelia* spp. positive ticks, none of the four investigate genospecies were detected. All 72 ticks collected from wildlife (one fox, two roe deer, two wolves and one deer) in 2021 resulted negative for *Borrelia* spp. at the molecular analysis.

Currently, the MLST analysis results are available for 7 ticks only (5 from humans and 2 from the field): seven different sequence types (STs) were detected. Nevertheless, the data are still insufficient to draw conclusions about the genotypic characteristic of the *Borrelia* population in the study area. Viable spirochetes were detected in 16 samples incubated in BSK-H medium, but the low concentration of *Borrelia* in ticks, together with the high concentration of contaminants and the long growth-times, have made the isolation of this pathogen difficult in pure culture up to now.

Although these results are to be considered preliminary, the data so far collected seem to confirm that Belluno Province is highly endemic for *Borrelia*. *Borrelia afzelii* was confirmed as the predominant genospecies. Noteworthy, *B. miyamotoi*, the etiological agent of tick-borne relapsing fever, was found in high percentage of ticks infected; this pathogen is still poorly explored and probably underestimated in humans. Moreover, data from field showed an increase of ticks' density in 2022 compared to 2021. This trend could be influenced by climate change, characterized by an increase of mean temperatures in alpine and pre-alpine areas. This rising tendency should be kept under observation since it represents a risk for humans to contract tick borne pathogens infections.

The present work is part of the research project IZSVE RC 08/2020 founded by the Italian Ministry of Health.

DISTRIBUTION OF TICKS AND RICKETTSIA SPP. IN WILDLIFE AND HUMAN POPULATION: ANALYSIS FROM 2018 TO 2021 IN ITALIAN CENTRAL ALPS

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Introduction

Ticks are important vectors of public health and wildlife pathogens. Tick-borne diseases include rickettsioses, which are considered emerging in Europe and endemic in Italy. The agents of rickettsiosis in Europe mainly belong to the Spotted Fever Group (SFG) which includes several species or subspecies of Rickettsiae. Among these the most frequently encountered is *Rickettsia conorii*, responsible for Mediterranean Spotted Fever (MSF) and isolated mainly from *Rhipicephalus sanguineus*. Recently, another rickettsiosis called SENLAT (Scalp Eschar and Neck Lymphadenopathy After a Tick bite) has been observed. The aetiological agent of SENLAT is *Rickettsia slovaca*, strictly related to the *Dermacentor* tick genus. Thanks to the collaboration established with the National Health Service, important information was obtained on the behavior of *R. slovaca* and its spread in the human and wildlife population in the period from 2018 to 2021, increasing the knowledge on a pathogen with a so far neglected impact on human health.

Material and Methods

During the period from 2018 to 2021, in the Italian Central Alps, a total of 943 ticks were sampled from 7 species of wildlife (roe deer, deer, fox, badger, chamois, beech marten, wild boar), received in the framework of the wildlife surveillance program, and 765 were collected from humans. Ticks recovered from humans were removed and sent to IZSLER by medical staff at the emergency room, through a partnership established between IZSLER and the National Health Service, otherwise ticks could be conferred to the laboratory directly by people. For each tick the developmental stage was reported and the species identification was performed using taxonomic keys. For DNA extraction, ticks were homogenized in PBS using a Tissue Lyser. The amplification was performed by endpoint PCR targeting *Rickettsia gltA* gene. PCR products were analyzed by 2% agarose gel electrophoresis and positive samples were subjected to Sanger sequencing.

Results and Discussion

Out of 943 ticks collected from wildlife 898 (95.2%) were *Ixodes ricinus*, 29 (3.1%) were *Dermacentor reticulatus* and 16 (1.7%) were *R. sanguineus*. Among them, 107 were positive for *Rickettsia* spp., in particular, 33 (30.8%) were *Rickettsia helvetica*, 23 (21.5%) were *Rickettsia monacensis*, 11 (10.3%) were *R. slovaca*, 2 (1.9%) were *Rickettsia hoogstraalii*, only 1 (0.9%) was *Rickettsia mendelli* and 37 (34.6%) were not identified. *R. slovaca* was found in 8 *D. reticulatus* (sampled from roe deer and deer) and 3 *I. ricinus* (collected from roe deer). All the *I. ricinus* reported positive for *R. slovaca* were associated with a concomitant presence of *D. reticulatus* tested positive for the same aetiological agent. This suggests that *I. ricinus* infection may have been facilitated by co-feeding.

Of the 765 ticks collected from people, 726 (94.9%) *I. ricinus*, 6 (0.8%) *D. reticulatus*, 5 (0.7%) *Ixodes hexagonus*, 2 (0.3%) *R. sanguineus* were identified and 26 (3.4%) could not be identified. Of these ticks, 84 were positive for *Rickettsia* spp., of them, 42 (50.0%) were *R. monacensis*, 27 (32.1%) were *R. helvetica* and 15 (17.9%) were not identified. No *R. slovaca* was found in ticks collected from human.

Unlike as observed in another study conducted in Euganean Hills Regional Park that found *R. slovaca* in wild boar ticks, in our study positivities were found in ticks sampled from deer and roe deer. Besides that, this data confirm that *Rickettsia* spp. is circulating among wildlife and human population and even if *R. slovaca* was found only in ticks in wildlife, this leads to the possibility of transmission to humans, particularly those who frequent wild environments. Wildlife population dynamics and climate change can affect tick population and the pathogens they transmit. For these reasons, it is necessary to collaborate with the National Health Service to maintain high surveillance against tick-borne diseases and to inform people about the risks associated with tick-bites and the prevention methods to be implemented.

ANTIBODIES AGAINST TICK-BORNE ENCEPHALITIS VIRUS AND OTHER ZOONOTIC FLAVIVIRUSES IN WILD UNGULATES FROM NORTHEASTERN ITALY

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Introduction

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 sentinels of virus circulation. This study aimed to investigate the seroprevalence of TBEV antibodies in wild ungulates from Friuli Venezia Giulia (FVG) and Veneto Alps.

Material and Methods

One hundred and eleven blood samples, collected from wild ungulates culled during the 2017 and 2018 hunting seasons, were tested for TBEV antibodies using a commercial ELISA kit. In particular, a total of 65 and 46 samples were collected from the FVG and Veneto regions, respectively. Positivity to the screening test were confirmed by the virus neutralization test (VNT) against TBEV, Usutu virus (USUV) and West Nile Virus (WNV).

Results and Discussion

A total of 34 out of 111 samples (30,6%) tested positive or borderline to the screening for the detection of TBEV antibodies with the following breakdown: 17 positive samples were from FVG (17/65; 26,2%), and 17 from Veneto (17/46; 37%). Except mouflon, all surveyed animal species showed a positive or borderline antibody response.

All ELISA positive samples from Veneto regions were confirmed for TBEV antibodies by VNT and one resulted also positive for WNV antibodies. Four out of 6 wild boars resulting positive at ELISA test in FVG were USUV positive, one was negative, and one was not eligible for VNT. Therefore, the 37% prevalence of anti-TBEV antibodies was confirmed in Veneto, while the prevalence decreased to 18,5% in FVG.

This serological survey shows a high seroprevalence of TBEV antibodies in wild ungulates from northeastern Italian Alps, confirming the endemicity of the virus in the studied area. Direct detection of TBEV in ticks and reservoir hosts rarely yields positive results, and the assessment of seroprevalence in wild ungulates, applying a proper sampling approach, can be considered a useful tool to investigate the epidemiological trend of virus presence over time. Furthermore, the detection of WNV and USUV antibodies in wildlife indicates the presence of these mosquito-borne zoonotic flaviviruses in alpine areas.

CYTAUXZOOM SPP. AND HEPATOOZOOM SPP. IN TICKS, WILDCATS AND DOMESTIC CATS FROM NORTH-EASTERN ITALY

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Introduction

Cytauxzoon spp. and *Hepatozoon* spp. are tick-borne pathogens infecting a wide range of mammals worldwide. Data on epidemiology, life-cycle and transmission between wild and domestic felids in Europe are still scant. To date, no arthropod vectors were found positive for *Cytauxzoon* spp., whereas feline *Hepatozoon* DNA was already reported in engorged *Ixodes* ticks, but their competence in transmission has not yet been proven.

In this study, *Cytauxzoon* and *Hepatozoon* were investigated in ticks, European wildcats (*Felis silvestris silvestris*) and domestic cats (*Felis silvestris catus*) from North-eastern Italy, known to be an endemic region for both protozoa.

Material and Methods

Nineteen European wildcats found dead from 2013, due to road accidents, in Trieste, Udine and Pordenone provinces (Friuli-Venezia Giulia region, North-eastern Italy) were included in the study and 40 domestic cats from the same provinces were sampled from 2019 to 2021 thanks to the aid of local veterinarian practitioners. DNA was isolated from wildcats blood or clot, lung, liver, lymph node, heart and spleen and from domestic cats K₃EDTA blood samples. A conventional PCR (18S-rRNA) was performed to detect both protozoa. Then, a nested PCR (Cytochrome B gene) was run to determine *Cytauxzoon* species.

Ticks were collected in public gardens and wooded areas in Trieste province (where sampled domestic cats were located). Sampling took place from April to September 2021 by dragging and flagging. Ticks were morphologically identified then stored at -20 until DNA isolation. Nymphs were grouped according to species and sampling date/site in pools (up to 10 individuals per pool), whereas adult ticks were examined individually.

DNA from ticks was isolated and submitted to conventional PCR (16S- and 12S-rRNA) to confirm morphological identification. Then, the same PCR protocol for 18S-rRNA gene was performed for the protozoa detection. Amplicons were sequenced and sequences were compared to those in GenBank® dataset.

The infection rate for pools was obtained using generalized linear modelling to calculate maximum-likelihood estimates of prevalence with EpiTool (<https://epitools.ausvet.com.au/ppvariablepoolsizes>).

Results and Discussion

Overall, *Cytauxzoon europaeus* was found in 4/19 (21.1%) wildcats spleens, whereas all other tested tissues and organs resulted negative. Besides, 6/40 (15%) blood samples of domestic cats were found positive to the same species.

Hepatozoon DNA was isolated in at least one organ of 8/19 (42.1%) wildcats: in particular, 2/8 hearts were found positive for *Hepatozoon silvestris* and 6/8 wildcats were positive for *Hepatozoon felis*, respectively in blood/clot (n=2), lung (n=1), liver (n=1), lymph-node (n=1) and heart (n=1). Eleven/40 (27.5%) domestic cats were infected by *Hepatozoon* spp. (i.e. *Hepatozoon felis*, n=2; *Hepatozoon silvestris*, n=9).

A total of 582 questing ticks were collected and identified as follows: 547 *Ixodes ricinus* (42 males, 25 females, 480 nymphs) and 35 *Haemaphysalis punctata* (1 male, 4 females, 30 nymphs). *Hepatozoon felis* was sequenced in 6/54 *I. ricinus* nymph pools, *H. silvestris* in 1, *Cytauxzoon* spp. in 5, corresponding to an estimated pooled prevalence of 1.3%, 0.2%, and 0.9%, respectively. Besides, *H. felis* was detected in 2 males, while *H. silvestris* in 4 males and 1 female. Adults and nymph pools of *H. punctata* were found all negative to both protozoa.

The results show that in Friuli-Venezia Giulia region, ticks, wild and domestic felids are infected by the same tick-borne parasites targeted by the present study (i.e., *Cytauxzoon* spp. and *Hepatozoon* spp.) suggesting a possible transmissibility, although the roles of both host species need to be clarified with further studies.

The detection of these protozoa in *I. ricinus* supports the hypothesis that these parasites are maintained during the moult from larvae to nymphs and from nymphs to adult, suggesting their potential role in transmission to mammals and their involvement in maintaining protozoa in the sylvatic cycle. On the contrary, the tick species *H. punctata* doesn't seem to play any role in these pathogens life cycle.

MONITO-RING A RINGING STATION OF MIGRATORY BIRDS: A SURVEY OF TICK-BORNE PATHOGENS IN NORTHERN ITALY

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Introduction

Intrapaleartic migratory birds follow many flyways to move throughout Europe, searching for a proper place to feed, mate, and raise their chicks. In Autumn, during the post-reproductive migration, many birds move to warmer lands, and thus the flux is mainly directed from Northern to Southern latitudes. It is widely known that, while migrating, birds could also carry hard ticks, which, in turn, could spread their associated pathogens in different locations. Although this phenomenon has already been described, few studies have focused on the description of the presence of hard ticks in migratory birds, with the aim also of describing the epidemiology of associated zoonotic pathogens. The main objectives of this research were to collect ticks from migratory birds to (i) quantify the infestation burden, (ii) identify the tick species, and then (iii) test all the specimens for tick-borne zoonotic pathogens.

Material and Methods

Ectoparasites were collected from migratory birds in Autumn 2019 and 2020 at the 'Malga Confin' ringing station, located in the Julian Prealps Nature Park (Northeastern Italy), along the Adriatic-Flyway route. Ringed birds have been carefully inspected for ticks and, for the infested ones, data have been recorded about bird species, age, sex, fat deposition, and muscle development. Ticks were stored in 70% ethanol until species identification and DNA and RNA co-extraction, followed by a molecular screening for tick-borne encephalitis virus (TBEV), *Borrelia burgdorferi* s.l., *Rickettsia* spp., *Ehrlichia* spp., *Anaplasma phagocytophilum*, and *Bartonella* spp..

Results and Discussion

A total of 3414 birds have been ringed and checked for ticks and 88 (2.6%) were found infested. The following species harboured ticks: black bird, European robin, brambling, chaffinch, song thrush, redwing, water pipit, and coal tit. Overall, 209 ticks were collected and identified as *Ixodes ricinus* (n = 207), *Ixodes acuminatus* (n = 1) and *Ixodes frontalis* (n = 1). All of them were immature stages, i.e., nymphs or larvae. *Ixodes* ticks carried different Rickettsiales zoonotic pathogens, in detail: *Rickettsia helvetica* (16/209), *Anaplasma phagocytophilum* (9/209) *Candidatus Neoehrlichia mikurensis* (2/209) and *Ehrlichia muris* (1/209). On the other hand, 114/209 ticks tested positive for *Borrelia burgdorferi* s.l. and many zoonotic species were identified as *Borrelia garinii*, *Borrelia afzelii*, *Borrelia valaisiana*, and *Borrelia miyamotoi*. Of note, statistical tests showed that birds infested with *Borrelia burgdorferi* positive ticks appeared to be more likely in a poor body condition, meaning low fat deposition and scarce muscle development. In addition, ticks were also found co-infected with *Borrelia burgdorferi* s.l. and *R. helvetica* or *Anaplasma phagocytophilum*. However, all tested ticks were negative for *Bartonella* spp. and TBEV.

This research provided some insights regarding the involvement of migratory birds in the epidemiology of tick-borne zoonotic diseases. On the one hand, the studied birds were poorly infested with ticks, but on the other side, the collected ticks showed a non-negligible prevalence of tick-borne zoonotic bacteria, particularly for *Borrelia burgdorferi*. In conclusion, migrating birds while moving between different lands and countries could, accidentally, also spread tick-associated pathogens. This study highlights the importance of monitoring wild avian populations and, thus, the important sanitary role that ringing stations could have in surveillance programs.

STUDY OF THE EFFECT OF BIRDS' POPULATION DYNAMICS ON WNV SPREAD THROUGH MATHEMATICAL MODELLING

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Introduction

West Nile Disease (WND) is a vector borne disease caused by West Nile Virus, a flavivirus belonging to the family *Flaviviridae*. Its biological cycle involves mosquitoes (mostly belonging to *Culex pipiens* genus) as the main biological vectors and birds as primary hosts. Even human and horses can be infected and although they can develop serious symptomatology, they can not transmit the infection and are thus considered as accidental dead-end hosts. WNV is considered one of the most world-widespread flavivirus representing a significant burden for public health. In Italy the first detection of the virus was in 1998 and since 2001 it is considered endemic, with a peak in circulation in 2013, 2015 and 2018. Despite that, there are still several knowledge gaps on WNV epidemiology, that hamper our capability to predict future outbreaks and define fully effective control plans. In particular, very few is still known about the role of different bird species, and consequently of the overall avian community, on WNV transmission and maintenance. Mathematical models are an useful, and often underexploited, tool to support field studies in investigating disease dynamics and control. We then propose a mathematical model to investigate the role of different birds' species and the effect of their population dynamics on the spread of WNV. Our aim is to investigate whether demographic characteristics of bird species affects WNV transmission to mosquitoes in order to explore the contribution to WNV spread played by birds species with different population dynamics.

Material and Methods

We theoretically compared how the population dynamics of five different bird species would affect WNV prevalence into mosquito population during summer season (April - October). To simulate WNV dynamics we adapted a mathematical model already validated in Veneto and Lombardy regions. The epidemiological model is a compartmental model based on a system of 11 differential equations aimed at simulating WNV dynamics between one competent bird species and one vector species. We assumed that the only vector is represented by *Cx pipiens*, whereas we assumed blackbirds, house sparrows, magpies, collared doves and hooded crows (one at the time) as the competent bird reservoir. Daily bird abundance for each investigated species was assessed by simulating its dynamics through a compartmental model based on a system of 4 differential equations representing birds' life stages. To inform the system we used data collected in literature and supported by expert opinion. Each birds' population dynamics was then included into the epidemiological model. Furthermore, to disentangle which of the demographic characteristics of birds affects WNV spread at most, we simulated other three theoretical scenarios. In the first scenario we fixed the initial number of subjects for each bird species to limit the effect of the initial population size of birds. In the second scenario we investigated the effect of the timing of the reproductive season. This was done by fixing all parameter and assuming the reproductive season to start in a different month for each "species" (starting in March, April, May and June); while in the last scenario we assumed four different lengths of the reproductive season (one-, two-, three- and four- months lengths) starting at the same time. To simulate the last two scenarios, we considered only blackbirds' parameters as model parameters rather than the five initial birds' species.

Results and Discussion

We found that the main demographic characteristic of birds affecting WNV dynamics is bird abundance since August to October. We found that the lower the competent bird population abundance, the highest the prevalence in vectors (estimated as the daily number of infectious mosquitoes over the total number of mosquitoes) is, and vice-versa. We furthermore showed that the prevalence in mosquitoes is not affected by the timing of reproductive pulse in birds. This finding, despite counterintuitive, is fully supported by the algebraical analysis of the R_t provided by previous works, thus highlighting the importance of investigating this complex system through modelling. Furthermore, this result needs to be taken into account when predicting the effects of strategies to control the spread of the virus. On the other hand, as our model does not consider the complexity of the avian community, further investigations are needed to fully comprehend the role of birds'. In conclusion, this study represents a first step in a better comprehension of the role of different bird species in WNV dynamics.

Sessione 2
**ECOLOGIA dei PATOGENI E GESTIONE SANITARIA
DELLA FAUNA**

Moderatori: Nicola Ferrari - Paola Beraldo - Walter Mignone

RELAZIONI AD INVITO

INFOFAUNAFVG: a centralized web database for wildlife health management

Paolo Tomé (Università degli Studi di Udine)

CONTRIBUTI LIBERI

Estimating red fox population density for *E. multilocularis* monitoring

Celva R., Crestanello B., Obber F., Trevisiol K., Agreiter A., Dellamaria D., Hauffe H.C., Citterio C.V.

Comparing faecal examination results with necropsy findings in the monitoring of red fox intestinal helminths with zoonotic and veterinary importance

Marchiori E., Obber F., Zarantonello A., Celva R., Marcer F., Danesi P., Massolo A., Cenni L., Citterio C., Cassini R.

Comparative assessment of *E. multilocularis* prevalence in red fox from the province of Bolzano

Obber F., Celva R., Da Rold G., Trevisiol K., Bregoli M., Danesi P., Cenni L., Rossi C., Capello K., Hauffe H.C., Massolo A., Cassini R., Agreiter A., Dellamaria D., Citterio C.V.

Presence of different canine distemper virus lineages in wildlife from north Italy

Trogu T., Castelli A., Canziani S., Tolini C., Carrera M., Sozzi E., Lelli D., Tosi G., Fiorentini L., Rugna G., Lanci D., Lavazza A., Moreno A.

Preliminary health surveillance records of European wildcat (*Felis silvestris silvestris*) in north-eastern Italy

Bregoli M., Beraldo P., Pesaro S., De Zan G., Sello M., Campalto M., Obber F., Danesi P., De Benedictis P., Filacorda S., Dorigo L., Lapini L., Gobbo F., Ceglie L.

Data on the parasitological status of golden jackal (*Canis aureus* L., 1758) in Friuli-Venezia Giulia Region (Italy)

Beraldo P., Pesaro S., Saccà E., Dorigo L., Lapini L., Bregoli M., Filacorda S.

Paratuberculosis in wild red deer (*Cervus elaphus*): ecological correlations to the probability of infection and contribution to pasture contamination

Nava M., Garbarino C., Ricchi M., Epis S., Bianchi A., Ferrari N., Corlatti L., Barsi F., Cortimiglia C., Luzzago C.

Comparison between major vessels and cavernous sinus blood for metabolic profiles in wild red deer

Comazzi S., Guanziroli S., Giordano A., Formenti N., Trogu T., Corlatti L., Nava M., Luzzago C., Ferrari N.

Asymmetric competition between the autochthonous deer ked *Lipoptena cervi* and the alien *L. fortisetosa* (Diptera: Hippoboscidae) in *Cervus elaphus*

Andreani A., Ponzetta M.P., Stancampiano L.

Detection dogs and African Swine Fever management

Fortebraccio M., Donnini S., Bottaro R., Viganò R.

INFOFAUNAFVG: A CENTRALIZED WEB DATABASE FOR WILDLIFE HEALTH MANAGEMENT

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Introduction

In 2017 the Autonomous Friuli Venezia Giulia Region and the Department of Agricultural, Food and Animal Sciences of the University of Udine (DI4A), signed a collaboration agreement to carry out scientific work with regard to wildlife recovery and surveillance. The scientific activity included both support and consulting for the Regional Forestry Service and the Wildlife Rescue Centres (CRAS), and the design of a novel information-database system to organize data on wildlife rescue activity and health management and surveillance. The DI4A developed a progressive web application, characterized by a Web Database and a WebGIS system, through the utilization of different open source software and named it "InfoFaunaFVG".

Material and Methods

InfoFaunaFVG is made of a Web Database and an integrated WebGIS system. The following open source software are used: Apache HTTP Server, Oracle MySQL, Symfony, Apache Tomcat, GeoServer, OpenLayers.

The access to the web application is possible, upon authentication, through a web browser or by installing the PWA on the computer or mobile devices.

In order to define detailed user profiles, different levels of use are assigned to each user depending on their operational level. Moreover, traditional functions of management software were developed: create, read, update, and delete operations, perform batch operations, create dynamic reports and filters and export data in Open Document format through dynamic reports.

InfoFaunaFVG has built in both a notification system, to send communications to every user at any level, and a log system, able to trace all the data input changes.

Results and Discussion

In short, operating from November 2019, InfoFaunaFVG currently (April 2022) contains a total of 40.175 records from 300 different users, including veterinarians, forestry guards, veterinary technicians and specialized personnel in animal rescue belonging to 16 different institutions. Among all species recorded, mammals are 40% (16.018) of the total, whereas avian species represent 59% (23.741), and others (reptiles and amphibians) 1% (416) respectively.

Two hundred twenty-six different species (175 avian and 51 mammals) are recorded. The wildlife species reported to be selectively culled were 42% (16732), 17% (7.829) found dead and 39% (15.614) rescued alive respectively. Apart from wildlife selective culling (11.249 avian and 5.483 mammals), roadkills were the main cause of death both for mammals (4.512) and avian species (226).

Data consistency and traceability was possible through regular data quality checks performed by the centralized coordination center at the University of Udine. To date, InfoFaunaFVG has proven to be a successful wildlife data repository system providing high quality consistent, accurate and traceable data capable of having a considerable impact on regional wildlife governance.

In conclusion, during the first two years and a half of its use, InfoFaunaFVG allowed the achievement of several objectives including: the recording of consistent, accurate and traceable data regarding wildlife, the optimization of regional investment, and resources and improvement of regional wildlife knowledge.

Considering the results of the first two years of its use, we conclude that InfoFaunaFVG has the potential to become the largest data repository for wildlife monitoring and surveillance in Italy.

The application of such integrated information systems on a larger scale at a national level is auspicious to best coordinate and design sustainable management plans and policy instruments to improve human-wildlife coexistence.

ESTIMATING RED FOX POPULATION DENSITY FOR *E. MULTILOCULARIS* MONITORING

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Introduction

The red fox (*Vulpes vulpes*) is the main definitive host for *Echinococcus multilocularis*, a small tapeworm causing a severe zoonosis in humans (alveolar echinococcosis). Fox is regarded as essential for the maintenance of foci in European endemic areas, pushing public health authorities to consider local management of fox populations as a strategy to control the parasite distribution; thus, knowledge of fox demographics is pivotal. Here, we estimated fox population density in two areas in the Province of Bolzano, Italy: one historically endemic for *E. multilocularis* (Alto Isarco), and another in which the parasite has never been detected (Val d'Ultimo). We then compared the results with the corresponding kilometer index of abundance (KIA), routinely used to monitor fox population fluctuations. In addition, we addressed the hypothesis that fox population density would be higher in the endemic area, compared to the naïve one.

Materials and Methods

Spotlight count data for 2018 (Alto Isarco) and 2019 (Val d'Ultimo) were obtained by counting all foxes identified while driving along fixed transects, during red deer census activities; this number was then divided by the distance driven in kilometers. The highest value was retained as the KIA for each of the two areas.

To estimate fox population density, we adopted a protocol for genetic spatial capture-recapture (genSCR). Fox stools were collected along parallel transects aggregated in different zones, which were distributed on the two study areas; each area was sampled three times (Alto Isarco: June-July 2018; Val d'Ultimo: July-August 2019). Fresh fecal pellets were genotyped at 21 microsatellite loci, considering a minimum of nine as the threshold for valid genotypes. Individual capture-recapture histories were then used in a spatially explicit Bayesian capture-recapture model implemented in OpenBUGS.

Results and Discussion

The highest KIAs were obtained on July 12, 2018 (Alto Isarco, 0.51 foxes/km) and March 29, 2019 (Val d'Ultimo, 0.18 foxes/km). For genSCR, 56 and 59 stool samples were collected from Alto Isarco and Val d'Ultimo, respectively. 39 and 33 valid genotypes were obtained, representing 31 and 25 individual foxes. Statistical analyses estimated fox population densities at 2.97 ± 0.64 ind/km² for Alto Isarco and 5.11 ± 0.97 ind/km² for Val d'Ultimo. Therefore, in our study, KIAs did not show a positive correlation with fox population density; in fact, the lowest KIA value was registered in Val d'Ultimo, where the highest fox density was found. Similarly, the presence of an *E. multilocularis* focus did not correspond to the area with a higher fox density, given that the Alto Isarco hotspot appears to be sustained by relatively low host population density. We conclude that fox population density is not the sole factor maintaining *E. multilocularis* foci, while further highlighting KIA as a relative indicator only.

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COMPARING FAECAL EXAMINATION RESULTS WITH NECROPSY FINDINGS IN THE MONITORING OF RED FOX INTESTINAL HELMINTHS WITH ZOONOTIC AND VETERINARY IMPORTANCE

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Introduction

Red foxes play an important epidemiological role for a number of helminthic infections of both zoonotic and veterinary interest (e.g., alveolar echinococcosis, toxocariasis, ancylostomatosis). Analysis of environmental fox droppings may represent a time and cost-effective way to estimate the presence and diffusion of targeted helminth species. Nevertheless, scarce sensitivity of coproscopy (isolation technique) associated with multiplex PCR for the detection of *Echinococcus* spp. eggs has been recently proven through comparison with necropsy findings. Other studies have previously reported the underestimation of helminthic infection occurrence in red foxes when evaluated by copromicroscopy, mostly using fecal flotation, and such low sensitivity seems to apply to all helminth classes. The reasons for such a low sensitivity have still to be investigated in depth. In this study, the sensitivity of fecal examination for relevant helminth species was studied by comparing the detection of eggs at copromicroscopy with isolation of adult parasites at necropsy.

Material and Methods

One hundred-fifty red foxes, found dead or culled during hunting season and population control campaigns in Bolzano Province in the period 2020-2021, were included in this study. For inactivation of *Echinococcus* spp. eggs, all carcasses were frozen at -80°C for at least 72h before necropsy. Parasites were isolated from the small intestine through a scraping, filtration and counting technique by using 1000 µm and 212 µm sieves. All isolated parasites were identified morphologically at species level and sex was determined for all mature specimens. Coprological examination was carried out on rectal content by means of a classical flotation technique (FT) using a Zinc chloride solution (s.g. 1,350) and a combination of copromicroscopy and multiplex PCR (CMPCR). Eggs were identified at the lowest taxonomic level possible, and sequencing was performed after multiplex PCR for species identification. The concordance and the level of agreement between necroscopic and copromicroscopic findings were evaluated using the parameter Kappa (k) (<https://epitools.ausvet.com.au/comparetwestests>). Using the results of the necropsy as our reference standard, the sensitivity of copromicroscopy was assessed for ascarids and Ancylostomatidae (only FT), and for Taeniids (both FT and CMPCR).

Results and Discussion

At necropsy, three species of nematodes (*Toxocara canis*, *Uncinaria stenocephala* and *Pterygodermatites* sp.) and four species of cestodes (*Echinococcus multilocularis*, *Taenia crassiceps*, *Taenia polyacantha*, *Mesocostoides* sp.) were identified. The FT allowed to detect eggs ascribable to ascarids, ancylostomatidae and Taeniids, besides the retrieval of *Trichuris* sp. and *Capillaria* sp. eggs, whose adult forms were not targeted by our scraping technique. Concerning nematodes, the overall concordance was 62.7% and the k was equal to 0.206 (95% C.I.:0.059-0.353) for ascarids, whereas Ancylostomatidae showed a slightly higher concordance (70.7%), but a similar k (0.151; 95% C.I.:0-0.322). In both cases, the agreement between the two techniques was poor, due mainly to the high number of infections undetected by the FT. However, the adult parasites were not retrieved in 14 and 17 individuals whose fecal samples resulted positive to ascarids and to Ancylostomatidae respectively. This finding may be partly due to bad preservation of carcasses, either to misdiagnosis at FT, mainly for Ancylostomatidae, whose eggs may resemble the ones of the herbivore strongyles, possibly present in red foxes feces due to necrophagy. The sensitivity was below 40% for both groups of nematodes. However, excluding single-sex infections and infections by solely immature worms, the sensitivity increased to 54.5% for ascarids and to 42.8% for Ancylostomatidae. No eggs ascribable to *Pterygodermatites* sp were reported at the FT; low resistance of eggs at freezing should be investigated as a contributing factor to this finding. Concerning cestodes, the comparison was possible only for Taeniids, since no eggs of *Mesocostoides* sp. were found by both FT and CMPCR. The concordance between necropsy and FT for Taeniids (including *E. multilocularis*) was 51.3%, with a very low k (0.092; 95% C.I.:0.029-0.154), whereas both concordance (58.7%) and k (0.220; 95% C.I.:0.127-0.313) had slightly higher but comparable values between necropsy and CMPCR. However, in the case of Taeniids, all positive fecal samples were confirmed by necropsy, and the very poor agreement is likely due to the low sensitivity of both coprological techniques (9.9% for FT and 23.5% for CMPCR). Out of the 19 samples positive at CMPCR, 13 were successfully sequenced as *E. multilocularis* (n=5; 3 coinfections), *T. crassiceps* (n=7; 1 coinfection), *T. polyacantha* (n=2; 1 coinfection) and *Taenia* spp. (n=2; 1 coinfection). All molecular identifications, except one, were confirmed by the presence of the specific adults at necropsy, and the presence of both species in case of co-infections. In conclusion, copromicroscopy correctly detected the presence of all helminths with zoonotic and/or veterinary interest circulating in the study area, that were specifically identified by the necropsy (i.e., *E. multilocularis*, *T. canis*, *U. stenocephala*). However, the performances of both FT and CMPCR in terms of sensitivity resulted very poor, and their use in surveillance programs can be promoted only if appropriate attention is given to the definition of the correct sample size (considering an imperfect test).

COMPARATIVE ASSESSMENT OF *E. MULTILOCULARIS* PREVALENCE IN RED FOX FROM THE PROVINCE OF BOLZANO

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Introduction

Echinococcus multilocularis is a zoonotic parasitic cestode affecting wild carnivores worldwide. In the EU, monitoring consists of assessing prevalence in red fox (*Vulpes vulpes*), which represents the main definitive host and is crucial for the maintenance of the sylvatic cycle. An adequately sized sample of foxes must be tested to assure the efficiency of the monitoring activity. However, sample size is dependent on parasite prevalence and monitoring is particularly time-consuming at the borders of its range, where prevalence is usually low. Since 2012 prevalence of *E. multilocularis* in the Province of Bolzano had been reported as increasing, but less than 5%. So far, positive foxes have been localized in two main hotspots: Alto Isarco and Val Pusteria. To improve the *E. multilocularis* monitoring strategy at the edges of its range while maintaining an acceptable cost/benefit ratio of surveillance effort, we tested sensitivity and specificity of two molecular tests (eggs isolation associated with multiplex CMPCR and quantitative PCR – qPCR) on fecal samples against what is considered to be the reference standard diagnostic, Scraping, Filtration and Counting Technique (SFCT) of the small intestine. Results were then applied on a real-case scenario to highlight the advantages of implementing the most sensitive test in terms of required sample size.

Material and Methods

Between 2019 and 2020, 123 fox carcasses were gathered across the Province of Bolzano and a faecal sample and the small intestine were collected. Screening for *E. multilocularis* were then carried out using three diagnostic techniques:

- CMPCR: 2g of faecal matter were treated by floatation, filtration and sieving to isolate Taeniidae eggs, which were used as matrix for DNA extraction. DNA amplification was carried out using multiplex PCR.
- qPCR: whole stool (0,2 g) DNA extraction using a magnetic beads protocol. DNA amplification was carried out using real-time PCR.
- SFCT: adult cestodes was separated using a three-step filtration (1000 µm, 212 µm and 75 µm mesh) of the contents of the intestinal lumen, obtained by scraping. Specimens were then counted using a stereo-microscope.

Sensitivity and specificity of the two molecular tests were calculated and compared to SFCT results. The agreement of each test with the reference standard was calculated using Cohen's K. True prevalence was estimated on a bigger sample (235 foxes for which only the two molecular copro-microscopic diagnostics were applied), using the adjustment to the Rogan-Gladen formula as proposed by Lang and Reiczig. The required sample size for *E. multilocularis* detection according to the true prevalence was calculated on a hypothetical epidemiological unit of 300 foxes, using the modified binomial approximation analysis method implemented in EpiTools.

Results and Discussion

Using SFCT, CMPCR and qPCR, 24/123 (19.5%), 5/123 (4.1%) and 23/123 (18.7%) foxes tested positive for *E. multilocularis*, respectively. The two molecular tests confirmed the reference standard outcomes in 5/24 (CMPCR) and 20/24 (qPCR) of cases, with an agreement (Cohen's K) of 0.3 and 0.82, respectively. Sensitivity was calculated as 0.21 for CMPCR, and 0.83 for qPCR, while specificity was 1.0 and 0.97, respectively. Using these values of sensitivity and specificity, true prevalence was estimated at 14.3% (CMPCR) and 14.2% (qPCR). Our results show that CMPCR performances were much lower than previously reported in the literature, suggesting that *E. multilocularis* prevalence in the Province of Bolzano has been underestimated up to now. Our results here allow us to label this Province as a highly endemic area (prevalence >10%). The higher sensitivity of qPCR means that, compared to CMPCR, sample size can be about one third smaller for *E. multilocularis* surveillance, which is especially relevant in areas at the margins of the parasite's range, where burden is often low and its distribution fragmented. We conclude that future strategies for *E. multilocularis* surveillance should be focused on a smaller scale, adapting to local fox populations and prevalence values. Another benefit of the adoption of a more sensitive diagnostic is that the required sample size for qPCR can be achieved using environmental faecal samples, which are often too degraded to be used for CMPCR.

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PRESENCE OF DIFFERENT CANINE DISTEMPER VIRUS LINEAGES IN WILDLIFE FROM NORTH ITALY

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Introduction

Canine distemper virus (CDV) belongs to the genus *Morbillivirus* (family *Paramyxoviridae*) and causes a fatal, highly contagious disease in wild and domestic carnivores; Canidae, Felidae and Mustelidae are the most susceptible families reported. Several outbreaks occurred in Italy in wildlife in previous years. Foxes, badgers and martens were mostly involved, showing an increase of mortality. Considering the potential impact on wildlife population dynamics, especially on endangered species, in case of unusual increases in mortality it would be appropriate to include CDV in the differential diagnoses for understanding the cause of mortality. Moreover, previous studies highlighted its ability to switch and infect different species, as well as to increase its virulence, which is particularly related to amino acidic mutation in residues 530 and 549 along the Hemagglutinin (H) gene. These characteristics could enhance the potential impact of the virus on wild populations, increasing mortality levels. A moderate mortality of red foxes in Emilia Romagna region was registered in 2021, thus CDV presence was also investigated in wild carnivores found dead.

Materials and Methods

Since the beginning of 2021, until early August, carcasses of 67 foxes and one badger were conferred to IZSLER. Animals in good state of preservation were submitted to necropsy and samples from brain, lungs, intestine, stomach and bladder were collected. Suitable samples were subjected to histological examination. Pool of viscera were used for viral genome extraction and subsequently analyzed through screening PCR to detect CDV presence amplifying a fragment of 287 bp of conserved nucleocapsid (N) protein gene. Complete H gene was then amplified in those samples positive to the screening RT-PCR. Sequences obtained were compared with sequences available in GenBank and phylogenetic analysis of complete H gene sequences was performed.

Results and Discussion

This study describes a small outbreak of CDV in Emilia Romagna region, showing a prevalence of 16% (ten foxes and one badger). Traumatic injuries were the main recognized lesions at necropsy, and most foxes did not show signs referable to infectious diseases. Few foxes presented melaena, gastric ulcers, catarrhal-haemorrhagic enteritis, spleen, liver and pulmonary congestion. Positive animals originated from two different provinces: one fox from Modena, in the Po Valley, while the remaining carnivores were from Rimini province. This area is characterized by a hilly environment, more suitable as fox's habitat, reaching high densities and thus facilitating pathogens spread.

Phylogenetic analyses showed the presence of different lineages between the two provinces: in the fox from Modena a CDV strain belonging to Europe/South America 1 lineage was recognized; in particular its sequence clustered with those of strains isolated in 2018 from alpine territory in North-Eastern Italy, and likely originated from Balkans. Oppositely, all positive animals from Rimini province belonged to Wildlife lineage. Analyses of mutations in residue 530 support the presence of different lineages: in fact, sequences from Rimini present aspartic acid (D), frequently associated to wild canine hosts, reflecting their affinity with Wildlife lineage whereas the sequence from Modena shows glycine (G), typical of dogs, indicating that it could have a common origin with one strain isolated from a dog in Hungary. At 549 residue, all the sequences show the mutation from tyrosine (Y) to histidine (H), usually associated to increased virulence and neurological clinical manifestations. It can be hypothesized that this mutation may have played a role in Rimini's outbreak, while the positive case from Modena seems to be more likely linked to the outbreak occurred in 2018 in the Alps, which showed a progressive evolution southward, reaching Emilia Romagna region.

This study highlights the endemic circulation of CDV in wildlife in North Italy and also indicates the presence of viral agents belonging to different lineages, which could also be highly virulent. Considering the co-presence of three lineages in North-Central Italy (beyond the Europe/South America 1 and the Wildlife, also Arctic lineage is present in wild carnivores), it would be interesting to continue monitoring the occurrence of CDV in wildlife and to further investigate the role of potential mutations on these populations. This represents an issue of central importance considering the presence, precisely in central Italy, of some endangered species susceptible to infection, such as the Marsican bear.

PRELIMINARY HEALTH SURVEILLANCE RECORDS OF EUROPEAN WILDCAT (*FELIS SILVESTRIS SILVESTRIS*) IN NORTH-EASTERN ITALY

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Introduction

The European wildcat (*Felis silvestris silvestris*) is of conservation concern. In the Friuli Venezia Giulia Region the wildcat is monitored according to the local health surveillance plan, along with other carnivores such as red fox (*Vulpes vulpes*), golden jackal (*Canis aureus*), grey wolf (*Canis lupus*), brown bear (*Ursus arctos*) and mustelids. Wildcat populations in the study area have been recently expanding as evidenced by the increasing number road-killed individuals. Besides parasitological investigations, very few data were previously available on the wildcats' health status in the study area.

Material and Methods

We carried out a total of 41 necropsies on wildcats during the period 2020-2022 (22 males and 19 females). Almost all of them were collected following car accidents (40), while the cause of death was unclear for only one individual. Among the processed specimens, there were four putative hybrids (ornamentation not typically *silvestris* and gut index towards domestic cat – *Felis silvestris catus*) that will be subjected to genetic testing for confirmation. We focused on pathogens at the interface with sympatric wild carnivore species, domestic cats and their preys including rabies virus, feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), feline parvovirus (FPV), feline coronavirus (FCoV), canine distemper virus (CDV), *Trichinella* and *Echinococcus multilocularis* (EM). We investigated rabies virus by IF and the other viruses by means of molecular methods. According to the EC Regulation 1375/15 the magnetic stirrer method was performed to detect *Trichinella* larvae by microscopy after the enzymatic digestion in muscle samples. Eggs of the cestodes were isolated from faeces with a sedimentation/filtration technique. The cestode species was determined through multiplex PCR, targeting and sequencing ND1 and 12S gene (Citterio *et al.*, 2021). We conducted further microbiological and histopathological analyses according to gross pathology findings.

Results and Discussion

All brains tested negative for rabies. At necropsy pathological findings but traumatic lesions included: lymphadenopathy (4); parasitic bronchopneumonia (5); liver degenerative lesions (4); hemorrhagic gastritis (1). The reportedly not road killed wildcat was in poor body condition and presented a severe hemorrhagic gastroenteritis; this wildcat was negative for viruses, however a hemolytic *E.coli* strain was isolated from the gut. None of putative hybrids was included within viral or bacterial positives. Spleen samples were negative for FIV and gut samples were negative to FPV and FCoV; muscle tissues were negative for the search of *Trichinella* as well as faecal/gut samples for EM.

FeLV was revealed in spleen samples of two wildcats (4.88%; CI_{95%} 0.00-11.12%), where we observed mild spleen enlargement in one of them; nevertheless this lesion was observed also in six FeLV negative ones.

We detected CDV from the lungs of one individual whereas the brain was negative, suggesting a recent infection (2.44%; CI_{95%} 0.00-6.91%).

The expansion of the species from mountain areas to the plain may lead to the increasing encounter of hybrids; comparison between typical *silvestris* individuals and hybrids could bring further insights at wild and domestic health interface.

FeLV was commonly evidenced in wildcats in Europe; on the other hand, an investigation in the close Slovenian wildcat populations failed to reveal evidence of FeLV infection. FeLV is generally considered as potentially maintained in wildcat populations regardless of domestic cats as possible source and a limiting factor for population growth; further data need to be collected in order to support these hypotheses in the study area.

Although CDV is not a typical felid pathogen, it was previously detected in Eurasian lynx (*Lynx lynx*) in the Alps, occurring during periodical epizootics in foxes and mustelids populations and it was spread in wildcats in Spain.

Our investigations highlighted for the first time the presence of FeLV and CDV in wildcat populations in north-eastern Italy.

DATA ON THE PARASITOLOGICAL STATUS OF GOLDEN JACKAL (*CANIS AUREUS* L., 1758) IN FRIULI VENEZIA GIULIA REGION (ITALY)

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Introduction

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Our investigations highlighted for the first time the presence of FeLV and CDV in wildcat populations in north-eastern Italy.

PARATUBERCULOSIS IN WILD RED DEER (*CERVUS ELAPHUS*): ECOLOGICAL CORRELATIONS TO THE PROBABILITY OF INFECTION AND CONTRIBUTION TO PASTURE CONTAMINATION

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Introduction

Paratuberculosis is an infectious disease mainly affecting wild and domestic ruminants. The disease is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Animals usually get infected through faecal-oral and congenital routes. The bacterium, excreted in faeces, is highly resistant in the environment; consequently, shared grazing between wild and domestic ruminants may represent a risk for MAP transmission. Wild ungulates, including red deer (*Cervus elaphus*), are susceptible and, besides transmitting the infection to conspecifics, may act as a source of infection for other species. Notably, the first cases of paratuberculosis in Italy were found in red deer in the south Tyrol sector of the Stelvio National Park in the early 90's. In order to evaluate possible ecological correlations about the probability to become paratuberculosis positive, all red deer killed during a culling program (2011-2022) were sampled for MAP analysis and biometric data, as well as culling locations, were recorded for each animal. Moreover, in order to evaluate red deer contribution to the contamination of alpine pastures, environmental faecal samples were carried out in the years 2020-21 and analysed by quantitative polymerase chain reaction (qPCR).

Material and Methods

The Stelvio National Park extends over 134,000 hectares in the central Alps. A large increase in red deer density occurred over the past decades (up to some 31 individuals/km² in wintering areas), leading the Park to start a culling program in 2011, with the aim to reduce the red deer density and its impact on agricultural activities, forest regeneration and biodiversity. Culling activities were carried out within the municipality of Valfurva, in the north-western part of the Park. A total of 1306 red deer, 791 females and 515 males, were culled. For each individual, ileocecal valve and mesenteric lymph nodes were sampled for MAP detection and biometric/ecological data (sex, age class, age, culling location, dressed weight, kidney fat index [KFI]) recorded. Environmental faecal samples, during the summer periods of the years 2020-21, were collected in alpine pasture areas throughout the Park in order to assess the role of red deer in the contamination of pastures. The areas were selected mainly on the basis of presence/absence of cattle in relation to the presence of red deer at different densities. A total of 1808 environmental faeces were collected, 470 and 1338 samples in the year 2020 and 2021, respectively. Each sample was weighed and geographical coordinates (elevation, slope, exposition) were recorded. All biological specimens collected from individual and environment were analysed by qPCR for MAP detection. Positive samples were cultured and when isolated, MAP field isolates were genotyped by MIRU-VNTR and SSR typing. The probability of positivity to MAP was analysed by a generalized linear model in relation to the different biometrics and environmental data collected as linear predictors.

Results and Discussion

Analyses of culled red deer returned a total of 121 positive subject for MAP out of 854 red deer sampled. The results of the statistical model suggest that there has been a decline in MAP positivity over the years in the last decade. These results are consistent with possible effects of density dependence in infection transmission, a concept already known for paratuberculosis. On the other hand, the mortality induced by the disease itself, exacerbated by winter starvation, might have contributed to the infection decline. Another consideration is that animals showing less dressed weight, have and higher probability to be infected by the disease, a concept which is consistent with paratuberculosis pathogenesis, which is associated by a body mass loss induced by the malabsorption of nutrients, in turns caused by intestinal lesions typical of this disease. Out of the 1808 environmental faecal samples collected, only 41 were MAP positive. Six strains were isolated and showed the INMV1 genotype, a genetic profile previously described in the area under study in red deer and in cattle herds in Italy. The low positivity in environmental sample may be due to the decline trend observed in red deer population and the type of sample analysed before mentioned. Analysis on faecal samples may produce, in some cases, negative results even for infected subjects because MAP can be intermittently shed in sub clinically infected deer. Overall, the environmental sampling suggests MAP contamination on pasture by red deer does not likely represent an important risk factor for the transmission of paratuberculosis in this area. The low prevalence of MAP found in recent years, at both animal and environmental levels, suggests how the reduction in density could have impacted the distribution of diseases in wild animals. Based on the large number of samples analysed in relation to the biometric data collected, the study amplifies the knowledge not only of the prevalence, but also of the epidemiology of paratuberculosis in the red deer population which inhabits the Stelvio National Park.

COMPARISON BETWEEN MAJOR VESSELS AND CAVERNOUS SINUS BLOOD FOR METABOLIC PROFILES IN WILD RED DEER

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Introduction

Metabolic profiles are useful for welfare monitoring in wildlife but they are often biased in alive animals by difficult sampling procedures, preanalytical errors and stress-induced alterations due to capture and restraint. Selectively culled wild animals may partially overcome this issue since they represent an easy source of blood in which stress-induced alterations are supposed to be minimal. However, blood collected post-mortem from major vessels or intracardiac/intrathoracic cavity (MV), which are the classic sites of blood sampling, often suffers from severe haemolysis, tends to clot and may be contaminated by other body fluids, thus strongly affecting results of laboratory tests. Recently, a new withdrawal site, the cavernous sinus of dura mater (CS), has been proposed in wild boar, red deer, fallow deer and chamois, since it provides an abundant high-quality venous blood sample. To date, this sample has been used only for serological tests and no data are available on the difference between blood samples collected from these two sites and the possible use of CS blood also for metabolic profiles. The aim of the present work is to investigate the concentration of major biochemical analytes in CS blood and to compare metabolic profiles from CS and MV blood in culled red deer, in order to propose a possible use of samples from this site also for evaluating metabolic status in red deer.

Material and Methods

A total of 127 blood samples from MV and 104 from CS were collected from 134 red deer of different gender and age, during a depopulation plan in the Stelvio National Park (Valfurva SO) in years 2016-2017. In 100 deer, samples from both the sites were available.

Haemolysis was evaluated using a visual colorimetric 7-tiers scale and samples equal or less than the value of 4 (corresponding to 200 mg/dl hemoglobin) were considered suitable for biochemical analysis. A total of 36 deer finally fulfilled inclusion criteria for both sample sites and were submitted to biochemical analysis for total protein, albumin, urea, triglycerides, total cholesterol, non-esterified fatty acids (NEFA), β -OH butyrate, total calcium and inorganic phosphorus. These parameters were chosen as mainly representative of protein, energetic and mineral metabolism. Results were statistically compared using analysis of variance, correlation and method comparison tests for coupled samples.

Results and Discussion

Haemolysis indexes were statistically lower in CS than MV samples (3.3 ± 1.4 vs 4.6 ± 1.4 ; $p < 0.001$) with 88% vs 44% of samples suitable for further biochemical tests. Statistical differences between the two sites were found for total protein, albumin, cholesterol, and calcium, while correlation between two sampling sites were found for urea, triglycerides, NEFA, β -OH butyrate, total cholesterol and total calcium.

A perfect agreement between the two sampling sites were found only for urea, NEFA and β -OH butyrate, while for total protein, albumin, cholesterol and calcium, CS showed lower concentrations with either constant or proportional biases. However, after correction for the concentrations of protein, the agreements for these analytes increased, thus indicating that the lower protein concentration is likely responsible for differences, as expected being cholesterol and total calcium bound to transport proteins in plasma. These differences are likely related to an increased concentration of water (and small solutes such as urea, β -OH butyrate and phosphorus) in CS blood resulting in an unpredictable dilution of samples.

Results support the possible use of CS also for evaluation of metabolic profiles but the insufficient agreement between CS and MV blood for some variables suggests that the two sites are not completely interchangeable and that some specific reference intervals should be calculated before interpreting metabolic profiles in CS.

The decreased concentrations of some protein-related analytes might be corrected for the concentration of total protein as inverse surrogate of the hydration level of samples. In contrast, the lower protein concentration (about 80%) in CS samples cannot significantly influence results of most serology tests but caution should be done for possible false negative results when titers are close to cutoff values.

Overall, CS is a promising sampling site also for the evaluation of metabolic profiles in culled red deer since it is easy to withdraw, minimally haemolytic and abundant. This leads to a higher percentage of samples suitable for most haematochemical procedures but results should be compared with adequate reference intervals and the biological meaning for evaluating welfare in wild deer should be adequately validated in different conditions.

ASYMMETRIC COMPETITION BETWEEN THE AUTOCHTHONOUS DEER KED *LIPOPTENA CERVI* AND THE ALIEN *L. FORTISETOSA* (DIPTERA: HIPPOBOSCIDAE) IN *CERVUS ELAPHUS*

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Introduction

Lipoptena cervi L. and *L. fortisetosa* Maa are hematophagous ectoparasites mainly infesting deer. They spend the entire life on a single host animal, feeding on its blood and reproducing on it through the adenotrophic viviparity strategy. *Lipoptena cervi* is a palearctic species, while *L. fortisetosa* is native to Japan but has largely spread from Asia to Europe being recorded in several countries, included Italy. Different hippoboscids species can infest the same geographical areas and have been found to share the same hosts, co-feeding on it also with ticks. *Lipoptena cervi* and *L. fortisetosa* can reach a very high abundance on the parasitised animal, reducing its health condition and altering its behaviour in case of severe infestations. These flies can occasionally bite also humans, with potential sanitary consequences since they can be possible vectors of harmful microorganisms. Many studies have been recently conducted on these species to map their diffusion and to highlight the possible health risk related to their attacks together with their vectorial capacity of pathogens. On the contrary, these species have not been enough investigated for their competition on hosts, especially on the mainly infested body regions, the neck and the groin areas.

The aim of the present study was to investigate the possible competition between *L. cervi* and *L. fortisetosa* on their favourite host in Italy, *Cervus elaphus*, in the Tuscan-Emilian Apennines. Additionally, the survey allowed to underline parasite abundance on the different host body regions, giving insights on their micro-niches preferences.

Material and Methods

Deer keds were collected in 2018-2020 from 75 and 104 red deer legally shot in Emilia-Romagna and Tuscany Regions, respectively. Sampling methods and standardized counting techniques are reported in Andreani *et al.* (2021. Animals, 11: 2794). For each host, data about sex (two missing values), age, altitude, longitude, and latitude were available. Each collected insect was classified and its localization in the neck or in the groin region recorded.

Statistical analyses were performed using STATA 12.1. Parasite abundance data were analyzed using multivariable negative binomial regression models. Preliminary, the influence of stationary variables (latitude, longitude, and altitude) on parasite abundance and the influence of host sex and age were tested for each species. Therefore, in order to evaluate the possible competitive interactions, two models were tested: one having *L. cervi* as independent variable and *L. fortisetosa* as additional covariate, and one having *L. fortisetosa* as independent variable and *L. cervi* as additional covariate. Lastly, spatial competition between parasite species was evaluated building four models, one for each parasite-species in each of the two examined body regions, including as covariate also its total abundance according to Stancampiano *et al.* (2010, Vet. Parasitol., 170: 88-95). The best models were selected with backward elimination and evaluated using likelihood-ratio test and AIC.

Results and Discussion

Two ked species were found: *Lipoptena cervi* (prevalence 98.6%; abundance 24.2; min-max 0-398) and *Lipoptena fortisetosa* (prevalence 72.1%; abundance 102.7; min-max 0-1844). Only the prevalence of *L. fortisetosa* was significantly higher in Tuscany (83.7%) than in Emilia-Romagna (56.0%) (Chi-square test 16.55; $p < 0.001$). As regards the infested body area, 82.7% of collected *L. cervi* were found in the neck while 75.9% of *L. fortisetosa* were found in the groin region.

Lipoptena cervi abundance was positively related to altitude ($p = 0.03$) and negatively related to the abundance of *L. fortisetosa* ($p < 0.001$), while *L. fortisetosa* was negatively related to altitude ($p < 0.001$) and to the abundance of *L. cervi* ($p = 0.037$) and more abundant in southern and western geographic areas ($p < 0.001$). The inclusion of host age, sex and of stationary variables in the models, controlling their effect as confounders, suggests that the competition between the two species at the population level is probably real. This is further confirmed by the analysis of spatial distribution of the parasites on red deer body areas. Both the abundance of *L. cervi* in the most infested area, the neck, and in the genital area are negatively related to the abundance of *L. fortisetosa* in the neck ($p < 0.001$) and in the genital area ($p = 0.008$), respectively. Conversely, *L. fortisetosa* is not influenced by the presence of *L. cervi* in the evaluated body regions ($p > 0.05$).

In conclusion, the results of the present survey indicate that the distribution of *L. fortisetosa* is mainly linked with stationary variables, while the distribution of the autochthonous *L. cervi* both in the single host and in the different geographic areas is strongly affected by the invasive alien species.

DETECTION DOGS AND AFRICAN SWINE FEVER MANAGEMENT

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Introduction

African Swine Fever (ASF) is a highly contagious and lethal viral disease that affects animal species belonging to the suidae family (domestic pigs and wild species). The economic losses that can result from outbreaks of ASF and that reverberate on the swine sector require investigation of all possible strategies useful for both prevention and emergency management. The presence of carcasses on the territory represents a major cause of environmental contamination, and their disposal is one of the most relevant objectives for the containment of the disease. In order to test strategies to support the prevention and control of ASF, we developed a pilot project using detection dogs trained to detect wild boar carcasses.

Material and Methods

In February 2022, two canine units trained within the pilot project "Use of Dog Units Trained in Wild Boar Carcass Detection as a Tool for African Swine Fever Prevention and Control" realized by Ente Nazionale della Cinofilia Italiana, under the patronage of Società Italiana di Ecopatologia della Fauna, Istituto Superiore per la protezione e ricerca ambientale, Legambiente, Università Federico II di Napoli, Dip. di Medicina Veterinaria, joined the monitoring teams in the province of Alessandria. The African Swine Fever infected area, identified and established following the Ministry of Health's executive order, initially amounting to 146 municipalities in the provinces of Alessandria, Genova and Savona, was divided into monitoring sectors. In each sector, search cells equal to 100 ha each were identified. The search for carcasses within the cells was organized to proceed from outside to inside the infected area. The two dog units deployed, searched the cells and mainly the habitats: forest, meadow, pasture, ditches and riverbanks. Dog tracks were recorded using GPS collars and the signs of wild boar presence (bones, feces, scrapings, etc), appropriately georeferenced with GPS. The bone findings were collected in special bags for subsequent laboratory analysis.

Dogs involved in the project were a Labrador retriever (male, 4 years old) and an Hannoverian Hound (female, 4 years old) both trained for 3,5 years in scent detection. Before the operational phase, dogs were trained and tested with sample of wild boars carcasses of different size, coming from different part of the carcass and different status of putrefaction. The training procedure used was odor imprint during which dogs learned to associate reward with the smell of boar carcass. The technique used is what is referred to in the jargon as "pairing": in the preliminary step inside a pvc container a piece of Kong (neutral odor already known to the dog) was placed together with a piece of boar carcass, the container is then placed in a line of containers all identical, in a first step all empty except the one containing the target. In the second odor imprint phase, only the piece of carcass was present in the container and the dog had to identify it both in a lineup containing empty cans except the one with the target and in a lineup in which all containers were filled with a distracting odor (e.g., a handler's object, toy, etc.) except the one containing the target. The test phase is aimed at evaluating the ability of canine teams to search for and detect wild boar carcasses in a realistic environment with common distractions. In addition, the likelihood that the dog teams will or will not recognize the target scent is tested. The test was not constructed to assess under what conditions the scent reaches the surface and it becomes possible to detect it. The test took place in an area of 25 hectares in centre of Italy (Tuscany) divided into work zones of the size of 5 hectares each. The test area was predominantly forested. The target odor was 8 wild boar carcasses of different environmental aging (72 hours, 48 hours, 24 hours, fresh). The testing phase included the following tests: line up search (odor identification); outdoor area search in restricted area (500-1000 mq); outdoor search in wooded area of 5 ha. A functional obedience assessment was also carried out. The carcasses for each area are in variable number 0-3, of variable freshness. Assignment of carcasses to areas is random in both age and numerosity just as the assignment of dogs to individual area is random.

Results and Discussion

A total of 14 transects were searched in 4 monitoring sessions in Alessandria Province in February 2022 by the two canine units (74,86 km traveled by the dogs). There were 10 search cells investigated. The duration of the search phase was 29 h 24 min, the resting time (i.e. the time in which the dog remained stationary because in the resting phase or in the phase of passive alert), was 10 h 15 min. The total effective search time was 19 h 08 min. Four bone specimens, reported by the dogs, were collected: no. 1 mandible and no. 1 skull, referred to the wild boar species and two long bone specimens not belonging to the target species. Laboratory tests for PSA positivity and genetics are ongoing. One wild boar lair and two stool samples were also indicated from the dogs. Monitoring, in the health field, conducted using detection dogs appears, therefore, to be a rapid and effective response to the problems associated with the need for animal carcass retrieval, which is the central point in a program in which early warning is essential to reduce the huge impacts this disease can cause to human activities as well as to the species and what depends on it. Detection dogs have a much broader potential for use than the use of human handlers, in terms of greater effectiveness (using the sense of smell, the dog can inspect the areas subject to investigation more quickly, including those that are difficult to access and walk); less invasiveness; lack of contact with the search target; possibility of more extensive, effective, and quicker coverage of the search area, especially in the case of dense vegetation; and potential reduction in the use of personnel.

Workshop
PESTE SUINA AFRICANA

In collaborazione con ATIt

Moderatori: Carlo Citterio - Michele Vicari - Enrico Merli

RELAZIONI AD INVITO

Controlling african swine fever in wild boar: what is really needed?

Vittorio Guberti (ISPRA)

African swine fever in wild boar: situation and perspectives in Italy and Europe

Francesco Feliziani (Cerep)

Un milione di cinghiali, la PSA... e adesso?

Enrico Merli (Regione Emilia Romagna – Atit)

CONTROLLING AFRICAN SWINE FEVER IN WILD BOAR: WHAT IS REALLY NEEDED?

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Introduction

African swine fever (ASF) is a contagious viral disease that recognises 24 different virus genotypes. Currently the infection is widespread in Africa, Eurasia (including South East Asia) and Central America. In Africa, the disease is sustained by all 24 genotypes while only the genotype II is responsible for the spread of ASF outside the African continent. The first appearance of genotype II occurred in Georgia (Poti area) in 2007 and from there the virus spread completely unexpectedly with a geographical expansion that brought the infection to the European Union in 2014 and to China in 2018 and to Haiti/Santo Domingo in 2021. The disease exclusively affects species belonging to the SUIDAE family and has massively used both domestic pigs and wild boars as epidemiological reservoirs, while other, rarer and more localized suid species (e.g. Bearded boar) are a likely to represent an epiphenomenon of the disease, also putting their conservation status at risk.

The infection in wild boar

Since 2014, when ASF entered EU territory, it has been that the wild boar could become the epidemiological reservoir of the virus, i.e. maintain it in the environment in the absence of susceptible/infected pigs. The biological cycle of infection recognizes two modes of infection: a. Direct (nose to nose) from infected and susceptible wild boar; b. Indirect from environmental contamination of the virus through the elimination of excreta and secretions of infected animals and from the slow decomposition of carcasses. The latter mode of transmission is very important during the cold season as it maintains the viability of the virus. The main risk factors identified for persistence are: high wild boar density; important forest connectivity (on the contrary, habitat fragmentation reduces the probability of persistence), hunting activity especially if conducted in ignorance of basic biosecurity rules; artificial feeding.

The dynamics of infection

The virus - in one of the possible ways - arrives in a free area (introduction phase) and if it finds a minimum number of susceptible wild boars it will spread (invasion phase) initially to a limited number of animals; then each of the infected animals will transmit the infection to other receptive animals (R_0) resulting in the epidemic spread of the virus (epidemic wave phase). During the initial phases (until the epidemic wave is over) the main mode of transmission is direct transmission (nose to nose). Two events always occur at this epidemiological moment, and a third is very likely. The first event is the continuation of the epidemic wave, which spreads as long as there is forest and wild boar; the second event is the endemic persistence of the infection behind the epidemic wave; persistence that does not seem to be affected by the low density of wild boar exterminated (about 80% mortality) by the disease. The third event is transmission by humans outside the infected area (anthropogenic spread).

This pattern of spatial spread of the infection has meant that - in the EU alone - the virus has spread to around 350,000 km² of forests inhabited by at least 1,000,000 wild boars.

Managing the infection in wild boar populations

It follows from the above that the management of ASF in wild boar is based on 3 main steps:

- a. Early detection through passive surveillance; the earlier the virus is found, the smaller the infected area and thus the fewer infected wild boars that need to be managed; early detection of the virus is the first real step towards eradication.
- b. Blocking the epidemic wave; blocking which can take place through artificial barriers (fences; highways etc.) or natural barriers (lakes, rivers etc.).
- c. Quasi-extinction of the boar population within the barriers once the virus has killed most of the boars. In addition to the quasi-extinction of the wild boar, it is essential to remove the infectious carcasses.

AFRICAN SWINE FEVER IN WILD BOAR: SITUATION AND PERSPECTIVES IN ITALY AND EUROPE

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Introduction

African Swine Fever (ASF) is a viral disease affecting suidae (domestic and wild), it is sustained by the only known DNA virus that is able to complete a life cycle in insects (soft ticks). The virus is endemic in Africa where more than twenty genotypes are present. A first pandemic wave was characterised by genotype 1; after more than 60 years, this is now dying out and the last trace is present in Sardinia. Another epidemic wave (Genotype II) started in 2007 in the Caucasus, but is now widespread in all five continents.

In Italy, apart from Sardinia, the virus is present in two areas: in the Apennines between Piedmont and Liguria and in Lazio.

Given the characteristics of this infection, control measures have been decided following European legislation with the aim to achieve the eradication as soon as possible. The level of alert remains very high even in regions that are still free of the disease.

Situation in Sardinia

For decades, the ASF problem in Sardinia was underestimated and only in the last 5-7 years has it been tackled with the right determination and appropriate resources. A pathway has now been shared with the European Union Commission that should lead to the achievement of free territory status by 2023. The fight against illegal wild grazing, an adjustment to acceptable biosecurity standards, hunting management measures and careful surveillance have allowed a favourable epidemiological trend to be set in motion.

Situation in Italy (free regions)

The ASF surveillance plan has been active since 2020; it is based on passive surveillance, stakeholder training and is supported by excellent information systems. Recently, the Italian Government has asked the regions to implement urgent intervention programmes for wild boar management based on ASF prevention and control measures.

Situation in Piedmont and Liguria (wild boar)

Unfortunately, the area affected by viral circulation has not been contained by the motorway barrier as initially hoped. Fences are being set up to contain the infected population with many practical and unfortunately bureaucratic difficulties. It is necessary to revise the strategy initially proposed, in order to define a plan to control the infected population and arrive at eradication soon as possible.

Situation in Lazio

In the municipality of Rome, ASF has been reported in wild boars living in urban areas. Citizens effectively report the presence of carcasses and the containment barrier represented by the Grande Raccordo Anulare seems to be adequate. However, isolated cases have been reported outside this barrier and the state of alert is high. A pig farm has been affected by the infection; in addition, there is a growing popular feeling of intolerance towards measures to prevent infection.

In the Province of Rieti, a case of ASF was reported in a wild boar that remained isolated.

Virus studies

Genetic analysis studies were conducted on the Italian isolates. It was not possible to trace the origin of the infection, but it can be reasonably assumed that the introductions in the North West Cluster and the Lazio Cluster are different. Studies to develop a safe and effective vaccine continue. Some promising news has unfortunately been downgraded. In Europe, some candidates are being tested as part of a Horizon 2020 project.

Conclusions

ASF is a disease that can spread like wildfire, but is capable of leaping and appearing anywhere. Its main characteristic is persistence, which is why every effort must be made to achieve eradication as soon as possible. Vaccines are not immediately available, and therefore action must be taken with primary prevention tools. Biosecurity and every effort to arrive at an early diagnosis of the infection are the pillars on which work must be done.

UN MILIONE DI CINGHIALI, LA PSA... E ADESSO?

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Introduction

Il cinghiale è una specie peculiare dal punto di vista zoologico: presenta caratteristiche che la distinguono nettamente dagli altri Ungulati (per es. la biologia riproduttiva, alimentare o il comportamento spaziale) e in generale manifesta un'elevata plasticità ecologica, che le consente di insediare popolazioni vitali in una molteplicità di contesti ambientali e interagire in modo complesso con le componenti biotiche ed abiotiche della matrice ambientale. Il risultato è una gestione complicata, tecnicamente e socialmente, in cui gli sforzi profusi dagli Enti preposti, sino ad oggi tesi soprattutto a limitare l'impatto della specie sulle attività antropiche, hanno dato esiti spesso discutibili e controversi. L'insorgenza di focolai di Peste Suina Africana in Italia rappresenta un elemento potenzialmente di forte impatto demografico sulle popolazioni di cinghiale, la cui reale entità dipenderà dai livelli di diffusione e letalità che assumerà il virus in un contesto profondamente diverso, dal punto di vista ambientale e climatico, rispetto a quelli finora interessati.

Materials and Methods

A partire dall'analisi delle più recenti acquisizioni sulla specie, si approfondiscono alcuni aspetti in materia di biologia ed ecologia del cinghiale (dinamica di popolazione, genetica, rapporti specie-habitat e comportamento spaziale), ritenuti cruciali per valutare i possibili futuri scenari di gestione faunistica e venatoria potenzialmente in rapporto all'evoluzione del quadro epidemico.

Results and Discussion

Il quadro che emerge lascia aperti importanti interrogativi circa la base di conoscenze disponibili per prevedere l'evolversi dell'epidemia e definire consapevolmente le misure di contrasto. Alla luce di ciò resta imprescindibile un approccio quantitativo ed *evidence-based* nella definizione delle strategie gestionali, che consenta di valutare oggettivamente i costi ed i benefici delle azioni intraprese e di modificare adattativamente le scelte operate.

Sessione 3
**CRAS: GESTIONE SANITARIA E RUOLO NELLA
SORVEGLIANZA EPIDEMIOLOGICA**

In collaborazione con SIVASZOO

Moderatore: William Magnone – Enrica Bellinello

RELAZIONI AD INVITO

Can wildlife rescue centers act as wildlife health surveillance centers?

Stefano Pesaro (Università degli Studi di Udine)

Health surveillance in zoos and cras: similarities and differences

William Magnone (Parco Natura Viva – Sivaszoo)

CONTRIBUTI LIBERI

Wildlife rescue centers as sentinels in wildlife disease monitoring: weaknesses and strengths.

Experience in the province of Piacenza

Rossi R., Ferrari F.

Contribution of wildlife to antimicrobial resistance (AMR) with a One Health approach: preliminary assessment of the use of antibiotics in the European hedgehog (*Erinaceus europaeus*)

Marliani G., Cenni O., Zaghini A.

Causes of admission, mortality and gross pathology in European hedgehogs in Piedmont region: preliminary data

Prandi I., Colombino E., Perotti M., Cardello M., Vacchetta M., Bertolotti L., Tomassone L., Mauthe Von Degerfeld M., Quaranta G., Capucchio MT.

Bat's surveillance: bat health vs human health

Colombino E., Lelli D., Quaranta G., Leopardi S., Guidetti C., Robetto S., De Benedictis P., Orusa R., Mauthe Von Degerfeld M., Capucchio MT.

Increase of passive surveillance for avian flu: The experience of collaboration with wild animal rescue centers (CRAS) in Piedmont region, northwestern Italy

Picco L., Radice I., Mandola M. L., Maglione D., Belvedere M., Renna G., Merlo W., Avagnina A., Milla F., Ercole G., Alonge S., Giammarino M., Ferrero E. M., Giovara G., Luciano R., Calligarich U., Vaschetti G.

CAN WILDLIFE RESCUE CENTERS ACT AS WILDLIFE HEALTH SURVEILLANCE CENTERS?

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Wildlife rescue centers (WRCs) are multifunctional structures diffused through all Italian peninsula. The theoretical function of these institutions is correlated to the direct and indirect health and medical management of wild animals found in difficult conditions. These functions can be resumed in: physical and medical rescue of sick subjects, monitoring of health status of wildlife populations, studies concerning the distribution of animals through territories, to increase skills and knowledge of students (veterinarians, biologists etc) and raise awareness among citizens on health issues and conservation of wildlife heritage. Regarding health surveillance of wild animals, to comply these fundamental activities, any territorial reality should have different technical characteristics and aspects: adequate numbers of WRCs and Zooprophyllactic Institutes, qualified personnel in WRCs (manager, biologist, veterinarian, veterinary technicians, volunteers), collaborations between different health institutions (competent ministries, local medical authority, veterinary research institutions, WRCs), uniformed and shared guidelines. Although these considerations, it can be obvious that the Italian situation appears to be very heterogeneous and complex. The target of this work is to report the legal, logistical management and economic aspects observed in Italy to find a possible shared solution with the aim of improving the level of sanitary efficiency and to resolve problems that prevent these structures to become real health surveillance centers.

I centri di recupero della fauna selvatica (CRAS) sono strutture multifunzionali diffuse in tutta la penisola italiana. La funzione teorica di queste istituzioni è correlata alla gestione sanitaria e medica, diretta e indiretta, degli animali selvatici che si trovano in condizioni di difficoltà. Tali funzioni possono essere riassunte in: soccorso inteso come recupero sul territorio e medico di soggetti malati, monitoraggio dello stato di salute delle popolazioni faunistiche, studi riguardanti la distribuzione degli animali nei territori, accrescere le competenze e le conoscenze degli studenti (veterinari, biologi ecc.) e sensibilizzare i cittadini sui problemi di salute e conservazione del patrimonio faunistico. Per quanto riguarda la sorveglianza sanitaria degli animali selvatici, per adempiere a queste attività fondamentali, qualsiasi realtà territoriale dovrebbe avere caratteristiche e un'organizzazione basati su: un numero adeguato di CRAS e Istituti Zooprofilattici, personale qualificato nei CRAS (direttore, biologo, veterinario, tecnici veterinari, volontari), collaborazioni tra diverse istituzioni sanitarie (ministeri competenti, autorità sanitarie locali, istituti di ricerca veterinaria, CRAS), linee guida uniformi e condivise. Nonostante queste considerazioni, possano essere ovvie, la situazione italiana appare molto eterogenea e complessa. L'obiettivo di questo lavoro è quello di riportare gli aspetti legali, logistici, gestionali ed economici osservati in Italia per trovare una possibile soluzione condivisa con l'obiettivo di migliorare il livello di efficienza sanitaria e risolvere i problemi che impediscono a queste strutture di diventare veri e propri centri di sorveglianza sanitaria.

HEALTH SURVEILLANCE IN ZOOS AND CRAS: SIMILARITIES AND DIFFERENCES

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Introduction

Zoological Gardens in Italy are those structures well identified on the territory and which have received a license satisfying the requirements of Law 21 March 2005 n. 73 "Implementation of Directive 1999/22 / EC on the custody of wild animals in zoological gardens". The European Directive aimed to promote the conservation of wildlife, enhancing the role of Zoos with regard to conservation and biodiversity. The Zoological Gardens changed in last decades and they are centre of conservation, education and research and all animals are under different levels or veterinary surveillance. The Wild Animal Recovery Centers (CRAS) are Required by Law 157/92 but there are many different application in Italy and the veterinary surveillance is also different.

Material and Methods

The characteristics of the Zoological Garden will be briefly described by the mandatory health surveillance point and a comparison will be done with the reality of CRAS on the Italian territory. In particular, the 3 main annex will be described.

Annex 1, letter e which prescribes the obligation to *"host animals in conditions aimed at ensuring their well-being and satisfying the biological and conservation needs of individual species, providing, among other things, to enrich in an appropriate way the environment of the individual areas of custody, depending on the peculiarities of the species hosted"*, clarifies all profiles related to the care, welfare, health and hygiene of animals;

Annex 2, letter f which prescribes the obligation to *"maintain a high quality level in the care and care of animals through the implementation of an articulated program of veterinary, preventive and curative treatments, and providing proper nutrition"*, determines the application protocols relating to the care of animals and veterinary profiles as well as the *post-mortem* protocol.

Annex 3 letters g and h, which *prescribe the obligation to "adopt appropriate measures to prevent the escape of animals, including to avoid any ecological threats to indigenous species and to prevent the spread of alien species"* and to *"dispose, in accordance with the guidelines set out in Annex 3, measures to guarantee the safety and health protection of the public and operators"*, describe all the protection and safety measures that must be equipped with Italian zoos.

Concerning CRAS, some health surveillance plans of some regions will be examined to underline their regional differences. These structures are often difficult to manage: they require constant, demanding work, with expert staff

In particular, the fundamental role in the epidemiological surveillance of many diseases, including zoonotic diseases and therefore with public health implications, will be stressed. Avian influenza, African Swine Fever, West Nile Disease are among discussed diseases and animal Health Law application on both zoos and Cras will be also discussed.

The important role in passive surveillance is one of the main aspect of CRAS because they receive animals found randomly on the entire provincial territory (and beyond), thus acting as a sentinel of the environmental health of the territory

CRAS can be also places of research in different veterinary field as well as Zoological Gardens.

Results and Discussion

Zoos and CRAS can have many similarities mainly on their role on health surveillance but there are many differences.

WILDLIFE RESCUE CENTERS AS SENTINELS IN WILDLIFE DISEASE MONITORING: WEAKNESSES AND STRENGTHS. EXPERIENCE IN THE PROVINCE OF PIACENZA

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Introduction

The wildlife rescue centers (CRAS) in Italy are a heterogeneous and not constant reality that provides for the collection, care, rehabilitation and release in the nature of wild animals found in difficulty. Law 157/92 defines the fauna as an unavailable heritage of the state and delegates its care to the regions. The regional administrations then delegate to the rescue centers, through dedicated agreements, permission for the activities above. It is therefore understandable how wildlife rescue centres and their staff are naturally predisposed to encounter animals affected by infectious, viral or bacterial, mycotic and parasitic diseases. The centres therefore, offer an opportunity for recognition and study of wildlife diseases, including zoonoses.

Material and Methods

For the preparation of this work, the entry registers of animals at the CRAS (considering an average of about one thousand animals admitted per year), the reports received by the Istituto Zooprofilattico di Lombardia e Emilia Romagna, reports of regional wildlife monitoring plans over the last five years were analysed. Therefore, the strengths and weaknesses that characterize a CRAS involved in a wildlife disease monitoring plan were considered.

Results and Discussion

The reality of the CRAS present on the Italian territory is extremely heterogeneous:

- from the operational point of view some structures are "specialized" only caring for a particular specie or class (only hedgehogs, only turtles, only protected birds, only mammals...) whilst others accept all authorized species.
- from the administrative point of view few structures are related to government agencies while most are founded by voluntary associations.

Piacenza rescue center treats all native species and it's run by a no profit volunteer association. The great strength of this rescue center are certainly the constant daily presence of a biologist who plays the role of manager and a veterinary director. To this is added the value of a 24/7 availability that guarantees direct contact with the population and institutions even in times and days of the year when public facilities are usually closed to the public. These factors, in recent years, and the integration of the rescue center between the figures present in the wildlife monitoring plan has led to the recognition on the territory of new species, illegal acts against animals and the identification of infectious diseases present or emerging. Often in the last period multiple conditions have coexisted. Some interesting cases were:

- the discovery of a carcass of golden jackal (*Canis aureus*), hit by a car, poisoned with anticoagulants and suffering from parvovirus
- a red deer (*Cervus elaphus*) victim of a poaching snare affected by *Mycobacterium avium subs paratuberculosis*
- an uncontrollable itchy red fox (*Vulpes vulpes*) suffering from Aujeszky's disease
- wild boars (*Sus scrofa*) with *Trichinella spiralis* and *T.britovi* were found over the years
- a wolf (*Canis lupus*) hit by a car, poisoned with a mix of anticoagulants and positive to parvovirus
- many birds affected by West Nile Virus

In conclusion, we believe that the rescue centers are an invaluable support to the monitoring of the state of health of the fauna and the emergency and spread of diseases but too often the exclusive reference to voluntary activity leads to an abuse of requests from institutional figures and a lack of professionalism of operators with poor skills in the handling and management of animals that can lead to the presence of nosocomial infections.

Finally, we believe that the presence of a staff trained to the strict ethics that governs the human-wildlife interaction is essential to avoid wrong environmental education and animal welfare harms.

CONTRIBUTION OF WILDLIFE TO ANTIMICROBIAL RESISTANCE (AMR) WITH A ONE HEALTH APPROACH: PRELIMINARY ASSESSMENT OF THE USE OF ANTIBIOTICS IN THE EUROPEAN HEDGEHOG (*ERINACEUS EUROPAEUS*)

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Introduction

Antibiotic resistance is an increasing pandemic. To date, it is estimated that every year in Europe at least 33,000 people die as a result of this phenomenon, which involves all the sectors where antibiotics are employed. For this reason, it is necessary to have a One Health approach that, through the collaboration of different industries and disciplines that acts on several levels (from local to global realities), has the purpose of balancing and optimizing the health and well-being of humans, wildlife, flora and ecosystems, recognising their interdependence. Wildlife, in particular, can play an important role as a reservoir and can become a carrier of resistant bacteria. To encourage research about the growth of this phenomenon, the study of small realities, such as wildlife rescue centres (CRAS), can be useful. This retrospective research aims to analyse the use of antimicrobials in these local realities. In particular, we focus on the European hedgehog (*Erinaceus europaeus*) because it is a synanthropic species that can easily come into contact with humans and pets and are commonly recovered in CRAS.

Material and Methods

This study has evaluated 582 clinical case sheets of European hedgehogs recovered in two CRAS in the Bologna district from January to December 2021. The information collected in the two centres has been harmonized and for all cases, we recorded the CRAS of recovery, the class of age, the use or not of antimicrobials, the type of antimicrobial, the reason for therapy and the outcome of their hospitalization (liberation or death). The analysis of data was performed through the software R, version 4.1.3. The percentage of animals treated with antibiotics, the type of antimicrobial employed and the reason for treatment have been calculated. A Chi-square test has been performed to analyze the correlation between the use of antibiotics and the class of age, the outcome and centres. In addition, the same test was employed to determine the relationship between the two CRAS and the class of age of hedgehogs recovered and the outcome. Significance was considered for $p\text{-value} < 0.05$.

Results and Discussion

The results show that there is no significant correlation between the considered CRAS and the class of age of animals recovered, the outcome and the percentage of animals treated with antibiotics. This suggests that the two centres had similarities in the characteristics of the hedgehog population that received and in their treatment procedures, even if in 2021 one centre recovered more hedgehogs than the other.

Considering the entire population, 21.6% of hedgehogs were treated with antimicrobials, of which in 81.7% of cases with fluoroquinolones, in 7.1% with a combination of fluoroquinolones and penicillin, in 4.8% with fluoroquinolones and local aminoglycosides, in 3.2% with penicillin and 3.2% with only local aminoglycosides. Excluding 145 individuals whose outcome was unknown, 41.2% of animals died during the recovery. In particular, there was a significant correlation between antibiotic usage and the outcome of death ($\chi^2 = 20.8$, $df = 1$, $p\text{-value} < 0.001$). Indeed, 61.7% of the animals that received antibiotics died, while 64.4% of animals that were not treated with antibiotics were reintroduced into the wild. Probably, the treated subjects were in more severe conditions, but a risk of antimicrobial resistance cannot be *a priori* excluded and further research is needed. In 41.3% of cases, antimicrobials were employed in presence of respiratory symptoms, while in 10.4% of cases the antibiotic therapy was made without the declaration of a clear clinical suspect of infections but in presence of poor physical conditions. Finally, a significant correlation was found between the class of age and usage of antibiotics ($\chi^2 = 35.8$, $df = 2$, $p\text{-value} < 0.001$), where 45.2% of animals treated with antibiotics were adults.

The study underlines several critical points. First of all, the first choice in antibiotic therapy is systemic treatment with fluoroquinolones. A comprehensive study of the pharmacokinetics of these antibiotics in this species could be useful to avoid the elimination of active metabolites after their reintroduction into the wild. In addition, fluoroquinolones and aminoglycosides, which in our case have been used only locally, are Veterinary Critically Important Antimicrobial Agents and their use should be parsimonious. However, the emergency nature of treatment and lack of time or economic funds for laboratory investigations are serious obstacles to the adequate use of antibiotics in these realities, and, as demonstrated by our results, their use can be preventive and not always justified by signs of infections. Finally, a more harmonized way of collecting data and registering treatment among CRAS could be useful for future studies.

These centres constitute preferential points of view for epidemiological investigations of antimicrobial resistance and its surveillance. The work carried out by CRAS has the fundamental purpose of safeguarding biodiversity and, considering a One Health approach, these realities should have a leading role in Veterinary Medicine.

CAUSES OF ADMISSION, MORTALITY AND GROSS PATHOLOGY IN EUROPEAN HEDGEHOGS IN PIEDMONT REGION: PRELIMINARY DATA

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Introduction

The European hedgehogs are nocturnal mammals declared as protected species due to population decline. Human activities, habitat/climate changes, road accidents, poisoning and predation have been suggested among the major causes of this decline. The aim of this study was to analyse the admission causes, causes of death and gross pathology reported in deceased hedgehogs admitted to Centro Animali Non Convenzionali (C.A.N.C. – Non - Conventional Animal Centre) of Turin University from January 2018 to July 2022. Hedgehogs died in 2022 in Centro Recupero Ricci LA NINNA of Cuneo were also examined.

Material and Methods

For this study, hedgehogs found in Turin and Cuneo province (Italy) were enrolled. For all the animals, sex, age, season and cause of hospitalization were recorded. All the deceased hedgehogs were necropsied, the main macroscopical lesions observed were reported and a cause of death was established. Aliquots of affected/selected organs including lung, heart, liver, spleen, small intestine, lymph nodes, kidney, urinary bladder, skin and brain, were collected and stored for further investigations (histopathological, microbiological and virologic investigations).

Results and Discussion

A total of 160 hedgehog were included in the study. Of these, 85 were males (53.1%) and 75 females (46.9%). Regarding age, the majority of the hedgehogs were adult (n= 79, 49.4%) or juveniles (n=78, 48.7%), while only 1.9% were neonates (n=3). Also, half of the deceased hedgehogs were hospitalized in summer (n=60, 37.5%) and spring (n=57, 35.6%) while few animals were brought in winter (n=5, 3.1%) and autumn (n=38, 23.8%). Trauma (n=63, 39.4%) and weakness (n=61, 38.1%) were the main causes of hospitalization, followed by random hospitalization of hedgehogs found in inappropriate places and brought to the centres by citizens (n=27, 16.9%) and respiratory/gastrointestinal symptoms (n=9, 5.6%). According to the necropsy findings, traumatic lesions (n=56, 35.0%) and infectious/parasitic diseases (n=47, 29.4%) were the main causes of death. Less frequently, hedgehogs died from starvation (n=15, 9.4%) and predation (n=9, 5.6%). Macroscopically, lungs were the most affected organs (n=117, 73.1%), mainly showing bronchopneumonia (n=102, 87.2%). Among the cases of bronchopneumonia, 34 hedgehogs (29.0%) showed pulmonary nematodes. In some cases, bronchopneumonia was associated with thoracic effusion (n=32, 20.0%). Lesions were recorded also in the intestine (n=29, 18.1%), stomach (n=27, 16.9%), spleen (n=22, 13.7%), liver (n=18, 11.2%) and brain (n=14, 8.75%). Particularly, segmental catarrhal (n=24, 82.7%) and hemorrhagic enteritis (n=5, 17.3%) were the main lesions recorded in the affected intestine while catarrhal (n=18, 66.7%) and hemorrhagic gastritis (n=5, 18.5%) were mainly recorded in the stomach. Splenomegaly (n=12, 54.5%) and discoloration (n=10, 45.5%) were mainly observed in spleen; lipidosis was the main lesion observed in the liver (n=9, 50%). In the brain, traumatic lesions such as subdural hematomas (n=7, 50.0%), hemorrhages (n=2, 14.3%) and hyperemia (n=5, 35.7%) were the main findings. They were generally associated with other traumatic lesions such as fractures of the forelimb (n=1, 0.6%), hindlimb (n=6, 3.7%), skull (n=2, 1.2%) or pelvis (n=1, 0.6%), amputation (n=6, 3.7%), skin hematomas (n=27, 16.9%) and lacerations/bruising (n=13, 8.1%) or liver rupture (n=2, 1.2%). These results are in accordance with literature, which consider debilitation, trauma and random finds as the main reasons of admission. Trauma represents one of the most common causes of mortality in hedgehogs and most of the time it is human-related. Infectious diseases mainly affecting lungs or gastro-intestinal tract are another important cause of death due to bacterial infection or parasites. In agreement with previous reports, we frequently detected lungworms; further studies are needed to understand the eco-biology of these parasites and the pathogenesis of their lesions.

In conclusion, our results emphasize that trauma is the main cause of death of hedgehogs and is frequently associated with fractures, skin lacerations and following infections. Histological and microbiological investigations are in progress to verify the potential role of infectious agents in causing hedgehogs mortality and contributing to their population decline.

BAT'S SURVEILLANCE: BAT HEALTH VS HUMAN HEALTH

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Introduction

Recently, the growing awareness of the role of *Chiroptera* as reservoir species for multiple infectious agents has increased the interest in bats biology and ecology. On one hand, the increasing human-bat interface has raised concern about the possibility that bats can represent a threat to human health. On the other, little attention has been given to the role of pathogens on bats' health and mortality. Thus, the aim of this study was to combine the evaluation of the main causes of death, gross and histopathological lesions in deceased free-ranging bats of North-western Italy as well as the investigation of the presence of emergent bat viruses that could represent a risk for human health.

Material and Methods

This study analysed the carcasses of bats found in Turin province (Italy), died in care or euthanized at the Centro Animali Non Convenzionali (CANC – Non-Conventional Animal Centre) of the Department of Veterinary Sciences, University of Torino. All the deceased bats were necropsied and samples of the main organs (patagium, lung, heart, liver, spleen, small intestine, kidney and brain) were submitted to histopathological examination. Also, aliquots of the small intestine, liver, spleen, lung, and brain were collected and submitted to biomolecular investigation for the identification of Rhabdovirus, Coronavirus, Mammalian orthoreovirus, Poxvirus and Kobuvirus. Statistical analysis was performed using R software.

Results and Discussion

A total of 71 bats belonging to 9 different species of the families Vespertilionidae and Molossidae (genera *Pipistrellus*=37, *Hypsugo*=28, *Vespertilio*=1, *Eptesicus*=1, *Myotis*=2, *Plecotus*=1 and *Tadarida*=1) were analysed. About 56.3% of bats died from traumatic lesions due to unknown trauma or predation, mainly showing patagium and skin lesions (n=23, 32.4%) and forelimbs fractures (n=15, 21.1%). These results are in accordance with previous studies conducted in Europe, which reported that almost three-quarters of all bat deaths can be attributed to trauma or cat predation. Also, previous studies reported that about 90% of the observed lesions in bats were in the upper extremities, probably due to the large and delicate surface of bat wings, which predispose them to injury. The second most reported cause of death was emaciation (n=13, 18.3%), followed by gastric distension (n=10, 14.1%). Microbats are highly susceptible to suffer from emaciation as a consequence of wing injury, chronic illness or disturbance during hibernation due to their small dimensions. Also, gastric distension has been reported to occur in captivity due to a tendency to overfeed these small mammals. Histologically, the lung was predominantly affected (n=24, 33.8%), mainly showing mild to moderate, multifocal to diffuse lymphoplasmacytic interstitial pneumonia. According to the literature, the lung is the most affected organ in bats due to its large respiratory surface, its thin blood-gas barrier and its greater pulmonary capillary blood volume adapted to fly. The second most reported lesion was acute to chronic dermatitis with different grades of hyperkeratosis in the patagium (n=23, 32.4%). It is well known that patagium lesions can easily get infected by a bacterial mixed population causing severe dermatitis that can lead to necrosis and loss of tissue. Splenic white pulp depletion (n=7, 9.8%), lymphoplasmacytic hepatitis (n=8, 11.3%) and liver steatosis (n=6, 8.4%) were also recorded. Further studies are needed to clarify the pathogenesis of these lesions, even though liver steatosis could be attributed to an unbalanced diet and overfeeding which is likely to occur in captivity.

Regarding viral isolations, Coronaviruses, Kobuvirus, Vaprio ledantevirus and Rabies Lyssaviruses were not detected. On the contrary, 12 bats showed PCR positivity for Orthoreovirus in the intestine and/or lungs and Hypsugopoxvirus was detected in 2 bats. In accordance with the literature, rabies has never been recorded in Italian bats and only low positivity for Coronavirus, Orthoreovirus and Hypsugopoxvirus have been previously detected in Italy, probably suggesting a low circulation rate of these viruses. Interestingly, Hypsugopoxvirus was associated with pneumonia while Orthoreovirus were associated with heterogenous microscopical lesions in liver, lung and spleen.

In conclusion, the present study highlighted that trauma is the main cause of death in North-western Italian bats mainly associated with forelimb fractures. Histologically, lungs are the most affected organs, suggesting that respiratory disease can be a serious issue for bat health. Considering viral isolation, Italian bats did not represent a threat for human health, even though bat surveillance is strictly recommended due to the increasing human-bats interface in urban areas. Further studies are still needed to clarify the role of viral pathogens in the determination of bat's pathologies.

INCREASE OF PASSIVE SURVEILLANCE FOR AVIAN FLU: THE EXPERIENCE OF COLLABORATION WITH WILD ANIMAL RESCUE CENTERS (CRASs) IN PIEDMONT REGION, NORTHWESTERN ITALY.

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Introduction

Wild Animal Rescue Centers (CRASs), as recognized sentinels of environmental health, play a crucial epidemiological role on animal health: the hospitalized animals - if appropriately subjected to sampling of biological material when entering the CRAS - can act as indicators of pathogens present on the territory. This work aims to support this theory, through the activation of the national project "increasing of the passive surveillance in avian flu": in this context, Piedmont Region (Prevention and Veterinary Sector) has established a relationship of active collaboration with the CRASs present in the regional territory, achieving the objectives set at national level.

Material and Methods

The project for the increase of passive surveillance for avian flu has been activated by the Italian Ministry of Health with the Decree of 14 March 2018 which, in agreement with the Minister of Agricultural Policies, distributed funds for poultry emergency required by law to the Regions. Following the risk assessment of introduction and spread of the disease, the Decree has assigned to regional administrations 10 million euros with the aim of strengthening the surveillance system for the prevention and control of avian flu. Piedmont Region issued a document (D.D. n.810 of 4/12/2018), allocating funds to the multizonal prophylaxis and veterinary police departments (PMPPV) and the Veterinary Services of ASL (Local Sanitary Authority) for the period 2019-2021. After that, ASL Prevention Departments cooperating with Wild Animal Rescue Centers have drawn up specific conventions with regional CRASs, regulating the cooperation between ASL and CRASs, through the collection of samples with a specific procedure: 1) the drafting of an operational protocol for sampling (carcasses and cloacal swabs), transport and lab tests (RT-Real Time PCR), agreed between Piedmont Region and the virology laboratory of the IZSPLV in Turin 2) the planning of meetings between ASLs official wildlife veterinarians and the CRASs staff; 3) the elaboration of final reports on the basis of a predefined model, in order to compare data collected.

Results and Discussion

A wildlife monitoring plan has been activated in Piedmont Region many years ago, also involving the regional CRASs and the ASL Veterinary Services on the basis of annual programs: dead animals in the CRASs are regularly sent to the Laboratory of IZSPLV in Turin to carry out biomolecular analyses required by international standards. In the specific case of avian flu, 142 samples were analyzed in 2019 from wild birds found in difficulty and delivered to regional CRASs (CRAS Tigliole d'Asti, CRAS Bernezzo, CRAS Racconigi, CRAS San Bernardino Verbano).

With the ministerial project focused on increasing the passive surveillance for avian flu, formally active in 2020 and 2021 but officially held in the three-year period 2019-2020-2021, specific agreements between Piedmont Region and CRASs have been established with the aim of encouraging an increase of collection of biological samples. As a consequence, a substantial increase of samples was recorded, having been sampled 142 samples in 2019 vs 338 samples in 2020 (+138%) and 410 samples in 2021 (+188.7% from the beginning of the project). An increase in sampled species was also recorded, going from 62 bird species sampled in 2019 vs 101 species monitored in 2020 (+62.9%) and 94 species in 2021 (+51.6% from the beginning of the project). The implementation of the sampling plan also led to an increase in the territories subjected to sampling: 47 municipalities in 2019 vs 93 in 2020 (+97.9%) and 98 municipalities in 2021 (+108.5% from the beginning of the project).

Therefore, the activation of specific protocols and agreements with CRASs has determined a significant increase in sampling activity in regional territories (number of samples, bird species and territorial area monitored), allowing the Public Health Service to encourage CRAS epidemiological role in passive surveillance of zoonotic agents. To date, all analyzed samples have been negative to molecular tests: considering the epidemiological trend of the disease, it would be useful to have more samples from aquatic bird species in the winter period, thus combining surveillance in CRASs with other specific capture activities, such as scientific ringing and hunting.

Sessione 4
SPECIE ALLOCTONE E PATOGENI CORRELATI

Moderatori: Nadia Cappai - Roberto Viganò

RELAZIONI AD INVITO

Invasive alien species and pathogens: patterns, impacts and solutions

Nicola Ferrari (Università degli Studi di Milano – SIEF)

CONTRIBUTI LIBERI

***Baylisascaris procyonis* and other endoparasites in non-native racoons (*Procyon lotor*) from the Arezzo province (IT): a preliminary survey**

Lombardo A., Brocherel G., Palmerini L., Donnini C., Giovannini S., Diano M., Cocco M., Falorni B., Gori R., De Liberato C., Iurescia M., Diaconu E.L., Smedile D., Cappai N., Mattioli L., Fichi G.

Containment of nutria through surgical infertilization

Serpieri M., Bonaffini G., Scandone L., Quaranta G., Ottino C., Prandi I., Mauthe von Degerfeld M.

Euthanasia in the wildlife: to keep running in the prairies

Franceschini F., Viganò R., Ferri M.

INVASIVE ALIEN SPECIES AND PATHOGENS: PATTERNS, IMPACTS AND SOLUTIONS

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Invasive Alien Species (IAS), organisms introduced intentionally or unintentionally by humans, represent one of the greatest threats to the conservation of biodiversity. At the same time, they play a great economical impact, with direct and indirect damages and cost of prevention and demographic control. The number of Invasive Alien Species is rising over the year and for these reasons European Community has issued Regulation N. 1143/2014 to face these issues.

Despite the above-mentioned conservation and economic impacts, the ecopathological aspects are still incomplete.

Indeed, the effect of pathogens on facilitating the establishment and spread of invasive alien species has been advocated, thanks to the parasite release. At the same time Invasive Alien species, by sharing pathogens with native species which suffer a greater detrimental impact, may impact biodiversity through the so-called “disease mediated competition”. Lastly, the role of Invasive Alien species on public health is still underestimated, due to low levels of knowledge about their pathogens and low awareness of these topics.

In the present presentation, the ecopathological investigation carried out in Italy in 2010 will be summarized, highlighting the impact on biodiversity, and public health, with specific attention, to the efforts directed to identify the priority species of interest and methodological approach to limit their health impacts.

BAYLISASCARIS PROCYONIS AND OTHER ENDOPARASITES IN NON-NATIVE RACOONS (*PROCYON LOTOR*) FROM THE AREZZO PROVINCE (IT): A PRELIMINARY SURVEY

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Introduction

The raccoon (*Procyon lotor*) is a carnivore native of North America, introduced in several Eurasian Countries, either deliberately or through accidental releases. Ecological impacts by non-native raccoons encompass the predation on native fauna, the competition with other autochthonous carnivores and the introduction and spreading of new pathogens, such as the helminthic zoonosis caused by *Baylisascaris procyonis*. In Italy, the presence of the raccoon was first reported in 2004 along the southern part of the Adda river and more recently (2013) in Tuscany, within the Casentino Valley. The European Union listed the raccoon among the invasive species of European concern (EU Regulation 1143/2014); in Tuscany an official eradication plan is currently ongoing, including a sanitary investigation of the carcasses in the context of a research program funded by the Italian Ministry of Health. In this scenario, in April 2021, *B. procyonis* was for the first time reported from Italy, in a raccoon culled in the Casentino Valley. The aim of this study was to further investigate the presence of *Baylisascaris procyonis* and other endoparasites of raccoons captured and culled in the Casentino Valley.

Material and Methods

Between January 2021 and July 2022, 59 free-ranging raccoons (28 males, 31 females, average weight 4.42 ± 1.72 kg) from the Casentino valley (Arezzo) were cage-trapped, euthanized and submitted for necropsy. Intestinal content and skeletal muscles (diaphragm and *tibialis* muscles) were collected for parasitological investigation. The intestinal content was examined for enteric and respiratory parasites: 1) macroscopic and stereomicroscopic inspection, 2) Baermann technique, 3) qualitative/quantitative egg/oocysts analysis (flotation with sucrose solution and Flotac®), 4) IF for detection of *Giardia* spp. and *Cryptosporidium* spp. oocysts. Skeletal muscles were submitted to artificial digestion for the detection of *Trichinella* spp. larvae.

Results and Discussion

At macroscopic evaluation of the intestinal content, 24 animals (40.7%) harbored ascarid nematodes morphometrically consistent with *B. procyonis*, with different parasites burdens (from one to over 100 adult specimens). The observed prevalence is comparable to the one reported in other European Countries. In 10 infected raccoons a partial intestinal obstruction was observed, caused by the high burden of adult parasites (over 100 specimens). Both male and female parasites were recovered. Parasites identification at species level was confirmed by molecular methods, using a next-generation sequencing-based approach, as described by Lombardo et al (2021). Faecal egg count showed abundant eggs shedding, up to 25.000 eggs/g, suggesting a high risk of environmental contamination. Other intestinal parasites, of minor sanitary relevance, were also recovered with low prevalence: *Cryptosporidium* spp. (1.7%), *Isospora* spp. (1.7%), Capillariidae (1.7%) and Ancylostomatidae (1.7%). All samples tested negative for *Trichinella* spp.

Adult *B. procyonis* colonize the small intestine of raccoons normally at subclinical level. In North American populations, prevalence in free ranging raccoons can reach 90%. However, birds and mammals other than raccoons, humans included, may act as paratenic or dead-end hosts and become infected with *B. procyonis* larval stage. In these hosts somatic migration of larvae can sometimes cause extensive tissue damage and granulomatosis, causing visceral, muscular, ocular or neural *larva migrans* syndrome. Death or permanent disability is a common outcome of neural *larva migrans*, especially in young children. The opportunistic and synanthropic behaviour of raccoons coupled with the high reproductive output of *B. procyonis* and the high resistance of its eggs to environmental degradation, result in a relevant risk of exposure for humans and domestic animals. Hence, the presence of infected raccoons in a natural area is likely to pose a public health risk, especially in a region like Casentino, which is geared towards ecotourism, hunting, and extensive farming. Further investigations on culled raccoons, paratenic hosts and environmental contamination are still ongoing, and will be important to better understand the risk for human and animal health in this area.

CONTAINMENT OF NUTRIA THROUGH SURGICAL INFERTILIZATION

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Introduction

The nutria (*Myocastor coypus*) is considered a pest in many regions, as it has potentially severe effects on ecosystem functionality, economy, biodiversity and public health: therefore, it has been included in the first list of “invasive alien species of Union concern” by Commission Implementing Regulation (EU) n. 2016/1141. Several containment programs have been made, pursuant to Regulation (EU) n. 1143/2014 and, in Italy, to D.L. 230/17.

D.L. 230/17 suggests various methods for the containment of this species, including eradication with the use of firearms, a method that presents some critical issues (recolonization of free territories by new individuals, animal escape after a shot) and which is inapplicable, also for ethical reasons, on the increasingly common urban colonies.

The remarkable spread of the rodent, however, makes eradication difficult to achieve, while the numerical management of the population is a more feasible target: among the proposed methods is the control of reproduction by surgical sterilization. A disadvantage of gonadectomy is the loss of normal hormone-dependent behavioral patterns, with marked alteration of the usual social hierarchies, especially in species with complex ones, such as nutria.

Conversely, “infertilization” methods by means of salpingectomy and vasectomy allow contraception without losing the hormonal component and, therefore, without altering the normal social dynamics.

The gonadectomies, carried out by the Centro Animali Non Convenzionali (CANC) of the Department of Veterinary Sciences of the University of Turin between 2006 and 2009, in collaboration with the CRAS of Bernezzo (CN), on nutrias (n = 23 subjects) of the Riserva Naturale Crava-Morozzo (CN) already highlighted the different results when compared with infertilization, in terms of behavioural changes.

D.L. 230/17 also prohibits the introduction or reintroduction, into the environment, of invasive alien species included in the list contained in the Commission Implementing Regulation (EU) n. 1141/2016; therefore, when planning surgery, it is currently not possible to move the animal from its territory, even if only to a veterinary service.

It is consequently necessary to design a strategy that allows the execution of clinical and surgical procedures under field conditions, and the reduction of captivity times with a prompt release of the animals.

Material and Methods

Between 2018 and 2021, n=78 nutrias (n=34 males, n=44 females) of various age (prepubertal and adults) underwent laparoscopic infertilization; of those, n=41 subjects (n=16 males, n=25 females) in 2018 and 2019 in Turin and n=37 subjects (n=18 males, n=19 females) in 2021 in Sesto San Giovanni (MI). We used a specifically equipped field-mobile unit to perform surgery and anesthesia.

We captured the animals with cage traps set near the colonies. In the order of capture, we immobilized the nutrias with a mixture of ketamine and medetomidine (6 mg/kg and 140 µg/kg, respectively) administered intramuscularly. Anesthesia was maintained with isoflurane in pure oxygen. Upon completion of the procedures, atipamezole was administered to the animals at doses 2.5 higher than those of medetomidine. Carprofen (4 mg/kg) was administered for its analgesic effects. Nutrias were placed in dorsal recumbency, and those who could undergo minimally invasive surgery (nonpregnant females) were placed in Trendelenburg position, and laparoscopic salpingectomy and vasectomy was performed using a 3-port technique and an electro-thermo-clotting-ablation device (Onemytis®), which employs Airplasma technology. Pregnant nutrias underwent laparotomic hysterectomy, but both ovaries were left in place.

The animals were released in the evening in the same place as capture. The animals were monitored after the procedures, with direct and indirect observation techniques based on a specific ethogram and using BORIS® software to analyze the typical behaviors on the territory of the urbanized nuclei, referring to previous personal experiences and in particular: primary and locomotive needs, social patterns, active or passive aggression.

Results and Discussion

Overall, the combination of medetomidine and ketamine, with supplementary isoflurane as required, provided a good-quality immobilization and anesthesia in free-ranging nutrias, adequate for performing surgical procedures under field conditions, whereas the use of atipamezole speeded recovery, allowing prompt release of the animals. Laparoscopic surgery allowed to minimize the extent of surgical damage and, presumably, the pain stimulus, thus obtaining a rapid recovery, with spontaneous feeding and normal activities. The monitoring carried out after the procedures showed no overt modification of the behavioral patterns typical of the species, with the ethograms highlighting normal activities: autogrooming, sleeping, feeding on land, feeding in water, matching, allogrooming, vocalizing, walk or swim, freezing in water, rubber, extra-specific competition, food hand, safe place, circle swim, rover.

The procedures described in this work complied with the laws that regulate the control of the spread of nutria and respected the sensitivity of public opinion. The control of reproduction through infertilization, therefore, is an effective method for the demographic containment of the nutrias on the territory, without changing the social patterns.

EUTHANASIA IN THE WILDLIFE: TO KEEP RUNNING IN THE PRAIRIES

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Euthanasia of an animal is a medical act by which the veterinarian decides to put an end to an unacceptable psycho-physical suffering of the animal, however, there are other cases permitted by law in which it is not practiced by a veterinarian.

Considering wildlife, euthanasia is generally practiced in two situations: in the case of an unfavorable prognosis for recovery and subsequent re-insertion in nature, or when there is a control of the species that has the aim of depopulation. In the first case the decision is the exclusive competence of the veterinarian, on the other hand, when it comes to controlling the species, the killing can be entrusted to trained people without any protocol shared with Veterinarians, so they can use firearm, captive bolt, CO₂, etc ..., always respecting animal welfare, as for example in the case of the euthanasia of the raccoon (*Procyon lotor*) and of the nutria (*Myocastor coypus*), which has long apnoea abilities not considered by the management protocols.

In wildlife recovery centres it can sometimes happen that the aesthetic sense prevails over the conservation of animal welfare, losing sight of the aspects of safeguarding the species. Certainly the issue of killing an animal is critical, but it is necessary to define the concept of euthanasia, literally "good death" (from Greek *eu-thanatos*), far from an unjustified killing, a crime that finds discipline in art. 544-bis of the Criminal Code which punishes any killing of an animal caused by cruelty or without need. It is therefore necessary to bring the veterinary profession to a reflection on the term "necessity", if "it is necessary" to try to provide manipulations, treatments, therapies and housing of wild animals which by definition live free and without any care by human being, or if "it is necessary" to put an end to the suffering of a subject in which it is assessed with knowledge and consciousness that every medical act can only and exclusively be a further form of stress and that therefore it is more proper to do euthanasia.

this issue must consider the freedom of choice of the veterinarian, who must not be influenced by public opinion, as stated in Art. 12 of the Code of Ethics "Duty of intellectual independence - In exercising his professional activity, the Veterinarian has the duty to maintain his intellectual independence and defend his freedom from external pressure or conditioning and commercial impositions. The Veterinarian must abide by scientific knowledge and be inspired by the ethical values of the profession and must not be subject to interests, impositions and suggestions of any kind".

In addition to these aspects, already problematic and sometimes divisive in themselves, it is necessary to pay attention to the issue of informed consent (Article 29 of the aforementioned Code of Ethics - Obligation to inform and informed consent in veterinary practice): how can this procedure be applied in the case of management of wild animals which according to article 1, paragraph 1 of the law 11 February 1992, n. 157 are "unavailable heritage of the state"?

Some operational protocols on wildlife euthanasia proposed by the Competent Authorities and by the Regions will be examined and discussed taking into account the regulations on animal welfare, bioethics and medico-legal and ethical aspects in order applicable in different situations. Actions following euthanasia must also be considered, such as the management of the carcass for consumption purposes or any disposal procedures, also from an animal health perspective.

These recommendations will be compared with the "Guidelines for the recovery of native wildlife" drawn up and approved by the SIEF in 1994, in order to motivate the assembly to draft new guidelines updated based on the need to manage not only the injured native wildlife, but also to apply management indications on invasive alien animals, also that subjected to depopulation or other forms of control (coypu, wild boar, corvids, etc ...).

Abstract – Poster

Contributi liberi

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TICK BORNE ENCEPHALITIS IN A ROE DEER: CLINICAL, PATHOLOGICAL AND VIROLOGICAL FINDINGS FROM THE FIRST CASE REPORT

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ZAMPERIN G.¹, FOIANI G.¹, VASCELLARI M.¹, DRZEWNIOKOVA P.¹, CASTELLAN M.¹,
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Introduction

Tick-borne encephalitis virus (TBEV) is a positive sense, single stranded, ~11 kbp RNA virus with Palearctic distribution. It is transmitted from ticks to different mammal species, and vice-versa, through tick bite, and it is the causative agent of Tick-borne encephalitis (TBE), an important zoonosis with potentially lethal outcomes. In central and western Europe, TBEV is carried mostly by *Ixodes ricinus*, which acts as both vector and reservoir for the virus. Large mammals such as roe deer (*Capreolus capreolus* L.) play a significant role for tick populations survival and abundance, representing important hosts especially for the nymph and adult phases; instead, their role in TBEV circulation is considered marginal, as viraemia after natural infection is usually too low to infect other ticks. We report the first clinical case of a TBE infected roe deer, found in known TBEV endemic area of Belluno province.

Material and Methods

On June 2nd, 2021, a female yearling roe deer was found by the Provincial Police in Modolo (Belluno 46°07'58.0" N; 12°15'11.3" E; 390 m a.s.l.), showing several neurological symptoms, such as ataxia, staggering movements, muscle tremors, wide-base stance of the front limbs, repetitive movements of the head, persistent teeth grinding, hypersalivation and prolonged recumbency. It was then culled and, at necropsy, 17 engorged *I. ricinus* ticks were removed from the animal, while no macroscopical brain lesions were found. After ruling out other possible infections, the presence of TBEV was investigated in both the roe deer and the ticks, targeting the 3' noncoding region through real-time PCR amplification. Whole genome consensus sequences were compared to related sequences available in GenBank, and a maximum likelihood phylogenetic tree comprehensive of the main TBEV subtypes was produced. The loci coding for the envelope protein (E) and nonstructural protein 5(NS5) were also sequenced and inspected, for known neuroinvasiveness and virulence determinants. A sample of the midbrain was prepared and stained with hematoxylin and eosin (H&E) for histological examination through optical microscopy. Immunofluorescence analysis for active microglia were also performed, by microscopy and subsequent image processing on a midbrain sample, after staining of the marker Iba1.

Results and Discussion

Molecular analysis on roe deer brain sample yielded positive TBEV results, while all ticks turned out negative. No other pathogens were found in the brain. Histological analysis of the midbrain are supportive of a neurotropic infection, highlighting signs of a moderate, multifocal encephalitis (perivascular cuffs, neuropil infiltrates of lymphocytes and histiocytes mixed with fewer eosinophils and rare neutrophils) in both grey and white matter, as well as gliosis, neuronal chromatolysis and rare microglial nodules in grey matter. Active microglia was further confirmed by immunofluorescence analysis. No viral protein, nor extensive neuronal necrosis and neuronophagy, was found. TBEV genome sequence isolated from the roe deer brain (10897 nucleotides) is deposited in GenBank (accession number OM084948); the strain belongs to the European subtype, grouping with a sample collected in 2018 from a pool of ticks in mount Calisio, about 100 km afar. The E and NS loci did not carry known virulence-linked mutations, while the antigenic domains I and II of locus E, a region involved in the viral membrane fusion with the host cell, showed amino acid differences worthy of further investigations.

In a public health perspective, the case herein described, as well as those quoted in the literature, cannot be used as an alert. Actually, even in the case of the emergence of TBEV, clinical episodes in animals, if present, would be, unfortunately, preceded by far by cases in humans. Nevertheless, cases in animals should be monitored and framed in a consistently mutating ecopathological scenario.

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DETECTION OF VECTOR-BORNE BACTERIA IN TISSUE SAMPLES FROM EUROPEAN HEDGEHOGS IN THE PIEDMONT REGION (ITALY)

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Introduction

Wild animals can act as important reservoir of zoonotic pathogens and play a fundamental role in their amplification and transmission. In particular, small wild mammals are involved in the emergence and diffusion of vector-borne pathogens (VBPs), such as the ones transmitted by hematophagous arthropods. European hedgehogs (*Erinaceus europaeus* Linnaeus, 1758) are small nocturnal and insectivorous mammals widely distributed in western Europe. They naturally inhabit rural lowlands and hilly areas but in last decades they have easily adapted to suburban and urban habitats (such as gardens and parks), where they have reached nine times higher densities than rural areas and nowadays are considered a synanthropic species. Hedgehogs are commonly infested with different ectoparasites, especially hard ticks (mainly *Ixodes hexagonus* and *Ixodes ricinus*) and fleas (*Archaeopsylla erinacei*, *Ctenocephalides* spp.), which can be vector of zoonotic pathogens to humans, such as *Borrelia burgdorferi* sensu lato (s.l.) complex, *Anaplasma phagocitophilum* and *Rickettsia* spp. Due to their close contact with humans and domestic animals, hedgehogs could increase their risk of direct or indirect contact with these pathogens and related vectors. Therefore, it is fundamental to gain a better insight into the role of hedgehogs in the cycle of these pathogens and in their diffusion in human environment.

Material and Methods

All animals included in the study were rescued in Turin province between August 2021 and May 2022 by C.A.N.C. (Non-Conventional Animal Centre), a unit of the University teaching hospital in the Department of Veterinary Sciences of Turin University, which heals and rehabilitates injured or sick wildlife. In May 2022, ten hedgehogs, which were humanely euthanized or deceased during hospitalization at the Centre, were submitted to complete necropsy and their organs were collected and stored at -20°C. If present, ticks and fleas on animals were collected and stored, and identified at stereoscopic microscope using taxonomic keys. Spleen and ear skin samples were submitted to DNA extraction and PCR was performed for the identification of *Borrelia burgdorferi* s.l. (5S-23S rDNA intergenic spacer), *Anaplasma phagocitophilum* (*msp 2* gene) and *Rickettsia* spp. (*gltA* gene). PCRs positive sample amplicons were purified and sent to an external service for automatic sequencing. The obtained sequences were analysed in BioEdit and compared with reference sequences deposited in GenBank.

Results and Discussion

One skin sample out of ten was positive for *Borrelia burgdorferi* s.l., which was identified as *Borrelia afzelii* by sequence analysis. Another skin sample was positive for *Rickettsia* spp.; the *gltA* sequence was 99% similar to *Rickettsia asembonensis*. We did not obtain any positivity from spleen tissues. We collected ticks and/or fleas from five animals. Ticks were identified as *I. ricinus* (nymphs) and *I. hexagonus* (nymphs and females). The rickettsia positive hedgehog was parasitized by fleas.

Borrelia afzelii is one of the main species responsible for Lyme borreliosis, the most common tick-borne disease in Europe and Usa. It was previously identified in hedgehogs tissues and their ticks. Skuballa *et al.*, in 2012, demonstrated that hedgehogs are reservoir hosts for *B. afzelii*, contributing to the spread of this zoonotic pathogen in urban and sub-urban areas. *Rickettsia asembonensis* is *R. felis*-like organism, with unknown pathogenicity to humans. Few studies have isolated this bacterium in fleas (*Archaeopsylla erinacei* and *Ctenocephalides felis*) but it is not still clear whether hedgehogs act as reservoir. To the best of authors' knowledge, this is the first report of *R. asembonensis* from hedgehogs' tissues. We did not detect *A. phagocitophilum* in our samples; this bacterium has been previously reported in hedgehogs' ticks and tissues (including skin and spleen). Our negative result may be attributable to the limited sample size tested so far.

Our research is in progress. The high number of hedgehogs rescued at C.A.N.C. will enable us to increase the sample size and the variety of tissues analysed. In addition, also vectors collected from hedgehogs will be tested and more VBPs will be targeted. This will enable a better understanding of the potential role of hedgehogs in the epidemiology of vector-borne zoonotic agents in the study area.

GASTRIC PARASITES IN NEBRODI BLACK PIGS: HISTO-MORPHOLOGICAL AND ULTRASTRUCTURAL INVESTIGATIONS

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Introduction

Intensive pig farming has reduced the impact of most of the parasitosis worldwide, especially of those parasites with an indirect life cycle involving arthropods and beetles. However, in some areas of Italy, extensive rural farming is still traditional. Thus, some of the “extinct” parasitic diseases might emerge and re-emerge in these territories. An example is the Nebrodi Natural Park, the largest Sicilian rural nature reserve, located in the northwestern part of the island and comprising about 86,000 ha. The Natural park’s biodiversity is rich in several free-roaming domestic and wild species, which live in promiscuity and share shelters, pastures, and watering points. In this multi-host ecosystem, the Nebrodi black pig, an autochthonous pig bred, lives in wild, free-range and semi-free-range conditions. Due to this peculiar husbandry, this pig seems to play a pivotal role in the epidemiology of most of the infectious and parasitic diseases endemic in the area, some of which have been linked more to wild boars than to farmed pigs. The aim of this study is to describe the gastric parasites observed in Nebrodi black pig stomachs in order to characterize them and study their ecology in the Nebrodi park multi-host ecosystem.

Material and Methods

Selected stomachs of Nebrodi Black pig carcasses collected during the abattoir surveillance within the Regional Project “Misura 16.1”, aging between eight months to 4 years, were submitted to a macroscopical evaluation. Gross gastric lesions were recorded, and samples of the stomachs were 10% formalin fixed to perform histological investigations. All the observed parasites were collected, fixed in 70% ethanol and submitted to morphological identification by means of light microscopy (LM) and scanning electron-microscopy (SEM). Microscopic observation by LM was performed after clarification with a lactophenol solution. Concurrently, fixation in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, at 4 °C for 2 h and dehydration in ethanol was performed prior to SEM observation. The parasites were then dried in liquid CO₂ in a critical point apparatus (Critical Point Dryer, model K850, Quorum) and coated with gold in an ion coating sputter coater (Ion Coater, model n. SPT-20, COXEM). The observation was performed with an EM-30 – COXEM Scanning Electron microscope and photographs were taken with a digital acquisition system for analog SEMs.

Results and Discussion

Most of the parasitized stomachs showed moderate to severe catarrhal inflammation, chronic granulomas in the mucosa and submucosa and/or fibrinous-suppurative sometimes haemorrhagic gastritis with mucosal erosions, ulcers and hyperkeratosis. Hypertrophic gastropathy characterized by a diffuse thickening of the gastric wall was frequently observed too. *Ascarops strongylina* was the most frequent parasite detected, few stomachs showed *Phisyccephalus sexalatus* infestation or granulomatous lesions due to *Simmondsia paradoxa*. Few reports are present in the literature about the gastric parasites in the wild boar and even less in feral pigs or pigs bred in a free-range system. However, this is the first-ever description by SEM and LM of these nematodes in free-roaming Nebrodi Black pigs. The detection of wildlife-associated parasites confirms the role of the Nebrodi Black Pig as a link between wild animals and livestock, crucial in the epidemiology of many infectious diseases in multi-host ecosystem, such as Tuberculosis, Brucellosis, Aujeszky disease, etc. Moreover, based on the present study, these parasites are involved in moderate to severe inflammation of the gastric mucosa with consequent potential impairment of the normal processes of digestion and absorption. These lesions can severely impair the weight gain, which is critical for the Nebrodi Black Pig, a notoriously slow-growing breed. Further studies are needed to clarify these parasites’ role in determining the Nebrodi Black pig’s gastritis and to know their biological cycle in the Nebrodi park.

EUROPEAN BROWN HARE SYNDROME AND RABBIT HEMORRHAGIC DISEASE VIRUS 2: SEROLOGICAL AND VIROLOGICAL SURVEILLANCE IN THE PROVINCE OF TRENTO, ITALY

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Introduction

European Brown Hare Syndrome (EBHS) and Rabbit Haemorrhagic Disease (RHD) are two distinct diseases caused by two lagoviruses, antigenically and genetically similar each other (EBHSV and RHDV). EBHSV typically affects some hares species (*Lepus spp.*) whereas RHDV affects rabbits (*Oryctolagus cuniculus*). However, in 2010 a new serotype strain called RHDV2 was reported in rabbits for the first time in France and then the virus spread worldwide. Several other lagomorph species including brown hare showed to be susceptible to RHDV2 with EBHS-like symptoms and lesions. First report of a RHDV2 outbreak in PAT was in 2012, in wild rabbits living in peri-urban areas close to Trento. We describe the results of passive and active surveillance of EBHS and RHDV2 on brown hares in PAT from 2012 to 2022.

Material and Methods

During the ten-year period, carcasses and organs (liver, spleen) of dead hares were submitted to IZSVe diagnostic lab for necropsy and EBHS passive surveillance; active surveillance was also performed on organs (liver, spleen and heart) which were collected on hunted hares. For serological analyses sera were obtained from cardiac clots when it was possible; from 2016, local hunting association was asked to use blotting paper to collect sera from hunted hares; 754 samples of sera were collected and analysed by using a competitive ELISA test specific for EBHSV antibodies. Sandwich ELISA for EBHSV was performed on 171 liver/spleen including both samples collected during necropsy or submitted by hunters. From 2012 a biomolecular method to detect EBHSV (One-step RT-PCR) replaced the ELISA for virus detection and 561 samples were analysed by using this method. RHDV2 was also investigated in brown hare: during 2016 and 2017 hunting seasons, 226 sera obtained from hunted animals (blotting paper) were tested by competitive ELISA specific for RHDV2 antibodies. In 2016, 205 sera were collected from hares coming from all PAT reserves participating to the survey, while in 2017, 21 sera were taken only from hares living in the areas where RHDV2 was circulating among wild rabbits. Moreover, in these last reserves, to investigate lagoviruses different from EBHSV, a broad-spectrum RT-PCR detecting all lagoviruses was used to test the organs of hunted hares (n.31). Such approach, i.e. PCR tests to reveal both EBHSV and other lagoviruses have been routinely conducted on dead hares from 2018 and in total 50 samples have been analysed from 2016 up to now.

Results and Discussion

Out of 754 sera, 303 (40.2%) tested positive for EBHS antibodies; seroprevalence fluctuated over time: 35% (2012), 55% (2013), 49% (2014), 17% (2015), 45% (2016), 31% (2017), 50% (2018) and 77% (2020). In 2019 and 2021 no samples were collected. From 2012 to 2018 low seroprevalence and low antibody titres (1:10 - 1:40) suggests low viral circulation; on the contrary in 2020, titres were slightly higher ($\geq 1:80$) in 39% of positive samples (20/51): this finding together with the high seroprevalence (77%) could indicate a quite recent increase of the viral circulation in the reserves where these samples were collected. This observation is supported by the fact that some positive cases have been detected in the previous season (2018-2019). Eight out of 732 (1.1%) samples of organs tested (by either ELISA or RT-PCR), resulted positive for EBHSV: 3 (3/110) in 2012-2013 (active surveillance), 1 (1/83) in 2013-14 and 4 (4/4) in 2018-19 (passive surveillance). Virological and serological data confirmed the endemic presence of EBHS in PAT. Serological analysis for RHDV2 conducted in 2016 showed that 69% of sera (156/226) tested negative, 21% (47/226) resulted positive with titers between 1:10 and 1:40 and 10% (23/226) was found not conclusive ($< 1/10$). In 2017, in the reserves where circulation of RHDV2 in wild rabbits was reported, all hares tested negative for RHDV2 antibodies: in this last sampling, small sample size could have affected the reliability of the results but a role of hare as reservoir with not detectable antibodies could not be excluded too. Out of 50 samples tested for lagoviruses 49 resulted negative for RHDV2 whereas one (a dead hare with typical lesions) was found positive in 2022. To the light of these results (low titers and no virus detection) it would be interesting to investigate in the future the role of brown hare as spillover host for RHDV2, considering the respective densities of wild rabbits and hares, their relative distribution and the level of existing sympatry.

ACTIVITY OF A UNIVERSITY CLINIC (C.A.N.C.) IN THE RECOVERY OF WILDLIFE

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Introduction

The Centro Animali Non Convenzionali (C.A.N.C. – Non-Conventional Animal Centre) is a unit of the Veterinary University Hospital in the Department of Veterinary Sciences of Turin University. It was founded in 2010 as an exotic animal practice. C.A.N.C. has also an active partnership with Turin and Biella provinces in order to cure and rehabilitate wild animals found injured or distressed on field. The centre is managed by two university professors, five veterinarians and two technicians and relies on the consultation of a pathologist (for in-vivo histological exams and for necropsies) and a Infectious Disease researcher. Furthermore, it avails of the collaboration of several students from the degree courses in Veterinary Medicine and in Production and Management of Domestic and Wild Animals, who choose to broaden their knowledge on the medicine and rehabilitation of these animal species. Even if C.A.N.C. is a unit of the Veterinary University Hospital, it operates as a wildlife rescue centre (WRC) and is ruled by the same laws. At arrival, each animal undergoes a complete visit by the veterinarian, who establishes the proper therapy and hospitalizes the patient according to its species, age and health status. Data related to each patient are registered in a specific computer program, developed in the centre using Access®.

Material and Methods

Data related to animals admitted from 2010 to 2021 have been processed in Access®. In particular, data referring to the total number of animals recovered each year, their Class, species and age and the outcome of their hospitalization were analysed.

Results and Discussion

32720 wild animals were hospitalised at C.A.N.C. during the period 2010-2021. In the first year, 124 animals were admitted, but their number increased each year until 5071 animals in 2021. The annual increase of admissions ranged from 1.53% to 1139.51% (average 115.91%). “Birds” is the main animal Class recovered each year (average 74.37%, followed by “Mammals” (average 23.59%), “Reptiles” (average 2.00%), “Amphibians” (average 0.03%) and “Fish” (average 0.01%). Most of the animals were rescue and brought to the centre by citizens (95%) and only a minimum percentage was recovered by police members, veterinarians of the sanitary service, firefighters or volunteers. Regarding the outcome of hospitalized animals, 3.38% died before or during clinical examination, 28.41% were humanly euthanized because afflicted with a pathology that prevented the reintroduction in their habitat, 36.15% died during hospitalization despite the therapies and treatments supplied, 28.82% were released or transferred to other centres (on average, 3.24% were still recovered when the statistics were calculated each year). According to their age, 66.04% of animals admitted were in the first stages of life (neonates and juveniles) and not able to survive alone in nature, while 33.96% were adults. Among young animals, 25.78% were released, 49.58% died and 24.14% were euthanized (on average, 0.5% were still recovered when the statistics were calculated each year).

The consistent increase of animals admitted each year witnesses the increased notoriety of the centre, that has become a reference point in the rescue of wildlife in the region. This can be ascribed to the citizens’ sensitivity in wildlife health (they rescue the majority of animals) and to the divulgation activities undertaken by the centre.

It is noteworthy that more than half of admitted animals were juveniles, not able to survive by themselves. Most of them, however, were not admitted for sanitary reasons but because citizens are not able to differentiate whether an animal is in danger or is just growing, according to the characteristics of its species and age. According to the information given by the rescuer, indeed, most of young animals were collected because the citizens believed they lied in unsuitable conditions (e.g., they did not see the parents or were worried about predators’ presence). However, 43.80% of them did not display any disease during clinical examination. Despite that, the liberation rate of young animals is limited. This can be ascribable also to some unwilling mistakes that can be made by the rescuer. For example, he waits too much time between the moment of collection and the admission at the centre and/or feeds the animal with inappropriate food.

Since most wild species have a seasonal reproductive cycle, in order to concentrate the births in spring, the activities at the centre are heavily unbalanced because most of arrivals are concentrated in last spring and summer. The young animals admitted require a huge employment of time and resources, putting under a strong pressure the organization of the centre during that part of the year.

These evaluations highlight the importance and needing of a proper education of citizens, in order to teach them to understand when a wild patient actually needs help and this would optimize the activities of a WRC. Therefore, it is fundamental that the operators of WRCs dedicate their activity, besides to the cure of animals, also to the education of people about the basis of biology and ethology of wildlife. Wild animals, indeed, have their own needing and should not considered nor treated as pets.

EXTERNAL FIXATION TO CORRECT A BILATERAL TIBIOTARSAL FRACTURE IN A COMMON BUZZARD (*BUTEO BUTEO*)

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Introduction

Fracture is one of the most common causes of adult raptors admission to wildlife rehabilitation centers, due to crashing into obstacles, hit-by-car or gunshot.

Fractures usually involve long bones, including ulna, humerus, radius and tibiotarsus. Bone exposure, involvement of a joint and the time between the traumatic event and hospitalization are factors that could worsen the prognosis.

The main goals in fracture management are stabilization of the limb and length maintenance, after obtaining the fragments alignment. Compared to mammals, surgical treatment of fractures in birds can be more challenging; the bones of avian patients are less supported from the surrounding vessels and soft tissues and have more brittle cortices.

Choosing the best treatment option can be difficult; among the surgical therapeutic methods, external fixation has some advantages, including fracture reduction with minimal damage to the bone vessels and surrounding tissues, without the need to place an implant on the fracture site. Maintaining vascularization is an important factor for achieving good bone healing in birds. This work describes the application of an external fixation system on bilateral tarsometatarsal fractures in a common buzzard (*Buteo buteo*).

Material and Methods

An adult common buzzard was brought to the Centro Animali Non Convenzionali (CANC) of the Department of Veterinary Sciences of the University of Turin in June 2021, after being hit-by-car in Orbassano (TO). On physical examination, the animal weighed 635 g, was alert and responsive but non-weight-bearing; both hindlimbs presented swellings compatible, during palpation, with fractures of the middle third of the diaphysis of the left tarsometatarsus and the distal epiphysis of the right one. The bird was administered lukewarm fluids (Lactate Ringer, 25 ml/kg SC) and carprofen (5 mg/kg IM). Isoflurane, delivered in pure oxygen through a face mask, was used to induce and maintain anesthesia to perform radiographic imaging, showing a comminuted fracture of the left tarsometatarsus and a simple transverse fracture of the right one, both without bone exposure. An interim stabilization was achieved through splinting, using Vet-Lite® casting material. On recovery, the animal was weight-bearing on hindlimbs.

After the general conditions were stable, surgery was performed. Anesthesia was induced by intramuscular administration of a mixture of ketamine and medetomidine (respectively, 7.9 and 0.16 mg/kg) and maintained with isoflurane, delivered in pure oxygen through a 2.5 mm Cole endotracheal tube. Feathers of the tarsometatarsal region were plucked and sterile preparation and draping were made with the animal in dorsal recumbency. Four stab incision were made on the skin laterally and medially on both tarsometatarsi to allow insertion of Kirschner wires (K-wires). A type II external fixator, consisting of 2 distal (1.8 mm) and 2 proximal (1.2 mm) K-wires, was applied to the left tarsometatarsus, inserted in lateromedial direction; a modified type III external fixator, consisting of 4 K-wires (1.2 mm), was applied to the right tarsometatarsus, with 2 proximal wires inserted lateromedially and 2 distal wires inserted in a cross-like figure (one lateromedially, one craniocaudally). The wires perforated both musculo-tendinous and osseous plans. X-rays were performed to assess correct positioning of the wires. The K-wires were connected using polymeric acrylic material positioned on medial and lateral aspect of both limbs and, also, on the cranial aspect of the right one; then, the wires were shortened, and a light wrap was applied on the fixators to prevent trauma. Upon completion of the procedures, atipamezole (0.47 mg/kg IM) was administered. The buzzard was administered carprofen (5 mg/kg q24h IM) and marbofloxacin (10 mg/kg q24h IM) for the following 7 post-operative days.

After the recovery, the animal was weight-bearing on both hindlimbs. During the following days, calcium carbonate was supplemented in the bird's diet, and the animal was exposed to ultraviolet wavelength rays, both with UVB-bulbs and direct sunlight. Ten days after the procedures, the buzzard showed spontaneous feeding, using its claws to grasp food.

Sixty days after the surgery, anesthesia was induced with the same doses to perform radiographic imaging and to remove the external fixators.

A flight rehabilitation program and live-prey tests were made in a flight cage during the following days; 15 days after fixators removal, the bird was released back into nature.

Results and Discussion

Type II and III linear external fixators have the advantages of being light-weighting and applicable with minimal damage to the surrounding tissues. Another advantage is the relative ease of removal of the fixators without causing further damage to the patient, thus allowing a good recovery and a prompt release into nature. In this patient, a linear modified type III fixator was applied on the right tarsometatarsus to further stabilize the little distal fragment. The surgical treatment allowed an immediate weight-bearing on both limbs, without complications and with minimal post-operative therapeutics and management. External linear fixators should be therefore considered in birds with bilateral tarsometatarsal fractures.

CONTROLLED REPRODUCTION OF A PAIR OF PEREGRINE FALCONS IN AN URBAN ENVIRONMENT

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Introduction

This work is the result of an observation of a couple of peregrine falcons naturally established in the historical center of the city of Jesi (AN). The peregrine female underwent an intervention of care and rehabilitation in captivity and, after the accident and the following recovery, it was possible to prove the reproduction and the success to a normal wild life.

Material and methods

In the end of 2017 a couple of adult peregrines suddenly appeared in the historical center of Jesi. A webcam in the nest box was positioned for monitoring the couple. Five-year observations allowed to follow the behaviour of this species of raptor in urban habitat, especially the hunting and mating activities until the reproduction. During a hunting session in 2019, the female has been found in the center of the city unable to fly. Diagnostic insights highlighted a muscle trauma. The raptor has been hospitalized and, in the first stage, manipulated with falconry techniques using hood and geti without damaging the plumage and legs, then rehabilitated in a large cage in order to restore the muscle tone and finally released with success.

Result and discussion

This project highlights the importance of monitoring a species such as the peregrine falcon after an intervention of recovery and rehabilitation in captivity. In birds there are a few studies verifying the conditions after the rehabilitation in a wildlife center. The positive result of the reproduction represents a success for the future conservation of peregrine falcon populations. Observations recorded in different moments of life of the animals, from mating season to weaning of young falcons, are fundamental to create a solid basis for a deep knowledge of species of conservation concern, including those that live in close contact with human. In the recovery of wild species, an interdisciplinary approach with the collaboration between biologists and veterinarians is the basis of a good job. In raptors, management techniques used in falconry can open new options in the rehabilitation of individuals injured without stressing them and better preserving their plumage, irreplaceable for a quick recovery.

OVERVIEW OF THE DATA RECOVERED FROM 2017 TO 2021 ON THE MOST FREQUENT CAUSES OF DEATH IN BIRDS IN THE AREA OF FERRARA

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Introduction

Wildlife Recovery Centers (CRAS) play an important role for health and rehabilitation of distressed wildlife species, which are often victims of trauma caused by humans in accidental or voluntary interactions. The CRAS aim is to reintroduce these specimens in their natural habitat. Out of the 97 centers in Italy, the Ferrara CRAS has been operating since 1997, safeguarding more than one hundred species including mammals, birds, and reptiles. Almost 99 bird species are hosted in the CRAS in Ferrara, until today. Wild animals are considered good biological indicators and, therefore, are included in infectious disease surveillance plans. The role of the Experimental Zooprophyllactic Institute of Lombardy and Emilia Romagna (IZSLER) is to contribute to the monitoring of the causative factors of zoonosis, such as the Avian Flu virus and West Nile virus, as well as to provide support to CRAS by subjecting the deceased animals to autopsy to investigate the causes of death. Considering the high number of avian specimens, conferred from 2017 to 2021, and once it was established that the causes of death were not due to an infectious factor, investigated under the Regional Wildlife Surveillance Plan, the aim of our study was to evaluate the susceptibility and the risk factor, for the species with higher mortality. To do so, it has been performed a retrospective analysis of the data on dead birds in the CRAS of Ferrara, carried to IZSLER for pathological examination during these years. For this purpose, it is important to highlight the crucial role of CRAS, both providing a first intervention on the specimens and collecting valuable information on their conservation status. This allows to consider a potential vulnerability, that is fundamental to the One Health approach.

Material and Methods

Between 2017 and 2021 a total of 2564 bird specimens, all from Emilia-Romagna, belonging to 99 different species, were conferred to IZSLER. The most common were: *Streptopelia decaocto* (12,4%), *Turdus merula* (8,6%), *Falco tinnunculus* (8,1%), *Apus apus* (7,7%), *Pica pica* (7,0%), *Columba palumbus* (6,3%), *Athene noctua* (4,9%). The avifauna specimens death with in our study, initially recovered and transferred to the CRAS in Ferrara, clinically examined in the center's outpatient clinic, where they were given the necessary care. Finally, the deceased specimens were delivered to the IZSLER headquarters in Ferrara, where the pathological examination was carried out.

Results and Discussion

From the clinical examination and from the pathological one it was found that in almost all the cases, the causes of debilitation and death were unnatural, and this could be, directly or indirectly, related to human activities. Among these, the most frequent events are related to traumatic events of collision, predation by domestic animals, and cases of intentional poisoning. In particular, necropsy revealed bruises and often complex multiple fractures, caused by traumatic impact events in 28,5% of total cases; lacerated-contused bite wounds associated with predation in 24,9%; cases of multi-organ hemorrhages and the presence of rat poison in the plumage related to poisoning in 2%. Moreover, in collision cases, which are the most frequent, injuries were detectable only internally, while externally animals appeared in good condition. According to the number of animals recovered, cases of death had a periodic trend, probably because some species are migratory in summer and, consequently, are found mostly in the summer months. Furthermore, during the breeding months the number of birds increases. In birds of prey, including *A. noctua*, an increase of deaths could be due to accidents with cars because of their approach towards urbanized areas, due to the simultaneous movement of their prey. Despite these data do not include all of the avifauna deaths that occurred in the area of Ferrara, they are, however, indicative of a phenomenon that occurred from 2017 to 2021, and which may have a resonance in the conservation status of the species investigated.

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