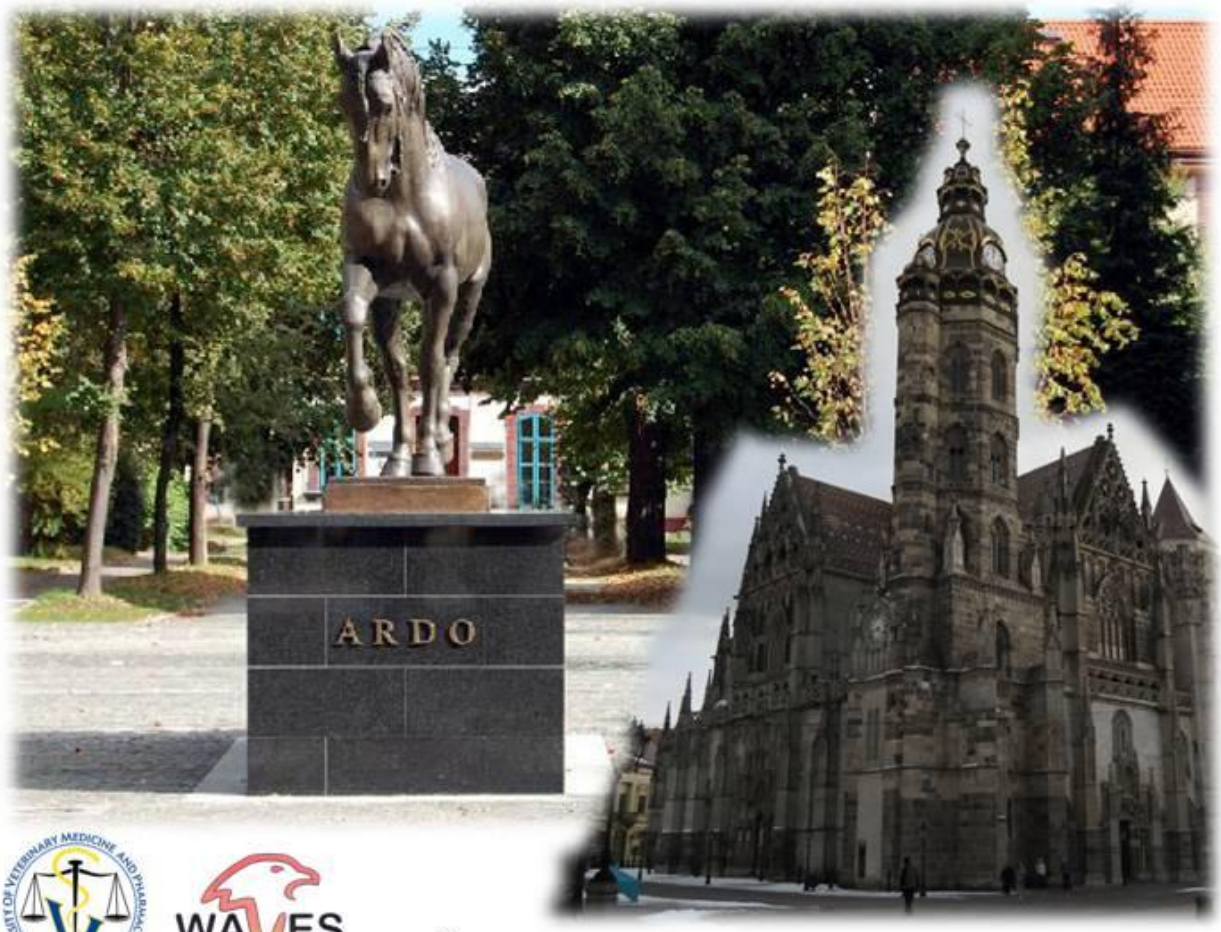
IX INTERNATIONAL SYMPOSIUM on WILD FAUNA

BOOK OF ABSTRACTS



KOŠICE (Slovakia) September 15 - 19, 2015

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ABOUT THE SYMPOSIUM

Dear Colleagues,

WAVES at the University of Veterinary Medicine and Pharmacy in Košice and the University of Veterinary Medicine and Pharmacy in Košice are pleased to welcome you in the IX International Symposium on Wild Fauna.

It has been only 10 years since Slovakia organized the 5th Symposium on Wild Fauna in Tatranská Lomnica, but during this period of time many species of animals became endangered.

That´s the reason why we need to pay even more attention to the protection of wildlife health. Climate change influence significantly the expansion and seasonal activity of vectors, the overpopulation of small rodents, and the survival of pathogens in the external environment, having thus direct negative effect to the health of animals. These abiotic factors are largely accompanied by biotic (anthropogenic) ones that are directly involved in the adverse bias on game and wildlife. Human pressure on the country caused by the intensification of agriculture, air pollutants from industrial activities, and the bloom of urbanization, transport, impassive interventions in the landscape and many other impacts cause rapid decline of several free living animal species.

Nature has ceased to provide conditions for the reproduction and survival of the game, decreasing thus its population.

These are the reasons why the role of all of us is to conduce to the improvement of the environment and thereby enhance the health of wildlife in order to preserve for future generations original gene pool game in superior landscape environment.

In this scenario, for main themes were selected for the Symposium:

1. Pathology and diseases control of wildlife animals
2. Breeding of Game animals
3. Ecology and veterinary aspect of endangered European fauna
4. Management and protected areas towards Europe 2020

I believe that our symposium will stimulate our foreknowledge in fruitful discussions and I hope that you will find new contacts for collaboration and friendship.

Prof. Valéria Letková, DVM., PhD.
President of WAVES at the University of Veterinary Medicine and Pharmacy in Košice

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THE LIMITS OF THE REGIONAL HUNTING CARD IN CONTRIBUTING TO THE CORRECT FAUNA-HUNTING MANAGEMENT: THE CASE OF THE LAZIO REGION (ITALY)

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ABSTRACT
The monitoring system of hunting currently adopted in Latium Region has proved largely inadequate for efficient wildlife planning the following reasons: it allows to verify aspects of qualitative / quantitative hunting only in retrospect; not conducive to suspension or extension of the harvest in real time; the implementation of the data is extremely laborious and for this reason carried out in a discontinuous manner; the results are not transparent and accessible in real time. It is proposed to implement a daily withdrawal data in an appropriate official web site.

INTRODUCTION
The law 11 February 1992, n. 157 "Regulations for the protection of homeothermal wildlife and for hunting", Art. 12, Paragraph 12, states: "As regards the exercise of hunting is [...] must hold a special card issued by the region of residence." The card should be properly filled out by hunters and returned to the province, which provide statistical analysis of the data. The results of the qualitative and quantitative analysis should form the base knowledge for the definition of hunting in the following season. The wildlife monitoring and analysis of badges should be the two key elements to program a sustainable harvest. In the province of Rieti screening of hunting badges is periodically performed by the DAPHNE University of Tuscia (Adriani et al., 2011). The dept. DAFNE have drawn the Provincial Hunting Plan (Amici et al., 2004, 2013) and performs the processing of the results from scrutiny of the badges. The results of these calculations should guide the drafting of the annual management plan. The potential effectiveness of this operation is severely limited by three main reasons:
1) incorrect or partial compilation by hunters (Adriani et al., 2008);
2) the territorial hunting district (ATC) does not take into account the results of the processing, that are limited to the calculation of the density of hunters and do not consider the withdrawal exercised (Adriani and Pettini, 2011; Adriani and Bonanni, 2010);
3) scarce reliability of information reported in the hunting badge. This paper aims only to deepen knowledge on this third point-

MATERIAL AND METHODS
The data processed in this study are drawn from regional hunting badges returned to the Provincial Administration of Rieti (Adriani et al., 2011). We have cheked if the ATC, when drawing the harvesting plan (Adriani and Pettini, 2011; Adriani and Bonanni, 2010) have considered data from hunting badges.

RESULTS
Hunting badges not provide the places of killing. The populations of hunting species characterized by low density and small home range may also be adversely affected by little withdrawals, such as: *Gruiformes* and *Charadriiformes* - Adriani et al., 2015 submitted. The analysis of badges at the end of the hunting season is not conducive to the monitoring of the harvest *in itinere*, but allows only the verification of the small amount of the harvest *ex post*. This procedure does not verify the possibility of exceeding the extent of the planned withdrawal. Surplus hunting bags of some species (submited

to planned withdrawal) are verified at the end of the hunting season. It should not be underestimated for those species undergoing decline (for example: *Alectoris graeca orlandoi*, hunted but not included in the hunting schedule of Lazio - Amici et al., 2007), which distribution area is unknown (i. e. *Lepus corsicanus* - Adriani et al., 2014) or in a not defined conservation status.

DISCUSSION

It is believed that the hunting badge currently used does not guarantee what is required by law, neither promotes a sustainable wildlife management, for the following reasons:

a) in the card is not included an indication of the planned sites of killing; The scale used is that of the ATC, far too wide to describe the spatial distribution of the harvest;
b) the paper badge does not allow the ongoing verification of the amount harvested. Even with the annual plans of settling fauna, you can not confirm the level of completion, if not final. Under such conditions, some species could suffer withdrawal unsustainable (both quantitatively as absolute density of withdrawal);
c) not all municipalities send badges to the province, and apply appropriate sanctions to defaulters;
d) the hunting badge that arrive in the province are processed intermittently over the years. This is because of the inherent excessive hard work that requires the implementation of the data on file. This problem is likely to worsen in response to the current dismantling of the provinces;
e) The ATC does not define the density of hunters in the areas of competence based on the size of the harvest denounced. Use badges simply to count those who have practiced as in the previous hunting season.

CONCLUSION

It is proposed to improve the system adopted: the paper badge, appropriately simplified, should remain for annotations and verification on the field. Reliefs should be entered into a daily official web site. This data should be accompanied by detailed instructions (ex. : name / coordinates of the harvest; species, sex, age, particularities of the slaughtered animal, etc.). This would allow the analysis of the dynamics involved. In cases where the right circumstances it would be possible to suspend and / or extend the practice of hunting. The summary of the data would be transparent and available for all institutions.

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For the contribution they have made to the realization of this survey, we thank: Prof. Andrea Amici, the Dept. DAFNE of the University of Tuscia, dott.sa Lorella Beccarini of the Province of Rieti.

QUALITATIVE AND QUANTITATIVE ANALYSIS OF GRUIFORMES AND CHARADRIIFORMES HUNTING IN THE PROVINCE OF RIETI (ITALY)

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ABSTRACT
This work presents the data analysis of the hunting activity in the province of Rieti as recorded by the hunters in their regional hunting badges. This study aims to: verify and assess the extent of the hunting of the Gruiformes and Charadriiformes species at the provincial level; evaluate the real utility of the badges records for the wildlife monitoring and the annual hunting planning.
Analysing the badges of the hunting seasons 2009 – 2010, 2010 – 2011, 2011 – 2012 and 2012 – 2013 we note that the killing of *Fulica atra*, *Gallinula chloropus*, *Rallus aquaticus, Gallinago gallinago*, *Lymnocryptes minimus* and *Vanellus vanellus* is totally negligible.
In view of the widespread tendency to not report all the animals killed, the numbers arising from the consideration of the regional hunting badges must be considered as the certain minimum bound on the hunting of a given species. As a consequence, the official database used by the public administration to plan the hunting seasons must be considered poorly reliable.

INTRODUCTION

The Italian law Febrary 11, 1992, n. 157 "Rules for the protection of omeotherm wildlife and for hunting", Art. 12, Paragraph 12, states: "As regards the exercise of hunting is [...] must hold a special card issued by the region of residence". Regional badges must be properly completed and returned at the end of the hunting season. The analysis of the data contained in the identification cards should constitute one of the elements of knowledge base for the programming of a sustainable harvest. Other fundamental information should come from the monitoring of the wild species of interest. Management activities should provide and maintain constantly updated species distribution, abundance, density and population dynamics of the target species. On this basis, and known the annual increase of each species it is possible to plan a conservative harvesting. It is known that in the province of Rieti hunting of *Gruiformes* and *Charadriiformes* involves a small part of the hunters. Nevertheless, the distribution and density of hunting may adversely affect the status of the populations locally. For these reasons it was considered important to check the trend of harvest from hunting badges issued in the hunting seasons 2009-2013. This is the first time that such a survey is conducted in the province of Rieti.

MATERIAL AND METHODS

The data processed in this study are drawn from regional hunting badges returned to the Provincial Administration of Rieti (Adriani et al., 2011a). The systematic collection of data was performed by DAFNE of the University of Tuscia in Viterbo. Processed data relate to hunting seasons 2009 – 2010, 2010 – 2011, 2011 – 2012, 2012 – 2013, and represent only a small part of hunting badges. In this paper were taken into account only those referring to 6 hunting species of *Gruiformes* (*Fulica atra, Gallinula chloropus, Rallus aquaticus*) and *Charadriiformes* (*Gallinago gallinago, Lymnocryptes minimus, Vanellus vanellus*).

RESULTS

Gruiformes

Common Coot (*Fulica atra*) - 13 heads: monthly average 0.65 (D.S.=±1.42); year average 3,25 (D.S.=±5.19).

Common Moorhen (*Gallinula chloropus*) - 33 heads: monthly average 1.65 (D.S.=±1,50); year average 8.25 (D.S.=±3,50).

Water Rail (*Rallus aquaticus*) - 42 heads: monthly average 2.10 (D.S.=±6.21); year average 10.50 (D.S.=±12.40).

Charadriiformes

Snipe (*Gallinago gallinago*) - 169 heads: monthly average 8.45 (D.S.=±8.31); year average 42.25 (D.S.=±26.25).

Jack Snipe (*Lymnocryptes minimus*) - 4 heads: monthly average 0.20 (D.S.=±0.62); year average 1.00 (D.S.=±1.15).

Northern Lapwing (*Vanellus vanellus*) - 20 heads: monthly average 1.00 (D.S.=±1.84); year average 5.00 (D.S.=±3.74).

Analysis of the data indicates that in the province of Rieti and the hunting of *Gruiformes* and *Charadriiformes* is not relevant.

DISCUSSION

It is well known that at national level the hunters do not to register all the harvested heads od wildlife. This phenomenon has been thoroughly studied in the province of Rieti for some hunting species i.e. ungulates (Adriani et al., 2008). Obviously, this behaviour is also recorded for hunting birds. Therefore the quantitative aspects of hunting of *Gruiformes* and *Charadriiformes* resulting from the hunting badges (Del Mese, 2012; Imperatori, 2013) is believed to be underestimated. The results should be interpreted as a certain minimum amount of the harvest. Therefore, concerning some precept of the Lazio Regional Law 17/95, it can be stated as follows:

1. Art. 20, Paragraph 6: the collection of data in regional badges requires considerable effort. The results produced take on a statistical value only if you know the average rate of registration of the heads harvested (Adriani et al., 2008).

2. Art. 27, Paragraph 1: the Territorial Hunting District determine the number of hunters permitted in their territories. This calculation is performed taking into account the density of hunters, based on the number of identification cards issued in the previous year. The focus should instead be planning for a sustainable harvest.

The problem of poor reliability of the official database also extends to other areas of wildlife management. In the province of Rieti we have studied the causes of mortality of some species not hunted. The results do not agree with the official data (Adriani et al., 2011b).

CONCLUSION

This study reveals two factors that are particularly important for proper planning of hunting: the qualitative and quantitative aspects of the annual planned harvesting of wildlife are based on statistical data not always reliable; hunters who do not register all the heads or not give back to provincial administrations the regional badges do not incur in appropriate penalties. None of this promotes ecologically sustainable wildlife hunting planning.

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For the contribution they have made to the realization of this survey, we thank: Prof. Andrea Amici, the Dept. DAFNE of the University of Tuscia, dott.sa Lorella Beccarini, Dr. Stefania Adriani and Silvano Colonna of the Province of Rieti.

ARTROPOD FAUNA ON VIVARA ISLAND, NAPLES, ITALY

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ABSTRACT
Vivara is a small island (33 ha) of the Gulf of Naples, included on Natura 2000 sites (SPA and SCI). Since 2002 has been transformed in a State Natural Reserve. Its megafauna, composed of small mammals, birds and reptiles, is mostly insectivorous. Information on Vivara Arthropods biodiversity is incomplete. In 2014 a census was carried out with the aim to establish a basis for future researches The results demonstrate the presence of 430 different species, 95% of which represented by insects (50% *Coleoptera* and *Lepidoptera*). Numerous new species were recorded while others, previously present, were no longer found.

INTRODUCTION
The first work on insect fauna of the island of Vivara dates back to about 26 years ago, when D'Antonio e Fimiani (1988) published a description of 229 species, which included 43 species of Lepidoptera belonging to 13 families. In the following years, the interest has focused mainly on butterflies. Since then a new list of *Lepidoptera* was published, with 85 species, 13 of which new (D'Antonio and Zeccolella, 2013). Although the efforts made with these studies, the information available on Vivara Arthropoda biodiversity is likely to be far from complete. In 2014 a census of Vivara Arthropod fauna was carried out with the aim to establish a basis for future researches on population dynamics and roles played in the island food webs. Its megafauna, composed mainly of small mammals (mice, rabbits, bats), birds (both migratory and resident) and reptiles (lizards, geckos and western whip snake), is mostly insectivorous relying on the rich Arthropod fauna here existent.

METHODS
Different approaches were used in order to sample the highest possible number of species. The daytime collection was done on the spot, with a net, or the entomological umbrella or by checking the plant litter on a white dish. For the night hunting (from sunset to midnight), a vertical white sheet was used, illuminated by two camping lamps (a 11W neon tube hanging from the top and 3x1W LEDS lamp resting on the floor in front of the sheet). For nocturnal moths, appropriate baits (foam rubber imbued with red wine, sugar and fruit peels) were used, hanging from tree branches along a circular path.
Specimens for which it was possible to make a reliable identification in situ were released. Others were collected and prepared dry (*Lepidoptera*) or preserved in ethanol at 80% (other groups); those immature, if possible, were reared up to adult to ensure correct identification.
For the determination of some *Lepidoptera* it was necessary to analyse the wings or the sexual organs. The wings, treated with a drop of ethyl acetate, were observed with a binocular microscope. For the study of the sexual organs, the abdomen was soaked in 10% KOH for a time dependent on its size, rinsed and dissected in distilled water. For females of some specimens it was necessary to colour the internal genitalia with Chlorazol black. After colouring, all the pieces were transferred into 70% ethanol through the series of alcohols (30% and 50%) and observed, if necessary, with a microscope.

RESULTS

As shown in Figure 1, (left panel) 95% of the species of Arthropods observed are *Insecta*. Within this group, the more represented order is that of *Lepidoptera* (right panel). Maximum of individuals abundance is registered in April-May, in coincidence with the period of maximum flowering on the island; 20 different families were recorded (*Micropterigidae*, *Adelidae*, *Eriocottidae, Tineidae, Psychidae, Gracillariidae* family, *Plutellidae, Gelechiidae, Blastobasidae, Lecithoceridae, Choreutidae, Tortricidae, Pyralidae, Crambidae, Pterophoridae, Geometridae, Noctuidae, Nymphalidae, Drepanidae, Erebidae*).

Interesting the fact that 27 new species were recodered and, also, that some species previously described were not found; in particular, it is noteworthy the absence of *Zygaena filipendulae* (L 1758), a butterfly reported to be common in the past (D'Antonio and Fimiani, 1988).

The second most abundant group of arthropods found in Vivara is that of *Coleoptera*, with 88 species; of these, 67 were found in present census, with 13 new. *Diptera* and *Arachnida* are scarcely represented.

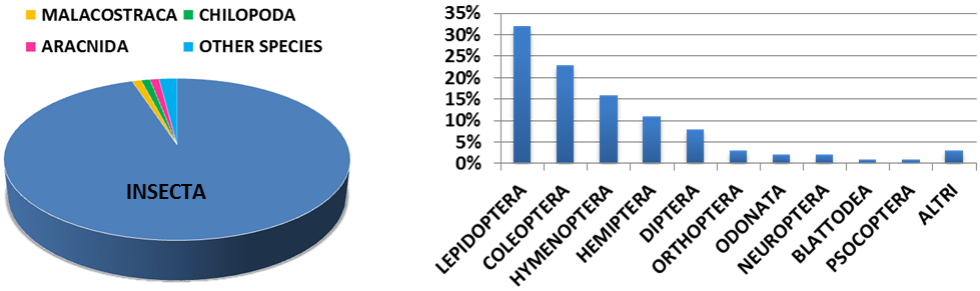

Figure 1. Composition of Arthropod fauna on Vivara Island. Left: distribution of species among the main classes of *Arthopoda*. Right: percent distribution of the major order of insects. Island.

DISCUSSION AND CONCLUSIONS

The absence of several previously reported Arthropod species is probably due to a problem of sampling rather than to a true loss of species. The identification of many species never seen before reinforces this point, suggesting that the Arthropod fauna on Vivara is not yet sufficiently explored and that the diversity of this group of animals is definitely underestimated. A further confirm comes from the scarcity of data on *Arachnida* and *Diptera*, groups usually very well represented. More targeted studies therefore must to be carried out.

However, the lack of *filipendulae*, a very common striking butterfly species, unlike to pass unnoticed, *Zygaena* may represent a warning: its larval stage feeds on herbaceous *Fabaceae* (D'antonio and Fimiani, (1988; Chinery, 1990) and these might have been victim of an excessive grazing by rabbits.

In conclusion, although the 2014 census on Arthropod fauna of Vivara has clearly enriched the knowledge on the biodiversity of this group of small animals, it has also evidenced that it is necessary to implement future efforts. In particular, more specialized and systematic actions should be carried out, taking into account the phenology of the various Families. A more complete picture of the Arthropod fauna on Vivara would allow estimates of its evolving trends in diversity and population dynamics with much advantage to the Reserve management.

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We thank the State Natural Reserve of Vivara Island, Procida who gave us the opportunity of carrying out this study.

CYTOGENETIC PROFILE OF A SPECIMEN OF FEMALE LION (*PANTHERALEO; 2N=38; XX*) OF THE ZOOLOGICAL GARDEN OF NAPLES

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ABSTRACT
African lion (Panthera leo; 2n=38) belongs to the family Felidae and his karyotype is similar to other felids (Tian et al., 2004). There are few studies about cytogenetic profile of lions and in particular there are no study about Chromosome Aberrations in this specie. Analyzing a specimen of 15 years old of the zoological garden of Naples we found an aneuploidy percentage of 23% and mean value of chromatid and chromosome breaks of respectively 0.10 ± 0.36, 0.04 ± 0.20. This results seem conflicting since they indicate an high aneuploidy with a very low level of chromosome damages but they could be explained by the age of the animal.

INTRODUCTION
African lion (Panthera leo; 2n=38) belongs to the family Felidae and his karyotype is similar to other felids (Tian et al., 2004)and consist of 38 chromosome subdivided in six groups: A, B, C, D, E, F.Pairs A1-A3, B1-B4, D1-D4 and sexual chromosome are submetacentric, pairs C1-C2 and E1-E3 are metacentric finally F1-F2 are acrocentric. There are very few reports about cytogenetic profile of this specie and in particular about Chromosome Aberrations thus aim of the work was to evaluate the cytogenetic profile of a lioness 15 years old of the Zoological garden of Naples by using RB-banding and Chromosome Aberration (CA) test.

MATHERIAL AND METHODS
Two cultures (with and without Aphidicolin), treated for RBA banding, of peripheral blood lymphocytes from a lioness of the Zoological garden of were set up in RPMI 1640 medium enriched with FCS (10%), L-Glutamine (1%) and Lectin (1.5%) and incubated at 37.5 °C for 72 h. BrdU (15 µg/ml) was added to cultures for RB-banding 4.5 h before the harvesting and Colcemid was added 1 h before harvesting. Hypotonic treatment (KCl 0.5%) and three fixations in Methanol-Acetic Acid (3:1), the final one overnight, followed. Three drops of cell suspension were dropped on cleaned and wet slides which were stained ten days later. Slides were observed about 24 h after staining, or later (1 week) under a fluorescence microscope, 100, 50 and 20 metaphases for aneuploidy percentage, CA test and RB-banding respectively were captured with a digital camera Nikon DS U1, transferred onto a PC and later processed by image analysis software.

RESULTS
Karyotype analysis was performed according to the karyotype proposed by Wurster-Hill and Gray (1973). The animal showed a chromosome profile 2n = 38, XX and no numerical or structural chromosome aberration have been observed.Aneuploidy percentage was 23% while mean value of chromatid and chromosome breaks were respectively 0.10± 0.36, 0.04 ± 0.20, fragments were absent as well as other kind of chromosome abnormalities (dicentric, centric-ring chromosomes, etc...).

DISCUSSION
The lioness showed a normal karyotype when compared with that proposed by Wurster-Hill and Gray (1973). As regard the evaluation of chromosome stability there was a higher aneuploidy percentage (23%) than that of specie like buffalo, dog, horse, cattle, wild boar and swine of similar age in which it ranges from 5% to 11%. Conversely chromatid and chromosome breaks are very low and there are

no numerical or structural chromosome abnormalities thus excluding the exposure to mutagens or genotoxic substances. Probably this condition is typical of this specie but to confirm this hypothesis it is necessary to analyze a greater number of animals.

CONCLUSION

To the authors knowledge this is the first report about the evaluation of aneuploidy percentage and CA test in lion. It is very interesting the high aneuploidy percentage value found in this animal in opposition to a very low number of chromatid and chromosome breaks. If these particular condition is confirmed for this specie it would be very interesting to evaluate the causes, infact aneuploids are produced by nondisjunction or some other type of chromosome misdivision. Instead, the high chromosome stability assessed with CA test make the lion a specie suitable as monitors for genotoxic events and toxicogenetics studies confirming the finding of Zamora-Perez et al., (2008) and Zúñiga-González et al., (2000) that found an high rate of spontaneous micronucleated erythrocytes (MNE) in peripheral blood.

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MAGNETIC RESONANCE AND COMPUTER TOMOGRAPHY OF THE BRAIN OF COMMON DOLPHIN (*DELPHINUS DELPHIS*) AND STRIPED DOLPHIN (*STENELLA COERULEOALBA*)

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INTRODUCTION

Magnetic resonance imaging (MRI) and Computed Tomography (CT) are increasingly used for the neuro anatomical descriptions of dolphin brain. Most existing descriptions in literature have used magnetic resonance images obtained ofbrains taken from dead animals.
In this poster presentation, we describe the bi-dimensional anatomy of the brain located "*in situ*", in its physiological position, of common dolphin (*Delphinus delphis*) and striped dolphin (*Stenella coeruleoalba*). For this purpose we have used anatomical slides as well as MRI and CT images.

MATERIAL AND METHODS

Post-mortem MRI and CT were carried out from the cephalic region of ten common dolphins (*Delphinus delphis*) and five striped dolphins (*Stenella coeruleoalba*). Magnetic resonance equipment was Signa Profile 3T (GE Health Care) and computed tomography equipment was a TomoScan M (Philips).
Afterwards, specimens were thaw and sagittal, coronal and axial anatomical sections were carried out. Within each section, all the anatomical structures of the brain that were possible, were identified and were correlated with the corresponding MR and CT images.

RESULTS AND DISCUSSION

MRI allowed the identification of most of the structures identified in the anatomical sections. In contrast, CT images provided a good definition of head bones, allowing three-dimensional reconstructions of the skull, but did not reach enough definition to allow the identification of brain structures.
According to this, MRI is a very useful technique for the pathologic evaluation of the brain in dolphins with the clear advantage of being applicable in live animals. Also, when used in dead animals, MRI would avoidthe need to open the skull, simplifying necropsy.
The comparative study including the three imaging techniques, anatomical sections, MRI and CT, constitute a reference guidefor interpreting topographic anatomy in other imaging studies carried out in these marine species.

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ANIMAL BIODIVERSITY CONSERVATION: THE CASE OF RED KIT (*MILVUS MILVUS*) IN LATIUM REGION (ITALY)

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ABSTRACT
The Italian forestry policies of last 20 years induced the presence of stored oak coppice due to stop to wood management (the rotation is usually 16 – 20 years) in many areas of Latium Region. As a consequence a modification of wood cover induced modifications of habitat suitability for many wild species. With the aim to comply with the European action plan for red kit (*Milvus milvus*) an appropriate habitat suitability model for nesting was computed in order to verify the compliance of silvicultural activities to the action plan. The model allows to identify in the surrounding of the cut, the availability of other area suitable for nesting of the species.

INTRODUCTION
The management of natural resources (as forest), should be based on a deep knowledge of the functionality of forest ecosystems and parameters of wild populations (distribution, abundance, density and structure). This is of great importance in Nature 2000 network areas where biodiversity conservation is the main aim. In this context, are very frequent and sudden the changes of habitat, that prelude in a modification of the richness of fauna leading to a simplification and therefore to loss of biodiversity. The complexity of the interactions between wildlife and natural resources has been investigated with a focus on wildlife population dynamics to identify the key mechanisms that influence the functioning of an ecosystem (Bieber and Ruf 2005).The forestry policies of last 20 years induced the presence of old oak coppice due to lengthening of cutting interval (usually 16-20 years). This scenario favoured some species that benefit of unmanaged forest area. The aim of this study was to produce a model able to predict the nesting suitability for red kite (*Milvus milvus*) in order to guarantee the conservation of the species Knott et al. (2009) and to comply with the action plan for the species. Red kite is classified as Near Threatened in the IUCN Red List Category (ver 3.1 - BirdLife International 2013). The suitable habitat is represented by broadleaf woods, mixed with farmland, pasture and heathland. For nesting a mature tree is chosen at an altitude between 300-1000 m a. s. l., preferably 500 – 600 m a. s. l. (Sergio et al., 2003). It feeds on a wide range of foods, but prefer carrion and small to medium-sized mammals and birds, sometime reptiles, amphibians and invertebrates. Migratory, is present on Tolfa Mountains wintering with a flock of 100 heads, but was also signalled as nesting.

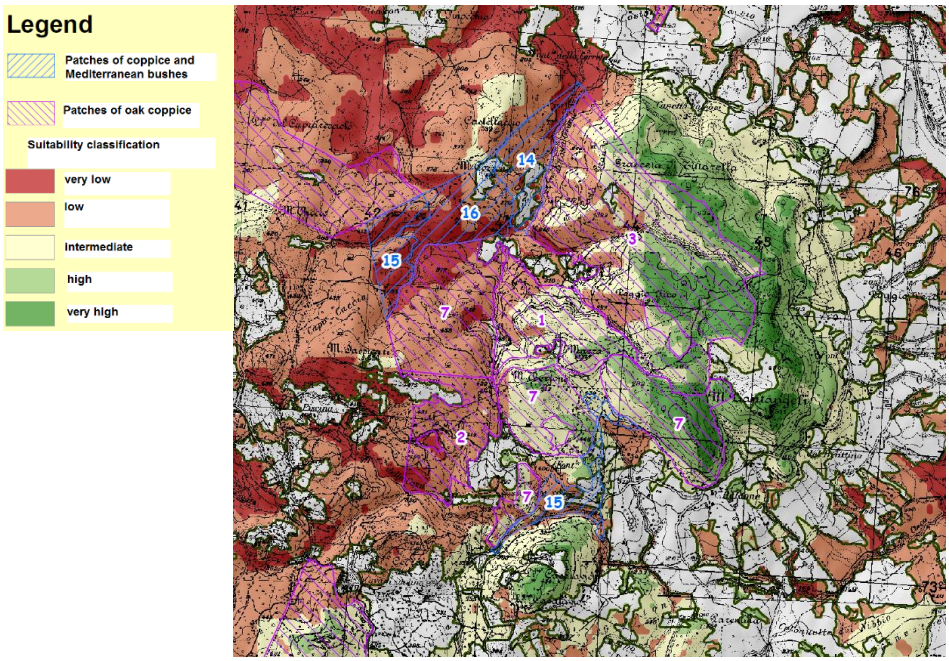
MATERIAL AND METHODS
The data processed in this study have been obtained by Land Use Map of Latium Region (CUS, 2010), additional layers are the Land Digital Model (minimal size of cell 40x40 m) processed in GIS. These data were used to produce a deterministic model (Amici et al., 2005) based on the principle of alternative available. The specific ecological requirements of red kite were considered for the construction of a Model in order to verify the existence of areas suitable for nesting in a spatial range which allows the choice of alternative sites (Minganti and Panella, 2012). In particular, they considered the wooded territories which were divided into five classes of suitability for nesting of the species, taking into account four factors: a) The species benefits from landscape diversity made up of forests, crops and pasture meadows (Knott et al., 2009); b) The presence of the red kite shows to be positively correlated to the existence of feeds reservoirs within 1 km (Sergio et al., 2003); c) The nesting occurs on mature trees, and the largest is usually selected, preferably at an altitude between

500 – 600 m a. s. l., with the upper limit 800 – 1000 m a. s. l.; d) favourite aspect for nesting site is NE-NO (Pandolfi, 2006). In addition, to compute fragmentation of the landscape the index of Shannon was included, classifying the land cover in three categories; agricultural systems, pasture-meadows, woods and shrubs, for a radius of 5 km. As regard altitude the maximum value was assigned to the class 500 – 600 m with decreasing values both upstream (up to 900 m above sea level) and downstream to 0 m above sea level. Aspect was given maximum score to NO, N, NE, and decreasing values going south. For the distance from water bodies it was assigned maximum value to a distance value up to 1 km and decreasing values with increasing distance.

RESULTS

The product of the various coefficients has allowed the realization of the layer of the suitability in a raster format, with values from 0 to 1 and the resulting map with reclassification into 5 classes of equal amplitude (Figure 1).

Figure 1 Detail of the map of the suitability of the site for nesting of the red kite (*Milvus milvus*) in the area of Tolfa, Latium Region, Italy



DISCUSSION

The Authors suggest that the availability of areas suitable for nesting, in an area surrounding the silvicultural intervention of 2 Km of radius around the potential nesting area, located within the surface to be cut, are necessary to ensure a sufficient level of protection of the species.
A map on the regional scale should be produced in order to preconize the future expansion of the species.

CONCLUSIONS

The map is consistent with the action plan for the red kite (Knott et al., 2009) in particular with expected result "2.1: Habitats supporting high densities of red kites are managed to ensure that conditions are maintained and, where possible, enhanced; a) Compile a catalogue of good agri-

environment schemes that are beneficial to raptors and grasslands; c) Promote crop mosaics by keeping field size below 100 ha".

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CONCENTRATIONS OF HEAVY METALS IN RED FOX (*VULPES VULPES*) FROM RURAL AREAS IN CAMPANIA (ITALY): PRELIMINARY RESULTS

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INTRODUCTION

Essential and non-essential elements occur naturally in the environment but various anthropogenic activities are the major causes of increase in environmental concentrations of heavy metals. Some elements are essential for biological functions, but heavy metals like cadmium (Cd) and lead (Pb) can create adverse effects on environmental and human health due to their potential toxicity and bioaccumulation in food chain. The impact of heavy metals on the environment can be a serious threat for ecosystem stability. The use of sentinel species can provide data to monitor the environmental quality and the contamination of their biological habitat. Some carnivores are at the top of the food chain and, therefore, could be very sensitive to any ongoing bioaccumulation process.

The red fox (*Vulpes vulpes*) is a highly opportunistic species, which adapt to a variety of environmental conditions and, in urbanized areas, it occupies small territories of 0.5 km^2 or less. For these reasons, the red fox could be a suitable bio-indicator for monitoring the presence of anthropogenic pollutants like heavy metals in cities, suburbs or rural areas, where it is exposed, at least in part, to the same contaminants as human beings.

The aim of the present study was to investigate the presence of Cd and Pb in liver and kidney of red foxes (*Vulpes vulpes*) from different rural areas in Campania (Italy).

MATERIAL AND METHODS

A total of 20 red foxes were collected during 2014 and 2015 from different rural areas in Campania (Italy). All specimens were hunted by quick-kill techniques (i. e. shooting), with the exception of four red foxes, which were killed during road accidents. Age was estimated by worn teeth. All animals sampled were adults. After the necropsy, samples of liver and kidney were collected from each fox, sealed in polyethylene bags, frozen at -20°C and kept at the same temperature until further analyses. Each tissue was homogenized by mixer and subsequently was digested in ultrapure 65% HNO_3 and H_2O_2 in a microwave digestion system. Concentrations of heavy metals were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) technique using a Perkin Elmer Optima 2100 DV instrument coupled with a CETAC U5000AT. The calibration curve and two blanks were run during each set of analyses to check the purity of the chemicals. Reference material (CRM DORM-4, NRC, Canada) were also included for quality control. All the values of the reference materials were within certified limits. Instrumental detection limits, expressed as wet weight (w. w.) and determined following the protocol described by Perkin Elmer ICP application study number 57 (Barnard et al.,1993), were 0.1 ng/mL for Pb and 1.8 ng/mL for Cd, while recovery values were: Pb 96%; Cd 99.

RESULTS

The results obtained showed the presence of Cd and Pb in all liver and kidney samples analyzed. Mean values of the Cd concentrations were of 0.059 mg/kg in kidney and 0.047 mg/kg in liver. The Pb mean concentration in the kidney and liver amounted to 0.061 mg/kg and 0.050 mg/kg, respectively.

Liver Pb concentrations obtained in the present study were similar to levels reported in rural red fox from Croatia (Bilandžić et al., 2010) and lower than those found in rural red fox from other European countries (Dip. et al 2001; Millan et al., 2008), and also lower then levels reported in other regions of Italy (Corsolini et al., 1999; Alleva et al., 2006). In kidney tissue, Pb levels were lower than those reported in rural red fox from Croatia (Bilandžić et al., 2010) and Switzerland (Dip et al., 2001).
In the present study mean Cd liver and kidney levels observed in rural areas are approximately comparable with those found in red fox from rural areas in Croatia (Bilandžić et al., 2010) and lower than those reported in red fox from Switzerland (Dip et al., 2001). Liver Cd concentrations observed in rural red fox in this survey were also lower than those found in Andalusia, Spain (Millan et al., 2008) and other regions of Italy (Corsolini et al., 1999; Alleva et al., 2006).

CONCLUSION

The preliminary results obtained in the current study showed the presence of Cd and Pb in all samples analyzed, underlying their presence in the environment. However the levels of Pb and Cd both in liver and kidney of red foxes analyzed, give evidence of low environmental pollution in rural areas in Campania Region (Italy).
Monitoring studies on heavy metals and other pollutants in a greater number of red foxes not only from rural areas but also from suburban areas with high anthropogenic activities will provide more detailed information on the role of this species as an indicator of environmental contamination and a clearer scenario of the distribution of inorganic and/or organic contaminants in their habitat.

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DYSMORPHOLOGY IN WILDLIFE AND THE ROLE OF REHABILITATION CENTERS

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ABSTRACT
Dysmorphism is the generic name for abnormalities of form and structure of an animal's body. In this work we describe 7 selected cases related to dysmorphological diseases as the results of 255 carcasses examined in diagnostic activities on alpine ungulates. Animals were found dead or recovered in wildlife rescue center (CRAS) of the Provincial Administration of Sondrio located in the central Italian Alps.

INTRODUCTION
Dysmorphism is the generic word for abnormalities of the form and structure of animal body, being used especially for congenital abnormalities, thus constituting an inborn error of development that it is not only synonymous with either "genetic" or "hereditary"(1). Therefore, there are congenital defects that have a genetic cause (the majority) while others have environmental cause (3). It is important to remember that the adjective "congenital" means born with the individual, regardless of the cause. The frequency of major dysmorphisms in humans is estimated in 3% of live births (4). In domestic animals, these numbers must be similar (4.7%), in wildlife is unknown.
The relief of dysmorphological diseases, which are rarely adaptive due to their nature, is especially complex in free-living animals. Wildlife rescue centers represent determining factor in collecting case studies. They provide an essential service for the environmental surveillance creating the basis for a network of passive monitoring of wild populations health. In particular, they provide important elements on demography and allow passive monitoring about infectious malformation and dysmorphogenic diseases. The Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna (IZSLER) collaborate with the private and public rehabilitation facilities in order to provide a diagnostic service and advice. In this work we report 7 selected cases of dysmorphism as the results of 255 cases examined in diagnostic activities on alpine ungulates in the last two and a half years.

MATERIAL AND METHODS
The populations of wild ungulates (estimated 19 000 heads) that live in the territory covered by this study (province of Sondrio) has undergone a certain natural expansion of indigenous individuals with small interventions of reintroduction and / or translocation of alpine chamois (estimated 9 000 heads) and roe deer (estimated 3 500 heads) made in the 80s. In the case of the alpine ibex (estimated 1 500 heads) the presence of the species is entirely attributable to a reintroduction project in the Italian Alps of individuals from the Gran Paradiso National Park.
Individual red deer (*Cervus elaphus N =56)*, roe deer (*Capreolus capreolus N=155*), alpine chamois (*Rupicapra rupicapra N=41*) and alpine ibex (*Capra ibex N=3*) founded dead (108 cases) from unknown causes or recovered alive and then died at the Sondrio CRAS (147 cases) were delivered to the IZSLER Diagnostic Section of Sondrio. They were examined to define the etiology by pathological, histological, bacteriological, parasitological and virological analysis.
During pathological examination we described and photographed the main pathologies and malformation evaluated macroscopically. In addition to the diagnostic methods described above, for the study of skeletal malformations, has been used radiographic examination via digital device.

RESULTS

Year	Species	Sex	Age	Dysmorphology profile
2013	Red deer	M	11 months	Bilateral microphthalmia
2013	Red deer	F	5 months	Microphthalmia with bilateral absence of the lens.
2014	Alpine chamois	F	3 days	Lethal Short spine
2014	Red deer	F	5 months	Microphthalmia with bilateral absence of the lens. Patent ductus arteriosus
2014	Red deer	M	3days	Multiple vertbral anomalies Clef palate
2015	Red deer	M	8 months	Dysplasia of the tricuspid
2015	Red deer	M	1 month	Microphthalmia with bilateral absence of the lens. Patent ductus arteriosus

Overall seven cases of dysmorphisms were found including one in chamois and six in red deer. Three malformations appeared simple while in four cases multiple malformations pertaining to multiple organs and systems were found. In two cases deformation of the spine were detected, in one associated with cleft palate, a single individual showed dysplasia of heart valves with left heart failure. The deformity most frequently encountered were bilateral microphthalmia (4,5) in red deer (four cases) with or without the persistence of the *ductus arteriosus* (two cases). All cases are related to individuals under one year of age recovered in the natural environment and hospitalized at the provincial CRAS. In three cases lesions resulted in death from direct cause or due to secondary complications.

DISCUSSION

All individuals presenting dysmorphogenic lesions delivered still alive at the provincial and CRAS were euthanized and/or come to death in the same structure. In all cases the diseases encountered were not compatible with the free-life.

The bacteriological, virological, parasitological and histological analysis have not determined any infective cause of dysmorphogeny. The high frequency of ocular abnormalities in red deer and the association of these defects of the arterial trunks, depose for a multifactorial cause. That finding is further supported by the description of similar cases of ocular dysmorphogeny in the same study area (3).

The relief of dysmorphisms in wildlife is difficult to implement, the creation of a network of local monitoring associated with wildlife rescue centers could collect useful epidemiological data.

CONCLUSION

This is the first systematic study on dysmorphogenic disease from wild ungulates populations. Relying on our experience we can assert that their incidence is particularly high in species like red deer.

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AVIAN BOTULISM IN ITALIAN WATERFOWL: CASE STUDIES AND DIAGNOSTIC TEST DEVELOPMENT

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INTRODUCTION

Avian botulism is a paralytic, often fatal, disease of birds that results when they ingest neurotoxin produced by *Clostridium botulinum*, a strict anaerobe bacterium that forms dormant spores in adverse conditions. Seven different neurotoxins are produced by strains of *C. botulinum*, designated types A to G. Researchers have also described a mosaic C/D toxin from outbreaks in birds. Birds are most commonly affected by type C and to a lesser extent type E and acquire the disease by ingesting directly toxin-laden food items or eating invertebrates (e.g. chironomids, fly larvae) containing the toxin. The carcass-maggot (fly larvae) cycle of avian botulism occurs when birds eat maggots that ingest animal carcasses with toxin. In this way, botulism outbreaks often become self-perpetuating. The disease can also be caused when *C. botulinum* colonize the intestinal tract of bird or secondarily infect a wound. Outbreaks and sporadic cases of botulism in waterfowl were encountered from 2008 to 2010 in central Italy lakes, reserves and coast.
In order to study avian botulism in Italian waterfowl, to evaluate public health risk and to replace the mouse test, the most widely used test for avian botulism diagnosis and neurotoxins detection, the Istituto Zooprofilattico del Lazio e della Toscana, *M. Aleandri* performed a research whose results are shown in this paper.

METHODS

The main morphometric, chemical, biological and anthropic characteristics of Albano and Bracciano volcanic lakes were evaluated using "LIMNO" a Italian National Research Council (CNR) database of Italian lacustrine environments and scientific studies on the subjects.
Visits were carried out for the detection *in loco* of physical and chemical water parameters and to check the presence of ducks in the lakes. Fish species and phytoplankton were sampled to search *C. botulinum* type C and D in Bracciano and Albano lakes and in Macchia Tonda reserve in compliance with CDC cultural method (Botulism in the United States, 1899-1996. Handbook for epidemiologists, clinicians and laboratory workers. CDC 1998, 15 – 21). Samples of death birds, maggots (diptera larvae) on the crops and near them and soil were collected during avian botulism outbreak in Albano e Bracciano lakes, in order to search neurotixin type C and D (CDC, 1998). Temperature, pH and dissolved oxygen in water were detected in different lakes points in order to check the favourable environmental conditions for vegetative *C. botulinum* forms development and toxin production and circulation. Symptomatic birds were identified during avian botulism outbreak in Albano e Bracciano lakes. The birds were inability to fly, had a flaccid paralysis of the limbs, opisthotonos, breathing difficulty and nictitating membrane paralysis.
Anatomopathological examination was carried out on dead birds. Liver, kidney and intestines were examinated for *C. botulinum* and neurotoxin (CDC,1998). Bacteriological, virological and toxicological analysis were conduct on samples to rule out other causes of death.
In order to replace mouse test a Polymerase chain reaction (PCR) method was performed. The genomic sequences were searched in GeneBank (web-site http://www.ncbi.nlm.nih.gov/nuccore; queries: *Clostridium botulinum* type D, mosaic CD, mosaic DC, bont/C, bont/D, bont type C, bont type D, cntA/C, cntA/D, chimericbont/CD, chimericbont/DC). A database were performed with GeneBank genomic sequences). The target gene sequences were inserted into a genome database. Clusters were obtained with the specific gene sequences encoding botulinum neurotoxins type C, type D, type

CD, type DC. Multiple sequence alignment of each clusters were obtained using the "ClustalW" algorithm.
Primer and probes were designed with Software Beacon Design 7.51 (Premier Biosoft, USA). To ensure that the system was working properly and confirms data reproducibility and quality, *C. botulinum* type B gene gyrB was use as control samples. Primers were amplified with *in silico* PCR using the software available on http://insilico.ehu.es/. Locked Nucleic Acids® (LNA®) hydrolysis probes were used. Mutiplex real time PCR probe was optimized with master mix (quantitec multiplex PCR master mix-Qiagen) at anealling temperature of 56°C and $MgCl_2$ final concentration of 9.5 nM. The amplifications were performed at least in duplicate using Stratagene mx3005p with filter setting: HEX, FAM, ROX, CY5, ALEXA350. A DNA template extracted from an overnight culture of *C. botulinum* from Italian botulism national reference center strains collection, was used to develop the protocol . Wild strains and naturally contaminated samples during avian botulism outbreaks were tested for *C. botulinum* and neurotoxins presence with PCR protocol and contemporary with CDC cultural method (CDC, 1998). PCR test performance (selectivity (inclusivity and exclusivity), dynamic range, limit of detection (*LOD*), relative accuracy, relative sensitivity and specificity) have been evaluated in accordance with the ISO 16140: 2005. Finally, the Italian botulism national reference center performed a ring trial in order to validate in-house the PCR protocol.

RESULTS
The main morphometric, chemical, biological and anthropic characteristics of Albano and Bracciano volcanic lakes were identified. A great water volume reduction there was in the last few years.
The Real time PCR analysis shown the following performance value:
-selectivity: inclusivity 100% and exclusivity 100%;
-dynamic range: at least a 6-log range for each target gene;
-*LOD*: *bont*/C 58 genomic copies, *bont*/D 43 genomic copies, *bont*/CD 58 genomic copies, *bont*/CD 221 genomic copies;
-relative accuracy: 100%;
-relative sensitivity: 100%;
-relative specificity: 100%.

PCR assay validation revealed good reproducibility and genes target identification in all used samples. Internal organs congestion (especially lung, liver and kidneys), stomach and early digestive tract contents absence were identified at avian gross necropsy during botulism outbreaks. Furthemore 145 samples were analyzed for *C. botulinum* neurotoxins detection and 59 (40.7%) were positive for neurotoxin type C. Among the samples tested, the highest percentage of positive was identified in maggots (*Sarcophaga* spp. n=3, 100%, *Musca* spp. n=1, 100%; *Lucilia* spp. n=7, 85.7%; *Fannia* spp. n=5, 80%; *Diptera* from gull corpse n= 2, 100%), mallards (n=7, 71.4%), duck (n=5, 60%) and gull (n=74, 33.8%).
Temperature, pH and dissolved oxygen in water were detected in Albano and Bracciano lakes during botulism outbreak. Mean temperature, pH and dissolved oxygen value were respectively 22-24°C, 8.5 and 0.2-0.7ppm. The death of fish was detected in all the outbreaks with the exception of Castel Porziano and Bracciano outbreaks.

DISCUSSION
Since the highest percentage of positive samples was identified in maggots, Diptera scavengers larvae could have a role in italian botulism outbreak epidemiology. The greatest number of deaths birds were recorded (60.3%) in the Anatidae family and Mallard species, was the most affected (50%). The performed PCR has proven to be a sensitive method for the detection of *C. botulinum* in the environment and in eco epidemiological study. During botulism outbreaks the overall *C. botulinum* prevalence in dead birds digestive system was 38.5%. *C. botulinum* was more frequently detected in large intestine (cecum) and less in stomach and small intestine (gastric content). This different

distribution may be due to a preferential growth of *C. botulinum* type C in the large intestine. It may also depends on its presence in lower gastrointestinal tract before birds death where *C. botulinum* arrives due to the vegetative cells and/or spores ingestion. After birds death *C. botulinum* reproduces in the caecum using animal tissues as nutrient medium. The research of *C. botulinum* in birds cloacal swabs and caecum samples had the same results, so cloacal swabs may be choice specimen in live birds suspected of botulism or could be used to study possible *C. botulinum* carriers in epidemiological studies.

CONCLUSION

The study elucidated part of avian botulism epidemiology in central Italy. In recent years the recurring outbreaks and the results of this study seem to indicate that in central Italy are now established climatic conditions favourable to the development of *C. botulinum* and neurotoxin production. The occurrence of epidemics seem favoured by the hight percentage of positive maggots, drought, water pH raising and dissolved oxygen reduction in the lakes. Finally the performed PCR has proven to be a useful method to replace mouse test.

GASTROINTESTINAL HELMINTHS IN FALLOW DEER *(DAMA DAMA)* BRED IN THE WILD STATE: PRELIMINARY RESEARCH IN CALABRIA REGION (SOUTHERN ITALY)

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INTRODUCTION

The Fallow deer (*Dama dama*) is an ungulate well adapted to the Mediterranean environment, common in many areas of Western Europe, particularly abundant in England. The total estimable Fallow deer population in Italy consists of about 21000 animals distributes mainly in the central northern Apennines (76%). The original range of *Dama dama* was in the eastern part of the Mediterranean basin. However the species nowadays has an early completely artificial distribution and the only original remaining population seems to be the nuclei of Duzlerçami present in Turkey (Termessos National Park). The origin of the Italian population is unknown. According to current knowledge, the species must be considered native to Italy. In Southern Italy, the Fallow deer is recorded in the Gargano area, in Basilicata and in Calabria. In this Region, the three small nuclei present are originated from animals escaped from enclosures in the Sila and Pollino National Parks and in the area straddling the Provinces of Reggio Calabria, Vibo Valentia, and Cosenza. In Calabria, like in other Italian Regions, *Dama dama* population is increasing more and more because of the lacking both of natural predators and check reproductions plans inside the faunal firms on the territory. In this Region it is illegal to hunt Fallow deer and killing planes for species control are not provided for by law up until today. Nowadays in the Province of Catanzaro there are 5 faunal firms that breed this ungulate and they are spread into the National Park of Sila and the neighboring territories. But there is almost no knowledge about the diffusion of helminths in the population of *Dama dama* living inside the five faunal firms. So the aim of our research is the acquisition of recent data on the prevalence of this parasitic group in the species *Dama dama* bred in the Province of Catanzaro. Our research has been done on the Fallow deer living in a faunal oasis belonging to the district of Catanzaro. We have used Flotac® dual technique that has underlined the presence of gastrointestinal endoparasites typical of ruminants, in the specie *Dama dama* bred in the wild state.

METHODS

Our research has been done, between September 2014 and March 2015, on the population living in Manulata Oasis, a faunal mountain facility belonging to the municipality of Petronà (Prov. Catanzaro). The tested animals have never received anti-parasite treatments inside the faunal firm, this emerged both from the pharmacological treatment registers and the working staff words that have confirmed that any parasitological plane has been followed because the administration of medicines should be very difficult to realize. The total population of Fallow deer that is 17 in total, 6 males and 11 females, between 1 and 7 years have been put to parasitic search. Endo-parasitic and parasitic elements research has contemplated the field sample of feces (copros) in two periods: P 1 (Sept. 2014) and P 2 (March 2015). The individual sample of copros (min 10 gr) has been done after a spontaneous defecation, with care to take the biological material from the apex of fecal heap, soon after the emission in order to reduce to a minimum the environmental contamination. All the samples together with identified form have been sent in simple vacuum envelopes to the Interdipartimental Centre of Services (CIS) Unit of Parasitic diseases - University Magna Graecia Catanzaro for the following parasitic researches. We have done coprological macroscopic test to search for adult nematodes. To search for and the count of parasitic elements such as eggs, larvas, oocisti, we have used Flotac® dual technique, coprological technique with a sensibility equal to 2 eggs/larvas/oocysts per gram of faeces (2 EPG/LPG/OPG). In order to search and count the eggs of

gastrointestinal light nematodes and coccidia oocyst, we have used one solution of flotation based NaCl (1 200 s.g.). To search parasitic elements with a greater specific weight we have used a solution based ZnSO4 (1 350 s.g.).

RESULTS

Our research has pointed out several species of endoparasites in the examined Fallow deers. Particularly among the gastrointestinal helminths, the most widespread are the Gastointestinal Strongyles (100% P1 and 100% P2), followed by *Capillaria* spp. (12% P1 and 35% P2), then by *Strongyloides* spp. (12% P1 and 18 % P2). In addition we have noticed some protozoa of Coccidia class (30% P1 and 60% P2). We report in succession the parasitic intensity averege expressed in EPG and OPG: Gastrointestinal Strongyles 18 EPG in P1 and 42 EPG in P2, *Capillaria* spp. 18 EPG in P1 and 22 EPG in P2, *Strongyloides* spp. 6 EPG in P1 and 10 EPG in P2, Coccidia spp. 30 OPG in P1 and 32 OPG in P2. We want also to specificy that we have not found any significant correlation among parasitic prevalences sex and ages in the examinated animals.

DISCUSSION

Our research has pointed out the presence of some helminths in the species *Dama dama* and want to be a preliminary phase for ulterior researches. In addition the Fallow deer has proved to be the final host of the most common ruminants endoparasites. It is difficult, or impossible, to compare our data, with previous results, because the researches on parasitosis through copromicroscopic exams on Fallow deer in Calabria Region are lacking or almost absent, but our results line up with the ones obtained in other region of Italy. According to us the sensible increase of parasites can be attributed to the season of takings, that is to the environmental temperatures linked to the place altitude (1 122 metres sea level), that could have a lot of influence on the biological cycle of helminths. In addition, the low values of middle parasitic intensity could depend on the acquisition of a certain immunity level already described in bovines literature, that should develop during the second and the third pasturage season in consequence of previous parasitic infestations. However the phenomenon should not exclude the presence of parasitarian elements in the environment.

CONCLUSION

The underlined parasitic picture is not grave but, in our opinion it could have possible consequences on the animal wellbeing. Indeed parasitosis cause some diseases with an often little obvious symptomatology, that gradually involve all the animals of the farming. Moreover we must take into account that parasites in relation both to the species and parasitic charge, provoke several damages to the animals, originated from the traumatic action of the parasite to fix itself to the tissues. Among the damages there are: anaemia, metabolic disorders, secondary bacterial infections, stress, etc. In this conditions there is a clear violation of some of the “Five Freedoms”- Brambell Report 1965, afterwards revised by the British Farm – FAWC 1993, such as freedom from pain, from diseases and from stress. People could easily respect these freedoms through prevention, a rapid diagnosis and giving suited medical cares to the animal. All these things are essential to guarantee a good health and to reduce the stress caused just by the parasitic traumatic action. On this subject it is important to underline that parasitic prophylaxis, should be difficult to realize by the operators of the centre, could be easily done, after a copromicroscopic diagnosis, with powdered anti-helminths and anti-protozoal, easily miscible to the food. On this subject one could say that species living in a natural state maintain and spread parasitic elements in the environment and they could be a potential sanitary risk for the auctoctonal wild species and for ovines, caprines and bovines, traditionally bread to a natural or semi-natural state. Because of social behaviour and ecology, the Fallow deer has a limited capacity of dispersal. So it can reach extremely high local densities (> 30 individuals/Km^2) causing considerable damages to the bush-wood and crops. High level of competition with the autochthonous Cervidae, Reed deer and especially Roe deer, is noticed; indeed, it appears to be better adaptable than them, at least in the Mediterranean environment. According to current

knowledge, the species must be considered native to Italy; the species currently has a completely artificial distribution. It would be good to intensify the scientific researches on this wild ungulate carrying out proper strategies for both monitoring and population control of the allochthonous groups naturalized in wild environment because “the biological invasions that is the expansion caused by man of animal or vegetable species out of their geographic range, represent one of the principal threats to the biodiversity preceded just by the destruction of the habitat”. Having said that should be a warning for people who manage the wildlife reserves on the territory. They could monitor the allochthonous species, also preventing the accidental escapes in order to protect both the wild autochthonous species and the ones of zootechnical interest of Calabrian territory.

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GASTROINTESTINAL HELMINTHS IN WILD BOARS (*SUS SCROFA*): RESEARCH IN CALABRIA REGION (SOUTHERN ITALY)

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INTRODUCTION

In Calabria, like in other Italian regions, Wild boar *(Sus scrofa)* population has had a considerable increase in the last few years. Nowadays the species living on the territory is no more the autochtonal *Sus scrofa majori* but it is composed by some subjects both imported from abroad that is *Sus scrofa raiseri* and in some cases by hybrids with pig *(Sus scrofa domesticus)* characterized by bigger dimensions, a bigger reproductive rate and a considerable phenotipic variability. In addition they have a greater adaptability towards more unfavourable habitat without natural predators. These factors have caused a strong expansion of the subspecies *Sus scrofa raiseri* in South of Italy too.
Several researches conducted in other countries too, have underlined that this wild ungulate is the final host for many endoparasites, some of which at zoonosis risk.
Considering the lacking knowledge about the diffusion of helminths in Wild boars population, the aim of this research has just been the acquisition of up to date data about the prevalence of this group of parasites in *Sus scrofa* species living in the Province of Catanzaro (Calabria, Southern Italy). The research has been done between September and December 2013 on a group of 60 Wild boars *(Sus scrofa)* killed during 2013-2014 hunting season. Through Flotac® dual technique, the research has underlined the remarkable diffusion of helminths in *Sus scrofa* of Sila and Presila in the Province of Catanzaro.

METHODS

The research has been done between September and December 2013 on a group of Wild boars killed during 2013 – 2014 hunting season. The animals, homogeneous for place of tacking, came from a geographical area of about 215 km^2 situated in Sila and Presila (Calabria Region, Southern Italy), exactly in the municipal land of Cicala, Taverna, Sorbo San Basile, and Carlopoli, belonging to the Province of Catanzaro at faunal hunting level. In each of these municipal lands, 15 specimen have been put to a parasitological search. The search of both parasites and parasitic elements has been done on the collection of fecal material (copros) in field. The individual collection of copros, min 10 gr, on the killed animals has been done from the fecal cruet during the evisceration. All the data concerning the animal (sex, weight, estimated age, hybridization signs, place and date of killing and GPS coordinates), have been transcribed on reports provided for the purpose. All the samples together with the respective reports have been carried in disposable hermetic containers at 4° C to the Interdepartmental Center Veterinary Service (CIS) for Human and Animal health Unit of parasitic disease University of Magna Graecia Catanzaro Southern Italy. On each of the 60 samples of copros belonging to 34 males and 26 females, different for ages, (20 between 1 – 2 years, 20 > 2 years, 20 > 4 years), we have done coprological macroscopic test to search adult nematodes and qualitative-quantitative microscopic test to search and count of parasitic elements (eggs, larvas). To carry out the latter ones we have used Flotac® dual tecnique, coprological tecnique with a sensibility equal to 2 eggs/larva for gram of faeces (2 EPG/LPG). In order to search and count the eggs of light gastrointestinal namatodes such as *Strongyloides ransomi, Physocephalus sexalatus*, etc, we have used one solution of flotation based NaCl (FS2 - s. g. 1200) and one solution based ZnSO4 (FS7 - s. g. 1350) for the search of the eggs of trematodes (*Fasciola hepatica, Dicrocoelium dendriticum*) for the Gastrointestinal Strongyles and for nematodes such as *Ascaris suum, Trichuris suis*, etc.

RESULTS

Our research has pointed out the presence of several helminths in the wild species *Sus scrofa* in Catanzarese Sila and Presila. The most widespread gastrointestinal helminths are the roundworms *(Ascaris suum)* present in 90% of the examined animals, followed by gastrointestinal strongyles (83%), then by *Trichuris suis* (38%) and finally by *Strongyloides ransoni* (15%).

Among the hepatic helminths, *Dicrocoelium dendriticum* was present only in 3% of the examined animals. We also must take into account the significant diffusion of *Metastrongylus* spp., helminths long at zoonosis risk, present in 60% of animals. We report in succession the average of the parasitic intensity expressed in EPG: *Ascaris suum* 282 EPG, Gastrointestinal Strongyles 132 EPG, *Trichuris suis* 20 EPG, *Strongyloides ransomi* 10 EPG, *Metastrongylus* spp. 22 EPG, *Dicrocolelium dendriticum* 5 EPG. We want to specify that we have not found any significant correlation among parasitic prevalence, sex and age of the examined animals.

DISCUSSION

Our research which wants to be a preliminary phase for ulterior researches, has pointed out the considerable diffusion of helminths in the wild species *Sus scrofa* of Sila and Presila, situated in the Province of Catanzaro. It is difficult, or impossible, to compare our data with previous ones because the researches on parasitosis through copromicroscopic diagnosis on *Sus scrofa* are lacking or almost absent in Calabria Region. Our results excepting for *Ascaris suum*, *Trichuris suis* and *Metastrongylus* spp., line up with the ones done in other regions of Italy. The clear prevalence of *Ascaris suum* and *Trichuris suis* on the other group of helminths, according to us, could depend on the family pig breeding practice on the territory. Actually in Sila and Presila belonging to the district of Catanzaro they both breed pigs in a wild state and a half wild one, overall in family farms. So the contact between Wild boar *(Sus scrofa)* and pig species *(Sus scrofa domesticus)* could have generate the diffusion of the typical helminths at direct cycle (roundworms and whipworms) in the area of our research and as consequence these parasitic prevalences. We can attribute the prevalence of *Metastrongylus* spp. to interspecific contacts between *Sus scrofa* and *Sus scrofa domesticus* and to the seasonal bronchopulmonary strongylosis coinciding with the period of our research. Our hypothesis is confirmed by the presence among the examined animals, six, that is 10 % of the total champion, of hybridism with *Sus scrofa domesticus.*

CONCLUSION

The underlined parasitological picture, attests that *Sus scrofa* is the final host of the most common endoparasites of the *Sus srofa domesticus* and represents an important reservoir for it. This picture, according to us, could have sanitary consequences because of the “frail” local zootechnical spinneret on a territory just characterized by a family agro-zootechnical micro-economy. So, zootechnical pig-breeding firms both of “genetic autochthonous type” (Black Pig of Calabria) and of other races bred in a wild or semi-wild state could be particularly stricken. We think it is right to intensify scientific researches on this wild ungulate carrying out both sanitary monitoring strategies and the curb on population of *Sus scrofa,* in order to preserve both the wild species and the domestic ones reared in Calabria. We cannot direct our efforts on the field of scientific improvement of calabrian agro-zootechnical sector and its consequent alimentary spinneret ignoring the sanitary monitoring of a species that in absence of any environmental competitiveness, excepting man, continue to be a reservoir for parasitic and infective agents.

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TAENIA HYDATIGENA LARVAE FORMS IN WILD BOAR HUNTED IN PORTUGAL

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INTRODUCTION

The wild boar (*Sus scrofa*) is an abundant wild species that is distributed throughout the national territory except for the coastal region. It is also one of the most important large game species hunted in Portugal.

Taeniid species can be considered relevant pathogens in human and animals, circulating between carnivorous definitive hosts and a variety of mammalian intermediate hosts. Wild boar can be considered as intermediate host of several *Taenia* species like *Teania solium*, *Taenia asiatica* and *Taenia hydatigena*. Scarce information is available about these parasites in wild boar. The aim of this study was to identify *Taenia* spp. larvae forms found in wild boars hunted in the northeast of Portugal.

MATERIAL AND METHODS

Taenia spp. larvae forms found in wild boar hunted during the hunting season 2011/2012 in Trás-os-Montes region were collected to containers with 80% alcohol. Molecular diagnosis by PCR was performed for identification of *Taenia* spp. larvae forms. All positive results obtained were sequenced.

RESULTS

Round fluid-filled vesicles were found in the abdominal cavity, more frequently in mesentery and occasionally in the surface of the liver, compatible with *Taenia hydatigena* larvae forms. The PCR assay targeting *Taenia hydatigena* yielded two presumptively positive samples. When analysing the nucleotide sequence similarity within *Taenia hydatigena* isolates, all were identical (100%).

DISCUSSION

In this study several metacestode (*Cysticercus tenuicollis*) of *Taenia hydatigena* were found in wild boar hunted in Portugal. *Taenia hydatigena* is a parasite with indirect life cycle with dogs, wolves, coyotes, lynxes and rarely cats as definitive hosts and sheep, goats, cattle, pigs and other domestic and wild cloven-hoofed animals as the usual intermediate hosts. Rabbits, rodents and humans can be also considered as intermediated hosts however they are rarely infected.

Most of *Taenia hydatigena* cysticerci infections are chronic and asymptomatic but heavy infections could be fatal with the destruction of liver parenchyma by larvae migrating and severe inflammation.

Hunting dogs can assume an important role in this parasite transmission to wild boar due the close contact between them during the hunting process, perpetuating the life cycle of *Taenia hydatigena* in wildlife.

CONCLUSION

To our knowledge, this is the first case of *Cysticercus tenuicollis* described in wild boar in Portugal. Measures to prevent this infection are required, including the correct disposal of potentially infected wild boar by-products as a veterinary health measure. These results also underline the importance of game examination after the hunting procedure in order to detect pathological processes, and the importance of a regular deworming of hunting dogs to minimize parasite disease transmission.

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SEROPREVALENCE OF PARATUBERCULOSIS AGENT IN EUROPEAN BISON (*BISON BONASUS*)

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ABSTRACT
The aim of the study was to investigate the population of European bisons in National Park Poloniny (north-eastern Slovakia). Over the years 2012 – 2014 in total 8 individuals were serologically tested for antibodies against *Mycobacterium avium* subspecies *paratuberculosis* using indirect ELISA. None of sample was positive for presence of antibodies against *Mycobacterium avium* subspecies *paratuberculosis.*

INTRODUCTION
Paratuberculosis, a chronic infectious disease of the intestinal tract of domestic and wild ruminants, has been described throughout the world since its discovery in 1895. This bacterial disease represent significant problem in animal and human medicine. The causative agent is *Mycobacterium avium* subspecies *paratuberculosis* (Map), a facultative intracellular acid-fast bacillus. European bison (*Bison bonasus*) as a wild ruminant can also act as a vector of the disease under natural conditions and contribute to the transmission. The presence of Map in bisons kept in zoo in Belgium was serologically confirmed in 2005 (Vansnick et al., 2005). Young animals are at highest risk. The infection is transmitted by faecal-oral route, contaminated food, water, objects or even milk, meat, semen and *intra utero* (Vansnick et al., 2005)

MATERIAL AND METHODS
Between 2012 – 2014, we examined blood samples from 8 individuals of European bison (*Bison bonasus*) from National Park Poloniny (Zvala, Ruske) over the age of 6 years. Blood samples were collected from the jugular vein after chemical immobilization of the game. After sampling blood serum was obtained by standard procedure and it was serologically analyzed in the laboratory of the Slovak State Veterinary and Food Institute. For the detection of antibodies to *Mycobacterium avium* subsp. *paratuberculosis* indirect ELISA method was carried out using commercial diagnostic kits i-ELISA paratuberculosis verification (Pourquier Institute, France) and ID Screen paratuberculosis Indirect (id. VET Innovative Diagnostics, France) according to the manufacturer's recommendations.

RESULTS
Serological examination of blood samples from 8 individuals of European bison has not detected the presence of antibodies against *Mycobacterium avium* subspecies *paratuberculosis* in any case.

DISCUSSION
Previous reports on the prevalence of Map in wildlife and livestock may vary considerably. The problem with diagnosis is due to the fact, that infected animals remain seronegative until an advanced state of infection (Probst et al., 2011). In Slovakia, paratuberculosis has increasing tendency in cattle without market milk production (yearly on pasture). Sharing the pastures with wild ruminants, cross-infection may occur.

CONCLUSION
No detection of antibodies against Map in European bison population is a positive phenomenon. On that basis it is not possible to exclude the presence of infectious agents in the environment and the risk of infection. Bisons infected with Map may remain undetected due to the remoteness of the herd, death in inaccessible pasture and efficient work of scavenging wildlife. Cross-border migration

of bisons represents the significant risk of transmission of zoonoses to the neighbouring countries and monitoring of the health status should be part of comprehensive epidemiological preventive measures at international level.

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WARBLE FLY, BOTFLY AND POSSIBLE CORRELATIONS OF SELECTED ENDOPARASITES IN CLOVEN-HOOFED GAME

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ABSTRACT
In 2005 – 2012 the warble fly and botfly and some kinds of the endoparasites were monitored in the cloven-hoofed game. In roe deer the prevalence of the warble fly was 50.39% and the prevalence of botfly was 44.09%. In red deer the prevalence of warble fly was 46.12% and the prevalence of botfly was 35%. The prevalence of other endoparasites in roe deer ranged between 4.00% and 92.00% and in red deer between 0.55% and 80.29%.

INTRODUCTION
Parasitic diseases of wild animals represent more than 2/3 of all diseases. With constant rising of the cloven-hoofed game numbers in the recent period (concentration rise), there is an assumption that the prevalence of diseases will rise. There are two types of bots in Slovakia – warble fly (*Hypodermatidae*) and botfly (*Oestridae*).

Warble fly type: *Hypoderma diana*
Hypoderma actaeon
Botfly type: *Cephenemyia stimulator*
Cephenemyia auribarbis
Pharyngomyia picta

Other (selected) endoparasites were assesed from the point of their classification (*Trematoda, Nematoda, Cestoda* and *Protozoa*).

MATERIAL AND METHODS
In accordance with the Hunting Act 274/2009 and amendments to certain laws 871 roe deer and 335 red deer were examined for warble fly within the cooperation with hunting associations in Prešov and Košice region. Also 186 roe deer and 60 deer were examined for botfly. After removal, larvae were held in 70% alcohol and then they were identified. The samples were examined for parasites using standard methods (flotation, sedimentation and larvoscopic).

RESULTS

class/endopar.	Warble fly	Botfly	Protozoa	Trematoda	Cestoda	Nematoda
Roe deer prevalence %	50.39	44.09	32.00	8.00	4.00	92.00
Red deer prevalence %	46.12	35.00	13.69	2.37	0.55	80.29

During the monitoring the weight of shot animals positively diagnosed with parasites the weight loss of roe deer was 21.8% lower than the weight loss of those with negative diagnosis. The weight loss in red deer was considerably lower (11.8%).

CONCLUSION

Based on the observed facts we concluded that the only proper maintenance of game can breed healthy animals with high breeding value.

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In authors

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IMAGING FINDINGS IN 20 FERRETS (*MUSTELA PUTORIUS FURO*)

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ABSTRACT
Mustelids are one of the largest family in the order Carnivora. Wild and pet ferrets share many anatomical similarities. In order to improve quality of ferret (and wild mustelid) veterinary care, veterinarians should be familiar with all aspects of veterinary medicine of this mammals, including Diagnostic Imaging. We present here the radiographic, ultrasonographic (US) and Computed Tomographic (CT) findings obtained from a caseload of ferrets admitted at the Interdepartmental Veterinary Radiology Centre, University of Naples Federico II. Twenty medical records of pet ferrets presented between January 2014 and June 2015, were retrospectively reviewed. Whole-body direct radiographs were performed in 4 ferrets, and contrast radiographs (intravenous urography) were performed in 3 cases. Abdominal ultrasonography was performed in 16 ferrets, and direct and contrast CT scan (head) was performed in one case. The imaging findings retrieved included: mesenteric lymphadenomegaly, splenomegaly (7/20; 35%), renal disorders (6/20; 30%), adrenal disease (4/20; 20%), uterine neoplasia (1/20; 5%), oral neoplasia (1/20; 5%), and multiple congenital malformations (1/20; 5%).

INTRODUCTION
Mustelids are one of the largest family in the order Carnivora. The family *Mustelidae* includes several species of mammal carnivores such as weasels, ferrets, minks, martens, otters, polecats, tayras, badgers, and many others. Among mustelids, ferrets (*Mustela putorius furo*) are the most common species currently kept as pets. Ferrets have been domesticated for approximately 2000 to 3000 years (Powers and Brown, 2012) and before becoming popular pets, they have been extensively used for rodent or rabbit control (hunting) for a long time. Ferrets have also been widely used in biomedical research in the fields of anatomy, reproductive physiology, endocrinology, virology and toxicology (Powers and Brown, 2012).
There are currently three living species of ferrets including the European (*Mustela putorius*) and the Siberian or Steppe (*Mustela eversmanni*) polecats and the black-footed ferret (*Mustela nigripes*). Wild and pet ferrets share many anatomical similarities and it is believed that pet ferrets may have originated from one or more of the above mentioned wild species (Powers and Brown, 2012).
In order to improve quality of the treatments, veterinarians should be familiar with all the aspects of veterinary medicine of this "exotic" companion mammals, including Diagnostic Imaging. Moreover many diagnostic procedures used for pet ferrets could also be effectively employed to care their wild counterparts. Therefore, we here present the Imaging findings obtained from a caseload of ferrets admitted at the Interdepartmental Veterinary Radiology Centre, University of Naples Federico II.

MATERIAL AND METHODS
Twenty medical records of pet ferrets presented to the Interdepartmental Veterinary Radiology Centre, University of Naples Federico II, between January 2014 and June 2015, were retrospectively reviewed. From each medical record the following data were retrieved: sex, weight, age, and presenting clinical signs (when available). The ferrets underwent radiographic, ultrasonographic (US) and Computed Tomography (CT) exams.

RESULTS

The age of the ferrets ranged from 0.25 to 9 years (mean of 3.6 years). Four ferrets were males (3 neutered, 1 intact), and ten were females (3 spayed, 7 intact). Whole-body direct radiographs (right and left lateral, and ventrodorsal views) were performed in 4 ferrets, and contrast radiographs (intravenous urography) were performed in 3 cases. Abdominal US was performed in 16 ferrets, and CT scan of the skull was performed in one case. General anaesthesia was performed in 4 cases (intravenous urography and CT). The presenting clinical signs included: alopecia (6/20; 30%), polyuria and polydipsia (4/20; 20%), abdominal mass (2/20; 10%), peritoneal effusion (ascites) (2/20; 10%), urinary incontinence (1/20; 5%), presence of bilirubin in the urine (1/20; 5%), mandibular swelling (1/20; 5%), hematuria (1/20; 5%), and dyspnea (1/20; 5%). The imaging findings retrieved included: mesenteric lymphadenomegaly, splenomegaly (7/20; 35%), renal disorders (6/20; 30%), adrenal disease (4/20; 20%), uterine neoplasia (1/20; 5%), oral neoplasia (1/20; 5%), and multiple congenital malformations (1/20; 5%).

DISCUSSION

The results of the present study showed that the most common Imaging findings were limphadenomegaly and splenomegaly. While splenomegaly is not necessarily a sign of disease, if no abnormalities are detected in the splenic parenchyma, the presence of limphadenomegaly (sometime associated with splenomegaly) is often associated with important gastro-intestinal diseases of ferrets, such as intestinal bowel disease (eosinophilic gastroenteritis, epizootic catarrhal enteritis, *Helicobacter mustelae*-associated gastritis, and proliferative bowel disease) as well as systemic diseases such as ferret lymphoma (Donnelly, 2013; Mayer and Robat, 2013). Renal disorders were the second most common finding of the present survey and included renal cysts, hydronephrosis and chronic interstitial nephritis. Our results are in agreement with previous reports indicating that renal cystic disease as well as chronic interstitial nephritis are commonly found on necropsy of geriatric ferrets. Conversely hydronephrosis is an uncommon condition that can be associated with ureterolithiasis, renal neoplasia and inadvertent ureteral ligation during ovariohysterectomy (Fisher, 2013). Adrenal disease was detected in 4 cases in which adrenal gland adrenomegaly was found along with focal increased adrenal width and thickness. In one case the adrenal gland enlargement was also associated with abdominal and pleural effusion. Uncommon conditions such as uterine and oral neoplasia were detected in one case each. To date this was the first report of oral melanoma in a pet ferret (d'Ovidio et al., 2014). A direct and contrast CT scan were also performed in the latter case, in order to detect any abnormality of the surrounding soft tissues as well as the underlying skull bones. Finally a case of rare multiple congenital malformations including renal dysplasia, caudal spinal agenesis and persistent cloaca was also documented. In the latter case ultrasonography, direct radiographs, intravenous urography, and a cloacography (injection of iodinated contrast medium into the abnormal anal orifice) were performed in order to characterize these rare complex uro-genital and skeletal abnormalities (d'Ovidio et al., 2015).

CONCLUSIONS

Imaging techniques provide useful important information in the diagnosis of many ferret diseases. The data collected from domestic ferrets represent an important source of imaging technique information, potentially reliable on other mustelids and small carnivores.

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BIODIVERSITY GOOD PRACTICE: FROGS SEDATION FOR BLOOD SAMPLING

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ABSTRACT
The knowledge relating to biodiversity are a central goal to prepare protocols of conservation to be applied on the most vulnerable species. Twenty one frogs *Pelophylax lessonae* captured into Serre Park of Calabria were submitted to an anaesthetic protocol with isoflurane dispensed in a closed box (40 cm x 20 cm) at a 5% concentration of oxygen (flow rate of 4 litres per minute). The initial breathing rate (83 breaths/min) stops after about 6 minutes and was absent for about 15 minutes; he gradually grows to return on baseline within 180 (light and middle frogs) and 228 minutes (heavy frogs).

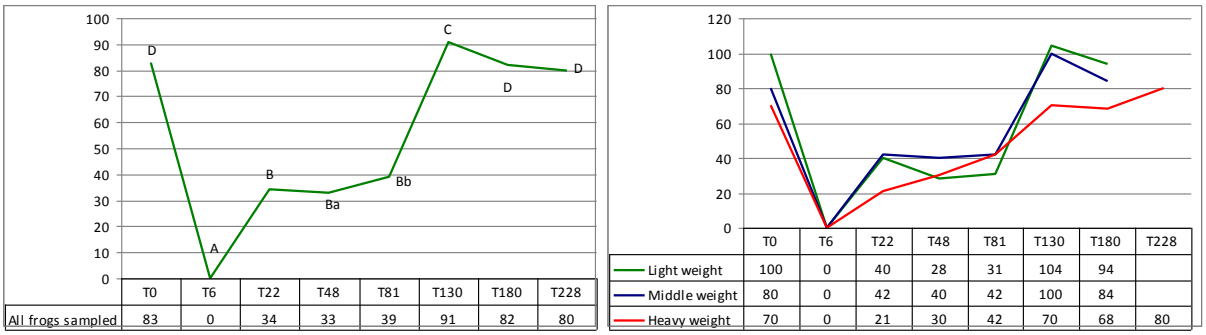
INTRODUCTION
The study of the physiology and the biological-behavioural functions of biodiversity present in natural areas, protected and unprotected, is a key objective to prepare protocols of conservation to be applied on the most vulnerable species. One of the aims of the project Angitola $FISH_2O$ (EFF Calabria Cod. 02/BA/12) was to identify the biodiversity in the SIC IT9340086 Lake Angitola, collect some species to be used as biological indicators, analyze the indicators and finally propose executive plans to build stations increase of aquatic biodiversity. The Lessona's frog (*Pelophylax lessonae*) has been one of the studied biomarkers. Amphibian populations have suffered severe depression caused by radical environment changes (1) and by the occurrence of emerging diseases such as those caused by ranavirus (2; 3) or other infections fault of introduction of alien species. In the past, but also today, for the diseases diagnosis on minor fauna, specimens championships were obtained by sacrifice. Recently, several non-cruel and/or non-lethal protocols have been proposed to find out the causative agents of diseases on small or very small animals (4). In order to not inflict unnecessary suffering on animals, taking samples for laboratory diagnosis (blood or small pieces of liver) must be practiced under sedation. While most of the authors indicates sedation as a prelude to euthanasia (5), in this paper we want focused the possibility of obtaining viable samples usable by laboratories without recourse to the animal suppression; though of particular biological importance, may also be re-introduced in the places where they were captured.

MATERIAL AND METHODS
In May 2014, 21 post metamorphosis frogs (*Pelophylax lessonae*) were captured in Lake Angitola (Serre Regional Park - Calabria, Italy). The capture was carried out during the project Angitola $FISH_2O$ (EFF Calabria Cod. 02/BA/12 – authorized June 28, 2013 prot. n. 756). The animals were placed in plastic box and transported (EC Regulation 1/2005 Annex I Chapter II, III) at the Dept of Veterinary Medicine and Animal Production (UNINA). After five days animals were transported into the surgical room of the Departmental surgery section where an anaesthetic protocol was applied in 16 steps. During sedation period, blood sampling was made, breathing rate was counted, and toe reflex was observed. Step 1: Preparation of the surgical room; Step 2: Manual individual pick up from the transport container and breathing rate count (RRC T0); Step 3: Single frog put into the induction

chamber; Step 4: Isoflurane dispensing; Step 5: Pending anaesthetic induction (RRC TI=T6); Step 6: Individual weight control over electronic scale; Step 7: Frog positioning on the surgical table; Step 8: Fifteen minutes waiting (RRC T20=T22); Step 9: Cardiac access by mesosternal puncture for blood sampling; Step 10: Twenty minutes waiting (RRC T40=T48); Step 11: Subdivision of the blood sample in capillary blood count and Eppendorf for infectious disease laboratory; Step 12: Thirty minutes waiting (RRC T80=T81); Step 13: Frog repositioning into individual box with bottom water before awakening; Step 14: Waiting for the awakening and excitation phase (RRC TE=T130); Step 15: Finger solicitation to cause reflex retraction of hind leg (RRC TR=T180-T228); Step 16: Frog repositioning into containment box.
Information and dosages of the drugs are derived from the University of Minnesota that stresses "no protocol guarantees the safety or effectiveness". Since the only reliable method of blood collection in amphibians is the cardiac puncture, and because the amphibians cardiac ventricle is close to *xiphisternum*, and since the latter position may vary significantly from species to species, routine sampling is practiced by prior dissection of sacrificed individuals (6). During steps 2, 4, 8, 10, 12, 14 and 16 breathing rate was noted. Isoflurane was administered with a precision vaporizer in a special box at the concentration of 5% in oxygen (flow rate of 4 litres per minute). To prevent the occurrence of traumatic injuries Lessona's frog was controlled by placing the subject in a suitable container, 40 cm x 20 cm.

RESULTS

The effect of deep anaesthesia was achieved on average, in all the subjects examined, in 5.96±0.98 minutes. Graph 1 shows that in this period the breathing rate significantly decrease to reach zero (83±12 *vs* 0; P <0.001) together with the complete disappearance the withdrawal reaction of the hind limb. After about 22 minutes from T0, the anaesthesia becomes shallow, the breathing rate starts to increase significantly (P <0.01) and, passing through a short excitatory phase (from T81 to T130; P <0.01), it reaches the initial values at 180 (light and middle weight frogs) and 228 minutes (heavy weight frogs) after induction (T6). In general, it was found that the heavier frogs showed a more pronounced and significant (P <0.01) decrease in breathing rate when compared with lighter subjects (Graph 2). Despite the trend of the 3 compared groups of frogs seem more or less overlapped between them, the heavier subjects presented a more prolonged anaesthetic depth and significantly different (P <0.01) from the other 2 examined groups.



	T0	T6	T22	T48	T81	T130	T180	T228
All frogs sampled	83	0	34	33	39	91	82	80

	T0	T6	T22	T48	T81	T130	T180	T228
Light weight	100	0	40	28	31	104	94	
Middle weight	80	0	42	40	42	100	84	
Heavy weight	70	0	21	30	42	70	68	80

Breathing rates trends: in all the examined frogs (Graph 1) and in the three weight groups (Graph 2).

DISCUSSION AND CONCLUSION

All the frogs start life as water tadpoles, breathe underwater through internal gills and their skin. When then later turn into land animals, mouth closed, they breathe air with the lungs and retain the ability to breathe through small blood vessels (capillaries) present under the outer skin layers. Both

the pre-metamorphosis, in the presence of the gills, both in the post-metamorphosis, in the presence of the lungs, breathing is controlled by the button throat that sucks air through the nostrils to the lungs. The exhalation is accomplished through contractions of the body. The anuri are provided with a respiratory system complex and may be anesthetized by immersion in the anaesthetic solution, applying anaesthetics on the skin or by entering them in an induction chamber with anaesthetic gas. Our work confirms that using an anaesthetic gas, total and complete animal recovery was reached also if in a long time (180-228 minutes). The amphibians pulmonary breath ceases altogether during anaesthesia and, in our sample, the cessation of the rhythmic breathing frequency had a duration ranging from the 6th minute to the 15th minute after induction, confirming that the skin breathing is sufficient to prevent hypoxia during clinical anaesthesia. The recovery was complete and the all animals regained their natural position in the awakening box. All the animals were re-released in an open environment after 7 days from the anaesthesia allowing us to say that the method of anaesthetic induction with isoflurane may be used safely even on vulnerable and endangered species. Anaesthesia with isoflurane on amphibians guarantees the four fundamental anaesthesia moments: 1) Induction (decreased skin movements in the golar region and slower rate of withdrawal reflex of the hind legs); 2) shallow anaesthesia (loss of righting reflex and disappearance of abdominal breathing); 3) Deep anaesthesia (No withdrawal reaction at pinch finger), disappearance of skin movements in the golar region and immobility of the subject; 4) Awakening in a reasonable time and total recovery of subjects.

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INVESTIGATION ON POTENTIALLY INVASIVE RABBITS AND RAT POPULATIONS ON STATE NATURAL RESERVE OF VIVARA ISLAND

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ABSTRACT
Vivara is a small volcanic island used by the Bourbon Kings as a hunting reserve (18th – 19th Century) which is why *Oryctolagus cuniculus* were introduced. The introduction of *Rattus rattus* is linked to the construction of a footbridge between Vivara and Procida. In the past decade an uncontrolled expansion of these two pest species was described. To assess the actual extent of their population, and to quantify their impact, a monitoring campaign was carried out during the 2014. With respect to previous reports, the results demonstrate a significant decline of the two populations. This reduction appears dependent on natural causes.

INTRODUCTION
The Management Plan of the State Natural Reserve "Isola di Vivara", drawn up in April 2012, reports (p. 33) "The mammals of Vivara are typical of insular environments and include a few species, mostly introduced by man. These entries were made voluntarily, as easily conceivable for the rabbit (*Oryctolagus cuniculus*), or involuntary, as easily presumable for the Black Rat (*Rattus rattus*), as often occurring when man occupies large or small Islands."
The Plan reports the officially recognized method for estimating rabbit population in a sample area but does not report data on the past or the actual consistency of the population; for the black rat neither the methods of evaluation nor the population consistency data are reported.
The Management Plan also recognises that an excessive presence of these two species might have important ecological implications of the labile insular ecosystem equilibrium: both rabbits and black rats are potential threaten for trees and shrubs and also for bird nestlings and small invertebrates. As an in-depth study of these species was among the priorities of the Plan, and considering the alarms raised by the population, we set a census counting methodology so to estimate the number of individuals present on the island.

MATERIALS AND METHODS
Within the project "Environmental Retraining of Vivara Island 2013 – 2014" the survey was carried out from June 12th, 2013 to May 30th, 2014. All signs of rat and rabbit presence (droppings, carcasses, bony remains, hairs, food residues, dens, etc.) were photographed and, when possible, collected. Areas in which their frequency resulted higher were reported on maps, in identical grid scale, using an autocad program. In these areas attractors (feed or food) and trail cameras set for night vision were placed.
Rabbits census was carried out by counting faecal pellets, using the method proposed by Taylor and Williams (1956) modified by Moreno and Villafuerte (1992). In particular, the density of rabbits (D) on the survey area is given by the formula D=d/r*t where “d” is the number of faecal pellets found in each site; “r” is the mean number of faecal pellets produced per day by an individual rabbit in the Mediterranean region (comparable to that reported for other regions; Moreno and Villafuerte, 1992; Wood, 1998); “t” is the number of days of faecal accumulation.
The two rat species (*Rattus rattus* and *Rattus norvegicus*) were surveyed using an indirect method based on the weighing of all the droppings found in the sampling areas; the selection of the number of droppings per day issued by an individual and average weight of daily excrements (different in the

two species). The application of a conversion formula allowed calculating the number of individuals who attended a certain area. The density of rats (D) on the island were determined according to the formula D=(n*ŵ/Ē)/ŵ where "n" is the number of droppings collected in the sampling area; "ŵ" is the weight (g) of droppings per individual (*Rattus rattus*: 15-30 g; *Rattus norvegicus*: 25 – 50 g) and "Ē" is the number of droppings produced per day per animal (*Rattus rattus*: 30-150 per day; *Rattus norvegicus*: 30 – 180 per day).

RESULTS

According to the calculations, the estimated population of rabbits on the Vivara Island surface is of about 14 adult individuals in an area of about 40 hectares that is 0.35 rabbits/1 hectare (Table 1).
The estimate on the different sampling areas reports the population of *Rattus rattus* amounting to 836 individuals and that of *Rattus norvegicus* of 322 individuals. Calculated per hectare, the three species counts an average of 0.35, 23 and 9 individuals respectively (Table 1).

Table 1. Number of rabbits (*O. cuniculus*) and rats (*R. rattus* and *R. norvegicus*) calculated on the whole island surface (~40 hectares) and on 1 hectare.

Specie	Vivara Island ~40 hectares Number of adult animals	Animal Density for 1 hectare Number of adult animals
Oryctolagus cuniculus	14	0,35
Rattus rattus	836	23.22
Rattus Norvegicus	322	8.93

Table 2. Number of rabbits (*O. cuniculus*) and rats (*R. rattus* and *R. norvegicus*) calculated on the different sampling area.

Sampling area	*Hectares*	*Oryctolagus cuniculus*	*Rattus rattus*	*Rattus norvegicus*
Olive grove	2.30	1.1633	0.0000	0.0000
Meadows	2.73	1.2157	0.0000	0.0000
Oak woodland	7.35	1.9073	3.5112	0.0000
Scrubland	10.50	3.2330	3.7620	0.0000
Halophile area	8.48	5.8758	3.8456	0.0000
Large cellar	1.00	0.3500	410.4760	131.1828
Napoleonic	0.95	0.3325	61.8640	11.9141
Summit area	1.12	0.3920	352.3740	178.9032
Vivara Island	34.43	14.4696	835.8328	322.0001

DISCUSSION AND CONCLUSIONS

The estimated population of wild rabbit in the Vivara Natural State Reserve indicates a minimum number of residents, a result in line with the IUCN that consider the *Oryctolagus cuniculus* as a nearly threatened species. A protection action seems therefore essential to save the few samples of wild rabbit still present in the protected area. Preservation of wild population appears essential for maintaining the island trophic chain and, in particular, for maintaining herbaceous species under control and sustaining the population of birds of prey. Conversely, the reserve should make an effort to remove from the population the individuals deriving from domestic breeds, introduced for hunting purposes, but more dangerous today for the phylosophy of extreme animalism and the animal charity. These are true aliens that may adversely affect the trend of the wild rabbit population.
The estimated population of rats indicates the presence of a relatively low number of individuals, for both black rat and grey rats. They presence is essential since they represent an important source of protein supply for birds of prey, such as the Barn Owl. As demonstrated in other areas, also on Vivara Island they tend to keep a grouped distribution so that in certain areas their presence is considerable

in other only occasional.
This study quantifies the number of individuals with a relatively high approximation but represents the first quantitative description of these populations present on Vivara Island. The results, though needing a check and an ongoing monitoring plan, allow to affirm that the black rat population on Vivara is not a threaten being in line with what described for other small islands of the Mediterranean Sea and definitively lower than that reported for big islands or continental areas.

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EUROPEAN GOVERNANCE FINALIZED TO BIODIVERSITY PRESERVATION TOWARDS THE 2020 TARGET

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INTRODUCTION

The report "Global Biodiversity Outlook" published in the framework of the Convention on Biological Diversity, confirms that environmental policies worldwide have failed by not getting the significant reduction in the rate of biodiversity loss planned by the year 2010.
The unordered exploitation of natural resources, the fast consumption of land and the incessant pollution of water and air, has exacerbated the critical points, it has long been highlighted by experts and scientists, beyond which it will be difficult, if not impossible, restore the original production state of "Earth system". The large-scale changing of ancient ecosystems as the rainforest and the coral reefs are caused by the impact of climate change, deforestation, fires, water eutrophication; by acidification of the oceans combined with the heating of the water; from non-sustainable fishing and by pollution from nutrients. It is not to be underestimated, however, the significant reduction in the cultural and economic value exercised by indigenous peoples, always accustomed to the use of renewable resources in the full respect of their resilience. With the "decade of biodiversity 2011-2020", proposed by the UN it takes note of the failure of years of false environmental policies and it seems groped to give additional time to the conservation actions, but aware that 2020 not will be the decisive year for restore of biodiversity as clearly indicated in the vision of the "Strategic Plan for Biodiversity 2011 – 2020 and the Aichi Targets" citing: “By 2050, biodiversity is valued, conserved, restored and wisely used, maintaining ecosystem services, sustaining a healthy planet and delivering benefits essential for all people.”
It appears quite clear that, if are not taken innovative measures, effective and coordinated action to reduce the pressure on biodiversity, we will not be able to get different results from those obtained so far, as well as a continued loss of biodiversity and worsening of climate change.
The Habitats Directive (together with the Birds Directive) forms the cornerstone of Europe's nature conservation policy. It is built around two pillars: the Natura 2000 network of protected sites and the strict system of species protection. All in all the directive protects over 1 000 animals and plant species and over 200 so called "habitat types" (e.g. special types of forests, meadows, wetlands, etc.), which are of European importance.

MATERIAL AND METHODS

The management and control of activities related to natural environments (protected and unprotected) and those of the agro-ecosystems have been analyzed. The 28 Countries of the European Union differently adopt the Birds Directive (Council Directive 79/409/EEC amended by Directive 2009/147/EC on the conservation of wild birds) and the Habitats Directive (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora, and Enlargement Changes).
The local and State regulations about management of wildlife (hunting and breeding) and of non-urbanized areas (agriculture, environmental tourism, protected areas, environmental improvements) do not always correspond to the suggestions of Directives.
The official EU sites were consulted and indicative data about the management of wildlife and habitats in each of the 28 Member States have been extrapolated.

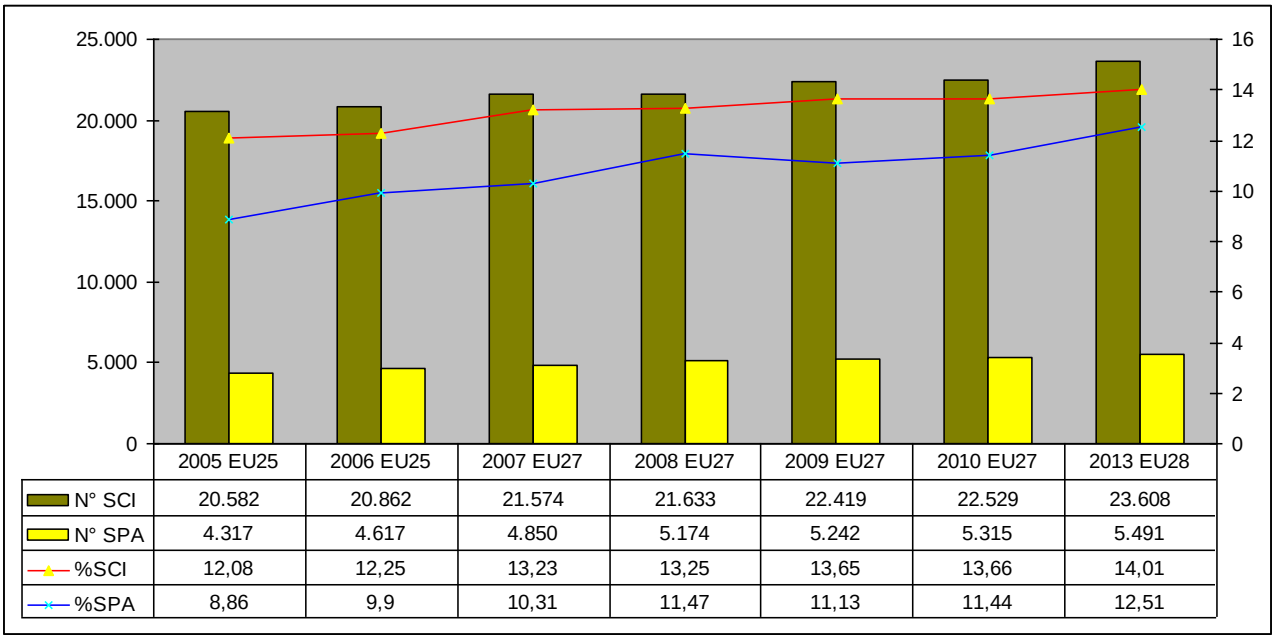
RESULTS

The obtained results have been generic and fragmentary, it is not possible to identify the management systems applied by each Member State to the protection of habitats and birds, and even to the exercise of hunting, breeding of wild species and the exploitation of natural resources.
However, what is clearly shown for each of the 28 European Union countries turns out to be the number and the areas in which it should apply the Habitats and Birds Directives.

- **Sites of Community Importance (SCI) and Special Protection Areas (SPA).**

On graph 1 we compare the official data about SCI and SPA, including both terrestrial and marine areas, for the different EU Member States. The results show a slight increase from the year 2005 to 2013, but considering that the member countries have become 28, it can be stated that all the countries, for operations of bird conservation and preservation of habitats, have only confirmed the proposed site from the start of the operation Natura 2000. If from the SCI and SPA surface are extracted marine areas, keeping only the terrestrial areas, we understand that 39% of the countries Members allocates more than 20% of the territory Agro - Forestry to SCI and SPA; 53% of member countries between 10 and 20% and two countries (7%) less than 10% (Table 1).

The current rate of soil loss by sealing through urban expansion and infrastructure in the Netherlands amounts to ca. 36 ha per day. In other countries of Europe, like Germany (120 ha per day), Austria (35 ha per day) and Switzerland (10 ha per day), similar soil losses by sealing through infrastructure and buildings have been observed. This urban sprawl increases the costs of urban infrastructure, traffic in urban areas, and energy consumption, and has negative effects on the quality of the countryside and the environment. This development is in direct competition with agricultural land uses and is threatening valuable agricultural soils all over Europe
(http://ec.europa.eu/agriculture/envir/report/en/inter_en/report.htm)
This consideration suggests that the year 2020 we will have to write down a further reduction of available habitat to biodiversity that will be replaced by pressing soil sealing and urbanization, as shown in the graphs included in the table 1.

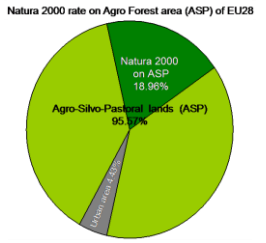


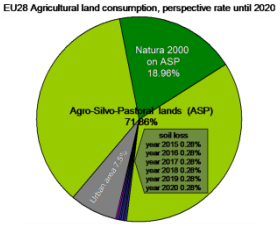
	2005 EU25	2006 EU25	2007 EU27	2008 EU27	2009 EU27	2010 EU27	2013 EU28
N° SCI	20.582	20.862	21.574	21.633	22.419	22.529	23.608
N° SPA	4.317	4.617	4.850	5.174	5.242	5.315	5.491
%SCI	12,08	12,25	13,23	13,25	13,65	13,66	14,01
%SPA	8,86	9,9	10,31	11,47	11,13	11,44	12,51

Graph 1. Number of SCI and SPA from 2005 to 2013 on the EU Member States)

Table 1. Total surface and land use (km^2) of 28 EU Member States.

MS	total area	%	Urban area	ASP	Nature 2000	%/ASP	%/Total	
DK	43.093	7,43	3.201,81	39.891,19	3.583,97	8,98	8,32	
UK	244.820	7,37	18.043,23	226.776,77	20.883,77	9,21	8,53	
LV	64.589	2,10	1.356,37	63.232,63	7.449,37	11,78	11,53	
LT	65.301	2,98	1.945,97	63.355,03	7.890,29	12,45	12,08	
FR	549.192	5,13	28.173,55	521.018,45	69.127,41	13,27	12,59	
MT	316	3,30	10,43	305,57	40,70	13,32	12,88	
IE	70.280	2,25	1.581,30	68.698,70	9.222,30	13,42	13,12	
SE	414.864	1,60	6.637,82	408.226,18	57.409,66	14,06	13,84	
FI	338.145	1,41	4.767,84	333.377,16	48.851,29	14,65	14,45	
CZ	78.866	6,35	5.007,99	73.858,01	11.061,53	14,98	14,03	
NL	41.526	12,24	5.082,78	36.443,22	5.563,06	15,27	13,40	
AT	83.859	4,87	4.083,93	79.775,07	12.559,48	15,74	14,98	
BE	30.528	20,67	6.310,14	24.217,86	3.882,99	16,03	12,72	
DE	357.031	8,41	30.026,31	327.004,69	55.142,12	16,86	15,44	
EE	45.226	3,03	1.370,35	43.855,65	8.076,35	18,42	17,86	
LU	2.597	5,80	150,63	2.446,37	469,48	19,19	18,08	
IT	301.333	4,89	14.735,18	286.597,82	57.137,06	19,94	18,96	
PL	312.685	4,00	12.507,40	300.177,60	61.059,32	20,34	19,53	
PT	91.990	3,36	3.090,86	88.899,14	19.009,51	21,38	20,66	
HU	93.030	6,01	5.591,10	87.438,90	19.949,74	22,82	21,44	
RO	238.391	6,29	14.994,79	223.396,21	53.787,67	24,08	22,56	
ES	504.782	1,99	10.045,16	494.736,84	137.365,09	27,77	27,21	
GR	131.940	2,92	3.852,65	128.087,35	35.761,28	27,92	27,10	
CY	5.736	3,30	189,29	5.546,71	1.628,01	29,35	28,38	
SK	48.845	5,80	2.833,01	46.011,99	14.441,54	31,39	29,57	
BG	110.910	4,10	4.547,31	106.362,69	38.066,47	35,79	34,32	
HR	56.594	3,05	1.726,12	54.867,88	20.674,70	37,68	36,53	
SI	20.273	2,73	553,45	19.719,55	7.673,22	38,91	37,85	
EU28	4.346.742	4,43	192.416,79	4.154.325,21	787.767,37	18,96	18,12	





- **Hunting.**

Sustainable hunting and the "Birds Directive"

The European Union recognizes the legitimacy of hunting and describes this as an activity that provides significant social, cultural, economic and environmental benefits in many part of Europe.
However, not to undermine the conservation efforts undertaken for some species (listed in Annex II) of the Birds Directive it is necessary to regulate the hunting activities in manner to ensuring sustainability towards habitats and animal populations subject to the hunting picking.
Facts: currently there is a factsheet that summarize the provisions relating to hunting according to the Birds and Habitats Directive, factsheet that indicates also the mutual benefits of Natura 2000 and the hunting activities.
Actual results: currently there isn't a document for single State Member of EU in which it is possible read the concrete scope of that card, and therefore this instrument would not reflect the real results of the theorized sustainability.

Strategy to achieve sustainable hunting

To achieve concrete results concerning the correct application of the habitats and birds directives, the focus was on the promotion of dialogue and cooperation between different interest groups.
- 2001, the Commission launched an EU 'Sustainable Hunting Initiative' (contribution to the understanding of the legal and technical aspects to develop conservation programs and sustainable hunting with the involvement of the scientific structures.
- 2004 BirdLife International and the Federation of Associations for Hunting and Conservation of the EU - have reached an agreement on ten points which will enable the correct application of Directive 2009/147 / EC consistently with the hunting activities.
- 2007 The Standing Committee of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern in 1979), has adopted the European Charter on Hunting and Biodiversity (Recommendation No. 128/2007).
Facts: currently there is a Charter that intends to strengthen the implementation and coherence of the instruments of global biodiversity and Europe.
Actual results: currently there is a Treaty open for signature by the member States, the non-member States which have participated in its elaboration and by the European Union, and for accession by other non-member States conducive to the sustainable hunting by Council of Europe. There isn't a final document signed for single State Member of EU, and therefore this instrument would not reflect the real results of the theorized sustainability.

EU Management plans for huntable bird species considered to be in unfavourable status

The "Birds Directive" allows for certain species (Annex II), to be hunted provided this is done in a way that will not jeopardize conservation efforts for the species.
The European Committee in collaboration with the ORNIS committee and NGOs (FACE, BirdLife International, OMPO, Wetland International) has developed specific management plans for huntable species considered to be in an unfavourable conservation status.
Facts: currently there are Management Plans that should be assist the Member States of their obligations under the Birds Directive.
Actual results: currently Management Plans are not legally binding documents nor do they engage the Member States beyond their existing legal commitments under this Directive. Therefore this instrument would not reflect the real results of the theorized sustainability.

- **Protected areas.**

Actually International Union for Conservation of Nature (IUCN) brings together States, government agencies and a diverse range of non-governmental organizations in a unique world partnership (over 1000 members in all, spread across some 140 countries) and represent the most authoritative organisation for the biodiversity protection reports. IUCN seeks to influence, encourage and assist societies throughout the world to conserve the integrity and diversity of nature and to ensure that any use of natural resources is equitable and ecologically sustainable. The World Conservation Union builds on the strengths of its members, networks and partners to enhance their capacity and to support global alliances to safeguard natural resources at local, regional and global levels. The World Commission on Protected Areas (WCPA) is the world's leading global network relating to protected areas. It comprises 1400 protected area specialists from over 140 countries. The IUCN Programme on Protected Areas (PPA) serves as the Secretariat for WCPA. WCPA's mission is to promote the establishment and effective management of a world-wide representative network of terrestrial and marine protected areas, as an integral contribution to the IUCN mission.
The world is on track to meet a 2020 target on the expansion of protected areas, but more work is needed to ensure areas of importance for biodiversity and ecosystem services are prioritized for

protection under equitably managed conditions, according to a new United Nations Environment Programme (UNEP) report released today at the International Union for the Conservation of Nature (IUCN) World Parks Congress. The report finds that 15.4% of terrestrial and inland water areas and 3.4% of the global ocean are now protected—highlighting growing global awareness of the need to safeguard the natural resources that will play a crucial role in the upcoming Sustainable Development Goals.

Effective management

Effectively managed protected areas conserve biodiversity and habitats. However, by 2013 only 29% of the total area of nationally designated protected areas had been assessed for management effectiveness. Lack of effective management remains one of the largest problems facing the current global protected area system. More management effectiveness assessments, plus a greater focus on measuring biodiversity and social outcomes, are needed.

Equitable management

There is weak reporting and little available data on equitable management, both of which need to be strengthened to provide meaningful assessments of how equitable protected areas and other kinds of conservation areas are managed.

Connectivity

Available evidence on corridors indicates they have a positive conservation benefit. Despite a growing number of large connectivity projects, there is little knowledge of the level of connectivity between conservation areas across the wider landscapes and seascapes. Connectivity principles should be better incorporated into national planning and climate change adaptation programmes.

Benefits to people and nature

Protected areas deliver numerous benefits for people and nature and need to be recognized as a proven and cost-effective natural way to deal with global challenges such as water provision, food security, disaster-risk reduction, and climate change mitigation and adaptation. This should be fully acknowledged by integrating protected areas into national planning and decision-making processes across all sectors.

- **Wildlife breeding.**

Captive breeding is the process of breeding animals in controlled environments within well-defined settings, such as wildlife reserves, zoos and other commercial and noncommercialconservation facilities. Sometimes the process includes the release of individual organisms to the wild, when there is sufficient natural habitat to support new individuals or when the threat to the species in the wild is lessened. Captive breeding programs facilitate biodiversity and may save species from extinction. Release programs have the potential for diluting genetic diversity and fitness.
Actually three types of wild breeding are recognised:

Meat production: Activities few developed in the EU countries so that the quantity produced does not is statistically reported if not minimally and generically.
In Italy, for example in the game section are included quail, pigeons, partridges, pheasants and red-legged partridges (the latter three grouped under "other game") for which a total recording of animals slaughtered 25 million, of which 24 million quails. The production amounted to about 4,000 tons, and the yield to 68.1% of the live weight. The average weight of each category is between 0.2 kg and 0.9 kg for the quails to the category "other game" in which the greatest weight is determined by pheasants. A special case is the wild boar.

Breeding of restocking game: Mainly the small game birds (pheasant, partridge, red partridge) and lagomorphs (hare and wild rabbit).
This type of farming is few or not controlled, it is not easy to estimate the real production in terms of numbers. These animals are brought into the public and privatet lands where hunting are practiced.

In some countries this action is only possible when hunting is closed, while in others even during the hunting season. Only in Italy it is estimated that each year are released throughout the country about 1 500 000 pheasants, 400 000 partridges; red-legs partridges 1 000 000, 1 500 000 hares.

Animal breeding used for ornamental, recreational and for the preservation of local traditions: This type of farming, in Italy, is not permitted for the hoofed species (wild boar, deer, bovidae) and, in the case of hunted species, may not be held more than a number different for each species and in different Regions. The subjects raised must be accompanied by appropriate health certification produced by the local competent Veterinary service. In some cases animals can be used for restocking, with the approval of the Province and the hunting territorial organisation (ATC). All birds reared belonging to wild species may be exposed in exhibitions and for singing competitions only if identified by irremovable and numbered ring. A comprehensive estimate of the number of these animals has never been done nor are available statistics about the number of species and birds growing in this form.

DISCUSSION AND CONCLUSION

The Strategic Plan of Decade consists of a shared vision, a mission, strategic goals and 20 ambitious but attainable targets. The Strategic Plan serves as a flexible framework for setting national objectives and regional and promotes the consistent and effective implementation of the three objectives of the Convention on Biological Diversity. The wide strategic plan and action integrates biodiversity considerations into the key issues of sustainable development and human security.

The media play a key role in transferring knowledge about the importance of biodiversity, reaching different audiences not referring to the typical coverage that takes place in scientific reporting (competences on environment and nature).

A key role is allocated to the local authorities that, although sensitive to issues relating to the management of biodiversity, they fail to approve operational plans and innovative laws diverging from the strict implementation of national legislation. Increasingly, implementation of Biodiversity Strategies and Action Plans takes place locally, normally unknown to the National Governments that also have an important role to implement the EU and international conventions.

To get a change of course is necessary that the different worlds involved in the management of resources and the environment, do not close objective only on own priorities and interests, but share common goals which, if brought to the attention of policy makers will be able to indicate and implement new strategies to reduce the loss of biodiversity without compromising the economic development. Starting from the causes of the failure to achieve the 2010 target and based on sustainable use of resources, the strategic planning of UN Decade, is an opportunity to reconcile development with the conservation of biodiversity and guarantee the maintenance of services provided by ecosystems, essential to human society and welfare.

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HAEMATIC AND PHYSIOLOGICAL PARAMETERS COMPARISON IN THE EUROPEAN HARES (*LEPUS EUROPAEUS*) FOR HUNTING TRASLOCATION IN THE NAPLES TERRITORY

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ABSTRACT
Since stress generates pathological, behavioral and physiological alterations, the study of this phenomenon it appears to be crucial and it is especially for wildlife during capture and hunting managing (Esposito 2005). With the aim to evaluate stress conditions in hares utilized for hunting traslocation, some haematic and physiological parameters were studied on 90 subjects from 2011 to 2014 in Hunting Territory of Naples. The effect of farm breeding origin and the sex on haematic and physiological parameters was measured and analysed. Glucose, CPK and AST were found to be good parameters to measure stress condition in hares better than cortisol.

INTRODUCTION
The interest shown by the European market, and mainly by the hunting, against Genres Lepus, Sylvilagus Oryctolagus has stimulated the field of animal production that, using the ancient experiences, has transformed the techniques and technologies applied to the Lagomorphs' breeding. Knowledge of living beings, arises from methodological application of the three disciplines and the highlighted differences, are used to draw up technical protocols customized for preserving operations, or man's use. The study of the life of the animal world in all its manifestations (zoology) is the basic element of knowledge when man decides to act on a zoocenosis, to use it or defend it (Esposito et al., 2005). The study of the morphological, physiological and behavioral characteristics of the animals, their diseases and attitudes, then it is essential to determine its functional value and commercial (zoognostics), which once defined, all information will be translated and aimed at the reproduction of animals, to improve species and their commercial utilization (sustainable animal production).

MATERIAL AND METHODS
The trial was carried out on breeding hares from different farms in Central and South Italy. Animals born and reared in cages, were utilized for restocking activities managed by Hunting Territorial District of Napoli Province (ATC NA) between April to July from 2011 to 2014 and monitored by Monitoring Center "CReM ATCNA". A total of 90 hares were sampled before being released on the territory of the Napoli Province. During the transport from the farms to the check point of ATC NA, animal pairs (one male and one female) were hosted inside the darkened wooden capture-boxes. For blood collection single hare was removed from the box, hand restrained and blood was drawn from the jugular vein using disposable syringes 1 ml (26G X 1/2"). Rectal body temperature was measured by digital thermometer; heart rhythm by phonendoscope auscultation and respiratory rate by visual observation. Sex and age were evaluated to confirm data reported on sanitary documents.
The tubes with lithium heparin, centrifuged at 2000 rpm, separated the blood plasma that was used for laboratory tests (glucose, creatine phospho kinase (CPK), creatinine, aspartate amino transferase, total protein). The cortisol concentrations (17-α-hydroxycorticosterone) were determined by RIA based on the binding of ^{3}H-steroid for competitive adsorption (Fenske and Schonheiter, 1991).

The effect of farm origin and sex on haematic parameters was evaluated by ANOVA. The physiological parameters between males and females within each of the three groups were compared with the Student t test.

RESULTS

The effect of the sex on haematic parameters (Table 1) consists on an higher and significant values of cortisol and glucose on the females respect to males, and *vice versa* higher and significant levels of the metabolite (creatinine, creatine phospho kinase, total protein) in males than females. Also physiological parameters show significantly differences between males than females: rectal temperature was more higher in males than females (P≤0.01); as well as respiratory and heart rate (P≤0.01).

The cortisol and glucose trend is the same among the three groups in comparison, although the differences between males and females are significant only on industrial group.

For the other metabolites are confirmed the differences between males and females as described for totals except for the semi-industrial group in which females have higher values of total protein and of rectal temperature.

Table 1. Different haematic and physiological parameters registered in the studied hares

Parameters	Familiary			Semindustrial			Industrial			Total		
	n. 15	n. 15	P≤	15	15	P≤	15	15	P≤	45	45	P≤
Haematic	Females	Males		Females	Males		Females	Males		Females	Males	
Cortisol µg/dl	14.85 ±3.01	14.09 ±2.31	NS	14.13 ±5.44	12.78 ±3.65	NS	14.76 ±3.04	12.56 ±0.96	0.01	14.58 ±3.92	13.10 ±2.57	0.01
Glucose mg/dl	240 ±15.68	210 ±17.98	0.001	217 ±47.95	233 ±54.43	NS	230 ±26.93	210 ±25.19	0.05	229 ±33.64	217 ±36.93	0.05
Creatinine mg/dl	0.82 ±0.04	0.88 ±0.05	0.01	0.80 ±0.16	0.86 ±0.18	NS	085 ±0.05	0.87 ±0.04	NS	0.83 ±0.10	0.87 ±0.11	0.05
AST U/l	155 ±7.83	137 ±20.36	0.001	142 ±26.98	173 ±91.39	NS	142 ±5,49	131 ±44.27	NS	146 ±17.26	146 ±61.35	NS
CPK U/l	1 997 ±499	2 653 ±462	0.001	1 942 ±589	2 558 ±755	0.05	1 870 ±427	2 450 ±489	0.001	1 936 ±501	2 554 ±588	0.001
Tot Protein mg/dl	4.81 ±0.63	4.96 ±0.58	NS	5.13 ±1.09	4.78 ±1.01	NS	4.50 ±0.51	5.13 ±0.64	0.01	4.81 ±0.81	4.96 ±0.77	0.01
Physiological												
Age months	6 ±0.12	6 ±0.12	NS	4 ±1.5	4 ±1.5	NS	7,2 ±1.0	7.5 ±0.9	NS	5.7 ±1.67	5.87 ±1.74	NS
Weight gramms	2 990 ±0.14	2 910 ±0.09	0.05	2 360 ±0.23	2 486 ±0.13	0.05	2 904 ±0.32	2 561 ±0.30	0.01	2 752 ±0.37	2 653 ±0.27	0.05
Rectal Temp. °C	39.50 ±0.48	39.85 ±0.85	0.05	39.88 ±0.32	39.64 ±0.55	0.05	38.63 ±0.78	40.15 ±0.44	0.05	39.34 ±0.75	39.88 ±0.53	0.01
Respiratory rate n./min	103 ±19	141 ±28	0.001	199 ±19	232 ±19	0.001	140 ±23	108 ±12	0.001	147 ±45	160 ±57	0.001
Heart rate n./min	104 ±6	155 ±22	0.001	224 ±21	259 ±18	0.001	128 ±28	120 ±31	NS	152 ±56	178 ±64	0.001

DISCUSSION AND CONCLUSIONS

Analysis of the results showed that, regardless of the type of management applied during the breeding of hare, the handling, transport and subsequent release, also in suitable areas under an environmental and vocation point of view for the specie, animals are stressed. As reported also by other authors (Paci et al., 2006) cortisol does not seem the best indicators of acute stress in hares. In

our study hormone appears to be higher in all subjects tested, although with a higher level in females than males. The fear, manipulation and possible trauma in the transport boxes seems produced muscle damage evidenced by the high values of CPK and AST. A clear metabolic response related to the state of the acute stress is witnessed by the high glucose levels in both males and females but statistically higher in the latter reflecting the more intense reactive response of the females.

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THE EFFECT OF AGE ON MEAT CHARACTERISTIC AND FATTY ACID PROFILE OF TWO HUNTED WILD UNGULATES; WILD BOAR (*SUS SCROFA*) AND FALLOW DEER (*DAMA DAMA*)

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ABSTRACT
The increase of wild ungulates populations induced an increase of harvesting and meat availability in the market and for home consumption. The aim of this study was to evaluate the nutritional quality of the wild boar and fallow deer meat at two different age classes, determining some physical characteristics and the fatty acid profile. Thirty-one wild boars and twenty-nine fallow deer were included in the trial. The age differently influenced chemical characteristics and FA profile of meat of the two species. The meat of fallow deer and wild boar is a low-calorie food that contains lower saturated fatty acid content than others species. Specifically, fallow deer muscle is a good source of long-chain n−3 PUFA, which are beneficial for human nutrition.

INTRODUCTION
In Italy, the wild ungulates populations has been growing since the 1990s. Wild ungulate, in particular wild boar and fallow deer could provide a noticeable amount of meat with remarkable nutritional characteristics (Quaresma et al., 2011; Ramanzin et al., 2010), although the variability of meat quality of these species is considerable in wild conditions, and it depends on many factors (Razmaite et al., 2012; Amici et al., 2015). However, the information concerning its nutritional quality, particularly of the lipid fraction, is scarce. The lipid fraction is of major importance to the characterization of meat nutritional quality (Wood et al., 2004).The aim of this study was to evaluate the nutritional quality of the wild boar and fallow deer meat at two different age classes, on some physical characteristics and the fatty acid profile.

MATERIAL AND METHODS
Thirty-one wild boars were subdivided in two groups on the basis of age, young and adults, estimated from tooth eruption. 15 wild boars were assigned to young (13.2 ± 10 months of age with a mean weight of 34.7 ± 12.84 kg) and 16 wild boars to adults (30.3 ± 10.93 months of age with an average weight of 65.86 ± 16.76 kg). 27 fallow deer were divided as explained above. 18 fallow deer were assigned to young group (13.1 ± 8.69 months of age with an average weight of 32.8± 11.9 kg) and 9 adults (26.3 ± 8.50 months of age with an average weight of 43.9± 9.41kg) were hunted during autumn in Central Italy. A portion of *longissimus thoracis* muscle from all the animals was taken and frozen at -80 °C in individual vacuum packing.Individual meat samples, after 2 months of freezing, were thawed out at 4°C for a night and after slightly dried to the surface, weighted to obtain the percentage of thaw loss by difference in weight. The shear force (WBS) was measured in four samples for each animal with a 1 x 1cm cross section and 2 cm of length, with a Warner–Bratzler shear attachment using an INSTRON 5543 texturometer (Chrystall et al., 1994). The lipids were extracted according to Folch et al., (1957). Statistical analysis was performed by GLM procedure of SAS, adopting a monofactorial model including age as factor and the mean values were compared by Fisher's LSD test, using $P<0.05$ as the significant difference level.

RESULTS ND DISCUSSION

The proximate composition is not affected by age of animals and showed 74.7% of moisture, 1.2% of ash, 2.6% of fat and 21.4% of protein for wild boar and 74.6% of moisture 1.38% of ash, 2.39% of fat and 21.32% of protein for fallow deer. No significant difference (table 1) was found for pH and percentage of taw loss, while younger animals showed meat more tender than adults as expected (P <0.001, P <0.05 respectively for wild boar and fallow deer).In fallow deer, fatty acids (table 1) showed in almost all data significant differences among ages except for monoinsatured fatty acids (MUFA), while in wild boar the difference was only in n-3/n-6 ratio (P <0.01). However, both young and adult wild boar showed low percentages of SFA (37.44% on average), a good P / S ratio (0.85) similar to the data reported in Sales and Radim, (2013). The meat of younger fallow deer had a higher nutritional value less (P<0.01) saturated fatty acids (SFA) and more (p <0.01) polyunsaturated fatty acids (PUFA), in particular n3 (6.39 vs 4.82%), also showed significant differences (p<0.001) for the P /S ratio. The high nutritional value of the meat is emphasized by all the parameters of fatty acids considered. The long- chain n-3 and n-6 PUFA contents in the muscle of wild boar were significantly lower compared to the contents in fallow deer muscle. The most abundant long-chain PUFA in the muscle samples in venison meat were 20:4n-6 followed by 22:5n-3 and 20:5n-3 particularly for deer (Quaresma et al., 2011; Dannenberger et al., 2013).

Table 1. Physical characteristics and fatty acids profile[(1)] of *longissimus thoracis* muscle of wild ungulates

	Wild Boar			Fallow deer		
	Young	Adult	Signif.	Young	Adult	Signif.
Physical characteristcs						
pH	5.62	5.63	ns	5.60	5.64	ns
Taw loss %	5.22	5.00	ns	9.23	10.19	ns
WBS (N)	3.73	5.81	***	3.36	4.35	*
Fatty acids						
SFA	36.72	38.17	ns	40.95	46.03	**
MUFA	31.44	30.32	ns	22.77	25.51	ns
PUFA	31.84	31.52	ns	36.28	28.46	**
n3	3.16	2.50	ns	6.39	4.82	**
n6	28.68	29.02	ns	29.89	23.64	*
P/S	0.87	0.83	ns	0.90	0.63	***
n3/n6	9.07	13.11	**	4.87	4.93	ns

Note: * p<0.05; **P<0.01; *** p<0.00; ns non significant; [1] The fatty acids were expressed as a percent of the total methylated fatty acids. SFA : Σ C12:0,C14:0, C 15:0, C16:0, C17:0, C18:0; MUFA Σ C16:1,C18:1trans-11, C18:1cis-9; PUFA Σ C18:2n-6, C18:3n-3, C20:3n-6, C20:4n-6, C20:5n3, C22:4n6, C22:5n3, C22:6n-3

CONCLUSIONS

The meat of fallow deer and wild boar is a low-calorie food that contains lower saturated fatty acid content than others species. Fallow deer muscle is a good source of long-chain n-3 PUFA, which are beneficial for human nutrition.

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TOXICOLOGICAL STUDY OF NEPHROTOXICITY OF HEAVY METALS AND BISPHENOL A IN COMBINATION

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ABSTRACT
Wild animals are exposed to harmful effects of various substances occuring in environment polluted by human activity. The aim of the work was to monitor effect of the toxicity of heavy metals cadmium chloride dihydrate, potassium dichromate, zinc sulphate heptahydrate and bisphenol A alone or in mutual combination on model cell line rabbit kidney cells (RK 13). The cell response was monitored by using real-time cell analyser xCELLigence system and metabolic activity of treated cells was examined by colorimetric MTT test. The results confirmed the importance of testing combined toxicity of chemical soccuring in environment.

INTRODUCTION
The wild animals are exposed to harmful effects of substances occurring in polluted environment. Many plastic products in environment contains substances with undesirable effects on live organisms. They impair environment, human and animal health by their presence in water, soil, plants. Bisphenol A belongs to the group of harmful substances arising from the rigid plastics – polycarbonates (Fernandez et al., 2007). Bisphenol A has become ubiquitous in the environment within the past 80 years because of its presence in a multitude of products including food and beverage packaging, flame retardants, adhesives, building materials, electronic components, and paper coatings (Flint et al., 2012). Important environmental pollutants are also heavy metals – naturally occurring elements. Their multiple industrial, domestic, agricultural, medical and technological applications have led to their wide distribution in the environment; raising concerns over their potential effects on human health and the environment. Their toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals. These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure (Tchounwou et al., 2012). The first target organ of heavy metals toxicity is kidney, because of its ability to reabsorb and accumulate divalent metals (Barbier at al., 2005). There are many physiologic a pathologic changes in cells (adherence and cell cycle changes, DNA impairment, apoptosis and necrosis) after toxic substances exposition. These changes differ according to origin, concentration of substances and time of cells exposure. The aim of the work was to monitor the nephrotoxicity of heavy metals *Potassium dichromate, Zinc sulfateheptahydrate, Cadmium chloride dihydrate and* carbon-based synthetic compound Bisphenol A. The response of model rabbit kidney cell line RK13 to exposure was monitored by real-time cell analyser xCELLigence system during the whole time of experiment.

MATERIAL AND METHODS
Real-time monitoring of cell changes was conducted by using xCELLigence system. The model cell line RK13 (rabbit kidney cells) were cultured for 24 h in 16-wells E-plate (Roche Applied Science, Germany), in EMEM medium (Minimum Essential Medium Eagle, Sigma Aldrich, Germany) with 10% of foetal bovine serum, in 37 °C and 5% humidified atmosphere. After 24 h cells were treated with bisphenol A (55 $mg.l^{-1}$), potassium dichromate $K_2Cr_2O_7$ (1 $mg.l^{-1}$), zinc sulphate heptahydrate ($ZnSO_4$.

$7H_2O$ (100 $mg.l^{-1}$), cadmium chloride dihydrate ($CdCl_2$. $2H_2O$ (10 $mg.^{-1}$) and their mutual combinations.The concentration were selected according to previous studies and IC50 determination Gold microelectrodes at the bottom of E-plates record resistance. The higher amount of adhered cells, the higher resistance and vice-versa. The resistance value means change in cell status (proliferation, adherence, morphology) and it is expressed in cell index (Ke at al., 2011). Cell index (CI) was recorded into curves by xCELLigence system each hour during the whole time of experiment that is a great advantage in comparison to other cytotoxic end-point analyses. Results were statistically evaluated by using GraphPad Prism 5.0 Software, Anova Dunnet´s comparison test. The results were expressed as mean (n=3) and $P < 0.05$ was considered to be significantly different.

RESULTS AND DISCUSSION

Kidneys are able to resorb and accumulate metals that leads to intoxication, renal impairment and nephropaties (Barbier et al., 2005). Model renal kidney cell line (RK13) was exposed to heavy metals zinc (100 $mg.l^{-1}$), chromium (1 $mg.l^{-1}$), cadmium (10 $mg.^{-1}$) in combination with bisphenol A (55 $mg.l^{-1}$) in concentration chosen according to previous studies and IC50 determination.

Exposed cells were monitored by xCELLigence system (72 h) that recorded cell changes into curves. CI values were expressed in % by using the control cells (without treatment). In all treatments were observed significant changes in comparison to control cells ($P < 0.05$). The most significant decreasing of cell index was observed in combination bisphenolA and zinc. This combination decreased CI to zero in first hours after exposure. Interestingly, this combination was more toxic to cell as bisphenolA itself. Regarding theseobservations we can assume that compounds with a relatively low toxicity may in some circumstancesaffectverysignificant toxic effectifthey occur in biological systemtogether. In assessing thesafety ofbiologicalsubstances it is very important to take into consideration the fact, that there is a possibility of combined toxicity in living organisms.

CONCLUSION

Results of the study showed that bisphenol A in combination with zinc significantly affect kidney cell behaviour. This study pointed out the importance of combined toxicity of substances occurring in environment, dangerous for wildlife.

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ANIMAL BIODIVERSITY CONSERVATION: THE BROWN BEAR IN THE NATIONAL PARK OF ABRUZZO LAZIO AND MOLISE

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ABSTRACT
In the period 2000 – 2014, this study estimates number of dead bears registered in the National Park of Abruzzo, Lazio and Molise. The mortality was compared to the number of damages attributable to bears. In 15 years of monitoring 39 dead bears were recovered: 2.6 subjects/year (4 – 5%) on the total of Marsicanus Italian population (47 – 61 heads). A negative correlation was found between the number of dead bears and the damages to crops or livestock, so to confirm the human action on the preservation and impairment of the population of this animal, which means a critically presence of the Italian plantigrade population.

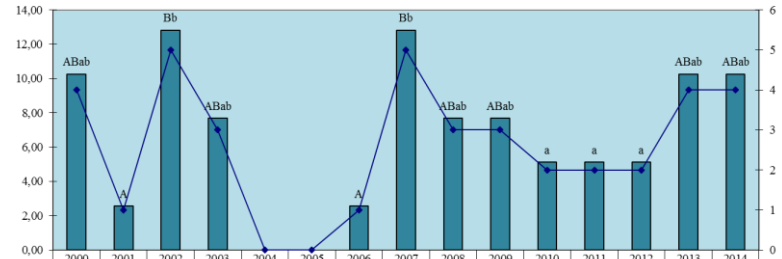
INTRODUCTION
When are considered species which play an important ecological role into a food web, as the carnivores, it is easier to understand the mechanisms that have resulted in their reduction, as well as it is also easier to verify the results of conservation efforts. The wolf and the bear suffered a population decline mainly because of intense persecution and due to the attitude of the humans considering large carnivores very negative when they share some territories. All conservation actions, therefore, depend on the degree of tolerance of humans penalized by the predation of livestock and related transactions to reduce the conflicts on the territory (Luck et al., 2003; Margules and Pressey, 2000; Purvis et al., 2000). This work aims to identify the number of Marsicanus bears who make up the population of bear in the National Park of Abruzzo, Lazio and Molise (PNALM), the trend of the population, the main causes of death and damage related to livestock.

MATERIALS AND METHODS
The study of available data highlighted the number of subjects of the *Ursus arctos* subspecies *marsicanus* in the area of PNALM. Data collection was carried out through direct cooperation with the official veterinarian and technicians of the Park. After the data collection, we proceeded to their statistical analysis through the chi-square test for comparison of qualitative data, the Student t test for quantitative data on mortality and damage to agriculture and animal husbandry. Finally to evaluate the relationships existing between the mortality and damage we calculated Pearson correlation.

RESULTS
The bear population within the PNALM would vary, according to the census in 2011, between 47 and 61 wild specimens, all of *marsicanus* subtype.
The Park also features of three Faunistical areas (Visitor Center of Pescasseroli; Faunistical area in Villavallelonga; Faunistical area of Campoli Appennino) all fenced. The first area hosted 1 Marsicanus

sterilized female; the second one hosted 2 Marsicanus brown bears, and the third 1 Marsicanus female and 1 male of European brown bear (castrated).
Figure 1 shows the mortality (number and percentage) of bears in the National Park during the time between 2000 and 2014. Figures 2 and 3 show the percentages of bears' death in male and female and the percentages of the main causes of mortality.

Figure 1. Number and percentage of dead Brown Bears into PNALM.



	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Death %	10,26	2,56	12,82	7,69	0,00	0,00	2,56	12,82	7,69	7,69	5,13	5,13	5,13	10,26	10,26
Dead N.	4	1	5	3	0	0	1	5	3	3	2	2	2	4	4

Figure 2. Sex percentage of dead brown bears into PNALM.

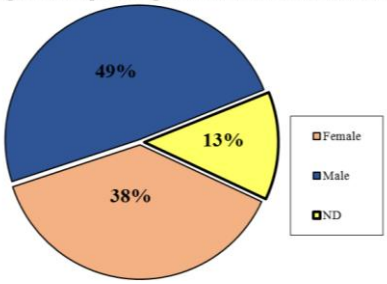


Figure 3. Percentage of causes of brown bears' death into PNALM

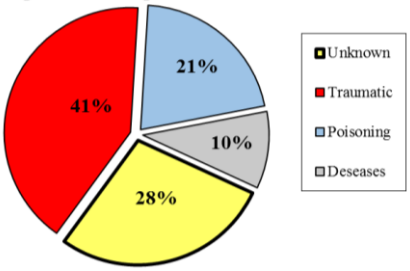
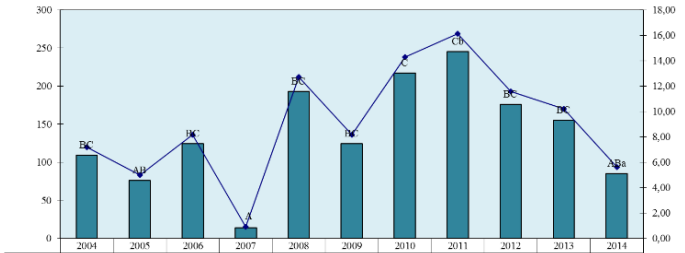


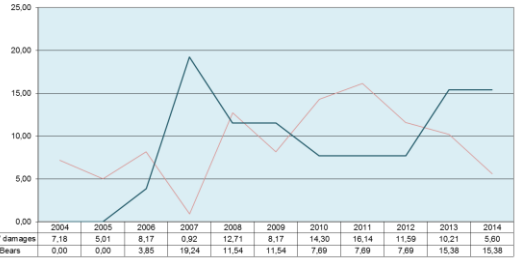
Figure 2 shows the percentage of males and females recovered in the period from 2000 to 2014 (September), calculated on the total number of carcasses or skeletal remains (n = 39). The figure shows a greater involvement of males although this result is not statistically significant.
The traumas are resulted the main cause of death (41% vs 28% vs 21% vs 10%; P<0.001 for the first two and P<0.05 for the last), related mostly to gunshot wounds, car and rail accidents, even if there is to specify that a large portion of deaths (28%) has to be attribute to unknown causes (Figure 3).
The Figure 4 shows the number of damage certified from predation: the year 2007 is the one with the lowest number of damage (14 complaints), while 2010 and 2011 resulted the years in which they have registered the highest and significantly number of certified damage (respectively 217 and 245 complaints).

Figure 4. Number and percentage of brown bears' damages into PNALM.



	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Damages N.	109	76	124	14	193	124	217	245	176	155	85
Damages %	7,18	5,01	8,17	0,92	12,71	8,17	14,30	16,14	11,59	10,21	5,60

Figure 5. Relationship between percentage of dead brown bears and brown bears' damages.



	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Brown Bears' damages	7,18	5,01	8,17	0,92	12,71	8,17	14,30	16,14	11,59	10,21	5,60
Dead Brown Bears	0,00	0,00	3,85	19,24	11,54	11,54	7,69	7,69	7,69	15,38	15,38

Figure 5 highlights the correlation between the number of damage from predation and mortality: it can be seen that to decreasing percentage of predator death, increases the number of certified damage.

DISCUSSION AND CONCLUSIONS

The number of dead bears in the park or in the Outer Protection Area (ZPE) appears to be constant over the years, with the exception of 2004 and 2005, years in which no dead bear was found. The largest number of dead bears was recorded in 2002 and 2007 (5 bears per year) followed by 2000, 2013 and 2014 (4 bears per year). The increase in mortality due to trauma, poisoning and disease, put in alarm the management bodies responsible for protecting these important carnivores.

The evaluation of the bear damages number recorded in the 2004 – 2014 period (Figure 4) shows that, over time, damages are repeated more or less with the same frequency. Significant differences ($P < 0.01$) were recorded for the years 2008, 2009 and 2011. Figure 5 shows that, considering the trend in the number of damages, the damage increases with decreasing the number of bears found dead ($r = -0.19$).

Overall, as reported by the EU-IUCN data (Osti, 1997; Febbo and Pellegrini, 1990), the bear population of PNALM can be judged stable. However, the small number that makes up the isolated core of Marsicano brown bear present in Italy and the mortality attributable to humans faults, oblige to retain the bears of PNALM critically endangered.

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ANIMAL BIODIVERSITY CONSERVATION: THE WOLF IN THE NATIONAL PARK OF ABRUZZO LAZIO AND MOLISE

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ABSTRACT
In the period 2004 – 2014, it is estimate the number of wolves found dead in the National Park of Abruzzo, Lazio and Molise, and the mortality results copared to the number of damages. The results describe an average of 9 wolves dead every year (24 – 30%) and confirm a good state of wild wolf population estimated at about 30 – 38 heads (5 – 6% of the Italian population). A negative correlation was found between the number of wolves death and the damage, so every year it was possible to describe an higher mortality corresponding to a lower amount of damage, reversed in the following year.

INTRODUCTION
Many human activities, previously compatible with the persistence of other alive species, have become threats to biodiversity and the anthropic influence acts directly on the species or habitats. The most worrying phenomena include the degradation, the fragmentation and the destruction, of some types of habitat with the consequently difficulty of many animal species to survive. To counter this phenomena, it is essential to start from the knowledge of endangered species by estimating the number that composes up the population and the elements that contrast their survival.
This paper aims to assess the actual presence of the wolf (*Canis lupus*) in the territory of the National Park of Abruzzo, Lazio and Molise (PNALM), and evaluate the critical points that emerged from the data relating to the damage caused by the canidae to the animal breeding.

MATERIALS AND METHODS
The experimental trial has been developed, in collaboration with the PNALM, using data and experiences on wolf population in the protected area. Data were collected on special forms in which it was reported: the number of wolves found dead in the park's territory; the cause of death; the sex; the damage to livestock predation; the finding place. The data collected have been ordered hierarchically, purged from aberrations and subjected to appropriate statistical analysis. Chi square test was used (for comparison of qualitative data different by significant probability for $P<0.05$ and $P<0.01$); Student's t test have been employed producing significant differences for $F = P<0.01$ (quantitative data on agriculture-livestock damages and wolf mortality). The Pearson correlation coefficient has been utilized to evaluate the relationships between mortality and the damages.

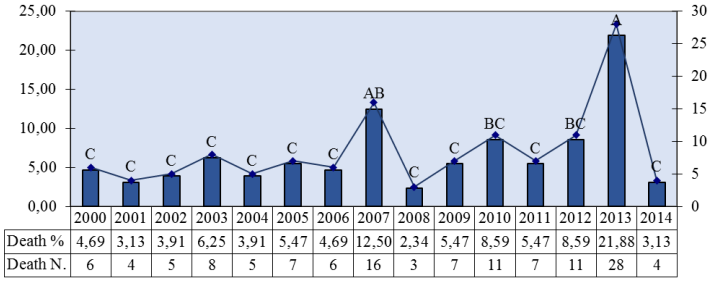
RESULTS
Figure 1 shows the mortality of wolves into the protected area in several years of measurement (2004 – 2014). In Figure 2 are illustrated mortality rates related to sex. Mortality incidence appears to be greater in males than females, although not statistically significant. The main causes of death (Figure 3) are related to the traumas that, in percentage, are greater than all other causes of mortality observed ($P<0.001$). A percentage of mortality of 9% is unknown. Figure 4 shows the number of damage from wolf predation estimated in PNALM. The percentage of wolves damages increases significantly from 2004 to 2008 ($P<0.01$) and then remains more or less constant in the

following years until 2014 in which there was a significant decrease of the damage (P<0.05). Figure 5 highlights the negative correlation between the number of damages from predation and mortality of wolves recorded from 2004 to 2014.

DISCUSSION AND CONCLUSION

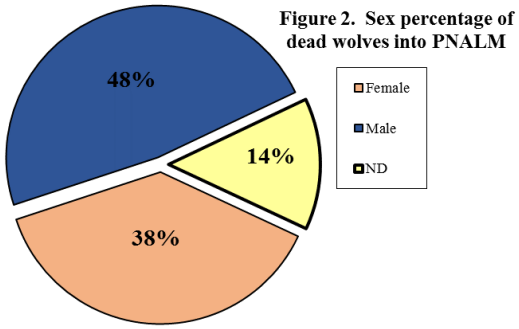
The examined data show that the number of wolves found dead inside the PNALM was rather stable. There aren't statistical differences among the years, with the exception of the years 2007, 2010, 2012 and 2013 (P<0.01) in which the number of deaths is greater than 10 and up to a maximum of 28 in 2013 (Figure 1). The mortality of wolves found is independent of sex (Figure 2) whereas the most recorded cause of death corresponded to traumatic events (P<0.001) due to road or rail accidents, gunshot wounds; traps (Figure 3). In relation to the livestock damages, Figure 4 indicates a biannual alternation with significant differences (P<0.01) in the years 2008, 2009 and 2011. The correlation between the amount of damage and the number of dead wolves is repeated every other year with the same frequency and it is negative (Figure 5): at the moment of the damages reduction, in the same year, we can describe an increases of the mortality rate of *canidae* (r = -0.018) and *vice versa*. Comparing our results with the official data concerning the wolf population into PNALM and those the EU-IUCN, we can confirm that the wild population of wolf leaving in the protected area is in good health and would range between 30 and 38 specimens.

Figure 1. Number and percentage of dead wolves into PNALM



	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Death %	4,69	3,13	3,91	6,25	3,91	5,47	4,69	12,50	2,34	5,47	8,59	5,47	8,59	21,88	3,13
Death N.	6	4	5	8	5	7	6	16	3	7	11	7	11	28	4

Figure 2. Sex percentage of dead wolves into PNALM



None of the subjects found dead belongs to the core present inside of a fenced structure of about 4 hectares (7 wolves including 4 adults and 3 puppies were born in the year 2014) or at the "Wildlife Park Visitor Center of Pescasseroli" where, currently, it has hosted an adult male found in difficulty, assisted, recovered and ready to be moved to more suitable accommodation.

Figure 3. Percentage of cause of wolf death into PNALM

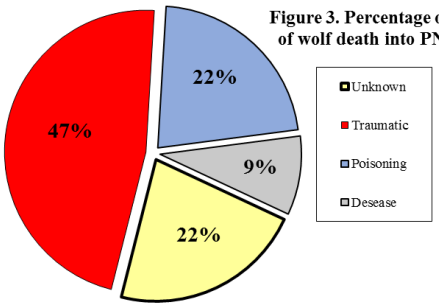
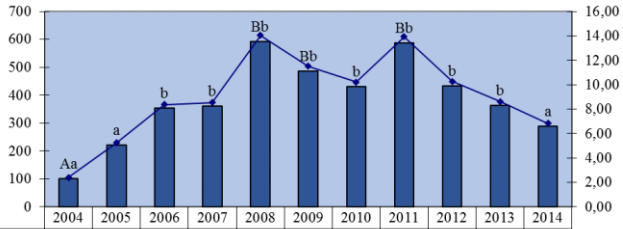


Figure 4. Number and percentage of wolf damages recorded into PNALM

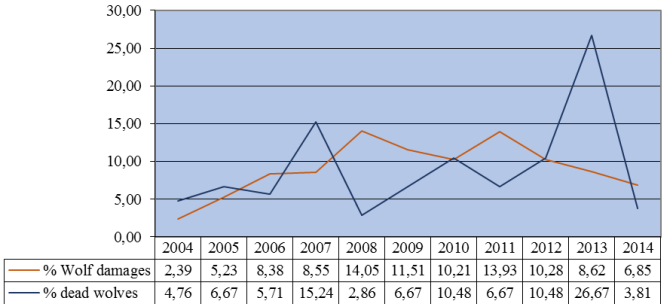


	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Damages N.	101	221	354	361	593	486	431	588	434	364	289
Damages %	2,39	5,23	8,38	8,55	14,05	11,51	10,21	13,93	10,28	8,62	6,85

The overall analysis of the wolf situation in the PNALM allows to estimate a certain stability of the wolf populations in the Park territory. Compared to Italian population, the wolves of PNALM

represent 5 – 6% of the national wild core, and confirm the data reported by the EU and IUCN that consider the population of *Canis lupus* (Gray Wolf) in a Status of least concern with a stable trend of the population that would be about 600 to 800 subjects in the whole Italian peninsula.

Figure 5. Relationship between percentage of dead wolves and wolf damages



	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
% Wolf damages	2,39	5,23	8,38	8,55	14,05	11,51	10,21	13,93	10,28	8,62	6,85
% dead wolves	4,76	6,67	5,71	15,24	2,86	6,67	10,48	6,67	10,48	26,67	3,81

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CORVIDS PRESENCE IN AGRICULTURAL, PASTORAL AND FORESTRY LANDS OF NAPLES

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ABSTRACT
The corvids cause extensive damage to agricultural crops, resulting in the loss of income by farmers. The evaluation of the number and structure of populations is one of the main actions proposed by ATC of Metropolitan city of Napoli before to designing and implementing any type of Hunting programming. Results of this study showed presence of hooded crow (*Corvus cornix*), jackdaw (*Corvus monedula*), jay (*Garrulus glandarius*) and magpie (*Pica pica*) on the lands classified as "Hill's farming" and "Mediterranean agriculture" of the Vesuvio and Sorrento areas.

INTRODUCTION
The evaluation of the number and structure of populations of different animal species, is one of the main actions for proper wildlife management. The management committee of a Territorial Hunting District can not ignore the knowledge of the presence and changes of status of wildlife populations before designing and implementing any type of Hunting programming (collection, re-peopling, translocation, management plans, environmental improvements, etc.). For these reasons the management committee of a Territorial Hunting District of Naples has organized a plan of survey and monitoring, (as reported on the new Wildlife and Hunting Plan of the Province of Naples 2013-2018 (paragraph 5.7 pg. 99) and reported in the Multiannual Program Plan of the same Naples ATC).

MATERIAL AND METHODS
As of August 2014, researchers and technicians began a census of the so-called "opportunistic" species and particularly of the corvids (hooded crow, jackdaw, jay and magpie). The areas East of the Province of Naples (Area 1 Vesuvio and Area 2 Sorrento) were studied.
Standard methods of census were used for the determination of hooded crow (*Corvus cornix*), jackdaw (*Corvus monedula*), jay (*Garrulus glandarius*) and magpie (*Pica pica*).
The contact points (sighting of individuals or of social structures) were transferred on maps (GIS service of Dept of Veterinary Medicine and Animal Production – University of Napoli Federico II).

RESULTS
Figures 1, 2, 3, 4 show the presence of hooded, jackdaw, jay and magpie respectively in the Sorrento and Vesuvio area.

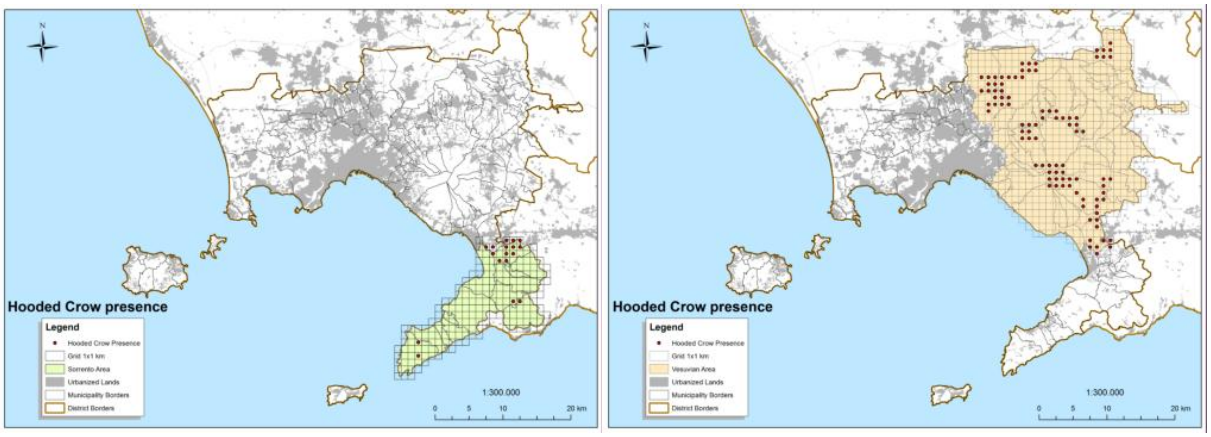

Figure 1. Sorrento and Vesuvio Hooded presence

The hooded crows (*Corvus cornix*) are distributed mainly around the Vesuvius Vulcan and the North-West part of this area, and on the top of the Sorrento area.
The jackdaw (*Corvus monedula*) are distributed mainly on the central part of the Vesuvius area, and on the peripheral North of the Sorrento area.

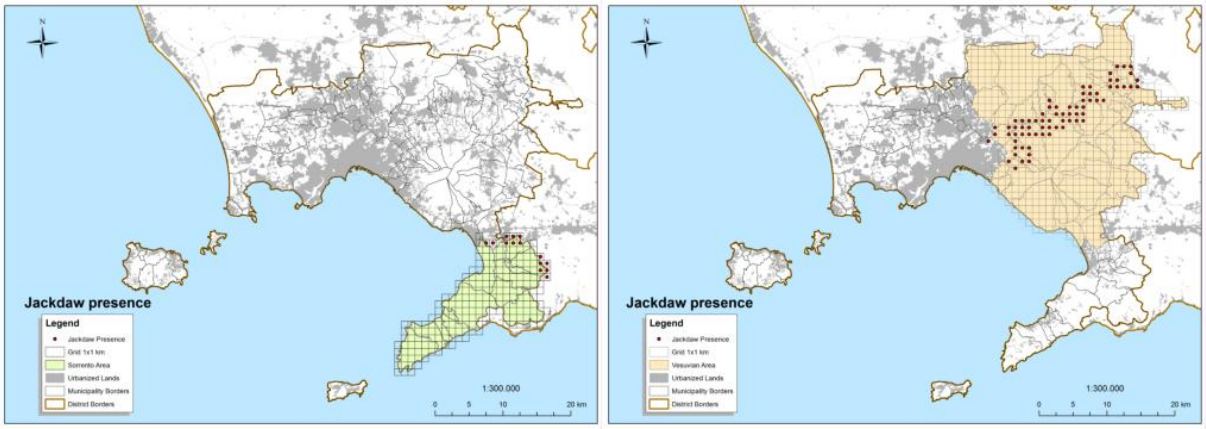

Figure 2. Sorrento and Vesuvio Jackdaw presence

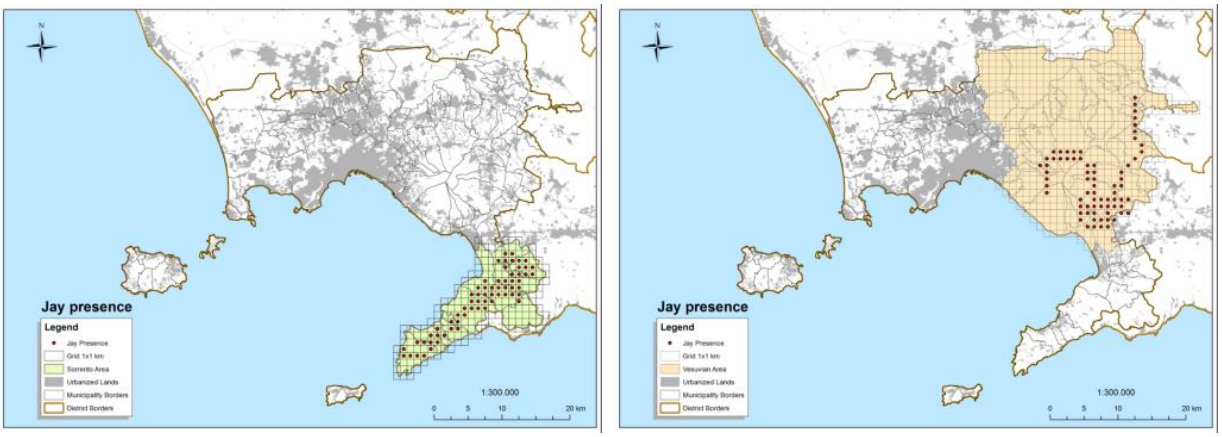

Figure 3. Sorrento and Vesuvio Jay presence

The jay (*Garrulus glandarius*) are distributed mainly on South-East of the Vesuvius area, and on the central part of the Sorrento area.
The magpie is spotted distributed on the plain lands both of the Vesuvius and Sorrento areas

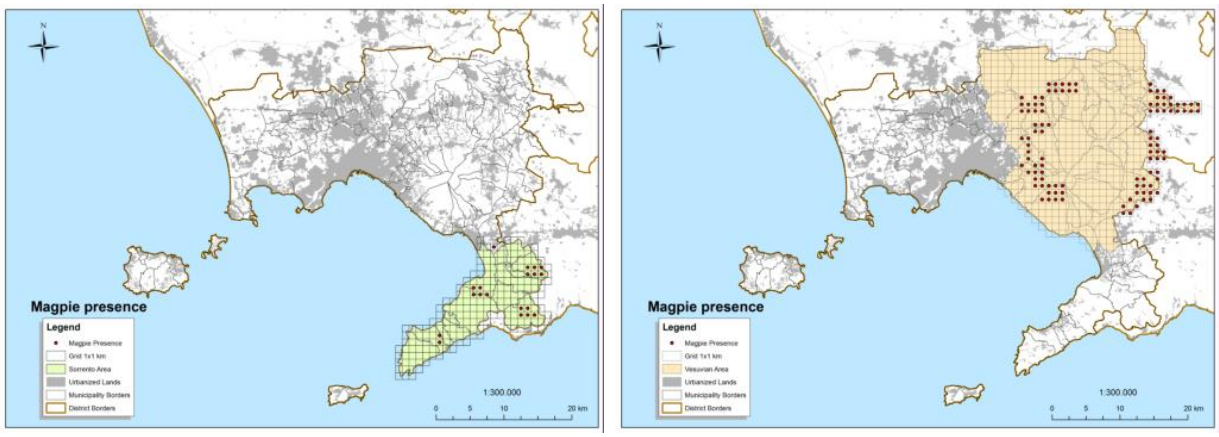

Figure 4. Sorrento and Vesuvio Magpie presence

DISCUSSION AND CONCLUSION

In recent years, with the increase of degraded areas and simplification of habitats, the corvidae seem to be overly present on our territory. Vesuvio and Sorrento areas have been identified, in the Naples plan for managing fauna of 2013 – 2018, according to the dominances vegetation: Area 1 predominant environment "Hill's farming" and Area 2 predominant environment "Mediterranean agriculture". It is no coincidence, therefore, that the Naples ATC has decided to wage a campaign of acquiring reliable data on these two species on which, if necessary, you will have to implement special control measures.

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ACKNOWLEDGEMENT

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THE PILOT STUDY ON ECOLOGICAL FACTORS INFLUENCING THE OCCURRENCE OF GASTRO-INTESTINAL PARASITES IN THE TATRA CHAMOIS (*RUPICAPRA RUPICAPRA TATRICA*)

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ABSTRACT

The pilot research on gastrointestinal parasite fauna of the Tatra chamois (*Rupicapra rupicapra tatrica*) as a significant representative of the Tatra Mountains endemic fauna species was initiated in 2013. In total 212 fecal samples have been examined to date for the presence of helminth ova and coccidia oocyst using standard flotation methods. The results revealed the overall prevalence of gastrointestinal parasites in chamois of the Slovak part of the High Tatra reaching 58.5%, whereas the faecal samples from the Western Tatra showed significantly lower occurrence of intestinal parasites. The initial research on gastrointestinal parasites of the Tatra chamois introduced one disputable finding - a relatively high prevalence of the genus *Moniezia* with nearly 30% of samples being positive in the Slovak part of the High Tatra that is significantly more in comparison with other European studies. Thus, further intensive research on is needed in broader temporal, ecological and zoological contexts to illuminate the influence of climate and microclimate conditions and intermediate hosts distribution on the presence of this parasite taxa.

INTRODUCTION

The Tatra subspecies of chamois (*Rupicapra rupicapra* Linnaeus, 1758) described in 1972 Milíč Blahout as the Tatra Mountains endemic subspecies - *tatrica* (*Rupicapra rupicapra tatrica* Blahout, 1972). The autochthonous population of the Tatra chamois is maintained in the territory of High and Eastern (Belianske and Western) Tatra, situated of 77.6% in Slovakia and 22.3% in Poland.

The chamois distribution area in the Tatra Mountains comprises habitats of alpine and subalpine vegetation zone and partly also the upper limit of the forest terrains and subnival. Chamois prefer the subalpine and alpine plant vegetation level that lie in very cold to slightly cold zones with an average annual temperature fluctuating between +3°C to -4°C. Horizontal expansion of Tatra chamois in the Slovak part of Eastern and Western Tatras is in the west - east 19º39'30 "E - 20º18'40" E and in south - north 49º08'18 "N - 49º15'35" N. In the vertical direction the Tatra chamois occur from the elevation of around 1 200 m a.s.l. to 2 630 m a. s. l., with a focus occurrence at altitudes from 1 700 and 2 200 m a. s. l. (Chovancová and Krystofik, 2013).

Chamois is a typical herbivorous cloven-hoofed animal living in open habitats of mountain ecosystems. Favorite biotopes of chamois in the Tatra are valley bottoms or mild grassy slopes with interspersed rocky cliffs and humid valleys. Due to derangement during the tourist season they move into inaccessible, although less nourishing areas in the exposed terrain.

Trophic base of chamois depends on food supply, which is in the alpine environment characterized by wide variations in connection with its seasonal availability. They forage mainly on evergreen perennials, grasses, herbs, but also on parts of trees, lichens, ferns and mosses (Newbies and Chovancová, 2010).

The natural factors affecting the numbers and health of chamois include carnivores, in particular lynx, less frequently wolves and rarely bears, foxes, golden eagles and ravens. The abundance of chamois is also affected by catastrophic events, extreme weather, anthropogenic threats, high traffic and increasing sport activities, and poaching (Chovancová and Gömöry, 1999). In terms of health of chamois game, an important role play parasitic infections that can significantly affect the condition of

the entire population. The aim of our work is to ascertain the distribution of parasites of the gastrointestinal tract in the current climate and environmental conditions of the Tatra Mountains.

MATERIAL AND METHODS

Chamois fecal samples were obtained during field data collection in various locations in the Tatra Mountains during spring and autumn 2013 and spring and summer 2014. In the area of Slovak High Tatra 164 samples from 24 locations were collected; 33 samples were sampled from the Polish part of the High Tatra and 15 samples came from Western Tatra. The presence of propagative stages of gastrointestinal parasites was investigated in the laboratory by standard coprological methods using Sheather and Faust flotation solution.

RESULTS AND DISCUSSION

The overall prevalence of gastrointestinal infections by parasites in the Slovak part of the High Tatra was 58.5%. Most frequently were present protozoa - *Eimeria* spp. (41.5%), of helminths tapeworm of the genus *Moniezia* (29.3%), and the family Trichostrongylidae (9.1%). Sporadically were detected eggs of *Nematodirus* spp. and *Capillaria* spp. Individuals from the Polish side of the Tatra Mountains were infected with *Eimeria* spp. (21.2%), *Moniezia* spp. (15.2%) and nematodes of the family Trichostrongylidae (9.1%). Examination of 15 faecal samples from chamois from the Western Tatra showed a significantly lower incidence of intestinal parasites; in one sample oocysts of *Eimeria* spp. and in another one strongylid eggs were present.

The pilot study on the incidence of gastrointestinal parasites in chamois raised one interesting question associated with relatively high prevalence of cestodes of the genus *Moniezia*. On the Slovak side of the High Tatra the propagative stages the parasites were present in almost 30% of the samples, which is significantly more compared with results from other European studies (Stancampiano et al., 2001; Hoby et al., 2006; Morrondo et al., 2010; Marreros et al., 2011). These differences may be due to the occurrence of suitable intermediate hosts (mites from the family Oribatidae) in the environment that is affected by the climate and microclimate conditions, and changes in habitat and land use. The post-calamity research in the High Tatra revealed after the storm in the year 2004 an increase in population density of soil mites (and overgrowth of especially oribatid species), but simultaneously confirmed the decline in species diversity (Kalúz et Ferenčík, 2008).

CONCLUSION

The results of our pilot epidemiological survey have raised questions whose clarification will require continuing intensive research on the abundance and distribution of chamois parasites, especially in the broader timeframe, ecological and zoological context.

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LIVER FLUKES IN WILD RUMINANTS IN SPECIFIC ENVIRONMENTAL CONDITIONS OF SLOVAKIA

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ABSTRACT

The aim of the study was monitoring liver flukes in wild ruminants including red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and mouflon (*Ovis musimon*) in selected regions of Slovakia. Between 2013 – 2015 we examined 572 faecal samples from wild ruminants using coprological methods and method of Kleimann et al. (2005). None of sample was positive for *Fasciola hepatica* and *Fascioloides magna*. 6.29% of samples were positive for *Dicrocoelium dendriticum*.

INTRODUCTION

Liver flukes in wild ruminants, occuring in Slovakia, are important parasites belonging to the genera *Fasciola*, *Fascioloides* and *Dicrocoelium*. Natural infections of *F. hepatica* are associated mainly with domestic ruminants. The presence of *F. hepatica* in cervids generally are not associated with clinical signs or significant pathology. Infections of *F. magna* occurs primarily in cervids and bovids with formation of thin-walled fibrous capsules containing adult flukes. *Dicrocoelium dendriticum* occurs in the bile ducts of a wide range of domestic and wild mammals including species of ovids, bovids, suids, equids, cervids, rodents, lagomorphs, primates, and camelids (Mapes 1951), particularly sheep and mouflon.

Liver fluke development is very dependent of the environmental characteristics. *F. hepatica* and *F. magna* are found mainly in aquatic and moist environments, suitable for the survival of their hosts (freshwater snails belonging to the family *Planorbidae* and *Lymnaeidae*). By contrast, the habitat of the small liver fluke *Dicrocoelium dendriticum* (aslo known as „lancet fluke“) is in dry lowland or mountain pastures representing adequate conditions for development of terrestrial snails and ants (Otranto et al., 2003).

MATERIAL AND METHODS

Between 2013 – 2015 572 faecal samples from wild ruminants (493 red deer – *Cervus elaphus*, 16 roe deer – *Capreolus capreolus*, 8 fallow deer – *Dama dama* and 55 mouflons – *Ovis musimon*) we examined. Samples were taken from 3 regions of Slovakia (Prešov, Košice and Žilina). For liver flukes detection we used coprological methods (sedimentation method and faecal sieving- staining method by Kleimann et al. (2005).

RESULTS

None of sample was positive for the presence of *F. hepatica* and *F. magna* eggs. In 36 samples from wild ruminants we isolated characteristic *D. dendriticum* eggs. The most of positive samples were from mouflons (32 from 55 faecal samples – 6.29%). 4 positive samples were from red deers. Positive samples came from 2 of 3 investigated regions (Prešov and Košice region).

DISCUSSION

Environmental conditions in Slovakia are ideal not only for the development of native species, as well as the introduced, as recorded by our previous findings – 14.03% prevalence of fascioloidosis in the south of the Slovakia and *D. dendriticum* infections in mouflons (prevalence 3.74%) (Oberhauserová et al., 2012). No *F. hepatica* cases in wild ruminants were recorded, although infections in cattle are known especially in the northern regions of Slovakia and pastures shared with wild ruminants.

CONCLUSION

The results of previous study revealed areas with favorable environmental conditions for *F. magna* development situated along the Danube river in the south of Slovakia. Although no infection of *F. hepatica* in wild ruminants was confirmed but cases of fasciolosis in domestic ruminants are found. *D. dendriticum* is fixed mainly on domestic and wild ovids. Depence of liver fluke incidence from weather factors indicate, that climate change have a marked influence on their evolution. Therefore, it is necessary to continue with monitoring the occurence of liver flukes in the territory of Slovakia.

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LOSS OF ROAD KILLED CLOVEN-HOOFED GAME IN THE REGION KOŠICE-OKOLIE

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ABSTRACT
The aim of the study was to evaluate loss of road killed cloven-hoofed game (red deer, roe deer, wild boars) in the region Košice-okolie for the period 2010-2014. During this period it was 787 times to road collision with cloven-hoofed game (with roe deer 41.68%, red deer 14.49%, wild boars 10.93%, undetermined game species 32.90%). At these collisions the loss arises sixty hunting organisations dealing with game. The total length of roads in the district Košice – okolie is 569.051 km.

INTRODUCTION
Slovakia has a network of routes falling between one of the densest route networks in Europe, despite fewer motorways compared with other European countries. Overall route lenght is 18 044 km. In district Košice – okolie total lenght is 569.051 km (I. class routes 64.168 km, II. class routes 105.511 km, III. class routes 399.372 km) and from 2014 is open expressway R4 with lenght 14.145 km. With the increase in traffic is also increasing the number of collisions between vehicles and game. Meat of road killed game is considered as inedible. One of the main causes of road collisions is a migration of game, because this routes form often hardly crossable obstacle especially at night, when game activity is the highest.

MATERIAL AND METHODS
Data we obtained from protocols "Record from traffic accident" and "Record of verification notices – harmful event" that provided us with District traffic inspectorate in Košice-okolie.

RESULTS
During reporting period 2010 – 2014 we found 787 times to road collisions with cloven-hoofed game. From 2010 the largest number of collisions were with roe deer (328 times – prevalence 41.68%) in comparison with red deer (114 times – prevalence 14.49%), wild boars (86 times – prevalence 10.93%) and undetermined game species (probably roe deer and red deer – 259 times – prevalence 32.90%).
In assessing the entire period, the most collisions with game were on first class routes (I/50, I/68) as this is the busiest roads linking surrounding districts (Prešov, Trebišov, Rožňava, Spišská Nová Ves, Gelnica and MR).

DISCUSSION
Increasing traffic volumes brings people a lot of benefits but on the other hand the increase number of collisions between vehicles and game. One of the main causes of road collisions is a migration of game, because this routes form often hardly crossable obstacle especially at night, when game activity is the highest. Another cause of collisions are food sources. Game from forest sites passes through roads to agricultural crops (Herz, J., 2014).
In 2009 the first module introduced ,,Damage to game caused by transport" within the nationwide record hunting statistics allowing to record game killed on motorways. To introduce the module was due to the growing number of traffic collisions with game, which were previously recorded only in the overall game mortality (Bučko, J., 2009).

Table 1 Evaluation of the loss of cloven-hoofed game in the district Košice – okolie in the period 2010 – 2014

Game species	**Route class**	Year						
		2010	**2011**	**2012**	**2013**	**2014**	**2010-2014**	**Total**
Roe deer	I. class	23	18	28	28	46	**143**	**328**
	II. class	8	11	19	12	26	76	
	III. class	13	26	25	20	25	109	
Total:		**44**	**55**	**72**	**60**	**97**		
Red deer	I. class	14	11	16	20	19	**80**	**114**
	II. class	3	2	4	4	8	21	
	III. class	2	5	2	2	2	13	
Total:		**19**	**18**	**22**	**26**	**29**		
Wild boars	I. class	4	7	6	9	17	**43**	**86**
	II. class	3	0	3	7	8	21	
	III. class	2	2	5	4	9	22	
Total:		**9**	**9**	**14**	**20**	**34**		
Undetermined game species	I. class	13	18	24	18	20	**93**	**259**
	II. class	23	19	15	20	23	100	
	III. class	11	19	10	12	14	66	
Total:		**47**	**56**	**49**	**50**	**57**		
Number of collisions for the whole period								**787**

CONCLUSION

Among the proposals for measures to reduce collisions of cars with game include warning for drivers to danger of collision with animals (warning signs, reflective mirrors), preventing the game pass through the road (fencing of dangerous sections of the road, reducing the quality of the environment in the road, agricultural management, odor fences), reduction of game, use of new information and command brands, game detection.

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ECTOPARASITES (ACARINA AND INSECTA) ASSOCIATED WITH BATS IN INTRODUCTORY STUDY IN SLOVAKIA

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ABSTRACT

This introductory research present the results of a survey carried out in Slovakia during last two years (2013 – 2014). A total of 356 ectoparasites were collected from 81 bats. Ectoparasites belonged to 5 families and 6 genera of ticks and insects.

INTRODUCTION

The possible role of bats as reservoirs of pathogens transmissible to man makes an understanding of the composition of their parasite fauna highly desirable. The arthropod ectoparasites of bats (687 bat insect species are known) belong to the Siphonaptera, Diptera, Hemiptera, Dermaptera, and Acarina (ticks and mites). *Argas vespertilionis* belong to the most important because its role as vectors/reservoirs of bacterial, viral, and protozoan pathogens is poorly understood; however, several pathogens as e. g. *Coxiella burnetii* have been detected in these ticks (Černý, 1972). Recently, ectoparasites of bats presented as vectors of pathogens with potential significance for people, especially those species that inhabit human dwellings. The aim of this paper is to extend the information about the ectoparasitic fauna of bats in Slovakia. This presentation shows the results of a survey carried out in Slovakia during last two years (2013 – 2014).

MATERIAL AND METHODS

Trapping bats was done in collaboration with members and staff SONU and members of State Nature Protection on the basis of obtaining permits – Ministry of Environment exemption from the Act. 543/2002 on nature and landscape protection in 13 areas, namely: Jelšava, Hodruša-Hámre, Voznica, Rakovnica, Rákoš, Kolbelárovo, Revúca, Kokava nad Rimavicou, Nitra, Šaľa, Nandraž, Družstevná pri Hornáde and Košice. Ectoparasites were collected from bats captured in mist nets. They were removed from the wing membranes by the means of tweezers and conserved in vials containing 80% ethyl alcohol and subjected to laboratory diagnosis by microscopic morphological appearance using available keys (Dusbábek, 1963; Filipova, 1977).

RESULTS AND DISCUSSION

A total of 356 ectoparasites were collected from 81 bats. Ectoparasites belonged to 5 families and 6 genera of ticks and insects. We recorded ticks *Ixodes verspertilionis*, *Argas vespertilionis*; mites *Steatonyssus occidentalis*, *Spinturnix mystacina*, *S. bechsteini*; bat fly *Stylidia biarticulata* (Nycteribiidae) and one species of fleas, *Ischnopsyllus intermedius*. On average, 92% of bats were infested by ectoparasites, with a mean intensity of 6.72 individuals per bat. The occurrence of *Ixodes verspertilionis* on a bat in Czechoslovakia published already in 1963 Dusbábek on seven species of bats and Ševčík et al. (2010) confirmed the finding and the other three species. We managed to get one female *I. vespertilionis* from *Myotis myotis*. Larvae of *Argas vespertilionis* were collected from *Pipistrelus pipistrelus* and *Myotis myotis* Šaľa and Hodruša Hámre. Mites of the genus *Spinturnix* that are localized mainly on flying membrane of bats we received from all the trapped animals. According to the Krištofík and Danko (2012), in recent years in Slovakia were recorded 14 species of mites of the genus *Spinturnix* on 24 species of bats from different parts of Slovakia. *Steatonyssus occidentalis* was diagnosed in three species of bats *Myotis myotis*, *Miniopterus schreibersii* and *Nyctalus noctula*.

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PLATYHELMINTHES OF OWLS (STRIGIFORMES) IN SLOVAKIA

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During the years 2012 – 2014 forty-five cadavers of owls (Order: Strigiformes) collected from the territory of Slovakia were examined for the presence of parasites of the phylum Platyhelminthes, which includes flukes (class: Trematoda) and tapeworms (class: Cestoda). Examined birds belonged to 7 species: northern long-eared owl *Asio otus* (L, 1758) (N = 13), little owl *Athene noctua* (Scopoli, 1769) (N = 1), Eurasian eagle-owl *Bubo bubo* (L, 1758) (N = 2), Eurasian pygmy-owl *Glaucidium passerinum* (L, 1758) (N = 1), tawny owl *Strix aluco* L, 1758 (N = 10), Ural owl *Strix uralensis* Pallas, 1771 (N = 15) and common barn-owl *Tyto alba* (Scopoli, 1769) (N = 3). The helminths were isolated from the intestines of owls during helminthological autopsy, than they were washed in tapwater and conserved in 70% ethanol. Before species identification they were stained in carmine and mounted in Canadian balm. For determination of flukes we used keys and papers by Dubois (1968, 1970), Gibson *et al*. (2002), Sitko *et al.* (2001), and Bray *et al.* (2008) and for tapeworms it was Khalil *et al.* (1994), Joyeux and Baer (1936).

Two species of trematodes typical for the order Strigiformes, namely *Strigea strigis* (Schrank, 1788) and *Neodiplostomum canaliculatum* (Nicoll, 1914) were detected in the intestines of 10 out of 45 bird individuals and 3 out of 7 owl species. The species *S. strigis* of the family Strigeidae Railliet, 1919 was found in following three hosts: *A. otus* (P = 15.4%), *S. uralensis* (P = 13.3%) and also in one of two *B. bubo* (P = 50%). The intensity of infection in all three hosts ranged from 2 up to 17 flukes. In one case this trematode was found in *S. uralensis* in mixed infection with *N. canaliculatum*. The second trematode identified in our material is the representative of the family Diplostomidae Poirier, 1886. Except of 5 of 15 *S. uralensis* (P = 33.3%), *N. canaliculatum* was aslo detected in one *B. bubo* (P = 50%), but only one individual of this fluke was isolated from the intestine of this host. The highest number of *N. canaliculatum* in one host was 23.

Regarding cestodes three different parasite species were confirmed in 6 bird individuals belonging to 2 Strigiform species. *Paruterina candelabraria* (Goeze, 1782) of the family Paruterinidae Fuhrmann, 1907 infected three individuals of *S. uralensis* (P = 20%) and also one *S. aluco* (P = 10%). The lowest intensity of infection was 1 (*S. uralensis*) and the highest intensity was 4 (*S. aluco*) cestodes in one host. One more cestode of the family Paruterinidae belonging to the genus *Spasskyterina* Kornyushin, 1989 (P = 6.7%) was identified in our samples and in one bird *Choanotaenia strigium* Joyeux & Timon-David, 1934 (P = 6.7%) belonging to the family Dilepididae Fuhrmann, 1907 was detected. In both cases these cestodes was found in the small intestine of *S. uralensis*. The mixed infection with two different species of cestodes in one host was not confirmed. The trematodes and cestodes parasitizing together in one host were found in two *S. uralensis.*

Platyhelminthes of owls in Slovakia have not been comprehensively studied yet and therefore all of above-mentioned species trematodes and cestodes have not been confirmed so far in Slovakia. All our findings of parasites represent new records for Slovak fauna.

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MONITORING AND CONTROL OF WILDLIFE DISEASES IN NATURAL CONDITIONS – A MODEL OF DEER FASCIOLOIDOSIS

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ABSTRACT

Wildlife diseases became increasingly important as potential threat to biodiversity, livestock and human health. Differently to health management in captive environment, control of diseases in free-ranging populations is related to numerous specific factors. Here we present control of wildlife disease using deer fascioloidosis in Spačva basin as a three-step example (treatment, pathological and parasitological analysis, epidemiological analysis). Deer fascioloidosis is an important disease of both wild and domestic ruminants with potentially fatal outcome, reduced survival rate and ability to cause economic losses in deer management. Fascioloidosis is a parasitic disease caused, by to Europe, non-native trematode *Fascioloides magna*. It was detected in Croatia during 2001, in Baranja region. Subsequently, the disease spread through the eastern parts of Croatia involving all three deer species and mouflons. In order to control disease a monitoring and control programme was launched using triclabendazole as a drug of choice. Triclabendazole was administered via medicated baits, as a single ingestion dose (calculated at 60 mg/kg of body weight) during early spring, following the termination of the hunting season. Following treatment, livers and faeces of all shot animals were analysed, showing the prevalence of 36.42%.As a further step, epidemiological methods were applied to understand disease dynamics at the population level. For that we used regression models (negative binomial and logistic) and evaluation of environmental factors using GIS program. Model for pathological lesions suggested that likelihood of lesions was dependent on age ($p=0.003$). We did not find any locality or sex related significant differences. This example showed that observed area represent one epidemiological unit with favourable environmental factors for maintaining the disease. Such conditions have significant negative influence on therapeutic effect.

RESCUE OF BATS – SOME ASPECTS OF REHABILITATION

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INTRODUCTION

All bat species and their roosts in the European Union are legally protected, by both domestic and international legislation. The protection of bats and their environment is also regulated by international agreements (Bonn Convention, EUROBATS Agreement and the Berne Convention). This conservation status makes bats an important object of veterinary medical care.
Bats can be found in all types of environment including human settlements. Many synanthropic bat species roost inside of human buildings year round. In Central European region individual bats occasionally enter houses, most often during summer evenings in mid-July and August. These wayward bats are usually pups that are just beginning to fly. Individual bat may enter house during the night hunt of insects attracted to light from the windows. Fortunately, these incidents can be dealt with quite easily. Large hybernating aggregations and behaviour called „autumn invasions“ when large groups of bats enter buildings are known in European bats.

MATERIAL AND METHODS

The occurence of bats inside the buildings was systematically recorded. We collected information provided by householders. Each case was investigated (reaction on public request, field investigation, catching of bats, rehabilitation of bats if needed, analysis of the problem, searching for solutions to avoid direct contact between humans and bats inside the buildings).

RESULTS

Since 2009 we have rescued 756 bats belonging to 5 species. Dehydration was the most frequent complication in bats found inside the buildings. Bats showing symptoms of dehydration and cachectic bats were treated subcutaneous with the combination of rehydration solution *Infusio Ringeri (IMUNA, Slovakia*) and DUPHALYTE A. U. V. INJ. (Pfizer Olot, Spain). The administration of 0.01 ml of DUPHALYTE per each g of bats' weight showed the best results. Bats were feeded by meal worms coated with the premix Roboran H plv. (UNIVIT, the Czech Republic). The dosage of of whole rehydration solution was 0.15 ml *pro toto* each 8 hours in small bats (Pipistrelle bats) and 0.5 ml *pro toto* each 8 hours in bigger bats (Noctule bats, parti-coloured bats).
We recorded 5 cases of bats attacked by dogs or cats. These cases were associated with traumatic lesions. After cleaning and disinfection of wound, systemic antibiotics were administered subcutaneously – Enrofloxacine and Amoxicillin Clavulanate were used.
The rescue of more animals during the hibernation peridode we have successfully used the method of induced artificial hibernation. After 7 days lasting hibernation in the refrigerator, animals were fed for 2 – 3 days and then returned into the hibernation box.

CONCLUSION

Bats used to enter the buildings through openings of ventilation, unsealed holes for antennae cables, elevator shafts, untightnesses in old windows and cracks between individual pannels. In cases of invasions into buildings intervention of trained specialists is needed. Openings in the outer shell of

buildings most advantageously sealed with polyuretane foam and air shafts should be covered with small wire mesh netting. The majority of the found cases were caused by self-trapping of bats (specimens or colonies) inside of the buildings. The availability of drinking water is the most important limiting factor for the survival of trapped bats in buildings.

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DIVERSITY OF THE OSTEAL BASE OF THE FOOT IN WILD ANIMALS

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INTRODUCTION

At first sight it would seem that all dry land mammals walk the same way. The manner of movement depends mainly on the tread. The structure of the foot is closely related to their environmental refuge and manner of obtaining food. From the perspective of the size of the treading surface we divide the wild animals into three types: *plantigrada, digitigrada, unguligrada*.

MATERIAL AND METHODS

In processing the matter, we worked with bones from the distal parts of limbs of these free range animals: Brown Bear /*Ursus arctos*, European Beaver/*Castor fiber,*Red Fox/*Vulpes vulpes,* European Bison*/Bison bonasus* and Deer Forest/*Capreolus capreolus*. Photos were taken with the cameraCANON Power shot SX40HS.

RESULTS AND DISCUSSION

Plantigrada: Tread with the entire foot up to the heel (*planta pedis* – foot). A large contact surface under laid with bones serves for stabilization when standing on the pelvic limbs (e.g. for sniffing the air, using the upper extremities for work, match...). The longer foot is an effective carrier of great body weight (bear) too. The movement of all bones and contact with the base of the foot increases energy demands and walking for them is more economical than running. The part of the leg which has contact with the bottom (in squirrels 31%), is capable of developing sufficient strength for leaps of great distance (squirrels, martens…). Although their natural manner of movement is to walk or jump, they are just as adroit at climbing and swimming. These mammals have retained the five-digit foot.

Digitigrada: Sometimes in literature named as *semiplantigrada.* The original five-fingered digitigrade foot underwent a series of changes related to the style and location of life. They use the thoracic limbs just for movement. The structure of the foot enables relatively great strength to be developed and high speeds reached, so many of those animals are predators. The main change concerned a reduction the contact surface of the foot. Only at the distal end of *metapodium* do carnivores tread, using a metacarpal pad *torus metacarpeus* and metatarsal pad *torus metatarseus*. The heel touches the bottom only when the animals are resting.

Since they move only on their fingers, being equipped with digital pads *torus digitalis,* they are quieter and less conspicuous. In the order digitigrade canivores there are differences between families canine beasts (fox, wolf), and the feline beasts (wildcat, lynx) in the ability to retract their claws.

Unguligrada: They tread on the ungual bone of one or more fingers, which are equipped with a strong capsule of hoof. The foot becoming upright was accompanied by other changes. The metapodium bones are extended and organized in semi-circular position. With cloven-hoofed types of animals, the main metacarpal and metatarsal bonesare fused.The bones metapodium II. and V. disappear completely, or are preserved in the form of proximal (plesiometacarpal – *Cervus, Dama*), or distal rudiments (telemetacarpal – *Capreolus, Alces, Rangifer).*

The fingers laying averted from the axis of the limb lose contact with the ground and hence their function. The loss of fingers can be seen not only paleontologically but also in recent species. The slow-moving *plantigrade* (bear, man) have on the ends of their extremities developed five fingers each. For the *digitigrades* the thumb becomes rudimentary, or missing. The number of fingers with the *unguligrade* depends on the suborder in which they are classified.

The short contact surface (ground contact) allows a small amount of expended energy to perform long strides and reach relatively high speeds at a gallop (70 – 90km/hr).

CONCLUSION

Good knowledge of the imprints of animal feet and a bit of hunting patience is suffice for us to gain an approximate image of what goes on in the territory. Specific tracks (in the sand, in clay ground, in the snow), characteristic of a certain species, apart from classification, give information as to the size, age, weight, and sex of the individual. More about animal can be gained from the trace track.

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HISTOLOGICAL FINDINGS OF THE MALE AND FEMALE REPRODUCTIVE SYSTEM OF THE RED PALM WEEVIL (*RHYNCHOPHORUS FERRUGINEUS)* IRRADIATED WITH MICROWAVES

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ABSTRACT
The Red Palm Weevil (RPW) is one of the major pests of palms, frequently leading to the plant death. Preventive measures used until now often have generated serious concerns related to environmental pollution and insect's resistance. In the present study, we investigated the effect of microwaves (MW) into the control of this pest. Adult female and male RPW were exposed at MW (2.45 GHz) for 5, 15, 30 sec. and were processed for anatomical and histological analysis. Histological lesions of male and female reproductive system were characterized by atrophy, degeneration and necrosis, which increased with the increase of irradiation time. These observations allow us to say that MW are a great way to fight against the action of the RPW because they reduce or remove completely the reproductive capacity of this dreaded insect.

INTRODUCTION
The Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Oliver) (Coleoptera), is a large polyphagous insect native to southern Asia and Melanesia and is one of the most important pests of numerous palm species worldwide. Today, RPW is widely distributed in Europe (including Italy), Africa, Oceania and Asia (Wattanapongsiri, 1966; Zhang et al., 2003; Al-Ayedh, 2008; Yuezhong et al., 2009). The RPW's invasive potential is a consequence of the elevated female fecundity, of the ability to complete several generations in a year even in the same tree, the ability of adults to fly for long distances, along with the tolerance to a wide range of climates due to the habit of hiding inside the host palm. Females are usually attracted by palm volatiles and lay several eggs in dying or damaged parts of palms, although undamaged palms could also be attacked. After a few days, eggs hatch into larvae which develop within the trunks of palms, frequently leading to the plant death. Different methods are used to control this weevil, including synthetic insecticides (Ajlan et al., 2000), trapping the insect (Oehlschlager et al., 1993), the use of plant extracts (Nassar and Abdullah, 2001), biological control (Wattanapongsiri, 1966; Murphy and Briscoe, 1999; Faleiro, 2006), the development of the Sterile Insect Technique (SIT) (Dick et al., 2005). Exposure to ionizing radiation is currently the method of choice for rendering insects reproductively sterile (El Naggar et al., 2010; Paoli et al., 2014). No researches until now have investigated the effects of MW on RPW. In the present study we investigate the anatomical and histological findings of the male and female reproductive system of the RPW exposed at MW (2.45 GHz) for different duration time (5, 15, 30 sec.) to trying to control the pest.

MATERIALS AND METHODS
28 adults RPW (14 females and 14 males) were reared into controlled conditions and exposed at 2.45 GHz for different duration time (5, 15, 30 sec). The insects, positioned in a Petri dish without the cover, were introduced in a customized microwave applicator fed by a magnetron (100 W). The applicator is a short-circuited rectangular waveguide connected with a cylindrical waveguide that allows calorimetric measurements through infrared camera without perturbing electromagnetic field distribution. In this way the surface temperature distribution of the insects was acquired during the exposure. After irradiation, as soon as dead, insects were observed under the stereomicroscope for identification of anatomical changes, and then were necropsied, after removing the cuticle, legs,

wings and rostrum. Dissected organs were processed for histological analysis, fixed in 10% formalin and embedded in paraffin wax. 5-µm-thick sections were stained with haematoxylin and eosin (H&E) and observed at light microscopy.

RESULTS

The reproductive organs of adult female and male of RPW exposed at MW (2.45 GHz) for different duration time (5, 15, 30 sec.) show no evident macroscopic changes.

Histologically: Germarium- The trophocytes and oogonia undergo vacuolar degeneration and/or pyknosis.

Vitellarium – The oocytes change shape, the cytoplasm of the follicular epithelial cells appears vacuolated and the nuclei become pyknotic. Yolk granules in the cytoplasm are not homogenous and germinal vesicle is severely degenerated and vacuolated. Increasing the irradiation time, the follicular epithelium cells become hyperplastic and separated from oocytes. Homogenization of cytoplasm and disappearance of the germinal vesicle is also detected. *Calyx*- The epithelium cells which line the calyx appears hypertrophic and hyperplastic. The cells undergo degeneration, desquamation and, in severe cases, pyknosis and karyorrhexis. The follicles also undergo degeneration and then atrophy and the substance “of unknown nature” present in the lumen is homogenous and with evident basophilic focal mineralized deposits, which consist largely of calcium salts.

Histological changes in male RPW consist of variable degree of degeneration. There is disorganization and exfoliation of germinal cells. Early changes in the germinal cells include failure of maturation of spermatozoa and degeneration of spermatids. When the degeneration is more advanced, the affected areas are more extensive and degenerative changes appear in the precursors of spermatids.The seminal epithelium becomes necrotic and desquamates. Progression of the changes results in loss of germinal cells.

DISCUSSION AND CONCLUSION

The histological lesions in irradiated male and female RPW are characterized by atrophy, degeneration and necrosis, and their severity increases with the increase of the irradiation time. According to the present results, there is a direct relationship between the MW irradiation time and the observed effects on the female and male reproductive system. At 30 sec. the effects are more evident than at 5 and 15 sec. but, according to Massa et al. (2011), the damage is not enough to cause death. These observations allow us to say that the MW could be a great way to fight against the action of the RPW because they reduce or remove completely the reproductive ability of this dreaded insect. We hope that the present results regarding the effect of MW irradiation on the adult female and male reproductive system of RPW could contribute to the success of MW against this pest.

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BLUETONGUE AND WILD RUMINANTS IN EUROPE: A REVIEW

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ABSTRACT

Bluetongue (BT) is an important vector-borne viral disease of domestic and wild ruminants caused by the Bluetongue virus (BTV), an *Orbivirus*of the Reoviridae family. To date, 26 serotypes of BTV have been described worldwide with five of them occurring in Europe. Several species of biting midges belonging to the genus *Culicoides* (Diptera: Ceratopogonidae) are involved in BTV transmission. Sheep often show clinical expression of the disease with severity varying according the breed, immunity and by the virulence of the BTV strain. Goats and cattle were considered as reservoir but the emergence of BTV-8 had revealed clinical illness and reproductive disorders in cattle, and fatal cases had been reported in Germany.

Regarding wildlife, BTV specific antibodies have been detected in wild ruminants in several European countries since the last decade. Nevertheless, most of seroprevalencehave been reported from samples between 2006 and 2008 during the wide occurrence of BTV-1 and BTV-8. Among the wild ruminants, the highest seroprevalences have been detected for red deer (*Cervuselaphus*) and fallow deer (*Damadama*). Red deer can be considered as wild reservoir of BTV as no clinical signs have been shown after natural or experimental BTV-infection, and long viremia duration (3.5 months) had been reported.However, it remains to know whether *Culicoides* spp. vectors can be infected by biting red deer allowing then BTV transmission from deer to deer. In the same way, the vector transmission from wildlife to livestock and from livestock to wildlife is a real gap in the comprehensive of BT epidemiology. Most BT-linked-knowledge about *Culicoides* spp. is from domestic livestock and little is known about vectors close to wild ungulates. The current vectors identified to play a role in BT transmission to livestock, are they also implicated to transmit to the wild fauna? After introducing actors of BT, virus-hosts-vectors, their interconnection in light of BT epidemiology is discussed highlighting knowledge and gaps.

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THE ROLE OF DIAGNOSTIC IMAGING IN LOGGERHEAD SEA TURTLE (*CARETTA CARETTA*) CONSERVATION

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ABSTRACT
The loggerhead sea turtle (*Caretta caretta*, Linnaeus, 1758) is an endangered species. Many countries have already developed and are currently running management and conservation programmes in order to reduce the anthropogenic impact on this species. In the present study, the role of Diagnostic Imaging Techniques in the successful management of sick or injured turtles rescued at sea is evaluated. 18 loggerhead turtles recovered off the coast of Naples by the Stazione Zoologica Anton Dohrn and admitted to the Interdepartmental Centre of Veterinary Radiology, underwent radiographic, ultrasonographic or CT exam. We found the following lesions: 8 foreign bodies, 4 lung injuries, and 3 fractures of the carapace. Diagnostic path and lesion aspects were discussed. Imaging techniques confirmed their relevance and their unique role in diagnosis or in confirming clinical suspect.

INTRODUCTION
In the last decade, the number of sea turtles in the need of veterinary treatment and specific rehabilitation as consequence of the interaction with fisheries, pollution and other human activities has drastically increased. The loggerhead sea turtle is the most common species in the Mediterranean Sea and in the South Tyrrhenian Sea and is listed as endangered in the red list of dying species by the International Union for Conservation of Nature and Natural Resources/Species Survival Commission (IUCN/SSC). Although the increased interest on these species, sea turtles still represent a challenge for veterinarians because of their peculiar anatomy and physiology. Injured sea turtles usually show only few clinical signs of illness and therefore diagnosis in this species is frequently poor. Diagnostic Imaging tehchniques play a crucial role by providing high quality images of the anatomical structures and organs, in a non-invasive way, which are essential for accurate diagnosis and can also help in understanding critical aspect of sea turtle biology (Valente, 2007; Maffucci et al., 2013). In the present study, we describe a caseload of loggerhead turtles admitted at the Veterinary Radiology Center of Naples during the last year.

MATERIAL AND METHODS
Between January 2014 and June 2015, the sea turtle rescue center of the Stazione Zoologica Anton Dohrn of Naples referred to the Interdepartmental Veterinary Radiology Centre of the University of Naples Federico II 18 loggerhead turtles rescued along the coast of the south Tyrrhenian Sea. The loggerhead turtles underwent to radiographic, ultrasonographic or CT exam. Whenever possible, life stage and gender were determined. The curved carapace length, measured at nearest 0.5 cm with flexible meter, was used to classify the analysed individuals in the following categories: early juveniles (>30cm), juveniles (>30 cm and <65cm), late juveniles and adults (>65 cm). Only adults could be sexed using morphological secondary characters linked to sexual dimorphism because these features appear late in the life (i.e. the males have a longer tail, a larger and curved claws, and a shorter plastron than females).

RESULTS

Our sample was composed of 2 early juveniles, 11 juveniles, and 5 late juveniles and adults; 9 individuals were sexed: 2 males and 7 females. All but one patients underwent to radiographic examination. As second level exam, CT was performed in 3/18 and US in 4/18. In one case it was performed only the US study. In 16 subjects the radiographic study included the dorso-ventral view alone. In 5 cases, the DV view was associated to a latero-lateral (LL) and/or a cranio-caudal (CrCa) views with the horizontal X-ray beam. CT was performed in 3 cases, with helical scans perpendicular to the sagittal axis. All the CT studies were performed on awake subjects. The US exam was performed in 4 subjects: in 3 cases using a prefemural acoustic window and in a hatchling directly over the cranial scutes of the carapace. Foreign bodies represented the most common lesion: 5 fishhooks, 1 radiopaque debris and 2 non-radiopaque foreign bodies (fishing line). Respiratory lesions were present in 4 cases and they were characterized by focal to total lung opacifications. In 3 cases there were fractures of carapace associated to vertebral fractures (2 cases) or right forelimb amputation (1 case). In one case, the US showed the presence of formed eggs in the oviduct of an adult female. In 2 cases, the suspected presence of a foreign body was excluded.

DISCUSSION

The diagnostic imaging techniques are particularly useful in chelonians, due to the presence of the carapace and plastron, which make clinical examination difficult to perform. Radiography is the most common and inexpensive diagnostic imaging method used in veterinary medicine, providing good overall information about the skeleton, respiratory system and radiopaque or radiolucent foreign bodies. Three views are used to examine chelonians: dorso-ventral using a vertical beam, latero-lateral and cranio-caudal views using a horizontal beam. Moreover, the radiographic exam is easy to perform because loggerhead sea turtles are usually collaborative and therefore it is not necessary pharmacological containment. The radiographic evaluation, as for other animal species, follows the same criteria or “Roentgen signs”: position, number, shape, size, and density. Ultrasonography is a non-invasive technique particularly useful for evaluation of soft-tissue organs. In chelonians, the carapace and the plastron represent a physical barrier for the ultrasound beam, however, it is possible to use various acoustic windows to study the organs inside the coelom, the most widely-used being the cervicobrachial and prefemural. In hatchlings and juveniles the carapace is not calcified, so it is possible to use the trans-carapace windows. CT study has a better contrast resolution compared to the radiography and permits to evaluate soft and hard tissues, but it is particularly reliable to respiratory and bone diseases diagnosis. CT is usually not time consuming and in most cases does not need sedation or narcosis, however, compared to radiography and ultrasound, it is more expensive.

Our results show that radiography is the gold standard for assessment of radiopaque foreign bodies and fractures. The main clinical problem of loggerhead turtles in our sample was the presence of a foreign bodies, mostly fishing hooks lodged in the oesophagus. The loggerhead sea turtles can ingest one or more hooks, and this is a common finding throughout the Mediterranean Sea. In our opinion, to identify the radiopaque foreign body it is enough the DV view but, if it is necessary a more precise characterization about the position inside the lumen of the oesophagus, the LL view with the horizontal beam would be useful too. When it is suspected the presence of a non-radiopaque foreign body (i.e. the fish line attached to the fish hook) the best technique has been demonstrated to be the ultrasonography. The ultrasound exam permits to assess the gastrointestinal tract (peristalsis, wall thickness and echostructure, type of contents, etc.). For the study of the lungs, radiography often represents the first step and it needs the DV view, with vertical X-ray beam, and the cranio-caudal view, with horizontal beam. These two views, usually, are reliable to assess the extent of lesions to the lungs. The LL view, with horizontal beam, has not the same usefulness, since the lungs are superimposed. CT is considered a second level exam, due to its greater costs and lesser availability compared to radiography, but its higher contrast resolution, that allows to visualize very small lesions without any overlapping through the anatomical structures. The ultrasound trans-carapace

examination was useful to reveal an characterize a pulmonary abscess in a hatchling in which the radiography showed a lung opacity. Traumatic lesions affect most commonly the flippers or the carapace and are generally the conseguence of entrapment in fishing lines and collision with boat propellers. Due to the dorsal position of the lungs, which are close to the carapace surface, traumatic injuries of the carapace often result in secondary pulmonary diseases. In these cases, the best diagnostic path is, first, radiographic exam, eventually followed by CT. The ultrasonography is useful in patients suspected to be ready for ovoposition as it allows to visualize the oviducts in order to assess the possible presence of eggs. In these cases, US must be considered as mandatory before admitting the patience to X-rays, in order to prevent possible damages to the embryos.

CONCLUSION

Diagnostic Imaging is an important resource to make diagnosis in species like the loggerhead turtles that exhibit few clinical signs of distress or illness compared to mammals or other better studied animals. Moreover, these techniques are essential to increase our knowledge on the anatomy and physiology of these endangered species in order to contribute to their effective conservation.

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IDENTIFICATION OF PATHOGENS IN LIVER OF WILD BOARS HUNTED IN LIGURIA, ITALY

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ABSTRACT
The aim of this study is to evaluate the health status of wild boars hunted in Liguria. During 2013 – 2015 years, 2 326 liver samples were analyzed for researching *Campylobacter* spp. and *Yersinia enterocolitica*; in this last case it was investigated the biotype: the 3.7% of 74 cases was classified as the pathogenic 1B biotype. These results showed a higher prevalence in Genova and La Spezia provinces for both pathogens. The monitoring of wildlife is important for verifying both the wellbeing status of the animals and the environment quality, as well as to prevent possible zoonoses to humans, safeguarding therefore the public health.

INTRODUCTION
During last decades, the growing of the wild boar population (*Sus scrofa*) in Liguria has induced a consequent interaction between animals (e. g. wildlife, pigs) and humans, also through meat consumption. The animal slaughter is frequently performed by hunters; in certain cases, the application of inappropriate hygiene procedures could determine a poor sanitation of meats (e.g. contamination by pathogens, such as *Yersinia* spp. and *Campylobacter* spp.), that may therefore affect human health (1-3). However, only few data on the presence of these pathogens isolated from liver and meat in wild boars are to date available (2). Owing to the above the aim of our study is to investigate the health status of wild boars hunted in Liguria.

MATERIAL AND METHODS
In the period 30 April 2013 – 30 April 2015, 2 326 liver samples were collected in Liguria (Imperia 546, Savona 604, La Spezia 203, Genova 973) and checked for the presence of *Yersinia enterocolitica* and *Campylobacter* spp. Furthermore 2280 liver samples (Imperia 546, Savona 604, Spezia 186, Genova 944) were tested for the presence of *Campylobacter* spp. The specimens were analyzed in according to ISO 10273-2003 and ISO 10272-2006 procedures, respectively; colonies suspected for the *Yersinia enterocolitica* presence were confirmed by biochemical tests, biotyping and serotyping using specific antibodies: anti-O8, O9, O3, O5 and O1, 2.

RESULTS
Regarding the analytical research of *Yersinia enterocolitica*, all samples from the Savona and Imperia provinces were negative, while 8 (3.9%) and 74 (7.6%) cases in La Spezia and in Genova, respectively , were positive. In the case of Genova, 96.3% of the posive samples belonged to the 1A biotype, considered as non-pathogenic for humans; the remaining 3.7% was classified as pathogenic 1B biotype. Concerning *Campylobacter* spp., 30 samples were positive (1.3%). This bacteria showed a non-homogeneous distribution, with the highest percentage of positive cases in Genova (2.5%) and La Spezia (2.1%), followed by Savona (0.3%) and Imperia (0.0%).

DISCUSSION

Yersinia spp. and *Campylobacter* spp. showed a higher prevalence in the provinces of Genova and La Spezia. These results can be explained either by: 1. primary infection of the animal, 2. secondary contamination of meat. The second one is the most likely cause and probably linked to poor hygiene applied by hunters at the time of evisceration and skinning.

CONCLUSION

Our results evidence the presence of *Campylobacter* spp. and 1B biotype *Yersinia enterocolitica* in the ligurian wild boars and environment, indicating a possible risk for public health. As previously suggested, this data may be related to the contamination of carcasses that could occur when animals are eviscerated and skinned under insufficiently hygienic conditions.

The wild boars may represent a transmission source of *Campylobacter* spp. and *Yersinia* spp. both for pets that humans through direct ingestion or handling of products obtained from these animals, or indirectly through the contamination of crops or irrigation water.

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SEROLOGICAL PREVALENCE OF AUJESZKY'S DISEASE VIRUS (ADV) IN WILD BOARS IN LIGURIA, ITALY

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ABSTRACT
The aim of this study is to evaluate the Aujeszky disease virus (ADV), characterized by a wide host range, in the wild boars in Liguria (Italy), whose populationhas increased during the last 50 years due to a lack of predators and competitors. During the hunting seasons 2013 and 2015, it has been possible to collect 2168 serum samples. A total of 625 specimens was positive for anti-ADV antibodies with a regional prevalence of 28,8%. In consideration of the possible negative health and economic impact in pig farms, the ADV in Italy is to date subjected to a National eradication program (DM 1/4/97).

INTRODUCTION: In the Liguria Region (North-western Italy), the wild boar (*Sus scrofa* L.) population has continuously increased during the last 50 years due to a lack of predators and competitors. These animals may act as reservoir for many types of pathogens, including those affecting farm animals; in particular, domestic pig and wild boar share various common etiological agents, such as Aujeszky's disease virus (ADV) (2). Recent studies highlight the infection of the Italian wild boar sustained by several pathogens (e.g. hepatitis E virus, *Trichinella* spp, *Salmonella* spp. and ADV (1). ADV, also known as pseudorabies, represents one of the most economically important infectious diseases in swine. Pseudorabies is caused by Suid herpesvirus type I, a neuroinvasive virus with a wide host range. ADV determines a high economic impact in pig husbandry, either through direct effects on health than on movements of the animals, as well as on trade restrictions. The consequences of ADV on the wild boar population dynamics are considered to be low, but ADV outbreaks with associated mortality have been also observed; in addition, the restriction of boar's trade flows may have negative effects on the production derived from hunting (4). Data about the prevalence of ADV in Liguria are currently poor or incomplete, except for a work focused on 430 blood samples analyzed between the hunting seasons 1992 – 93 (3). In order to improve the knowledge in this field, the aim of the present study is to investigate the serological presence of specific antibodies anti-ADV in ligurian wild boars during two consecutive hunting seasons.

MATERIALS AND METHODS
From 30 April 2013 to 30 April 2015, according to the "Monitoring Plan for health monitoring and epidemiological surveillance of wildlife in the Liguria Region", 2168 blood samples were obtained from wild boars hunted in Liguria (Imperia, Savona, Genova and La Spezia provinces). Blood specimens (10ml), collected from the jugular vein or hearth in tubes without anticoagulant were investigated for the presence of specific antibodies anti-ADV. Non-heparinised blood samples were stored at room temperature for 2 h and centrifuged at 2,500 rpm for 15 min; serum aliquots were stored at −80 ◦C until they were tested. The presence of antibodies was evaluated by ELISA kit.

RESULTS
A total of 625 serum samples were positive for anti-ADV antibodies with an overall regional prevalence of 28.8%; the positivity rate for each district is reported as follows: Imperia 19.6%, Savona

20.4%, Genova 40.4%, La Spezia 34.3%. Within the provinces of Genova and La Spezia, a growing W-E gradient was notified, with peaks in areas bordering the Parma province (45% Sturla, 42.3% Aveto), where an intense pig farming activity is carried out.

DISCUSSION

These results show that the western ligurian provinces (Imperia and Savona) were characterized by a lower prevalence (19.6% and 20.4%) compared to the eastern ones (Genova and La Spezia).In particular, the highest value (44.4%) was achieved in Genova. In the territories of Imperia and Savona, a prevalence increase from coastal to mountain areas was observed. This finding could be explained either by a greater amount of animals hunted in the mountain zones and/or in the proximity to the province of Cuneo, characterized by presence of a significant number of farmed pigs. It's possible conclude that the prevalence data here obtained (28.8%) is significantly increased respect to the 9.3% value reported in a previous research (3).

CONCLUSION

Our results suggest a clear prevalence's increase in Liguria of ADV compared to other studies. This growing could be explained by a high persistence of this virus in the environment, its transmission mechanisms (both horizontal and vertical) as well as an enlargement of wild boar populations living close to the urban areas. For these reasons the ADV in Italy is to date subjected to a National eradication program (DM 1/4/97).

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A STUDY OF ORAL AND DENTAL DISEASES IN A POPULATION OF EUROPEAN POLECATS (*MUSTELA PUTORIUS*)

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BACKGROUND
Polecats (*Mustela putorius*) belong to the family Mustelidae and are widespread through most of Europe. According to the literature, viral, bacterial and parasitic infections, as well as traumatic injuries are common amongst this animal species. However, dataonoral and dental diseases of free-living European polecats are rather rare or lacking.

OBJECTIVE
To investigate oral and dental diseases in free-living European polecats.

METHODS
In this study, 31 European polecat skulls from a museum collection (Slovenian museum of natural history) were examined by means of visual inspectionand dental radiography for presence and number of teeth, tooth form and number of roots, attrition/abrasion, dental fractures and periapical disease, bony changes consistent with periodontitis and any other findings.

RESULTS AND CONCLUSION
Variations in occlusion and number of roots were noted in comparison to published literature on ferrets and polecats. Palatal root of maxillary first molar tooth was commonly observed to penetrate the orbit. Periodontal disease, attrition/abrasion and dental fractures, especially of canine teeth, have been observed in theskulls examined. Periapical disease associated with dental fractures was rather rare, but it was observed to be as severe as causing fenestration and osteomyelitis.In conclusion, clinical and radiographic examination of our polecat skullsrevealed some differences in their dental anatomy and pathologyin comparison with the data published before.

THE EPIZOOTIOLOGICAL SITUATION OF RABIES IN CENTRAL EUROPE

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ABSTRACT
Foxes are the only known reservoir for rabies in Europe, and raccoon dogs are important transmitters, while other carnivores play a less important epidemiological role. The expansion of raccoon dogs and their movements after hibernation are risk factors for rabies reccurrence.

INTRODUCTION
Rabies is an important zoonotic viral disease, which causes encephalitis in mammals. It is transmitted through contact with saliva from infected animals; rabies is always fatal. Rabies virus belongs to the order Mononegavirales, the family Rhabdoviridae. Rabies virus (RABV) is the first of 14 lyssavirus species (Dietzgen et al., 2011), with additional genetic evidence of a not yet isolated lyssavirus named Lleida bat lyssavirus (LLEBV) (Ceballos et al., 2013). According to their viral genetic distances and antigenic differences, two major phylogroups have been defined. Phylogroup I comprises the following species: RABV, European bat lyssavirus 1 (EBLV-1) and European bat lyssavirus 2 (EBLV-2), Duvenhage virus (DUVV), Australian bat lyssavirus (ABLV), Aravan virus (ARAV), Khujand virus (KHUV), Bokeloh bat lyssavirus (BBLV) and Irkut virus (IRKV). Phylogroup II comprises Lagos bat virus (LBV), Mokola virus (MOKV) and Shimoni bat virus (SHIBV). The remaining viruses, West Caucasian bat virus (WCBV) and Ikoma lyssavirus (IKOV) cannot be included in either of these phylogroups and have been temporarily assigned to putative phylogroups III and IV (EFSA, 2015; ECDC, 2015).

MATERIAL AND METHODS
The occurrence of rabies in animals, excluding bats, in Europe is provided. The paper summarizes the informations obtained from Rabies Bulletin Europe, WHO.

RESULTS AND DISCUSSION
The incidence of rabies in both domestic and wild animals in EU has drastically reduced over the past decades after systematic oral vaccination campaigns, and rabies cases have disappeared in western, northern and most of central Europe. About 83 million Euros of EU contributions were needed between 2005 and 2012 for oral vaccination programmes in wildlife in MSs and bordering areas of neighbouring countries. Mass vaccination of pets provides a first line of defence to prevent rabies in humans, whereas oral vaccination of foxes and raccoon dogs has proved efficient for the long-term control and elimination of terrestrial rabies (EFSA, 2015; ECDC, 2015).
Between 2003 and 2014, the total number of rabies cases at EU level in wild animals (carnivores and ungulates) decreased very significantly, from 3 748 cases (2 500 cases in foxes and 1 000 cases in raccoon dogs) to 215 cases (200 of which were reported in foxes) in 28 countries of EU (Rabies Bulletin Europe, WHO). The reported cases in domestic animals (dogs, stray dogs, cats and livestock) dropped from 800 cases to 65 cases in 2014, mostly reported in Romania (41 cases) and Poland (16 cases).
After implementation of an improved ORV programme in 2000 based on aerial distribution of vaccine baits, Slovakia did not reported any rabies cases between 2006 and 2012. From 2010, the vaccination

area was reduced and ORV activities were restricted to eastern regions of the country bordering infected areas in neighbouring countries. Although vaccination has decreased the number of rabies cases, it has also contributed to a rise in fox density. In 2013, three new cases in foxes were detected along the Polish border. The current campaigns cover the entire territory of the Slovak Republic, except for the areas bordering the Czech Republic and Austria and part of the area bordering Hungary. They involve annual aerial and manual bait distribution in spring and autumn. Monitoring involves testing of hunted foxes and raccoon dogs after vaccination. In Europe the role of rabies reservoir is played mainly by red foxes (*Vulpes vulpes*). ORV of foxes and/or raccoon dogs is the most effective method of eliminating terrestrial rabies in wildlife (EC, 2015). The strategy of ORV initially applied to red fox proved to be effective in raccoon dog populations in several countries (Finland, Baltic countries and Poland) where raccoon dog populations play a significant role in rabies epidemiology (EFSA, 2015).

CONCLUSION

In Europe, oral immunisation by means of vaccine baits has been found to be successful in eliminating terrestrial wildlife rabies in most cases. The analysis of the dynamics of rabies cases and vaccination areas confirms that ORV is an efficient method for rabies control in wildlife, including areas with both foxes and raccoon dogs. The ultimate success of ORV campaigns requires both a long-term strategy and cross-border cooperation (EC, 2015).

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"MAZE.ROE" PROJECT: STUDY OF GUT MICROBIOTA AND DIET COMPOSITION OF ROE DEER (*CAPREOLUS CAPREOLUS*) IN THE ITALIAN ALPS

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ABSTRACT
The MAZE.ROE research project aims at characterizing the gut microbiota and the diet composition of roe deer (*Capreolus capreolus*) in the Italian Alps, by applying a metataxonomic approach coupled with high-throughput sequencing (Illumina). A total amount of 384 fecal samples, collected from roe deers in the Trento province (years 2013-2014) and the Valle d'Aosta region (2013), were selected for the study. Preliminary results obtained during a pilot study on a limited number of fecal samples deriving from 3 roe deers individuals confirmed the feasibility of the whole project, which is currently in progress.

INTRODUCTION
Roe deer (*Capreolus capreolus*) populations in Italy have been expanding during the last 30 years (Carnevali et al. 2009). This herbivorous species is involved in the transmission cycles of several infectious and parasitic diseases, including zoonoses (Carpi et al. 2009, Solarczyk et al. 2012); nevertheless, the role of roe deer as a reservoir of intestinals pathogens has not been investigated until now. Concurrently, both the ecological impact on vegetation and the diet composition - considered as a proxy of its health status- of *C. capreolus* remain largely unknown. The MAZE.ROE project therefore intends to study the gut bacterial community (microbiota) and the diet composition of roe deer in the Italian Alps, by applying a metataxonomic approach coupled with high-throughput sequencing (HTS). Herein, the genereal outline of the project and the results of a pilot study performed on a limited number of samples are reported.

MATERIAL AND METHODS
Rectal fecal samples, ear biopsies and hair samples were collected from 19 roe deer individuals captured and radio-collared in the Trento province, NE Italy, in 2013-2014, and preserved at -80°C. After radiotracking of roe deers, a total amount of 341 fresh fecal samples found on the ground were putatively attributed to the marked indiduals according to their location and collected as follows: 2-3 sampling sessions per 3 sampling seasons (early summer, mid summer, late summer) of each year. Ground-collected samples were splitted into 2 aliquotes each and preserved in silica gel and RNAlater, respectively, and then freezed at -20°C. Sixty-six fecal swabs were also collected from hunted roe deers in Valle d'Aosta Region, NW Italy, in 2013, and preserved at -80°C, as an outgroup for the gut microbiota characterization. A total amount of 384 fecal samples (ground samples plus fecal swabs), including some replicates and negative controls, were finally selected for the study.
For the metataxonomic phase, the V3-V4 hypervariable regions of the bacterial 16S rRNA gene (Schwartz et al. 2014) and the P6 loop of the trnL(UAA) intron of cpDNA (Taberlet et al. 2006) were selected as barcodes for bacteria and vascular plants, respectively. As genus- and species-level indentification based on the P6 loop is problematic for some plant families, ITS1 for Asteraceae, Cyperaceae and Poaceae, and ITS2 for Rosaceae were chosen as additional barcodes (De Barba et al. 2014). A reference database of plant species barcode sequences, based on a checklist of local plant

species prepared during the field phase of the study, was obtained through a search on GenBank public database. Given their lower cost and the high number of reads provided, Illumina MySeq for microbiota and Illumina HiSeq 2000 for plants were chosen as HTS platforms.
To obtain individual roe deer identification for individual life-history analyses and verify whether ground-collected fecal samples belonged to the putative individuals, a panel of 24 nuclear dinucleotidic microsatellite markers and the amelogenin (AMEL X,Y) gene (Gurgul *et al*. 2010) were tested on genomic DNA extracted from ear biopsies/hair samples and rectal fecal samples. Genotyping data were analysed using GeneMapper software v3.7 (Life Technologies, USA).
To evaluate the feasibility of the whole study, a pilot study on a limited number of samples, belonging to 2 roe deer individuals (1 adult male and 1 adult female) from Trento province was performed; an adult female from Valle d'Aosta was also included in a few analyses. Three commercial kits (Thermo Scientific KingFisher Cell and tissue Kit, QIAmp DNA stool Minikit from QIAgen and Mag-Bind Stool DNA kit from Omega bio-tek) and protocol modifications for genomic DNA extraction were tested on both silica gel- and RNA-later preserved fecal samples, to simultaneously recover host, bacterial and plant DNA. Extracted DNA was quantified on a spectrophotometer. Amplifications of roe deer mtDNA D-loop control region and of bacterial and vascular plant barcode regions were performed on thermocyclers. PCR products were separated and visualised using QIAxcel (QIAgen, Germany). Whereas fragments of the expected size were obtained, amplicons were purified and sequenced on an ABI 3130XL genetic analyzer (Life Technologies, USA). In the case of microbiota and plants, several overlapping sequences were expected. Sequences obtained were maually checked using Sequencher software (Gene Codes Corporation, USA). In the case of any clear sequence fragment, a BLAST search on GenBank was performed for taxonomic identification.

RESULTS AND DISCUSSION

For what concerns genotyping for individual identification, 2 STR loci out of 24 did not amplify and were discarded, while 4 more loci revealed to be monomorphic. The remaining 18 loci turned out to be polymorphic (3-10 alleles); data analyses is currently in progress.
During the pilot study, all DNA extraction kits and protocols allowed to simultaneously obtain host, bacterial and plant DNA, however the Mag-Bind stool DNA kit (Omega bio-tek, USA) gave more satisfactory results in terms of DNA yeld, costs and the possibility to be coupled with an automated extractor. As RNAlater-preserved samples performed much better than silica gel-preserved ones, especially in the case of the diet component, the former were chosen for the whole study. Amplification of the host control region provided the expected fragment; one of the haplotypes obtained was highly similar to the one of a *C. capreolus* individual previously found in the Trentino province (Vernesi et al. 2002). Amplification of bacterial and plant barcode sequences was also successful and several overlapping sequences were obtained from most samples. On the microbiota side, the BLAST search on GenBank allowed to identify *Pseudomonas* sp. in a few samples from Trentino. Interestingly, the sample from Valle d'Aosta was characterised by a unique pattern of overlapping bacterial sequences. For what concerns diet composition, a few sequence fragments deriving from Trentino samples revealed the presence of *Fragaria* sp., *Vaccinium myrtillus*, *Rubus chamaemorus*, *Rubus* sp.

CONCLUSION

The pilot study demonstrated the feasibility of the MAZE.ROE project. Analyses are currently in progress and the HTS phase will be soon performed.

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POPULATION TRACKING OF *LACERTA VIRIDIS* AND *PODARCIS MURALIS* IN NATURAL RESERVE OF DOMICKÉ ŠKRAPY

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ABSTRACT

Monitoring programs such as are important for wildlife protection, tracking of population if is maintained for a longer period. In our paper, two lizard species were monitored for three years on the same habitat. *Podarcis muralis*, the only representative of the species *Podarcis* in Slovakia and the biggest lizard in Slovakia *Lacerta viridis.* Since *P. muralis* and *L. viridis* have similar demands on the environment, it often happens, that their areas of habitats overlap. Suroundings of cave Domica, natural reserve Domické škrapy is a great example of such habitat. The geological base consists of Triassic limestones. Typical inhabitants from class of reptiles are: *P. muralis (PM)*, *L. viridis (LV), L. agilis (LA)*, *E. longissima (EL)*, very rare is *Ablepharus kitaibelii (AK)*. This location was regularly monitored in three years (2013 - 2015). In our work, we describe the population found within this time period. Monitoring of reptiles is very dependable on the weather, therefore sometimes if the weather is not perfect for reptiles, no speciement could be found. That is why, the numbers of found species vary from year to year. We found in total within three years 22 specimens of LV, 20 specimens of PM, 14 specimens of LA and 1 specimen of EL.

REPATRIATION OF PRZEWALSKI´S HORSES (*EQUUS PRZEWALSKII* POLIAKOV, 1881) TO MONGOLIA

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HISTORY

Next year it will be 20 years from the first repatriation of Przewalski´s horses from European Zoos to Mongolia. This species disappeared from the wild at the turn of the 60th to 70th years of the last century. The last wild Przewalski´s horses had been seen in 1966 at China, in Mongolia 1968-1969. Extinction came very quickly and unexpectedly (competition ever more numerous herds of domestic livestock, severe winters and a lot of snow in the 40s and 50s, various armed groups- amusement in shooting in herds at water-lock).

THE GOAL OF ZOOLOGICAL GARDENS

Early 80s of last century – annual increase in the world population was between 10 – 12%. The total number was quickly close to the limit of 500 individuals. First reflections on the return of the Przewalski´s horses to the wild.

INTERNATIONAL COOPERATION

1985 at the invitation of Academy of Science SSSR, The Food and Agriculture Organization of the United Nations (FAO) and United Nations Enviroment Programme (UNEP)- international conference, which was to consider and approve the plans for relocation of population from ZOO to the Mongolian reserves.
The Plan: relocation of herds for 1 – 2 years in the Ukrainian Askania-Nova. Then the relocation to Mongolia, at that place for 3 – 4 years in a large acclimatization paddocks. After that relocation to the wild nature.

DISAPPOINTMENT

Lack of funds from FAO and UNEP Problem was the lack of preparation of Mongolians (unable to build acclimatization paddocks or legislative protection area). What was agreed in Moscow didn't come true. Zoological gardens were not able to organize the action. The arrival of private foundations.

PRIVATE FOUNDATIONS

In 1985 Christian Oswald Stiftung (COS) organizes the first transport to the acclimatization station Jimsar. The Netherlands foundation of Boumans (FRPH) expect for Germans, comes with their own plans. In the year 1990, the VII. national symposium for the saving of PH meets in Leipzig, the meeting however ended without accepting a single final dokument.

ZOOS EFFORTS

Zoos united to EAZA tried to prepare their own project on the autumn of 1990, the commission EEP for breeding PH was created, because of the clear financial and organizing potential superiority of privat organizations, the efforts of the zoo ended with no result on the June of 1992, 2 transports

landed in Ulanbatar, one to the reservation Hustain Nuruu and the second to the national park Gobi The return of PH to Mongolia became a reality.

PRESENT TIME

Nearly 2000 PH are living on the whole world. 1 quarter of that lives freely in Mongolian and Chinese reservations another 300 horses lives inside the Ukranian Askania National park Hortobágy is currently the biggest breeder of PH's (about 150 horses).

LIVING IN THE WILD

Fear from wolf packs-horses living in captivity for 12 generations preserved their basic instincts and were able to align (loss 20%). Parasitic infections - genus *Babesia* and *Piroplasma* (age is important) - optimal age 1 to 3 years Infanticide - when the harem is overtook by a new stallion.
The terrorism of young horses by grown stallions that the dominant stallion can't fend off
Limiting factor - severe winters – dzud.
It's nearly certain that dzud in the year 1969 killed the last wild horses living in the mountains Tachin Sar Nuruu.
On january and february in the year 2010, 98 PH feel victim to it. That's about 2/3 of a promising growing reintroducting population.
Importance - preserve a big and reproducible population - have a steady supply of animals coming to the Zoo.
Financial difficulty - in the year 2007, the last transport was cancelled, because the price for transporting one horse was 30 000 dollars.

TRANSPORT ORGANIZED BY ZOO PRAHA

The transport this year to the area Khomiin Tal was organized by the CZ airforce
4 horses - 3 from the Zoo Praha and 1 from Zoo Košice.
The goal of the first czech transport was not only to reinforce the population, but also to catch the attention of the czech and world public to the faith of the last wild horse species.

PH BREEDING IN ZOO KOSICE

Zoo Košice breeds PHs since 1991(Titán, Trója, Ulita).
The first foal was born in 1994 (Klára).
A sum total of 10 females and 1 male were born.
Currently Zoo Košice breeds 4 females.

ZOO KOŠICE IN THE REINTRODUCTION OF THE PH

In the year 2000, a female horse named Babeta was transported to the national park Hustain Nuuru. Babeta gave birth to 8 foals.
The mare Klara lives in the national park Hortobagy since 1999. She gave birth to min. 5 foals.
In the year 2011, the mare Cassovia was transported to Mongolia (Khomiin Tal), in the year 2012 she gave birth to her first cub.
In the year 2012 - was sent to Mongolia (Gobi B) mare Agnes, which has also foal.

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SEROPREVALENCE AND RISK FACTORS ASSOCIATED TO *NEOSPORA CANINUM* AND *TOXOPLASMA GONDII* INFECTIONS IN HUNTING DOGS FROM SOUTHERN ITALY

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ABSTRACT
Blood samples were collected from 398 hunting dogs of 19 different breeds. The sera were screened by indirect fluorescence antibody test (IFAT); a titre ≥ 50 was considered positive. Antibodies to *N. caninum* and *T. gondii* were detected in 14.8% and 23.6% of dogs respectively, with co-infection in 6.3% of tested animals. The old age (p = ≤ 0.05; OR = 2.65) represented a risk factor for *T. gondii* infection. None tested variables represented a risk factor for *N. caninum* infection. Our results showed that hunting dogs in Southern Italy could be considered to be in risk for both protozoa infections.

INTRODUCTION
Neospora caninum and *Toxoplasma gondii* are related coccidians that until year 1988 were regarded as the same organism. Dog, coyote and dingo are the definitive hosts of *N. caninum* that can shed environmentally resistant oocysts. Domestic and wild felids represent definitive hosts for *T. Gondii*, while dogs can serve as intermediate hosts. In Europe, and extra-european countries, there are only few studies focusing on *N. caninum* and *T. gondii* seroprevalences in hunting dogs (Collantes-Fernàndez et al., 2008). Hunting dogs may have a higher level of exposure to these two protozoans than other canine populations. It could be caused by their closer contact with wooded and rural areas contaminated by *T. gondii* oocysts, and their frequent contact with intermediate hosts represented by wild animals (birds, wild boars, foxes and hares). The aim of the present survey was to determine the seroprevalence of *N. caninum* and *T. gondii* in hunting dogs from Southern Italy and assess risk factors related to these protozoan infections.

MATERIAL AND METHODS
The survey was conducted on 398 hunting dogs from 76 municipalities located in two provinces (Avellino and Salerno) of Campania region in Southern Italy. A questionnaire was submitted to the hunters in order to obtain information about age, gender, breed, size, cohabitation of several dogs (packs of dogs), living environment (urban or rural), contact with other domestic animals and hunted species. Furthermore, a complete clinical examination was performed on each animal. The presence of antibodies to *N. caninum* and *T. gondii* was detected by indirect fluorescence antibody test (IFAT) using a commercially available *N. caninum* and *T. gondii* antigen IFR and anti-dog IgG FITC conjugate (VMRD, Pullman, USA). A titer ≥ 50 was considered positive for both parasites. The seroprevalence according to the various characteristics of the canine population was calculated with an associated 95% confidence interval (CI). Differences in prevalence between these various groups were assessed by Pearson Chi-Square test; p value ≤ 0.05 was considered significant. The variables were then

applied to binary logistic models in order to find risk factors associated with the seroprevalence for each parasite.

RESULTS

Overall, the average age of the hunting dog population was 3.5 years. Animals were divided by age into four groups: < 2 years, ≥ 2 – 4 years, ≥ 4 – 7 years and ≥ 7 years. There were 232 males and 166 females, including 19 different breeds. Almost all dogs lived in rural environment (391/398) and the pack size was variable from 1 to more than 13 dogs. Regarding the type of hunting, the most hunted species were represented by boars (179/398) and game birds (177/398). Clinical examination of the seropositive dogs did not show any evidence of abnormalities referable to the two protozoan infections. Antibodies to *N. caninum* and *T. gondii* were found in 59 (14.8%; 95% CI: 11.3 – 18.3%) dogs, with titers ranging from 50 to 3200, and in 94 (23.6%; 95% CI: 19.4 – 27.8%) dogs, with titers ranging from 50 to 1 600, respectively. The co-infection of both protozoa was found in 6.3% (25/398; 95% CI: 3.9 – 8.7%) of dogs. The older age (≥ 7 years) ($p \leq 0.05$; OR = 2.65) was the only risk factor for *T. gondii* infection, while none of the observed characteristics represented a risk factor for *N. caninum* infection.

DISCUSSION

To the best knowledge of the authors this is the first study focused on *N. caninum* and *T. gondii* prevalence in the population of hunting dogs in Italy. The finding of a lower rate for *N. caninum* than *T. gondii* is in agreement with the general trend reported in dogs of other countries (Langoni et al., 2013). Previous studies showed a significantly higher *N. caninum* seroprevalence in farm, stray and hunting dogs than in household dog (Maia et al., 2014). Similarly, in our study hunting dogs showed a higher infection rate for *N. caninum* than previous data (6.3% – 11.6%) in household dogs in Italy (Cringoli et al., 2002; Capelli et al., 2004). Hunting dogs are particularly susceptible to *N. caninum* and *T. gondii* infections for their outdoor lifestyle, and the habit of hunters to eviscerate carcasses in the field and feed the dogs with raw organs from the prey. Furthermore, neosporosis and toxoplasmosis are widespread in wild animals in Italy (Zanet et al., 2013). Regarding *T. gondii*, there are not wild felines that may contaminate the wooded environment in Southern Italy; however, we have to take into account oocyst excretion in the faeces of stray cats whose number is considerable. Our findings of a gradual increase of seroprevalence for both protozoa with age is in agreement with other reports (Paradies et al., 2007), although in our study the increasing age was a risk factor only for *T. gondii*. The documented higher seroprevalence in older dogs suggests that the infections are mainly maintained by horizontal rather than vertical transmission. Our results confirm that the detection of clinical disease is uncommon, affecting especially the puppies in the case of *N. caninum*. In most cases, adult dogs, sometimes even with high antibody titres, are asymptomatic; in these animals can be observed sporadically a reactivation of the subclinical infection with the onset of neurological or generalized signs, following immunosuppressive conditions, prolonged therapy with glucocorticoids and infections of various etiologies. Maia et al., (2014) in a recent seroprevalence survey in Portugal have reported the detection of neurological or muscular symptoms in three dogs infected by *N. caninum*. However, these authors have not specified the age of the animals and have not confirmed the presence of the parasite through PCR analysis or muscle biopsy.

CONCLUSION

In conclusion, hunting dogs in Southern Italy can be considered to be in risk for both protozoa infections, and they may play an important role in the transmission cycle of *N. caninum* between wild animals and livestock.

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MOLECULAR SURVEY OF LEISHMANIA INFECTION IN RED FOXES (*VULPES VULPES*) FROM SOUTHERN AND CENTRAL ITALY

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ABSTRACT
The aim of this study was to investigate on the prevalence of *L. infantum* infection in foxes living in two distinct areas of southern and central Italy. Sixty eight carcasses of foxes were subjected to PCR analysis. At external examination no clinical signs of leishmaniasis were recorded. Ten foxes were found to be positive by *Leishmania* nested-PCR, of which 4 in spleen, 2 in lymph nodes and 4 in both organs. Our results show that in southern and central Italy foxes may me subclinically infected by *Leishmania*, but their importance as reservoirs for the maintenance of the infection remains to be determined.

INTRODUCTION
Leishmania infantum is endemic in the Mediterranean basin. Domestic dogs act as the main reservoir hosts and may suffer severe disease, characterized by chronic evolution of viscero-cutaneous signs. Campania region, and some areas of central Italy, are historically considered territories of elevated endemicity for both human and canine leishmaniasis (CL); however limited information is available as regards potential wild reservoirs of *L. infantum*. The red fox (*Vulpes vulpes*) has been suspected to have an epidemiological role as a source of CL cases occurring in rural or peri-urban areas (Millán et al., 2014). Therefore, the aim of this study was to investigate on the prevalence of *L. infantum* infection in free-ranging foxes living in two areas in southern and central Italy.

MATERIALS AND METHODS
The first area of study was represented by the Picentini hills, a mountain range in Campania region of southern Italy, part of the Apennines. The area is located in the Salerno and Avellino provinces. The second area of study was represented by the southern part of Lazio region and sampling has been carried out in the provinces of Latina and Frosinone. Hunters were invited to bring carcasses of foxes, either shot or found dead because of vehicular accidents, to the Avellino and Latina sections of the Istituto Zooprofilattico.
Carcasses were weighted, examined externally and submitted to necropsy. The body condition was scored from 1 to 5 during necropsy. Whenever possible, spleen, lymph node and liver samples were obtained from each animal and stored at -20°C pending *Leishmania* examination. Frozen vulpine samples were thawed and total genomic DNA was extracted from approximately 20 mg fragments of liver or spleen tissue, and from whole lymph nodes using Easy-DNA kit (Invitrogen) following the manufacturer's instruction. DNA was subjected to two consecutive PCR amplifications using the kinetoplastid-specific primers R221 and R332 in the first run, and the *Leishmania* genus specific primers R223 and R333 in the second run. Positive *Leishmania* nested-PCR samples were afterwards identified at species level using ribosomal internal transcribed spacer-1 (ITS-1) PCR-RFLP analysis by HaeIII enzyme.

RESULTS

Overall, sixty eight foxes were examined; 53 animals were shot between October 2012 – January 2013, while 15 vehicle-crashed foxes were found in a period between June and September 2013. From an examination of teeth, animals were classified as puppy (3), young (12), adult (49) and old (4). Thirty nine were males and 29 females, with a mean weight of 4.4 kg (range 0.9 – 7.0 kg). As regards geographical distribution, 48 animals were from Campania region and 20 were from Lazio region. At external examination no clinical signs of CL were recorded, except for frequent weight loss that could be attributed to other causes (e.g. intestinal worms). Likewise, no classical CL lesions such as spleen, liver or lymph node enlargement, nor renal macroscopic alterations were detected at necropsy. All target tissues were available for the examination in 43 foxes; spleen and lymph nodes were available in 2 cases, spleen and liver in other 2 cases, and spleen in 21 cases. Ten foxes (14.7 %) were found to be positive by *Leishmania* nested-PCR, of which 4 in spleen, 2 in lymph nodes and 4 in both organs. All liver samples were found negative at the analysis. *Leishmania* typing by ITS1 PCR-RFLP analysis of all positive samples confirmed *L. infantum* as the aetiological agent. As regards the estimated age of positive animals, 2 were puppies, 7 adults and 1 an old adult. Seven infected animals were recorded as presenting a poor body condition score (=1).

DISCUSSION

In our study, that confirmed indisputably the identification of the agent as *L. infantum*, 4/10 infected foxes where detected in January, indicating the persistence of the infection after the sand fly season, that in southern peninsular Italy occurs from the end of May through the end of October. The two positive puppies showing an apparent age of less than 2 months were detected early in June and in mid August, respectively; as regards the former case, a maternal transmission could also be hypothesized because sand fly infectious bites are estimated to increase in incidence starting from July. Our results showed a rate of *Leishmania* infection in foxes lower than previous data reported in Italy (range 40 – 52%) (Dipineto et al., 2007; Verin et al., 2010). Different rates of *Leishmania* infection have been reported in foxes from other Mediterranean regions endemic for CL, from very high in central Spain (74.6%) (Criado-Fornelio et al., 2000) to moderate (8.7%) in southern France (Davoust et al., 2014). In our study the low infection rate and the lack of the clinical disease support the hypothesis that foxes do not play a major role as reservoirs in endemic areas (Millàn et al., 2014). This latter conclusion was strongly suggested by Courtenay and colleagues (2002) as regards a different fox species in Brazil. A population of crab-eating foxes (*Cerdocyon thous*) was studied longitudinally by means of xenodiagnosis and quantitative PCR of skin samples. Results showed that *L.infantum*-infected foxes, which typically presented asymptomatic infections, were non-infectious for the vector (*Lutzomyia longipalpis*) and exhibited parasite loads similar to those detected in non-infectious subclinically infected dogs.

CONCLUSION

In conclusion, in southern and central Italy foxes may me subclinically infected by *Leishmania*, but their importance as effective reservoirs for the maintenance of CL remains to be determined.

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NON-INVASIVE METHODOLOGIES TO EVALUATE STRESS ON HARE (*LEPUS EUROPAEUS* PALLAS, 1778)

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ABSTRACT
In all living beings, stress generates different types of alterations: pathological, behavioural and physiological. In higher animals, especially wildlife, stress can suggest a physiological response-behaviour in relation to a target biological-ethological. In order to make an assessment of metabolites of stress is desirable to identify a bloodless method that allows to evaluate the activation of the axis Hypothalamic-Pituitary-Adrenal (HPA) by monitoring the cortisol on organic substrates. The results show differences but the reliability, practicality and utility of using faeces, hair and blood for the measurement of cortisol and estimation of stress in European hare.

INTRODUCTION
To measure the degree of animal welfare, the scientific community accepted two different methods: 1) Evaluation of the behaviour of animals by comparing the biological behaviour with the one standardized in 5 freedom criteria, and 2) assess the state of stress through the evaluation of hypothalamic-pituitary-adrenal axis (HPA). The methodologies currently applied and recognized include the measurement of corticoids from blood, faeces, urine or saliva (Cook et al., 2000). In animals, the sampling of hairs is an excellent method slightly invasive, easy to perform, without any problem of shelf life since it does not influenced by the variations of the water content. On the other hand, however, the hairs do not lend themselves to the study of the effects of acute stress because they are not able to provide indications about the daily or hourly fluctuations of the metabolites that use the hairs as site of accumulation (Koren et al., 2002). The behavioural characteristics of the hares for restocking induce this species in very violent defensive reactions, responsible of poussée stressful very difficult to study, and due to identify as pathological conditions (Esposito et al., 2001; 2005).

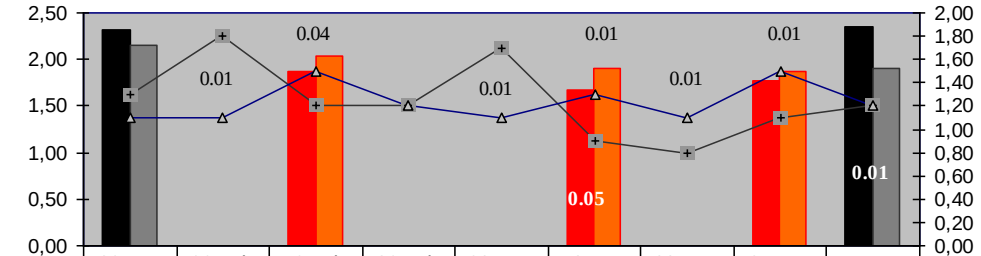
MATERIAL AND METHODS
In a farm of in cage's hare located in the San Felice a Cancello (Caserta, Italy), 20 hares (*Lepus europaeus*) aged between 6 and 8 months with equal distribution between the sexes (10 females and 10 males) have been tested. In a period of 90 days (16 January – 15 April 2014) samples were collected from organic substrates. For this aim cortisol levels were evaluated on 40 samples of hair (cut two time at T10 and T90); on 180 pools of faecal samples (pick up at intervals of 10 days: T10, T20, T30, T40, T50, T60, T70, T80, T90), and on 60 blood samples (collected three time during the trial (T30, T60, T80). Each animal was taken manually from the cage housing, placed in a carrying case to be taken to a special room equipped for manual measurement. On each specimen was evaluated: the general health situation, rectal temperature, cardiac and respiratory frequencies. At time ten (26.01.2014) and repeated at T90 (15/04/2014), the hair samples were obtained by shaving an area of about 3x5 cm from the jugular region and the nape region. Following the first shaving it was performed a control of re-growth of hair every 10 days in conjunction of which it made the blood sample with 1 ml syringe 26G X 1/2 ". On plasma samples of approximately 1 ml, obtained after suitable centrifugation, it was carried out the determination of cortisol. The hair samples were placed

in bags PPL individual, labelled and stored at room temperature until the arrival at the laboratories of the Department of Veterinary Medicine and Animal Production - UNINA. The hair samples, after being weighed, were divided into two aliquots: one for the determinations of cortisol and one for the measurement of growth. The hair sample of each animal was picked up with tweezers for eyebrows curve (94 mm) in such a way as to obtain for stripping, the hair whole outfitted with hair bulb. The morphological measurements were made with program" Imaging Processing and Analysis" in Java (Image J). The faeces produced by each individual in the 24 hours were picked up manually and individually, equipping the bottom of the cages with a lightweight net. Faecal samples, cleaned of food debris were collected in PPL bags, marked, labelled, weighed and stored -80° C until analysis. Samples of hair, blood and faecal pellets were processed for the dosage of cortisol (17-α-hydroxycorticosterone). The concentrations of the steroid were determined by RIA based on the binding of ^{3}H-steroid for competitive adsorption (Fenske and Schonheiter, 1991). All concentrations were expressed in pg/mg of hair and fecal, and in µg/dl of plasma (SEAC, Radim Group, UK). The extraction of cortisol by the hairs was performed by following the directions suggested by Koren et al. (2002). The Method of extraction from faeces was done by changing the suggestion by Schatz and Palme (2001). Cortisol was determined, both in the hair, blood and faeces according to the method proposed by Tamanini et al. (1983).

RESULTS

The results obtained demonstrated a reaction of the subjects to the manipulations which they have been subjected to data collection. The cortisol measured in hair during the experimental trial, shows that males hare get stressed more than females. However, the determinations of faecal cortisol allow to signal a state of stress higher in males than in females only in an initial period. While females have reacted in a more accentuated than males in half of the trial. The males and females, both stabilize stress reaction towards the end test. The differences recorded between the collection of stool, are conditioned by generic events occurring in farm (handling of weaned, allocation on carrying case for sale of restocking subject, etc.).

Figure 1. Cortisol response and trend curves in the different tested substrates (Feces pg/mg, Hair pg/mg, Blood µg/dl*10) in caged housing hares



	26 Jan	06 Feb	16 Feb	26 Feb	08 Mar	18 Mar	28 Mar	07 Apr	14 Apr
M	2,32		1,88			1,66		1,78	2,35
F	2,16		2,04			1,90		1,88	1,90
M	1,30	1,80	1,20	1,20	1,70	0,90	0,80	1,10	1,20
F	1,10	1,10	1,50	1,20	1,10	1,30	1,10	1,50	1,20

DISCUSSION AND CONCLUSIONS

The results confirmed what the present study aim to demonstrate. To measure cortisol as an indicator of the HPA axis in the European hare, the hair sampling represents a reliable and useful practice bloodless method to measure the stress condition in a period of middle-long time. The objective was achieved through validation of the determination of cortisol in the hair by comparing the values obtained from the already known method of determination in the stool and blood. Our

results show a low negative association between cortisol concentrations in hairs and those measured in the stool and blood. This correlation seems to support the hypothesis that both faeces/blood and hair are valid samples to measure cortisol and describe the adrenal lagomorphs activity, like what happens to other animals (dog, cat, primate). In conclusion, although it is only right to continue the investigation of the field, we can state that the use of hairs for the determination of cortisol as an indicator of stress in hare It is perfectly stackable with others biological substrates already tested such as blood, saliva, urine and faeces and the evaluation of physiological parameters (body temperature, heart rate and breathing, evaluation of blood parameters, etc.). However, it remains yet to be identified in the specific case of a hypersensitive animal as the hare, the method with the minimum stress during the translocation in natural environments.

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BREEDING OF GAME ANIMALS UNDER THE BIOTECHNOLOGY CONTROL

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ABSTRACT
One of the main objectives of our work was to collect available scientific and technical information concerning purpose and particularities of game animals, mainly red deer, fallow deer and mouflon breeding and reproduction, assisted reproduction, possibilities of long-term archiving of sperm using cryo-conservation and verification of this knowledge in practice. Other intentions were the verification of practices for hormonal preparation of females in controlled reproduction and its relation to mating season - rut, and also the results evaluation of mating with selected sires on the intensity of the growth of progeny and production improvements monitored over 3-year period. From the presented results we found out, that the optimal dose of PMSG hormone to synchronize oestrus for farmed game animals for red deer and fallow deer 200 IU and for mouflon 350 IU. As we detect, 62% of the observed animals were positive pregnant, with the deposition of insemination doses to the uterus body. We used two main methods, trans-cervical for red deer and laparoscopic intrauterine for mouflon and fallow deer. Small weight differences were found between progeny born from natural and artificial insemination. We described the methods and reasons for semen collection at breeding males wherein the positive results of the processes have been tested in praxis. Utilisation of proven breeding males with known and recorded information about their performance lead to improvements inbreed values of their offspring, wherein semen collection and cryo-conservation from quality proven breeding animals can save gene pool quality for the next artificial insemination and prevent inbreeding. This work was also financially supported by EU, project code ITMS: 26240220080.

INTRODUCTION AND GOALS

Farming of game animals is a global agriculture model, mainly for production of the best quality of venison and other products include breeding animals, trophy stag and fawns. We can classified the all product during live: velvet, milk, trophy and after slaughter as venison, skin, tail, sinew, glands and intestinal organs. If you would like to produce controlled animal products, you have to use all kind of biotechnology technics as assisted reproduction (artificial insemination, embryo-transfer), cryo-conservation of cells, DNA controlled breeding and safe your genetics for other generation. This work optimized the collection of semen from stag, hormonal synchronization of hinds before artificial insemination and also all process of insemination of females and placement of semen dose. As additional work we also optimized production of embryos of red deer and mouflon for better "genetic jump" of breeding animals under DNA control. The main goals of this work is application of reproduction and genetic research knowledge to real practise of game animals farming in Europe and use all biotechnology technics for farmers and their increase quality. Methodical and applied goals of all biotechnology technics of reproduction system consisted of hormonal synchronization, determination of time the ovulation and effects of place for better result of artificial insemination, collection, examination and processing include archive of semen doses of male as cryobank and storage for future.

MATERIAL AND METHODS

As experimental animals as partial part of this research were used animals from I. Insemination and breeding station of red deer, fallow deer and mouflon in EU - Xcell Slovakia Breeding Services,

officially registered number SK – ISJ 001 (approved by State Veterinary Authority of Slovak Republic). For our experiment we used totally 60 hinds and 5 breeding stag of red deer (*Cervus elaphus*) and 15 mouflons (*Ovis musimon*). The main experiment had done during 2012 – 2014 and all experimental animals were breeding in the same environmental conditions, include alimentary, veterinary care and welfare conditions.

Female reproduction methods of red deer and mouflon contain of hormonal synchronization of hinds. We used for synchronization combination of intravaginal tampons (CIDR®G, Eazi-Breed™, Pfizer, UK) as progesterone treatment and PMSG (*Gonadotropinum sericum equinum*) in two levels of international units. The CIDR (Controlled Internal Drug Release) tampons contain 0.3 g of progesterone. We prolong the luteal phase and control the follicular growing. The tampons we have leaved in vagina for 14 days, recommended after first 7 days change for a new one. After this CIDR period we take out and do the intramuscular injection application of Folligon® 1000 UI, Intervet International, Netherland, which contains 1000 IU of PMSG (Pregnant Mare Serum Gonadotropin). We controlled the result as reaction of ovaries, cervix of uterus, and also external expression of ovulation (external reproduction organs). We also determinated of time the ovulation and definition of place for better result of artificial insemination. Physiology of estrus was examined through rectum; the palpation of ovaries, uterus and also use ultrasonography USG ALOKA SSD-210DX with endorectal probe V561 (Hitachi Aloka Medical, Japan). We checked the reaction of *Corpus luteum* (CL) and other internal and external symptoms in 6 times from 48 hour till 58 hour after PMSG intramuscular injection. Consequently we used for this work two different places for force in the insemination dose – cervix of uterus and body of uterus. We used the same quality of ID (insemination dose) which contains concentration 140 mil. ml^{-1}, pejet volume 0.25 ml and motility minimum standard was 50% of progressive motility after thawing. We controlled the pregnancy on 35^{th} day after artificial insemination and used USG ALOKA SSD-210DX.

The main purpose of male reproduction is controlled reproduction, using more breeding males in the same herd and assures the variability of genes. The wide variability the offspring provide the long – term production of good quality of animals without inbreeding depression. Collection, examination and processing of semen doses of donors primary serve for cryobank as storage of the best genetics of red deer, fallow deer, mouflon and other wild and farmed species. Archiving in liquid nitrogen (-196^{o}C) is sufficient for storage semen doses, embryos for long time. What is different for all species is processing, ingredients of dilution medium and freezing of sperms. We optimized in this work medium and all processing of red deer and mouflon semen and use for artificial insemination. All collection and processing of semen contains: preparing collected stag, handling in crush, collect semen to sterile dose and laboratory processing as examination, dilution, preparing extender medium, filling of semen straws, equilibration, freezing and archiving. The semen was examined macroscopically and microscopically. Macroscopic examination involves density, consistency, colour, aroma and pollution. Microscopic examination contains mainly concentration of sperm ($mil.ml^{-1}$), total sperm (mil.), viable sperm (mil.), total motility (%), progressive motility (%), local motility (%) and immotile (%). For all microscopic analysis we used examination software Sperm Vision® Therio, CASA (Minitübe GmbH, Germany).

RESULT

Conclusions linking intensive animal husbandry biotechnological processes for stag (red deer) include: average of total volume of ejaculate: 5.7 ml; pH 6.85; average of concentration of sperm: 2 856 mil. ml^{-1};average of total number sperm: 16 279.2 million; total motility: 95.3%; progressive motility: 88.7%; pathological sperm: 11.3% of this 6.6% local motility and 4.7% non-motile. Standard quality of semen dose after defrosting: average 51.4% of progressive motility with total viable sperm for one AI 17.4 million.

Bio-technics for females (red deer, mouflon) contained synchronization of oestrus, superovulation, laparoscopic and transcervical AI, ET. Efficiency of AI 62%, optimized progesteron treatment for synchronized of ovulation: 14 days, 200 IU (red deer)/ 350 IU (mouflon) of PMSG. We optimized the

time for AI: interval 54 – 56 hours after PMSG, CIDR out. For profit of embryos and superovulation: combination of progesteron treatment and FSH/LH: 14 days in total number for red deer 500 IU and for mouflon 400 IU.

CONCLUSION AND RECOMMENDATION

The basic utilization of all results is application to practical farming of game animals in Europe. Also these results can help to save a lot of good and extraordinary genetics from farm and wild population. General advantages of artificial insemination are biological and genetic aspects, zoological and health factors, herd and offspring planning, export and import of semen / embryos.

ACKNOWLEDGEMENT

Big and sincere acknowledgement to my teacher and friend MVDr. Libor Čunát from Czech Republic. His great contribution in the field of biotechnology sheep and goats is irreplaceable, he applied the laparoscopic insemination and embryo-transfer of sheep and goat in practice and help us to understand very sensitive work with animals.

WHAT IS THE MOST APPROPRIATE WILD SPECIES FOR SURVEYING THE SYLVATIC CYCLE OF *TRICHINELLA* SPP. IN THE NORTHWEST OF THE IBERIAN PENINSULA?

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ABSTRACT

During the period 2006 – 2014, a total of 6 046 wild mammalian species have been examined for monitoring the presence of *Trichinella* spp. in Galicia (NW Spain). Animals belonged to seven wild carnivorous and omnivorous species, including autochthonous (4 533 wild boars, 1 195 red foxes, 96 Iberian wolves, 28 badgers and 12 European otters) and allochthonous species (150 American mink and 32 raccoons).

Larvae of *Trichinella* were isolated by artificial digestion from 13 muscle samples (one wild boar, nine foxes and three wolves), with a global prevalence of 0.22%.

Our results suggest that, among the wild mammalians present in northeast areas of the Iberian Peninsula, red fox is the recommended species for surveying the sylvatic cycle of *Trichinella* spp., because it is a common and abundant game species and, for this reason, the possibility to obtain a large number of samples is easier than in other hosts. In addition, this wild canid is widely distributed, adapted to sylvatic and synanthropic habitats and, moreover, capable to use a varied range of trophic resources, including rodents and carrions.

INTRODUCTION

Trichinellosis is one of the most important zoonosic parasites. Infections due to members of the genus *Trichinella* has a wide distribution (Pozio et al., 2009). In Europe, more that 150 mammalian species has been reported as carrier of *Trichinella*, including red fox (*Vulpes vulpes*), wild boar (*Sus scrofa*), wolf (*Canis lupus*) and other carnivorous and omnivorous species (Pozio, 2007).

In some European regions, *Trichinella* spp. spreads almost exclusively in the sylvatic cycle, with the red fox often playing a significant role as reservoir host (Hurníková et al., 2006). Even though *Trichinella* infection does not induce a symptomatic disease in foxes and other wild carnivorous, carcasses of these hosts can increase the biomass of the parasite in the environment, representing a risk for wild and domestic animals, and indirectly for humans (Miller et al., 2006; Blaga et al., 2009). In this context, the presence of the Iberian wolf in Galicia (NE Spain) potentially increases this risk.

The aim of the present study was to determine the prevalence of *Trichinella* spp. in wildlife from Galicia and, moreover, to propose the most appropriate host species to investigate the sylvatic cycle of *Trichinella* spp. in the northwest of the Iberian Peninsula.

MATERIAL AND METHODS

A total of 6 046 muscle samples (diaphragm, tongue and semimembranous-semitendinous muscles) from seven wild carnivorous and omnivorous species (Table 1) were collected during 2006 – 2014 in Galicia (NW Spain). Wild boar, fox and most of the wolf samples were from hunted animals, whereas samples of protected species (Badger and European Otter) came from carcasses conserved in Wildlife Rescue Centers of Galicia. In the case of raccoons and American minks, both of them allochthonous

species, animals were captured in Galicia and subsequently sacrificed, according to official programs for controlling invasive species.
Samples were examined by a pooled artificial digestion method with 5 samples per pool (European Commission, 2005; Gamble et al., 2000). When a pool was positive, 20 g from each sample of this pool were individually digested. *Trichinella* larvae were identified by a multiplex PCR analysis according to Pozio and La Rosa (2003).

RESULTS AND DISCUSION

Larvae of *Trichinella* were isolated from one wild boar (prevalence 0.02%), nine foxes (0.75%) and three wolves (3.13%), with a global prevalence of 0.22%. The only species detected in foxes and wild boars was *T. britovi*, whereas *T. spiralis* was also identified in wolves; concretely, one wolf was positive for *T. britovi*, another one for *T. spiralis* and, in one wolf, it was detected a mixed infection by both *Trichinella* species.
In Europe, *T. britovi* is the most frequent species in wildlife, and the prevalence of infection in wild boar is generally similar to that described in our study (Kirjušina et al, 2015). Moreover, specifically in Spain, mixed infections *T. britovi/T. spiralis* in wild boar have also been reported (Rodríguez et al., 2008).
With regard to wild canids, *Trichinella* prevalence in red fox from Galicia is lower than other previously reported in our country (Pérez-Martín et al., 2000). In our study, the higher prevalence was found in wolves, but it is lower than those described in some endemic areas from European countries (Teodorović et al., 2014). Despite the potential broad host spectrum for *Trichinella* spp. in wildlife, canids are the most important reservoir species of *T. britovi* (Pozio et al., 2009); in fact, the increase of *Trichinella* spp. prevalence among wild boar has been related to a concomitant increase of the carnivore populations (Kirjušina et al., 2015).
In many European countries, trichinellosis has been declared a disease that must be monitored, and for European Union Member States, this is obligatory (Directive 2003/99/EC). According to our results, wolf can be regarded as a good indicator species for monitoring the sylvatic *Trichinella* cycle in the northwest of the Iberian Peninsula. However, in our opinion, red fox is probably a better species for surveying the natural nidality and dispersion of *Trichinella* spp., because of its abundance and wide geographic distribution; moreover, despite fox and wolf are game species in Galicia, and both are in the top of the trophic pyramid (fox just below the wolf), the election of fox versus wolf is due to the fact that it is easier to obtain a large amount of fox samples per year, whereas the number of wolves hunted per year is scarce (no more than 9 – 10 every year).

Table 1. Prevalence of *Trichinella* spp. in wildlife from Galicia (NW Spain).

Family	Host species	Period	Nº samples	Total samples per Family	Nº *Trichinella* positive samples	Prevalence (%)
Suidae	Wild boar (*Sus scrofa*)	2008-2014	4 533	4 533	1	0.02
Mustelidae	Badger *(Meles meles)*	2006-2014	28	190	0	0
	European otter *(Lutra lutra)*	2010-2014	12		0	0
	American mink *(Neovison vison)*	2006-2014	150		0	0
Canidae	Red fox *(Vulpes vulpes)*	2006-2014	1 195	1 291	9	0.75
	Iberian wolf (*Canis lupus signatus)*	2006-2014	96		3	3.13
Procyonidae	Raccoon (*Procyon lotor)*	2013-2014	32	32	0	0
TOTAL			6 046	6 046	13	0.22

CONCLUSION

The red fox (*Vulpes vulpes*) is the most appropriate wild species for surveying the sylvatic cycle of *Trichinella* spp. in the northwest of the Iberian Peninsula, because of its wide geographic distribution and abundance. Moreover, given that fox is a game species, it is easy to obtain a large amount of samples per year.

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SANITARY INSPECTION OF LARGE WILD GAME OF EXTREMADURA

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ABSTRACT
Extremadura is an autonomous community of western Spain and is one of the most important reserves for big-game hunting in Europe. The aim of the work was to evaluate the appropriateness on consumption of meat from large wild game, after a post-mortem inspection of carcasses, conducted in the traditional Spanish «Montería» hunting way. A total of 1 107 carcasses were examined, of which 729 were seized. The major cause of seized has been the presence of sign of tuberculosis. The results indicate the endemic character of zoonoses that require deepening research in epidemiological data analysis.

INTRODUCTION
Spain, represent one of the major game reserves of the Europe and above all it take place in Extremadura, Castilla e La Mancha and Andalusia. In these areas is possible to meet large and small wild animals like: roe deer, deer, chamois, wild boar, rabbits, hares, partridges, quails and pheasants.The "Montería" is the traditional way of hunting large wild game (wild boar, deer and mouflon) in areas called "manchas", through "recovas" of dogs and battsem who raise their game to direct it towards the hunters placed in fixed points. From November to February is allowed to organize hunting of large wild game known as "Caza mayor". According to regulation (EC) No. 854/2004 all large wild game in Spain are subjected to a routine meat inspection in order to detect pathological-anatomical organ alterations due to disease. According to the Hygiene packet (852/2004, 853/2004, 854/2004) and by local rules the veterinarian of the *Equipo de Atención Primaria* (EAP)" must be present at the end of hunting game to guarantee the suitability of the meats to human consumption. Just like as other products of animal origin, large wild game may present microbiological and chemical risks and be carriers of zoonoses disease. The aim of the work was to evaluate the appropriateness on consumption of meat from large wild game, after a post-mortem inspection of carcasses, conducted in the traditional Spanish «Montería» hunting way in Extremadura.

MATERIAL AND METHODS
1 107 carcasses have been inspected by the Competent authority, in accordance with the Regulation 853/04 and 854/04in Cáceres, Spain. In particular, 588 wild boars, 488 deer, 19 mouflon sheep and 12 fallow deer have been inspected. Moreover it has been evaluate the trajectory of the projectile to verify the possibility of a perforation of the gastrointestinal organs that may lead to a carcass contamination and the extent of injuries caused by the bite of dogs. Furthermore, in wild boars, have been done research studies of Trichinella.

RESULTS
Details of causes of seizures have been reported in Table 1. A number of 1 107 carcasses have been inspected ,of which 34.14% are suitable for human consumption and 65.85% not suitable. Have been excluded from human consumption meats from carcasses with injuries caused by hunting dogs (10.11%), visible signs of deterioration (3.52%), abscess (1.8%) and neoplastic processes. Moreover, have been seized also the carcasses with gunshot perforations (0.18%).One case reported the

presence of Cysticercosis. Rather high has been the percentage of animals seized for the presence of tuberculosis lesions (47.96%).

Table 1. Post mortem inspection: Eligibility to the consumption and cause of seizes of the exanimated carcasses.

Post-morteminspection	Male deer	Female deer	Boars	Male mouflon sheep	Female mouflon sheep	Male Fallow Deer	Female Fallow Deer	TOTAL
Suitable for human consumption	103	75	183	6	4	4	3	**378**
Deterioration	5	14	15	3	1	1	0	**39**
Tuberculous lesions	74	125	330	1	0	1	0	**531**
Abscesses	6	4	10	0	0	0	0	**20**
Bited dog	28	31	46	2	2	3	0	**112**
Cachexia	0	12	1	0	0	0	0	**13**
Trichinella	0	0	3	0	0	0	0	**3**
Processes Neoplastic	4	4	0	0	0	0	0	**8**
Trajectory of the bullet.	0	2	0	0	0	0	0	**2**
Cysticercosis	0	1	0	0	0	0	0	**1**
Total Examined	220	268	588	12	7	9	3	**1 107**
Seizures For Species	117	193	405	6	3	5	0	**729**

DISCUSSION

In the carcasses exanimated has been reported a high percentage of seizes for suspected tuberculosis (47.96%) of which: 62.14% were wild boars, 37.47% deer and 0.18% fallow deer and mouflon sheep. It is possible to hypothesize the endemic nature of the tuberculosis that presumes the importance of knowledge by competent authority of the epidemiological data and implements measures of eradication of this pathology.

CONCLUSION

The spread and the recent increases of hunting in Extremadura caused growth of species population in the areas used for hunting, situation that increases the diffusion of diseases among these species (some of them with zoonotic character) and causes contaminations and infections to consumers. In order to ensure health and safety to consumers and to obtain epidemiological data on which base eradication campaigns to eradicate these zoonotic diseases in the country, it is necessary to intensify the monitoring and inspection of meat from the carcasses. As the hunting game in Spain represents a significant source of income, thanks to the continuous influx of people, the Junta of Extremadura may implement all possible way to eradicate these zoonosis from big wild animals, also limiting the trasmittion of infection to humans.

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PELOPHYLAX LESSONAE BLOOD COLLECTION BY ULTRASOUND-GUIDED CARDIAC PUNCTURE

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ABSTRACT
The use of amphibians for experimental purposes is the cause of death of the samples. In recent years Protected Areas managers ask to reduce the catch of these important animals of water-ecosystems. To know more details of the biology and the health of the frogs it is necessary to investigate the captured subject without killing them. Our work used an anesthetic protocol with isoflurane and described the echo-guided cardiac puncture to collect enough blood to be sent to laboratories. The ultrasound has allowed us to describe the heart rate and the relationship with the respiratory rate during the anesthetic induction.

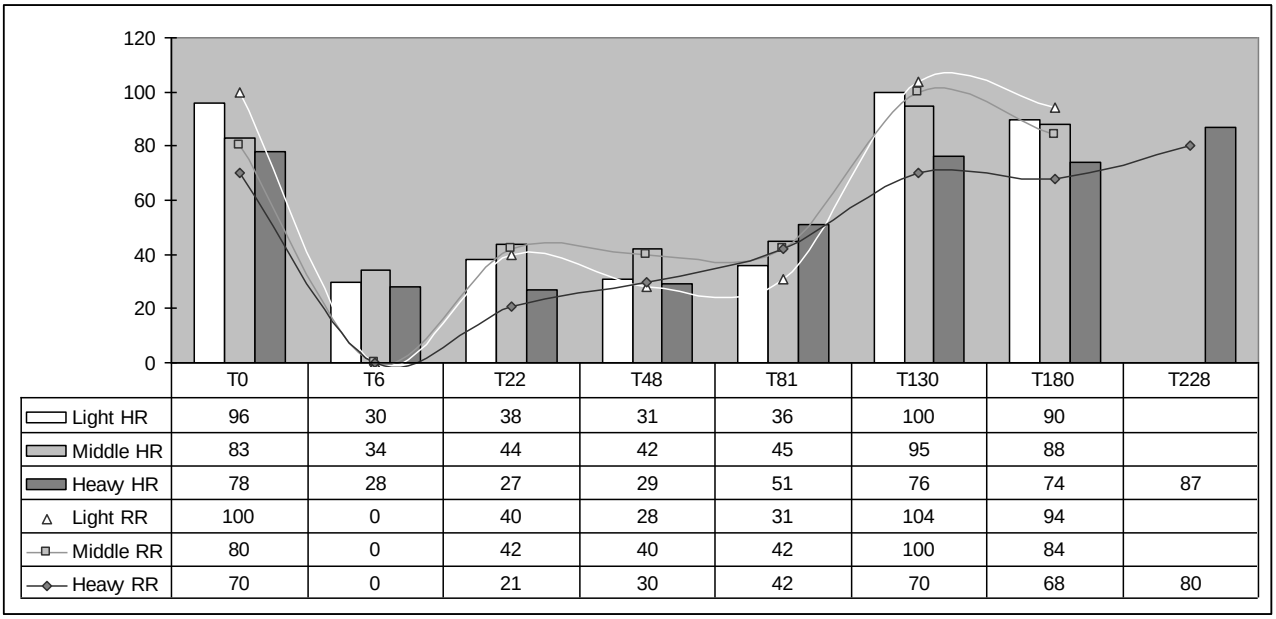
INTRODUCTION
Historically, frogs and toads were used extensively for experimental purposes: as the subjects for the study of helminth parasites (1); as the first simple diagnostic test for human pregnancy involving the injection of a sample of the patient's urine in Bufo (2); routinely for demonstration of nerve-muscle transmission on the isolated sciatic-gastrocnemius muscle, and for acetylcholine assays on the isolated rectus abdominis muscle (3, 4); as producer of particular skin immuno-protein(5); in the recent as subject for toxicological studies of environmental pollutants (6). In the past, but also today, all the above mentioned laboratory practice were obtained by the sacrifice of specimens. Given the recent concerns about the decline or total disappearance of some species of frogs, the collection and movement of native frogs in most Countries is subject to various legislative laws and regulations. Protection activities needs more information about amphibians management. The Protected Areas (National and Regional Parks, Wildlife Service, etc.) require knowledge about biology and environmental practice, included study and research. Recently many Parks have focused their attention to survival biodiversity and the role on the net food of many small animals as frogs. Recently, several non-cruel and/or non-lethal protocols have been proposed to find out the causative agents of diseases on small or very small animals (7, 8). In order to not inflict unnecessary suffering on animals, all frogs investigated by us were submitted to a sedation protocol (9). In this paper we used ultrasound guide to obtaining sufficient quantity of blood samples by our laboratories without recourse to frogs suppression in order to detect physiological parameters and microbiological/virological information about studied animals.

MATERIAL AND METHODS
During the activity of zoological identifcation of biodiversity into SCI IT9340086 Lago dell'Angitola (EFF Calabria "Angitola FISH2O" - Cod. 02/BA/12), twenty one adult frogs (*Pelophylax lessonae*) were captured (Park authorization June 28, 2013 protocol n. 756) in two places of Regional Natural Park of the Serre (Calabria, Italy) identified as "Pool" (38°44'21.90''N - 16°13'38.51''E) and "Bath" (38 ° 44'21.08''N - 16th 13'44.25''E). After five days from the arriving to Department of Veterinary Medicine and Animal Production (EC Regulation 1/2005 Annex I Chapter II, III) animals were transferred into the surgical room, submitted to an anesthetic protocol with isoflurane (9),and with the ultrasound guide (*Esaote* Mylab30gold with a 7,5 MHz linear probe) blood collection was effected by cardiac puncture.

When an adequate sedation of frogs was sure, confirmed by cessation of throat movement, closure of eyelids, lack of righting reflex, and lack of withdrawal to toe pinch, were carried swabs oral, cutaneous dorsal and abdominal for the determination of the bacteriological mapping.
Ultrasonography was made to evidence cardiac area, and heart rate was registered.
When ultrasound image showed the needle near the ventricular heart cavity, the cardiac puncture was effected on all the frogs using a separate syringe for each frog.
To ensure a physiological skin humidity, after the cardiac puncture (average of 7 minutes) the frogs were put on individual box with adequate shallow water to avoid the drying of frogs.

RESULTS

Heart rate may be monitored during anesthesia by direct observation (ventral midline, caudal to the shoulders), with ultrasonography detector. Because typical breathing of frogs (pulmonary and/or skin) normal values for heart rates have not been published. Our observations permit to signal different responses at different times of anesthesia. Graph 1 shows heart and breathing rates from time zero to time of induction and from the period of depth anesthesia to the awakening time. All the three groups of frogs (light, medium and heavy weight) show a significant decrease of heart rate when breathing rate was set to zero ($P<0.001$).
In general the heavy weight frogs shows a more marked bradichardia respect to the light and middle weight frogs at the time 6, 22, 48 and 81. The heart rate increased for the light weight frogs and middle weight frogs at time 130 when number of heart pulse were stabilized. Heart rate reach the starting number at 180 minutes, time of awaking. On the contrary heavy weight frogs reach heart rate similar to the starting moment at 130 minutes, but they need more time to awaking (T228).



	T0	T6	T22	T48	T81	T130	T180	T228
Light HR	96	30	38	31	36	100	90	
Middle HR	83	34	44	42	45	95	88	
Heavy HR	78	28	27	29	51	76	74	87
Light RR	100	0	40	28	31	104	94	
Middle RR	80	0	42	40	42	100	84	
Heavy RR	70	0	21	30	42	70	68	80

DISCUSSION AND CONCLUSION

The use of ultrasonography has allowed us to collect sufficient quantity of samples for laboratory (0.5 cc) but mainly to monitor the heart rate of frogs used.
For their characteristic to breathe through the lungs and through the capillaries under the skin, the evaluation of respiratory rate it is not a good parameter patient monitoring in anesthesia. Pulmonary respiration will cease during anesthesia in amphibians and can not be used to monitor anesthetic depth. Cutaneous respiration is sufficient to prevent clinical hypoxia during anesthesia and control cadenced with ultrasound system has allowed to control the triggering of bradycardia and its duration before awaking.

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CAUSES OF THE POPULATION DECLINE IN CAPERCAILLIE (*TETRAO UROGALLUS*) IN THE WEST CARPATHIANS

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ABSTRACT
From 1981 – 2014, population dynamics of the capercaillie *Tetrao urogallus* L. was studied on forty-three leks in the West Carpathians (Slovakia). Results demonstrate a marked decrease (>50%) in numbers of cocks and hens on twelve monitored leks (28%) and a slight decrease (<50%) on ten display grounds (24%). During the study period, capercaillie cocks became extinct on seven (16%) leks and in their surroundings. More or less constant numbers were found on only twelve leks (28%) and a slight increase occurred on only 2 leks (4%). Several factors have contributed to the dramatic recent decline in capercaillie population in the mountains of the West Carpathians. Habitat deterioration has probably played a main role.

INTRODUCTION
Most Slovakian data concerning the population dynamics of the capercaillie come from hunting statistics (Bancík, 1969; Ferianc, 1977). Only few serious population studies have been made on this endangered grouse species in the West Carpathians (Saniga, 1996, 1999).
This paper reports on the findings of a twenty-five-year capercaillie population study in the mountains of central Slovakia (West Carpathians). This study is aimed at: (a) monitoring population dynamics of the capercaillie on leks and their surroundings; (b) chick losses during the summer in this forest-dwelling tetraonid; (c) evaluation of the sex ratio in the chicks; (d) evaluation of the relationship between nest and chick losses and predaceous factors; and (e) explore reasons for the persistent downward trend in numbers that was documented over the study period.

MATERIAL AND METHODS
The field work was conducted in the mountains of central Slovakia (Veľká Fatra Mts., Malá Fatra Mts., Kremnické vrchy Mts., Starohorské vrchy Mts., and Nízke Tatry Mts., 18°50' - 19°10'E; 48°47' - 49°19'N) from 1981 – 2014. A total of forty-three leks were monitored during the spring display season. Altogether 842 evening and 1 337 morning observations were carried out on the forty-three leks during the spring display season.

RESULTS AND DISCUSSION
Results demonstrate a marked decrease (>50%) in numbers of cocks and hens on twelve monitored leks (28%) and a slight decrease (<50%) on ten display grounds (24%). During the study period, capercaillie cocks became extinct on seven (16%) leks and in their surroundings. More or less constant numbers were found on only twelve leks (28%) and a slight increase occurred on only two leks (4%).
Comparing the number of cocks on forty-three leks with the proportion of old-growth forest (over 80 years old with suitable spatial structure) within 1 km radius of a lek, a statistically highly significant correlation between the amount of old-growth forest and the number of cocks attending a lek was found (Pearson correlation coefficient $r = 0.725$, $p < 0.01$). This supports Wegge and Rolstad's (1986) findings that leks surrounded by a high proportion of old-growth forest supported more males than leks in fragmented areas.

When old natural forests are fragmented by clearcuts, the landscape loos qualities which are very important to this tetraonid. Transformation of the forest landscape from old-growth forests to clearcuts and younger stands auguments the number of small rodents. This presumably favours higher densities of generalist predators (especially marten and fox), which prey on capercaillie eggs and chicks (Rolstad and Wegge, 1989). Furthermore, the fragmentation of continuous forest habitat and its replacement with young stands unsuitable for capercaillie disrupts the social organization of capercaillie populations, particularly the formation of lek communities (Klaus and Bergmann, 1994).
Predation appears to be of major importance in limiting numbers of birds, including capercaillie. Out of 75 capercaillie clutches 49 (65%) were destroyed. Main mammalian egg predators of the capercaillie were found stone marten (*Martes martes*), pine marten (*Martes foina*), mustelids (*Mustela* sp.), and red fox (*Vulpes vulpes*) (altogether 18%), wild boar (*Sus scrofa*) (6%), and braun bear (*Ursus arctos*) (4%). Main avian egg predators were corvid birds, particularly jay (*Garrulus glandarius*) and raven (*Corvus corax*) (altogether 18%).
In the years with very cold weather during May (heavy snowfall), nests were destroyed by snow cover and abandoned (21%). Four clutches (8%) were found abandoned, their hens probably having been predated by goshawk (*Accipiter gentilis*), golden eagle (*Aquila chrysaetos*), and ural owl (*Strix uralensis*), or by some of the mammalian predators - lynx (*Lynx lynx*), red fox (*Vulpes vulpes*), martens (*Martes* sp.). The damage agent was not known in 12 destroyed and abandoned nests (25%).
Out of 23 perished adult capercaillies, hitting fences was a common cause of death to 11 (48%) capercaillies. Fences used in mature forests where browsing by deer is preventing the growth and development of natural regeneration do present a considerable hazard to capercaillie, especially when a fence runs through a forest. Two capercaillies were found to be killed on cables of the ski-lifts.

CONCLUSION

Several factors have contributed to the dramatic recent decline in capercaillie population in the mountains of the West Carpathians. Habitat deterioration has probably played a main role. The correlation between the amount of old forest and the number of cocks attending a lek has been significant. The presence of older trees has appeared to be important for capercaillie in West Carpathians, as elsewhere. The recent decline in numbers has also been associated with an increase in rainfall and snowfall in early June. The number of rain- and snow-days in this crucial period, when most of the chicks hatch, has been inversely associated with capercaillie breeding success. Predation has appeared to be of major importance in limiting numbers of capercaillie populations. Fences have also been an important cause of capercaillie mortality. The future of capercaillie populations in the West Carpathians will depend on the way in which the forest resources will be used and also on the effects of air pollution on forest health, ground vegetation and the abundance of insects available to chicks during the first weeks of their life. Habitat improvement via forest management practices should be the most successful way to save the species.

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THE OCCURENCE OF CULICOIDES MIDGES (DIPTERA: CERATOPOGONDAE) IN ZOOLOGICAL GARDEN IN KOŠICE

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ABSTRACT

The aim of this study was to determine the occurrence and host preference of *Culicoides* species in enclosures with different animals in zoological garden Kavečany – Košice. On three nights in summer 2014 a total of 3 168 *Culicoides* of 20 species were captured. The most abundant species were *C. obsoletus/C. scoticus* (30.4%), *C. pulicaris* (26.4%) and from other species dominated *C. furcillatus* (30.7%). The trappings in June and July were significantly more numerous than the sampling in August. The enclosures with ruminants provided more suitable biotope for *Culicoides* with the highest species diversity and abundance at antilopes and takins. Less attractive environment for *Culicoides* was near birds (ostrich, waterbirds, penguins) and small amount of *Culicoides* were acquired from enclosures with carnivores (lynx, wolves).

INTRODUCTION

Culicoides biting midges are primarily mammaliophilic and ornithophilic, although some feed on reptiles and frogs. They are responsible for the transmission of economically important viruses as Bluetongue and Schmallenberg in ruminants, and serve as vectors of avian trypanosomes and haemosporidian infections as well as vectors of filarial worms in mammals. Whereas the ZOO concentrates different kind of animals the aim of this study was to determine the occurrence and host preference of *Culicoides* species in enclosures with different animals.

MATERIAL AND METHODS

The survey was carried out from June to August 2014 using Mini UV light traps during one night every month. The traps were located in enclosures as closest to animals as possible and operated from late afternoon to the next morning. The insects were collected into water solution with few drops of detergent and transferred the following morning to 70% alcohol for storage purposes. The midges were identified to species level by examination of wing markings and further morphological characters (Delécolle 1985; Mathieu et al. 2012).

RESULTS

During three nights a total of 3 168 *Culicoides* midges of 20 species were collected. Proportionately the most abundant species were *C. obsoletus/C. scoticus* (30.4%), *C. pulicaris* (26.4%) and from other species dominated *C. furcillatus* (30.7%). The trappings in June and July were significantly more numerous (with the mean catch per trap per night 265 and 279 *Culicoides* respectively) than the sampling in August (mean 112 *Culicoides*/trap). The highest species diversity was observed in June (14 species). The ruminants attracted more *Culicoides* and the enclosures with these host provided probably more suitable breeding site for *Culicoides* since the most abundant samples were from paddocks with antelopes (87.2% of midges caught in June) and takins (63.8% of midges in July). The trap based at the antelopes caught the greatest total number of *Culicoides* (n=1 156), representing 10 different species. The highest species diversity (11 species, n=890) was observed in paddock with takins. *C. pulicaris* definitely prevailed at the antelopes, constituting more than 54% of all midges caught and the abundance of *C. obsoletus/C. scoticus* was 20%. In the enclosure with the takins dominated *C. furcillatus* (59%) followed by *C. obsoletus/C. scoticus* (24%). In August was the biggest the sample from enclosure with Przewalski´s horses (66.7%), but none trap was situated in vicinity of

ruminants during this night. *C. obsoletus/C. scoticus* were by far the most commonly trapped species (85%) in this paddock.
Less attractive environment for *Culicoides* was in enclosures with birds (ostrich, waterbirds, penguins) and small amount of *Culicoides* were acquired from enclosures with carnivores (lynx, wolves).

DISCUSSION
Except the wildlife animals which can be the reservoirs of pathogens for domestic animals and play important role in epizootology of diseases transmission, likewise the ZOO animals have to be taken in account. To our knowledge there are very few papers dealing with this topic in Europe. This our pilot study indicates that *Culicoides* vectors are common in enclosures with different animals in zoological garden. Although in comparison with the available results of Vilars et al. (2011) research conducted in Chester Zoo in north-west England, where over 94% of all the *Culicoides* trapped were females of the Obsoletus group, in this our study the principal vectors from both Obsoletus and Pulicaris groups comprised over 60 %. *C. furcillatus* is among the common species in ZOO, but the role of this species in pathogens transmission is unknown. The midges are principally concentrated at ungulates. We can assume that the reason is probably the accessibility of breeding sites in habitats containing mud rich in dung near water reservoirs, from moist soil or maize silage residues (Uslu and Dik 2010; Zimmer et al. 2013) or that these animals are the largest bait and the most attractive host as mentioned Viennet et al. (2013) and Elbers and Meiswinkel (2014).

CONCLUSION
Based on these results we conclude that *Culicoides* midges are common not only in habitats with farm animals but also in the environment of ZOO. The role of animals living in ZOOs in *Culicoides*-transmitted pathogens can not be neglected and it is necessary to consider the risk of transmission of pathogens between these animals and wild animals (red deer, roe deer etc.) as well as to farm animals.

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DIAGNOSTIC IMAGING IN EURASIAN SPOONBILL (*PLATALEA LEUCORODIA*): MAGNETIC RESONANCE VERSUS X-RAY

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INTRODUCTION

The Eurasian Spoonbill (*Platalea leucorodia*) is a bird of the order of *Ciconiiforms* family *Threskiornithidae*. In the Iberian peninsula, it inhabits western wetlands of Andalusia, Galicia and some other wetlands of the Cantabrian-Atlantic coast. This species is listed in the Red Book of Birds of Spain as "vulnerable" species and is listed as "special interest" in the National Catalogue of Endangered Species (1, 2, 3). This situation together with the lack of references on clinical aspects of the species justifies a particular interest in each of the individuals received.

MATERIAL AND METHODS

An adult of Eurasian Spoonbill 1.750 g weight and flightlesswas referred to the Veterinary Hospital of the University of León by the Local Environmental Services (Junta de Castilla y León). On examination, it was alert and reacted to external stimuli but an asymmetry was observed in the position of the wings, both at the station (with the animal standing) and walking; right wing was slightly drop with respect to the left with the carpus slightly lower and the tip of the remiges (flight feathers on the wings) located lateral and ventral to the rectrices (flight feathers on the tail) rather than relying on them. It also presents a punctiform wound at the junction of the neck with the thorax, on the ventral midline, with broken or missing plumage in an area of approximately 10 x 5 cm. A recent hematoma was observed beneath this area as well as external parasites of *Anatoecus* spp.

RESULTS AND DISCUSSION

Radiographic study discarded bone fractures and changes in the air sacs or coelomic organs. X-ray images were compatible with second-degree contusion in the right brachial and pectoral region. A routinely clean the wound was carried out and the animal washospitalized and kept under careful observation.

Despite the favourable evolution of lesions, the animal remained unable to take flight and maintained the positional asymmetry of the wings after 72 hours. A new examination was performed; the symmetrical retraction of both wings after passive extension (regarding both time and shape) was confirmed although there was a remarkable resistance to passive extension of the right glenohumeral joint and moderate or mild resistance to passive extension of the right elbow joint. So, it was decided to perform a MRI study using a closed 3.0 T HDx (General Electric, GE) equipment, with a superconducting magnet (4,5).The MR study started with a three planes localizer, followed by sagittal, coronal and axial slices in T2 and proton density with fat suppression. The most illustrative were coronal and axial slices, because in them the damaged region can be compared with the healthy contralateral. T2 hyperintense regions were observedin periarticular regions in both sides, although it occupies most of the medullary cavity in the right humerus. This made us to suspect of a subendostial accumulation of fluid. The images obtained with fat suppression allowed a better discrimination between damaged and healthy areas.

According to these results, we can confirm that MRI study allowed the visualization of anatomical structures in multiple planes and three-dimensional reconstruction andprovided more accurate and comprehensive information than other imaging techniques (5, 6). Several lesions that would have been imperceptible by conventional radiographic study in this Eurasian spoonbill were revealed using MR.

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CONSERVATIVE TREATMENT OF A TARSUS-METATARSUS FRACTURE IN LONG-EARED OWL *(ASIO OTUS)*

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INTRODUCTION

The eared owl, *Asio otus*, belongs to the *Strigiformes* family and is a strictly nocturnal bird. The distribution of the population is circumpolar, mainly in central North America, Eurasia, and Atlantic and Mediterranean islands (Khris Valero, 2003). The "NOCTUA" program revealed a negative growth in the population of this specie during the last years of the present and past century.

We present a clinical case of a male juvenile eared owl referred to the Veterinary Hospital at the University of León by the local environmental veterinary services.

MATERIAL AND METHODS

The specimen had been located few hours before arriving at the veterinary hospital on the side of a road close to a small village in the province of León, completely unable to fly.

On examination, the specimen seemed little reactive and lethargic, with dry and damaged plumage. He was able to stand, mainly on his left claw. The right wing was tilted to the left, with a noticeable asymmetry in both carpal bones and in the end of the flight feathers of the wings. When palpating the breastbone, the poor state of the muscles of the area could be detected.

When manipulating the right leg and claws, the specimen showed pain with an increased defensive reaction.

During the examination of the two wings, a partial cut about 3 cm with a triangular shape could be seen in the palette of the 5th, 6th, 7th and 9th flight feathers, while the 8th flight feather was completely cut at its proximal third.

There were no more significant findings according to routine protocols for bird examination.

A whole-body X-ray study was performed under general anesthesia with inhaled isoflurane. The anesthesia was induced and maintained after oxygenating the specimen. X-rays were taken with Sedecal equipment (A6501-02 model) and digitally processed. The patient was positioned lying on his right side to obtain a lateral projection. This was the first projection taken since it was considered the most stressful position for the animal. A second radiograph was taken with the specimen on the supine position, obtaining a ventro-dorsal view.

After the radiological study, the oxygen mask was kept until the animal recovered consciousness and muscle tone. At this point, the owl was place in a dark cage with an oxygen tube until complete recovery.

RESULTS AND DISCUSSION

X-ray study revealed a fracture at the distal metaphysis of the right tarso-metatarsal bone, not displaced, that separated the three articular facets of the distal tarso-metarsal bone from its diaphysis. There were also some noticeable deformities in the phalanges of both wings and slightly low radiodensity of long bones of the wings, both findings compatible with a metabolic bone disease at an early stage.

With all these data, the following diagnosis was proposed: distal metaphyseal closed fracture of left tarso-metatarsal bone, mild metabolic bone disease, moderate dehydration, partial amputation of flight feathers, capture stress and possible capture stress myopathy. Our suspicion was that the animal had been taken away from the nest and kept in captivity for a long period that gave rise to the incipient bone disease.

A conservative treatment was chosen for the fracture. It consisted in whole leg immobilization (femoral-tibio-tarsal joint, tibio-tarsal-tarso-metatarsal joint and tarso-metatarsal-phalangeal joint) with a light splint and bandages.
The positioning of the splint was carried out under general anesthesia in an open circuit T de Ayre using oxygen mask with 4% isoflurane. This concentration was reduced to 2% after 8 minutes. The splint was home-made, using an X-ray film cut in a rectangle and folded in the longitudinal axis, creating a V-section.
Typically, the treatment of choice for this type of fracture is based on external fixation (Vilaplana Valverde, 1987). However, external fixation was not suitable in this particular case due to the location of the fracture, as there was not enough space at the distal end of the fractured bone. On the other hand, intra-bone marrow fixation techniques, is generally inadvisable in birds. It is also contraindicated in either epiphyseal or metaphyseal fractures of bones with three articular facets in the epiphysis. Moreover, this type of fixation is contraindicated in animals with calcium metabolism impairment, increasing bone weakness and compromising calcification of the callus.

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SEPARATION OF NICKEL-BINDING PROTEINS

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ABSTRACT
Nickel-binding serum proteins of human and 11 animal species were determined using a combination of metal affinity chromatography and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Blood sera of human, cattle, sheep, red deer, mouflon, fallow deer, horse, pig, wild-boar, brown bear and pheasant were tested. Ni^{2+} bound to tetradentate ligand NTA (nitrilotriacetic acid, Ni-NTA) immobilized on agarose beads was incubated with animal sera to capture nickel binding proteins and subsequently proteins were eluted and fractionated on SDS-PAGE. Results showed an array of nickel-binding proteins with varied molecular masses in different animal species. It is noteworthy that, unique nickel binding ~42 kDa protein observed in the serum of bear was not present in any of the tested sera from other species.

INTRODUCTION
Serum proteins have been fractionated on the basis of their electrical charge into five classical fractions: albumin, α_1-, α_2-, β-, and γ-globulins. The major protein constituents of serum include albumin, immunoglobulins, transferrin, haptoglobin, and lipoproteins [1]. In addition to these major constituents, serum also contains many other proteins [2].
A number of biologically important Ni-binding sites have been identified in studies of the proteins and enzymes that bind metals [3]. Watt and Ludden [4] define and categorize nickel-binding proteins as (1) proteins involved in the recognition, binding and transport of nickel into the cell; (2) proteins involved in binding and sequestering the nickel once it enters the cell; (3) proteins required for the insertion of nickel into the nickel enzyme and (4) the nickel enzyme.
The aim of the study was to compare nickel-binding serum proteins in different animal species.

MATERIAL AND METHODS
Human, animal (cattle, sheep, red deer, mouflon, fallow deer, horse, pig, wild-boar and brown bear) and pheasant sera samples were purchased from Sigma-Aldrich. Samples were filtered through 0.22 µm syringe filters (Minisoft), pooled, aliquoted and stored at –80 °C until use.
Albumin depletion
Serum was incubated in NaCl (0.1 M) and cold ethanol with gentle rotation at 4°C for 60 min. Samples were centrifuged at 16 000 g for 45 min at 4°C. Supernatant was transferred into fresh tubes and pellet (# 1) was retained for further processing. pH of supernatant was lowered to 5.7 with cold Na-acetate (pH 4) and incubated with gentle rotation at 4°C for 60 min. After centrifugation supernatant containing albumin was removed and pellet (# 2) was resuspended in RNA free water and mixed with first pellet.
Metal affinity binding chromatography and protein fractionation
The albumin depleted proteins were incubated with metal affinity Ni-NTA beads (Talon, Clontech, Mountain View, USA) under the native condition as per manufacturer's instructions. After two washings with native wash buffer (0.05 M NaH_2PO_4 x H_2O, 0.3 M NaCl, 8 M urea, 10 % glycerol, 1 % Tween) bound proteins were eluted with elution buffer (NuPAGE® LDS Sample Buffer, pH 8.4).

Detailed protocol of pull-down assay is described in our recent publication [5]. Proteins were separated by SDS-PAGE on 10 % polyacrylamide gel. Serum proteins were visualised using Coomassie Brilliant Blue staining.

RESULTS

Electrophoresis showed the presence of Ni-binding protein bands in serum of each animal species (Fig. 1). The molecular weights of first band in each animal, except pheasant and fallow deer, were determined at over ~100 kDa. Second protein bands, except wild-boar, pheasant, cattle and horse, were determined at ~60 kDa. Third protein bands were observed in each case at ~57 kDa. In sample of brown bear ~42 kDa Ni-binding protein was observed but not in other animal species.

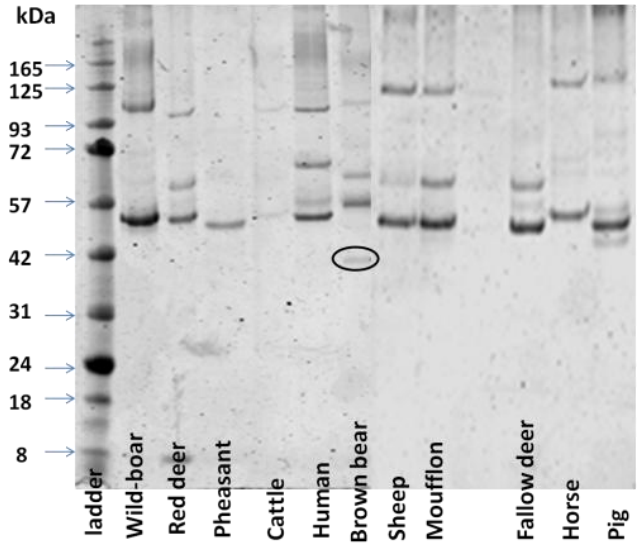

Fig. 1 SDS-PAGE analysis of sera Ni-binding proteins. Lane Ladder – molecular mass marker; Lane Brown bear – ~42 kDa

DISCUSSION

Wang et al. [6] used metal affinity chromatography to enrich a fraction of human serum proteins on immobilized columns loaded with cadmium, nickel, zinc, copper, or lead in bis–Tris saline and these proteins were identified using LC–MS/MS. They identified 20 Ni-binding proteins, e. g. alpha-2-macroglobulin, serotransferrin, apolipoprotein A–I, ceruloplasmin, apolipoprotein B-100, 47 kDa protein, hemopexin, transthyretin, histidine-rich glycoprotein, alpha-1B-glycoprotein etc. Similar results were also observed in other studies [7, 8]. However, affinity of serum proteins to Ni^{2+} in animal species has not been described in such a detailed way.

CONCLUSION

Results showed an array of nickel binding proteins with varied molecular masses in different animal species. It is noteworthy that, unique nickel binding ~42 kDa protein observed in the serum of bear was not present in any of the tested sera from other species.

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ENAMEL THICKNESS OF BEAVER'S INCISORS

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INTRODUCTION

The beaver is Europe`s largest rodent. Beavers are well known for constructing dams a cross water ways to form ponds. Therefore they need strong incisors, which are specialized for gnawing. So the constant growth of their incisors not only provides lots of building material, but also keeps their teeth short and sharp. A cross-section of a beaver's incisor shows three layers: an inner core of pulp, a surrounding layer of soft dentin, and a layer of hard enamel which covers only the front surface of the tooth. Enamel in particular is the layer responsible for the hardness of the tooth.

MATERIAL AND METHODS

Incisors inside the jaw were scanned by X-Ray Computer Tomography (CT). Since the density of the tooth layers was different, the thickness of enamel incisors were measured. The measurement was carried out on *corona dentis* at a length of 42.41 mm in 5 points. Software Volume Graphics V Gudio MAX 2.2 was used.

RESULTS AND CONCLUSION

In our study, we found out that the enamel of a beaver's incisors is 0.26 mm thick. Since rodent enamel is very similar to the enamel of man, this study may contribute to a better understanding of the mechanism of human dental caries.

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WILD RUMINANTS AS POTENTIAL RISK FACTOR FOR TRANSMISSION OF ANTHELMINTIC RESISTANT PARASITES BETWEEN SMALL RUMINANT FARMS

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ABSTRACT

The study was carried out to demonstrate a possible role of wild ruminants in spreading of anthelmintic resistant nematodes between flocks of sheep and/or goats. A benzimidazole resistant strain of *Haemonchus contortus* was passage through three mouflons (*Ovis musimon*) and subsequently used to infect two worm-free lambs. As the parasite isolate was passaged, the level of its benzimidazole resistance was estimated using an in vitro Egg hatch test in all, used hosts: sheep, mouflons and lambs. The level of ED_{50} did not change significantly, ranging from 0.71 to 0.78 $\mu g.ml^{-1}$ thiabendazole.

INTRODUCTION

The issue of the anthelmintic resistance has emerged first as sporadic findings at the beginning of the 1960's, slowly reaching the current state, when it presents a serious economic threat, especially in the countries with an intensive farming. At present, the problem anthelmintic resistance is of great concern especially in the herds of livestock. While the cross-transmission of gastro-intestinal nematodes between wild ruminant and livestock have been confirmed, there has been no investigation into whether could act as potential vectors of anthelmintic-resistant nematodes between sheep farms. Given the lack of treatment in wild ruminants, this would indicate nematode transmission from livestock to wild ruminants and raise the possibility that they can transfer anthelmintic resistant strains between farms.

MATERIAL AND METHODS

Three mouflons (*Ovis musimon*) aged between six and twelve months were placed into clean pens.All animals received treatment with ivermectin subcutaneously at dose of 0.4 mg per kg of body weight. When no eggs had been found in their faeces by sensitive flotation method (MAFF, 1986) for 30 days, each of the animal was infected with 5 000 L3 larvae of *Haemonchus contortus* strain. The strain was resistant to fenbendazole and isolated from the sheep farm in the Baton Rouge area of Louisiana, USA (Hembry et al., 1986). Egg hatch test was performed as previously described Coles et al. (2006). Pure thiabendazole (TBZ) was dissolved in DMSO. Assays were carried out using Sterilin 24 multiwell plates (Linbro plate, Flow Laboratories). Approximately 200 eggs per well were incubated for 48 hours at 26°C in serial concentrations of TBZ. The final concentrations of TBZ used were 0.1, 0.3, 0.5, 0.7 and 1.0 $\mu g.ml^{-1}$. Results of the test are presented as ED_{50} values, which is the concentration of TBZ required to kill 50% of the eggs. The data were analyzed by a logistic regression model (Dobson et al., 1987).The test was carried out with two replicates for each drug concentration plus control well and was repeated five times during patency.

RESULTS

Twenty five, thirty five and forty five days after infection individual rectal faecal samples were collected from all experimental animals to determine eggs per gram (EPG). All animals were positive on EPG on day 45 post infection. Faecal samples for in vitro Egg hatch test was collected on five separate days (Day 45, 48, 55, 58, 62) post-inoculation. The mean $LD_{50}\pm$ SD was 0.71 $\mu g.ml^{-1} \pm 0.12$ TBZ. The infective larvae obtained from coprocultures of faecal samples were then used to infect a pair of lambs. After the parasites reached patency the Egg hatch test has been performed and showed ED_{50} values 0.78±0,09 $\mu g.ml^{-1}$.

DISCUSSION

The results indicate that mouflon is able to transmit anthelmintic resistant nematodes and could spread anthelmintic resistance in those regions where it exist. The experiment also showed that the change of host species did not have any significant influence on level of benzimidazole resistance of *H. contortus*. However, further research is required to investigate the possible role of different species of wild ruminants (*Capreolus capreolus*, *Dama dama*) in transferring anthelmintic resistant nematodes between farms.

CONCLUSION

The study confirmed that *Haemonchus contortus* maintained the level of anthelmintic resistance during passage through *Ovis musimon* and change of host species (transfer from mouflon to sheep) did not have any significant influence on level of resistant alleles within population.

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RELATIONSHIP BETWEEN LIVESTOCK FARMS AND LARGE GAME REGARDING THE PRESENCE OF BOVINE TUBERCULOSIS – A SPATIO-TEMPORAL APPROACH

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INTRODUCTION

Bovine Tuberculosis is a serious animal health problem. Eradication of bTB in cattle is a priority in the EU (Martínez-López et al., 2014). In Portugal, despite the efforts made over the last years to eradicate bTB, several livestock hotspots remain infected. In some regions, difficulties in eradicating bTB in cattle may be related to the occurrence of the disease in large game species living in areas around herds. The severe problem of tuberculosis in large game in these regions, was already identified by the National Veterinary Authority which, in 2011,stated them as “Epidemiologic Risk Area for Bovine Tuberculosis in Large Game” and published an internal law (Edital n. º 1) describing mandatory rules for each driving hunt organized within these areas. Idanha-a-Nova (IN), is one of the counties included in the risk area, due to the high infection rate in wild boar and red deer as previous presented by Vieira-Pinto et al. (2011). In Portugal epidemiological studies at the interface between livestock and large game concerning to bTB infection remain scarce. Thus, address how animals and the disease are geographical distributed as well as identify interspecific interactions can be of extreme importance to better understand transmission between wildlife reservoirs and sympatric livestock populations and implementation of cost-effective control and mitigation strategies. This way, the aim of this study was to investigate spatiotemporal distribution of bTB in sympatric livestock and large game (from 2010 to 2014) from IN and analyse associated disease distribution.

MATERIAL AND METHODS

We performed the study in IN County located in the central-east Portugal (lat. 39º 55'N: long. 7º 14'W, figure 1) with 1412.7 Km2 and 10561 inhabitants. This county has a considerable hunting activity and domestic animal production (bovine and small ruminants, especially sheep), mainly based on outdoor extensive production that is of major economic importance. A GIS project was created, for the years 2010 up to 2014, with data concerning to bTB prevalence in wild boar and red deer, and herds status from IN recorded by the Regional Veterinary Authorities. Tuberculosis-like lesions (TBL) in ungulate populations have been employed as a criterion to estimate and evaluate disease distribution and prevalence (Vicente *et al*., 2007). Carcass evaluation was always performed, after each driven hunting actions on hunted game animals, by the same veterinary official from the IN Town Hall. All hunting spots were georeferenced, by means of Google Earth analysis and polygons creation, in order to update the GIS project with information regarding bTB prevalence in large game and to enable descriptive statistics analysis and geoestatistical analysis calculation (Soares, 2006). Using the percentage of bTB compatible lesions calculated to each sampling plot and geoestatistical analysis (performed using Geoestatistical Analyst 2.0 for ArcGIS 9.x. ArcInfo version) continuous maps related to disease spread were created, in order to extent the results to all study area and to create continuous disease intensity maps represented by a colour intensity gradation (Schröder, 2006).They were used various types of statistical analysis: Pearson correlation, regression analysis, Student´s t test and inverse distance weighted interpolation according to the different type of variables and analyses.

RESULTS AND DISCUSSION

In this study we attempted to analyse the spatial variation of the bTB in cattle herds throughout IN in the period 2010 – 2014 in order to further assess its relationship with spatial distribution of bTB in large game. It was observed a decrease from 9% in 2010 to 2.5% in 2014.From the positive herds, after 2010, 14 were newly-infected and 30 lost thebTB positive status. From those, 12 herds were positive for over than one year: 8 (two times), 3 (three times) and 1 (four times).When analysed the spatial location of positive herds for over than one year, it was noticed that 8 (57%) were located at southern Idanha-a-Nova County precisely where hunting areas presented higher percentage of hunted animals with bTB compatible lesions. These herds include all the cases of persistent infection 3and 4years and of re-infected 2times in alternate years. These results are in accordance with the studies developed by Mendoza et al. (2006) in Extremadura (Spain) that pointed out the transmission of bTB from the red deer and wild boar to cattle as the responsible for the frequent re-infection of cattle herds. Geoestatistical analysis also shown that for the years 2010, 2011 and 2013, estimated bTB prevalence in large game in geographical areas of positive herds was higher than in geographical areas of negative herds, been these differences statistically significant (p-value < 0.05), both for red deer and wild boar. For the years 2012 and 2014, it was noticed the same pattern, but the differences were not statistically significant. These results substantiate our earlier suspicion, enabling to state that positive herds in IN County located in areas were bTB estimations evidenced higher values (Southern and western areas) tend to be more propitious to TB infection. Nevertheless, in this study, bTB prevalence in large game seems to be a more severe problem, than the one observed in bovine herds and development of bTB in game in IN appears to be independent of the continuous decrease in the disease in cattle. Presently, these wild species seems to have the ability to maintain the disease in the absence of livestock and this trait can identify them as potential reservoir for the disease (Gortázar et al. 2005; Corner 2006). This study demonstrate the potential of these tools to analyse spatial distribution of animals and bTB infection as well as identify interspecific interactions that can be of extreme importance to better understand transmission between wildlife reservoirs and sympatric livestock populations and implementation of cost-effective control and mitigation strategies.

CONCLUSION

To the best of author's knowledge this is the first study in Portugal that uses information over an extensive period of time for evaluate spatiotemporal dispersion of bTB in large game and livestock (2010–2014) and to assess the relationship between them. Results enabling to state that positive herds in IN County located in areas were bTB estimations evidenced higher values (Southern and western areas), considered of priority intervention, tend to be more propitious to TB infection (statistically significant for years 2010, 2011 and 2013; years 2012 and 2014, it was noticed the same pattern, but the differences were not statistically significant). However, it is simplistic to assume that infection in cattle is due entirely to an inherent reservoir in wildlife. Although we found evidence of transmission to cattle, we cannot rule out the existence of other possibilities responsible for cattle infection. Also, the high number of recurrence, persistence and new infections suggests the necessity to look for alternative, more cost-effective TB prevention strategies. We would like to underpin the usefulness of GIS technology as an essential component of modern disease surveillance systems. GIScan be an important scientifically based tool to continuing understanding and monitoring TB transmission and persistence in order to help to cost-effectively control and eradicate this complex zoonotic disease. Methods and results presented in this study may support policies to better prevent and control bTB in the IN and in other regions with similar epidemiological conditions.

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MONITORING OF AUJESZKY DISEASE IN WILD BOARS OF THE VENETO REGION, ITALY, 2012 – 2015

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INTRODUCTION

Porcine herpesvirus 1, also known as Aujeszky's disease virus (ADV), is the causative agent of Aujeszky's disease (AD). The virus belongs to the *Herpesviridae* family, *Alphaherpesvirinae* subfamily, *Varicellovirus* genus and it causes one of the most important and widespread diseases affecting domestic and wild pigs. ADV is a neurotropic virus, infecting both the respiratory tract and the central nervous system where it remains latent especially in the trigeminal ganglia, the olfactory bulb, the tonsils and the sacral ganglia. Members of the family *Suidae* are considered the natural hosts although the virus can infect a wide range of dead-end hosts, with the exception of humans and major primates. As a cause of heavy economic losses to the pig industry, AD is a notifiable disease included in the OIE list.

As wild boars could play a role in the transmission of ADV to domestic pigs, the European Union guidance to the Commission Decision 2008/185/EC (SANCO 3023/2008) recommends the monitoring of wild boar populations while ensuring stringent biosecurity measures in order to prevent the transmission of ADV between wild animals and domestic herds. In European wild boar populations, seroprevalence ranges between 0.3% and 66% (Müller et al., 2011). In Italy, AD seroprevalence varies from 0% to 51% and high percentages of positive samples for viral antigen were also recorded (e.g. 41% in tonsillar tissue; Lari et al., 2006).

Many European Union countries and regions have gained the AD free status or have implemented compulsory control programs (article 9 of the Council Directive 64/432/EEC). In such framework, the Veneto region (North-East of Italy) needs information on the epidemiological situation regarding AD in the local wild boar population in order to be included in Annex II of the Commission Decision 2008/185/EC. The present study evaluates the presence of AD in the wild boars hunted in the territory of the Veneto region through a serological survey on serum and meat juice, plus a direct search of the virus in tonsils and trigeminal ganglia.

MATERIALS AND METHODS

From 1st January 2012 to 30th April 2015, 252 wild boars were hunted in four provinces of the Veneto region (Belluno, Treviso, Verona and Vicenza). Animals were divided into 4 age classes according to Matschke, 1967.

Sera collected after centrifugation of cardiac clots were diluted 1:25 and tested for Abs presence by the Ingenasa Ingezim ADV total 1.1 ADV.K1 kit.

Masseter muscle was cut into pieces, frozen at -20°C and thawed repeatedly in order to collect the *meat juice.* Antibody quantification was performed using the Ingenasa kit (final dilution 1:10; Natale et al., 2012).

Viral DNA from tonsils and trigeminal ganglia was extracted using "High Pure PCR Template Preparation kit" (Roche Diagnostic) for the detection of a 338 base pairs fragment through a PCR end-point protocol (Katz and Pedersen, 1992). The reaction target is situated in the genomic portion US8, codifying for the glycoprotein E that allows to differentiate between vaccinated and infected animals.

RESULTS

The distribution between different sex and age classes of wild boars harvested in the period 2012 – 2015 is shown in table 1 and 2, respectively. Table 3 shows the positivities detected in the same period.

Table 1. Wild boars harvested and analysed for AD since 2012 in the Veneto region, stratified by sex.

Year	Females	% of females	Males	% of males	Not determined (ND)	% of ND	Total
2012	73	52.1%	67	47.9%	0	0.0%	140
2013	21	43.8%	27	56.3%	0	0.0%	48
2014	5	22.7%	17	77.3%	0	0.0%	22
2015	18	42.9%	17	40.5%	7	16.7%	42
Total	**117**	**46.4%**	**128**	**50.8%**	**7**	**2.8%**	**252**

Table 2. Wild boars harvested and analysed for AD since 2012 in the Veneto region, stratified by age class. Class A: absence of molar teeth, less than 6 months of age. Class B: 1 molar tooth, 6 months – 1 year of age. Class C: 2 molar teeth, 1-2 years of age. Class D: 3 molar teeth, more than 2 years of age.

Year	A	% of A	B	% of B	C	% of C	D	% of D	ND	% of ND	Total
2012	7	5.0%	36	25.7%	71	50.7%	26	18.6%	0	0.0%	140
2013	7	14.6%	19	39.6%	18	37.5%	4	8.3%	0	0.0%	48
2014	0	0.0%	10	45.5%	11	50.0%	1	4.5%	0	0.0%	22
2015	2	4.8%	19	45.2%	16	38.1%	3	7.1%	2	4.8%	42
Total	**16**	**6.3%**	**84**	**33.3%**	**116**	**46.0%**	**34**	**13.5%**	**2**	**0.8%**	**252**

Table 3. Province, age category and sex of hunted wild boars tested positive for AD in the period 2012 – 2015. In addition, analytical technique and matrix of samples tested positive are specified.

Year	Province	Age category	Sex	Analytical technique	Sample matrix
2012	Treviso	A	M	PCR	Tonsils
2012	Vicenza	B	F	PCR	Tonsils
2013	Treviso	C	F	Antibody quantification	Muscle (Meat Juice)
2013	Verona	C	M	Antibody quantification	Muscle (Meat Juice)
2015	Treviso	C	M	Antibody quantification	Muscle (Meat Juice)

DISCUSSION AND CONCLUSION

The variability in the distribution of wild boars between sex and age classes throughout the years was expected as the samples were collected from wild boars hunted in the framework of harvest plans. With regard to the survey conducted in the Veneto region, 0.79% of wild boars hunted were tested positive for Ag and 1.19% were tested positive for Abs. Interestingly, data from the neighboring Lombardy region are similar: from 2011 to 2012, 2.7% and 0.1% of wild boars resulted serologically and virologically positive, respectively (Cordioli, 2013). Both regions were recently colonized by the wild boar, which populations have relatively low densities with a patchy geographical distribution (ISPRA, 2005 http://www.isprambiente.gov.it/contentfiles/00004300/4327-banca-dati-ungulati-1.pdf/view).

The findings confirm the presence of ADV in the study area. Even though analyses on virus isolation and typing are still ongoing, it is possible to affirm that the presence of ADV in wild boar populations

poses a potential risk for pig farming. Although ADV affecting domestic pigs belongs to different genotypes and subtypes in respect to wild boar AD viruses (Müller et al., 2011), the risk of spillover from wild to domestic animals is consistent, as suggested by experimental studies (Müller et al., 1998, Hahn et al., 1997). Nevertheless, the focal distribution of positive wild boars in the Veneto region seems to represent a risk mainly for open-air pig farms and small-scale pig production, where biosecurity measures are less stringent.

INFLUENCE OF CLIMATIC AND LOCAL ENVIRONMENTAL CONDITIONS ON DEER: COMPARISON BETWEEN AIR TEMPERATURES MEASURED BY A WEATHER STATION AND GPS COLLARS

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INTRODUCTION

Climatic and local environmental conditions, particularly during summer and winter (Anderson et al., 2009), affect critically the habits of wild ungulates, conditioning their ranging behaviour, the size and distribution of the home range (Rivrud et al., 2010) as well as their movement rates in different land cover types (van Beest et al., 2013). Knowing the exact local meteorological conditions could help understand and predict the extent of these effects; GPS collars are frequently equipped with a temperature sensor that provide the animal-level environmental temperature monitoring. However, several bias connected with the features of the sensor construction might exist. In this work we tried to test the reliability of the sensors comparing them to weather station data.

MATERIAL AND METHODS

Twelve hinds (*Cervus elaphus* L.), aged 2 – 9 years, were immobilised with a dart-gun around the Acquerino-Cantagallo Natural Reserve (Tuscany, Central Italy) and fitted with GPS/GSM radiocollars (Vectronic Aerospace GmbH) provided with sensors measuring, hourly, environmental temperature. Hinds were monitored from January 2009 to December 2010. In the same territory, air temperature data were obtained from a reference weather station (altitude 890m a. s. l.; 44°00′03.7″N, 11°00′35.7″E) representative of the average weather conditions (Hydrologic Service of Tuscany Region). Correlation between weather station and collars temperature data was tested with a statistical way, Pearson's correlation coefficient.

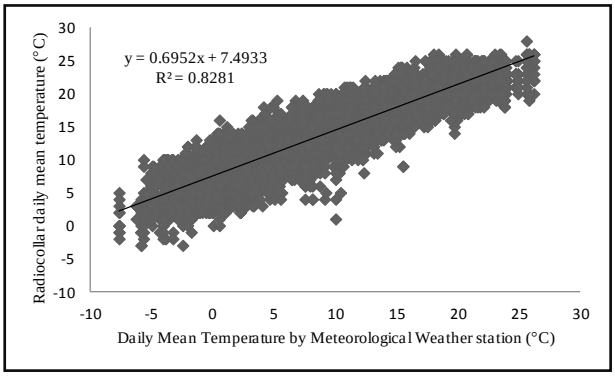

Figure1. Correlation between daily mean temperatures measured by GPS collar (N = 12) and those measured by a weather station.

RESULTS

The comparison between the temperatures registered by GPS collars and by weather station showed a significant linear correlation (Fig. 1) with an $R^2 \approx 0.83$. When focusing on the temperatures registered by the collars of two hinds selecting different habitats, both cases shown in the overall collar temperature pattern are nearly identical to the weather station temperature. However, collar temperatures were always higher than the weather station temperature (Fig. 2).The lowest differences between the two sets of data occur when considering MinDT, while the variance (Δ)

increases progressively in AveDT and peaks in MaxDT, with the highest Δ in autumn and winter (Fig. 2).

DISCUSSION

The study has shown that the environmental temperature measured by the GPS collar was highly correlated with the air temperature measured typically by a weather station. However, the GPS collar temperature was on average higher than the weather station temperature, prevalently due to the animal's body temperature, fur colour, animal behaviour, its activity in different land covers. The main effects were strongly related with the solar radiation contribution (Berger and Courtiol 2013), so the maximum Δ occurred during periods with shorter day length (autumn-winter), when red deer is more active during mid day hours (Carranza et al., 1991); on the other hand, during summer, the animals shown a greater activity during dark hours.

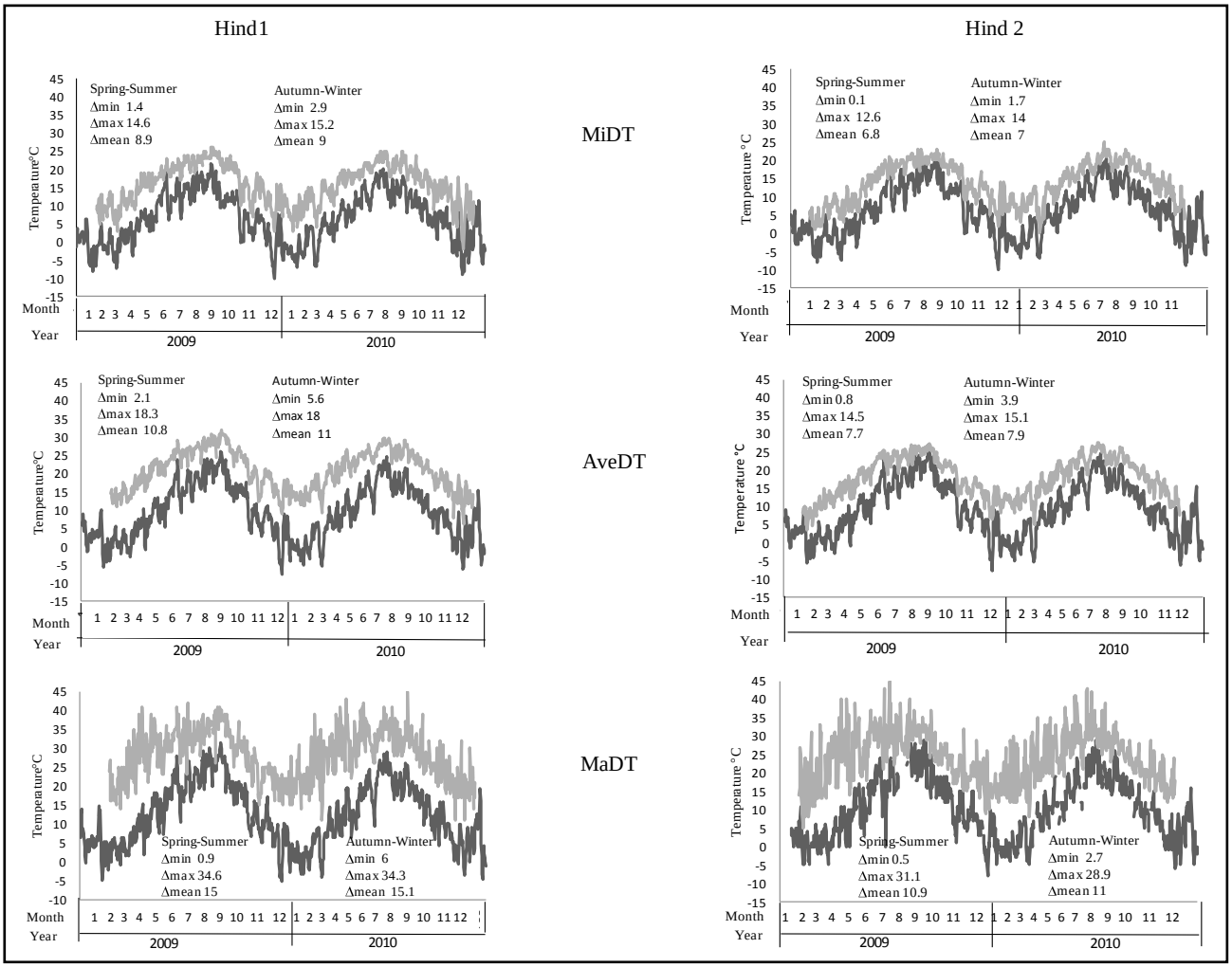

Figure 2. Trend of minimum daily temperature (MinDT), average daily temperature (AveDT) and maximum daily temperature (MaxDT) measured by two GPS collars (light grey line) fitted on adult hinds and by meteorological weather station (dark grey line).

CONCLUSION

The high correlation found could allow the use of the data recorded by sensors in GPS collars, through proper data adjustment after identification of a correction factor. The availability of the

animal-level environmental temperature parameter might represent a useful tool for studying the behaviour of wild ungulates, especially when representative data from weather stations are not available or lacking. Moreover, opportune improvements of the collar design and ergonomics would allow a reduction of the effects of solar radiation on the sensor measurement.

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