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**ISoWiF 2019**  
**BOOK OF ABSTRACTS**

XI<sup>th</sup> INTERNATIONAL SYMPOSIUM  
ON WILD FAUNA

The final version  
will be available soon

**25 to 28 September 2019**  
**Viterbo, ITALY**

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ON WILD FAUNA**

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Book of abstracts

ISoWiF 2019

**11<sup>th</sup> International Symposium on Wild Fauna  
Viterbo (Italy), September 25<sup>th</sup> – 28<sup>th</sup>, 2019**

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Editors

Andrea Amici, Luigi Esposito, Luigi De Grossi, Paolo Viola, Riccardo Primi, Settimio Adriani

*Waves Italia* - is a non-profit organization- whose purpose is to enhance the scientific and technical training of its members and other professional associations related to the management and conservation of wildlife in the Euro-Mediterranean Countries

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## Wildlife management

### *Main lectures*

#### **Rock partridge population biology in the Alps: results from a radio telemetry study**

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**Abstract:** Rock Partridge *Alectoris graeca* is endemic to southern Europe, occurring only in the Alps, the Apennines, Sicily and the Balkans. Its population is known to have widely declined throughout its range since the 1950s', as a result of habitat loss and degradation and in some countries of overhunting (Bernard-Laurent and De Franceschi 1994, Griffin 2011). Populations of the Alpine subspecies *Alectoris graeca saxatilis*, in particular, were becoming more fragmented and smaller on the western edge of the species' range in the French Alps (Magnani *et al.* 1990, Deloche and Magnani 2002, Buffet and Dumont-Dayot 2011). Included in the Annex I and II of the 2009/147/ECC Directive (ex ECC Directive 79/409), Rock partridge, recently uplisted to "Near Threatened" by Birdlife (2012), requires to be preserved and needs a careful management of its habitat and population. Moreover Rock partridge is a game species whose hunting in the French Alps is allowed under harvest quotas. Despite the high patrimonial and hunting value of this mountain wild game, no field study at all have investigated the species dynamics and spatial ecology. Aiming to improve management, an intensive field study started in 2012 in the French Alps, conducted by ONCFS, to get data on demographic traits and on and patterns of movements. 179 radio-tagged birds were monitored by telemetry from 2012 to summer 2019. As the study is still in progress, we present preliminary results which have to be confirmed by future statistical analysis. Spatial ecology: As resident species, rock partridges were not known hitherto to migrate or move long distances. Radiotelemetry of birds revealed that a high proportion of the population migrated to distances. Main results about spatial ecology of our rock partridge population are presented here in a poster. Demographic traits: Components of breeding success, survival and causes of mortality were studied from radio-tagged birds. Data about reproductive success originate from 97 records of nesting attempts and 45 known-age broods. Some females, lay in 2 nests, one incubated by herself and the other one by her mate (Bernard-Laurent *et al.*, 2017). Clutch sizes and hatching success were recorded, as well as the variation between incubating sexes. Rock partridge lay large clutches (mean 10 eggs): clutches incubated by males are larger than those incubated by females. The proportion of incubating males was rather high, but varied markedly between years from 33 to 88%. The nest survival rate was 0.58 [0.46-0.68]. Nest success was higher for females than for males. Predation of eggs by small and medium-size mammals was the the main cause of nesting failures. Sex-ratio of chicks at hatching is unbalanced in favour of females. Survival rate of chicks until 1 month old, is 0.26 [0.22-0.31] all years combined. It varied between years. Predation by raptors was the major cause of death of partridges more than 2 months old, with raptors accounting for 85% of identified predation events, while predation by carnivorous mammals was low. Seasonal mortality was highest first in winter and second during the breeding season. The annual survival rate of adult birds was 0.35 [0.28-0.42] with no significant difference between sexes. Young birds had a lower survival rate than adult ones (0.29 vs. 0.35). Rock partridge is therefore a short-lived galliform bird which exhibits high reproductive potential. Demographic models are

ongoing to help making decisions for managing partridge populations. Impact of local climatic factors: Alpine species are exposed to a highly seasonal climate. Several biological traits of rock partridges make them very sensitive to climatic events (Bernard-Laurent et Léonard 2000), much more than grouse species. The availability of resources in winter decreases for rock partridge with increase of snow cover because the species feed only on herbaceous food. Winter severity (particularly height of snow cover) has been shown, on our study area, to reduce winter survival and occurrence of double-nesting behaviour in spring. Weather conditions in spring also affect components of reproductive success (productivity and offspring survival) before and after hatching. Breeding phenology was highly dependent on the timing and conditions of the growing season, as food availability determines body condition. Our work allows us to better understand the mechanisms underlying the effect of climate change on population dynamics and to highlight the process of extinction and recolonization which were observed on alpine populations from the 1970s'. Conservation strategies and practices could thus arise from this work, in order to maintain Rock partridge populations and more broadly biodiversity in mountainous ecosystems.

**Keywords :** life-history traits, spatial ecology, climatic factors, *Alectoris graeca saxatilis*

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## **Game management in protected areas: the case of Castilla y León (Spain)**

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**Abstract:** Hunting in Castilla y León and other Spanish Autonomous Communities is going through a critical period due to the large number of legal actions that environmentalist and animalist organizations are taking against current regulation and practices. The views on hunting from urban context leads to disagreements between rural and urban population, with a greater influence of urban society on the political debate. This requires new approaches that bring the contrasting positions closer while at the same time guarantee the necessary hunting activity. In the region of Castilla y León, hunting has always been considered an important resource for rural development, as well as a tool to control populations of certain species such as rabbit, wild boar or some ungulates. Therefore, in our protected areas and Natura 2000 sites, hunting in general allowed, except in specific areas within them. In fact, only in National Parks, with their own national regulations, game activities are banned, although population controls are allowed with the support and participation of local hunters. Wild boar is currently one of the species of highest concern due to the increasing damages it produces in agriculture field and to the transmission of diseases to livestock. Specific national plans have been established to control wild boar populations. Special attention has been given to the control of African swine fever transmitted by this species, with specific plans coordinated at the European level.

**Keywords:** hunting, game species, wild boar population control, protected areas

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## Ungulate management in Europe: a world-wide problem

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**Abstract:** Ungulates in Europe has experienced in the last century a great increase that brought them back to areas where they had been absent from centuries. Higher and higher local densities posed serious questions about their management in many countries where they become overabundant. The huge change in European economies, that moved people from the countryside to urban areas and the subsequent rewilding of most European environments had probably the most relevant role. Ungulate reintroductions and legislative changes in many countries (i.e., a more conservative oriented management) contributed to this process. At present some species are still increasing their numbers and distribution in Europe, reaching the role of pests, like wild boar in most of Europe, and many European management systems are unable to deal with this overabundant presence. Indeed, main management problems are the impact on agriculture, forestry and conservation habitats, the disease diffusion and road traffic accidents. At the same time, the consequences of global change are already exerting a negative impact of some sensitive species like mountain ungulates that are showing signs of decrease in numbers and fitness. Moreover, a general problem caused by the mismatch between birth date and vegetation phenology touched also very common and widely distributed species, like roe deer. Within this frame it is necessary to develop a vision that allows to consider the wide presence of ungulates as an opportunity and a resource rather than a nuisance, and to conserve species and population in face of a changing environment and human society. Finally, the generalized decrease in number and increase in mean age of hunters poses further problems, related to the actual possibility that recreational hunting could still retain an efficient role in ungulate management. A common European strategy, able to set priorities respecting local tradition is badly needed to reach this result.

**Keywords:** global change, hunting, management problems, overabundance, ungulates

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## Monitoring Eurasian woodcock (*Scolopax rusticola*) in Lazio Region with pointing dogs: a scientific program

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**Abstract:** The Eurasian woodcock (*Scolopax rusticola*) is classified as Least Concern (LC) by the International Union for Conservation of Nature (IUCN) and since t 2017 it was listed among the species not currently of Conservation Concern (non-SPEC). The species is of considerable and increasing hunting interest in the entire distribution range, but demographic data are lacking and not useful for evaluating populations' dynamics. This generate uncertainty about the present conservation status of the full migrant populations of the species. Accordingly, woodcock is classified as Data Deficient (DD) in the Italian Red List of threatened species. With the aim of promoting a useful advancement of knowledge, the Superior Institute for Environmental Protection and Research (ISPRA) has arranged a specific protocol for monitoring the dynamic of the Italian wintering population with the help of specialized pointing dogs. In the year 2018, the Department of Agriculture and Forestry Science (University of Tuscia - VT) planned a three-year monitoring program in Lazio region coordinating all ten (10) regional territorial hunting organizations and 25 regional and state protected

areas (74% of total). Compared to the ISPRA's standard protocol, this monitoring program includes important experimental variants, which consist, principally, of temporal and spatial extensions. These variants were positively received by ISPRA because they make possible the use of abundance data also for the assessment of: *i*) the "refuge effect" over the spatial distribution pattern, *ii*) the capacity of the abundance index variations to describe the trend of the pre-nuptial migratory movements, *iii*) to test the reliability of the distance sampling method for density estimation. Two hundred thirty-four (234) sample areas (SU) of about 100 ha each, potentially suitable for the diurnal rest of the woodcock, were identified in Lazio region equally distributed between hunting (H) and non-hunting (NH) areas. These SU were proportionally stratified among 5 phyto-climatic bands in order to consider environmental and climatic variability. A minimum inter-distance of 500 m was also ensured between nearby samples. Three counting sessions in the period 20 December - 31 January and one session per week between 1 February and 15 March have been scheduled. The standard duration was fixed equal to 3 hours. In order to standardize the detection method, the crew should consist of a maximum of two detectors with two pointing dogs. Only detectors and dogs enabled at the end of specific courses and tests were admitted. The species abundance index (ICA) was determined as the number of woodcocks seen during a counting session of 3.5 hours (ICA<sub>3.5</sub>) to ensure data comparability with previous surveys performed abroad. ICA was also corrected for the number of detectors and dogs (ICA<sub>3.5c</sub>). All statistics were performed with STATISTICA 7. Descriptive statistics were firstly computed on January only that can still be considered representative of the wintering phase according to the current Key Concepts. Furthermore, both descriptive and variance analysis were performed using management type (H or NH), month or decade lapses as grouping variables and factors. For bureaucratic reasons, the first year (2018) of survey began on January 15<sup>th</sup> and, due to the unavailability of a sufficient number of enabled detectors (122) and dogs (189), it was possible to investigate only 62.4% of the total SU. Moreover, only the 32% of the 904 planned counting sessions have been completed. The main causes of this poor monitoring effort were adverse weather conditions, diseases and injuries that affected both detectors and dogs. Overall, 251 woodcocks were counted, and all the resting points were georeferenced and archived for successive analysis. In January, the total ICA<sub>3.5</sub> (mean ± SE) was  $1.15 \pm 0.19$  and  $1.23 \pm 0.26$  in H and NH areas respectively, without significant differences between that ( $t = -0.22$ ;  $p = 0.83$ ). Overall, ICA<sub>3.5</sub> gradually decreases from January to March ( $0.9 \pm 0.14$ ) but a significant effect of the time was not recorded. In the same time lapse, ICA<sub>3.5</sub> gradually decreased in H areas while showed stability in NH areas. These different trends resulted in significant differences ( $t = -2.54$ ;  $p = 0.012$ ) between management types only in March when ICA<sub>3.5</sub> was  $0.53 \pm 0.12$  and  $1.26 \pm 0.25$  respectively in H and NH areas. Unfortunately, the monitoring effort was lower than expected, fluctuating and sometimes unbalanced in months, decades and types of management. However, these preliminary results highlighted the need to increase the number of detectors and specialized dogs with the aim to ensure compliance with the experimental design. For the same reasons, in the case of adverse weather conditions, the programmed count must be carried out promptly in the following days. In effect, the 7-day notice requested by the Region in the case of count postponement, often prevented the recovery of the lost session. Furthermore, a poor ability to use satellite tracking tools has been detected. Specific workshops are scheduled to limit this gap.

**Keywords:** monitoring, abundance, dynamic, wintering, woodcock, *Scolopax rusticola*

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## Oral presentations

### Settlement sharing by crested porcupines, badgers and red foxes

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**Abstract:** In Italy crested porcupine (*Hystrix cristata*), badger (*Meles meles*) and red fox (*Vulpes vulpes*) coexist and overlap their home-range. Normally, these species use settlements for refuge, diurnal rest and reproduction. Settlements (“Sett”) is a ground holes cluster connected to a one or more hypogean burrow that are not individually recognizable. Settlements in a hilly area of 1,552 ha in Crespina-Lorenzana, in the Province of Pisa, were detected and geolocated. For each sett the geographical coordinates, orientation, number of ground holes and general environmental characteristics were recorded. Ten settlements were continuously monitored by camera-traps in order to establish between these three species who inhabit the sett. Daily time of permanence of one of the three species in burrow was the criterion by which inhabitation was establish. Overall, 45 sets, for a total of 190 ground holes, were detected, geolocated and described. The mean of ground holes for sett was  $4.2 \pm 4.06$ . The 89% of setts were detected in wooded areas near ecotones. The other setts were located in cultivated and uncultivated areas (6.6%) and woody covered man made floodplain (4.4%). The 57.7% of setts were oriented to North-West (n=26) and the 42.3% to South-East (n=19). The monitored setts were peacefully shared by the three species at different times. The porcupine resulted the main and faithful inhabitant of all monitored settlements. The porcupine inhabitation in each sett resulted to vary from 1 day to more consecutive months. Single and paired badgers have inhabited 5 of the monitored setts for short and reiterate periods (1 to 6 days) mainly in autumn and winter. In one occasion, a social group of three individuals stably inhabited a set for three months. The cohabitation in the same sett was recorded only between porcupine and badger in autumn and winter (October-March) for short periods (1 to 3 days) also in presence of porcupine cubs. The red foxes inhabited the settlements only in spring for breeding. No aggressive behaviour among these three species was detected, but a general mutual avoidance. Foxes were detected eating porcupine carcasses twice. In both the cases, the red fox extracted the porcupine carcasses from the burrow. This suggest that the crested porcupine is not a common prey of the red fox and badger.

**Keywords:** *Hystrix cristata*, *Meles meles*, *Vulpes vulpes*, Underground burrow, Inhabitation, Co-habitation, Scavenging carcasses, Camera-trapping

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### Morphological plasticity of captive rock partridges (*Alectoris graeca graeca*): searching exportable criteria for sex determination

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**Abstract:** the unfavourable conservation status of the rock partridge (*Alectoris graeca*) (SPEC 1) requires integrated strategies that include both *in situ* and *ex situ* conservation. When wild founders are not available, a restocking or reintroduction plan should perform, assuming a similar mortality rate between two sexes, the translocation of sex balanced groups of hand-reared birds to pre-release

acclimatization sites at a very young age. The age suggested for the translocation of other similar Galliformes is 42-56 days post hatching. Therefore, in the case of sexually monomorphic species, as the rock partridge, the availability of early sex determination method is crucial. Molecular methods are already available, but when any discriminant morphological trait is detectable, this approach is still convenient because cheap and expeditious. However, the morphological plasticity to different environmental stimuli should be considered if the target is an exportable sex determination method. In order to identify early and exportable sex determination morphological criteria and methods, considering also the potential effect of different rearing conditions, we recorded the measures of five body traits (BTs) on two groups of related chicks (first generation captive) of Apennine rock partridge (*A. g. graeca*) grown under antipodal rearing condition: intensive (G1) and semi-natural (G2). Measured body traits were body mass (BM), tarsus length (TL), tarsus depth (TD), tarsus width (TW), head length (HL). Starting at 21 DPH and continuing up to 42 DPH, the birds were weighed and measured fortnightly by the same researcher. Partridges were weighed with an electronic balance ( $\pm 0.01$  g accuracy). All linear measures were taken to the nearest 0.01 mm with a digital calliper. After, we tested BTs for the robustness respect to the rearing system (R) and for the discriminant capacity regardless the rearing system (DR). We considered a body trait as R morphological criteria when it does not show significant differences between groups by sex and DR when it shows significant differences between sexes in all the possible combinations. Statistical analysis was performed with STATISTICA 7 and the 1% significance level ( $p = 0.01$ ) was adopted. We proved that few morphological traits are reliable exportable criteria for accurate sex determination because contemporary R and DR; only these were processed by Discriminant Analysis (DA) for model development. We developed the best exportable sexing model at 42 days after hatching when DA retained only SL as variable in the analysis. This model assessed the discriminant function (sexing model) with the highest canonical correlation (0.89) and accuracy (100%) regardless the rearing system. Because the extreme simplicity, this model can be applied directly by the owner at farmer level.

**Keywords:** morphological plasticity, rearing systems, sex determination

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## **Estimation of nutritional requirements of wolf (*Canis lupus* L.) living in Monti Aurunci Park**

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**Abstract:** The wolf (*Canis lupus* L.) is a predatory carnivore, which eats essentially different preys (Boitani *et al.*, 2003). Feed resources affect the extension of hunting area as reported by many authors (Vilà *et al.*, 1995; Okarma *et al.*, 1998), which estimated that in Europe mean hunting area of wolf varies from 170 to 240 km<sup>2</sup>. In wild animals, feed intake is regulated mainly by satisfaction of energy requirement (NRC, 2006) as consequence the micro- and macro-nutrients needs are strictly related to the energy ones. As described by Stevens (1988), wolves' digestive tract is particularly short and characterized by a simple stomach and a short intestinal tract. In carnivores the retention time of digesta in the gut varies from 23 to 25 hours in function of meal's moisture (Banta *et al.*, 1979). All these characteristics indicate that wolf's energy requirements to support maintenance, growth, reproduction, lactation and physical activities are particularly high. In absence of adequate energy supply, animal performance will be suboptimal, and a depletion of energy and nutrient stores will



result. Young (1944) evidenced that after seven days of fasting a wolf loses about 17% of its live weight. Carnivores obtain energy mainly from lipids and proteins, hence no specific carbohydrates requirements are indicated for these species (NRC, 2006). However, the presence of carbohydrates in the diet, during specific life stages, such as growth, pregnancy, lactation allow to preserve essential nutrients (e.g. amino acids and fatty acids) for other functions. Aim of the present study was to estimate the number wolves in relation to of Esperia ponies' carcasses found in Monti Aurunci Park area. To calculate daily metabolizable energy requirement (REM) of wolves the equation proposed by NRC (1974) for domestic carnivores was used:  $REM = 132 \text{ kcal EM/LW}^{0.75}$ . Considering a mean live weight (LW) of 30 kg, the energy requirement for maintenance corresponds to 1692 kcal/d. This value could be increased up to 3384 kcal/d during specific life stage or when weather conditions are extreme. In function of this energy supply the following amounts of nutrients needs to be daily guaranteed: 42-85 of crude protein (CP), 23-46 g of ether extract (EE), 1.7-3.4 g of Ca, 1.3-2.6 g of P, 1.7-3.4 g of K; 254-508 mg of Mg and 338-676 mg of Na. The chemical analyses of a mix of horses' meat and bowels (30:70) revealed: 235 g of CP, 68 g of EE, 0.23 g of Ca, 2.16 g of P, 2.08 g of K; 0.182 mg of Mg and 1.95 mg of Na, and the energy density is 1336 kcal ME/kg. As consequence, in order to satisfy the energy requirement a wolf of 20-30 kg has to eat from 1.266 up to 2.533 kg/d of this kind mix. These quantities of horses' bowel and meat mix satisfy the wolves requirement of fat and P, albeit by the CP, Ca, Mg, Na and K balance resulted negative. From these results, it is possible to speculate that a wolf needs to eat an average of 1.900 kg/d of horse's carcasses to satisfy its energy requirements.

**Keywords:** energy, protein, horse's meat and bowel, feed intake, nutritional behavior

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## **Follicular and oocyte population in relation to pregnancy status in African buffalo (*Syncerus caffer*)**

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**Abstract:** The African Buffalo (*Syncerus caffer*) species has been recently listed as near threatened by the International Union for Conservation of Nature (IUCN, 2019) with a current estimated population decline of 31.2%. One of the factors contributing to the decline in population is agriculture and, in particular, the diseases control programs that lead to population reduction, due to the culling of carriers of infectious diseases transmittable to the livestock. In this scenario, ex-situ conservation programs are among the best tools for wild fauna preservation. In vitro embryo production (IVEP) is undoubtedly the most competitive to enhance offspring from selected female donors. The morphological aspect of cumulus-oocyte complexes (COCs) is correlated with the developmental competence in many species, including cattle and domestic buffalos. To our knowledge, the follicular and oocyte population in African buffalo has not yet been investigated. The aim of this work was to describe the follicular population and oocyte recovery of wild African buffalo in pregnant and non-pregnant animals, in order to evaluate the potential for IVEP in this species. Ovaries (n = 52, of which 21 from pregnant and 31 from non-pregnant females) were collected at the Hluhluwe–Imfolozi nature reserve (South Africa) from buffaloes culled within a Tuberculosis monitoring control program. The ovaries were isolated from carcasses and transported to the lab in physiological saline at 36°C within 2 h from death. The follicles on the surface of the ovaries were counted and aspirated by syringe, then COCs were counted

and classified according to morphological criteria, with grade A and B considered suitable for IVEP. Differences in the number of follicles/oocytes were analyzed by Student's t test, while the percentage of good quality COCs between pregnant and non-pregnant buffaloes was analyzed by Chi Square test. A higher number of total counted follicles (mean  $\pm$  SE:  $23.2 \pm 2.0$  vs  $16.8 \pm 1.8$ ;  $P < 0.05$ ) and recovered COCs (mean  $\pm$  SE:  $16.7 \pm 1.9$  vs  $12.1 \pm 1.6$ ;  $P < 0.05$ ) was recorded in non-pregnant compared to pregnant animals. However, in the non-pregnant buffaloes the percentage of good quality oocytes (A and B) was lower than in pregnant ones ( $38.8$  vs  $57.6$ ;  $P < 0.01$ ). Regarding the quality categories no differences were found in the number of grade A ( $2.2 \pm 0.4$  vs  $2.3 \pm 0.6$ ) and B ( $4.3 \pm 0.6$  vs  $4.7 \pm 0.7$ ) between non-pregnant and pregnant animals, whereas higher ( $P < 0.01$ ) number of grade C ( $1.7 \pm 0.3$  vs  $0.8 \pm 0.2$ ), D ( $3.2 \pm 0.5$  vs  $1.5 \pm 0.3$ ) and E ( $3.1 \pm 0.5$  vs  $1.4 \pm 0.4$ ) was observed in the non-pregnant. These preliminary results demonstrated that the number of follicles and COCs recovered from African buffalo is higher than that of domestic buffalo, suggesting a potential use of IVEP for propagation of valuable germplasm in this species. Further studies are, however, needed to assess the blastocyst yields after in vitro fertilization.

**Keywords:** *Syncerus caffer*, follicle, oocyte, pregnancy status, South Africa

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### Reflexion about current challenges in wildlife welfare

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**Abstract:** Wildlife welfare should deserve increased attention and it must be seen as a priority during research, management and conservation activities. The procedures adopted during direct or even indirect interaction with the wild animals must be innocuous and for this reason it is not presently admissible only to affirm that they are carried out with a high ethical standard. The wildlife research faces up to physiological and behavioral variation in a great diversity of vertebrate species. In this sense, the objective interpretation of its welfare requirements is complex, demanding a huge work to be done. Additionally, the notorious and increasing climate change could impact wildlife welfare in many ways, with a high number of species at risk of extinction, and other target study populations be in a stress condition. We should recognize the usefulness of reflecting on the application of the European Directive 2010/63/EU and its transposition to each of the member states, directed to all those carrying out research activities with live vertebrates and cephalopods. This directive lays down the obligation to submit any study project to the institutional Animal Welfare Body and if “involving the use of animals in procedures” also to the National competent authorities. The project evaluation must include verification of application the “three Rs” principles of humane experimental design (Russell and Burch 1959): replacement, reduction, and refinement; ensure that staff are competent with specific training/accreditation for responsible and executors; verification the scientific justification or a harm-benefit analysis with regard to animal suffering (requiring assessment of pain, suffering, distress, and lasting harm imposed on research animals) and the predicted gain for society. Besides this legal framework, public interest and ethical debate is increasing regarding the procedures involving free-living wild animals. Certainly, the editors of scientific journals will also help to reduce possible mistreatment of wild animals, requiring authorization to carry out the research work before the publication of the articles. Thus, the objectives of this work are: a) to stimulate the reflection about

wild animals welfare; b) highlight the challenge of using improving procedures (e.g new technologies or less invasive practices in individuals and populations); c) to warn about the lack of studies in this area that need to be promoted; d) to remember that if we improve wildlife welfare practices we will have better scientific results; e) discuss the potential of an interdisciplinary and integrated approach to the concept of "One Welfare" where the relationships between animal welfare, human health/wellbeing and conservation of the environment / biodiversity should be explored.

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**Keywords:** animal welfare, behavior, ethics, research, welfare assessment, wildlife

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## **Study of wolf's diet in the Baixo Sabor Basin (Portugal): conservation implications**

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**Abstract:** The Iberian wolf (*Canis lupus signatus*) is a subspecies classified as “Endangered” in the Portuguese Red Data Book (Cabral *et al.* 2005) and legally protected since 1988. The studies about food habits in Portugal, based on the analysis of scats, shows a strong dependence on domestic animals and it is the primarily reason for persecuting wolves. Diet studies are important in the demand of conservation tools, which minimize conflict with different stakeholders. In the Baixo Sabor basin (northeast of Portugal) we conducted a sampling between 2015 and 2017, looking for wolf scats along transects with a minimum length of 2 km, following rural and forest roads. Three replicates per year (spring, summer and autumn) were performed and, additionally, a sampling was made in the winter of 2017 in the most promising transects We collected 94 samples of which 78 were confirmed by molecular identification (89%) as being of wolf, belonging to a single pack (Mogadouro Sul). To assess diet composition we separate the macroscopic components, mainly hairs, and exclude the occurrence of vegetation, fruit, invertebrates or soil. We developed a reference collection of hairs of the most common domestic and wild prey in the area. Were compared the cuticular impressions on slides to the reference collection, through the optical microscope. The results, expressed as percent frequency of occurrence (hereafter FO) and classified according to the proposal of Ruprecht (1979), showed that domestic animals were the most frequent food category of wolf diet, with a special incidence in goats which accounted for about 52 % of the FO of domestic animals. Wild ungulates, such as roe deer and wild boar, although both are present throughout the study area, represented only 21.7 % FO of the same. Seasonal analysis of the diet revealed a major consumption of domestic animals in summer and fall. Goats consisted in a regular food resource during the year and sheep had more importance in summer (basic resource) and constituted a regular resource in the remaining seasons. Dog was always present in the wolf's diet, except in winter. In a trophic level, the pack presented a low diversity and breadth of the food niche, with a degree of specialization in the domestic ungulates, mainly goats, in part consequence of its size. These feeding habits can generate a threat for the pack conservation, due to the persecution that may be object. We suggest some recommendations in order to conservation the unique pack observed in the area.

*Special thanks to EDP Produção for authorizing the use of the scats for the purpose of this study.*

**Keywords:** *Canis lupus signatus*, wolf, diet, Portugal, scats analysis, conservation

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## Using camera trapping to estimate wild ungulates density in Amiata Mountain, Central Italy

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**Abstract:** Wild animals monitoring is crucial for improving management strategies aiming to favor vulnerable species or to limit the problematic ones. Traditional method based on direct counting require a great organizational and operational effort. Regardless the counting scheme adopted no representative results could be reached without a great number of voluntary detectors contemporary in the field, in particular when the focus species is erratic and widely distributed. When the species is of hunting interest, trained hunters are commonly involved, but their demographic trend is negative and the availability of free labor is progressively decreasing. More in general, the contemporary presence of many detectors can affect the detection probability with the risk of biased results. For all these reasons, an alternative way to count wild animals is camera trapping. Camera traps are standardized respect to the view ability, and allow us to perform animal counting during both night and daytime, in any weather conditions and in environmental contexts where direct observation would be very difficult. For many years, camera trapping had an important role in determining relative abundance index. In the last decade, some models have been published to estimate population density starting from the images captured by camera traps without the need of individual recognition. Among these methods, the Random Encountered Model (REM), that is based on the gas model theory, was tested successfully in wild boar (*Sus scrofa*) density estimation and with encouraging results on roe deer (*Capreolus capreolus*) density estimation. REM rescales linearly the trapping rate ( $y/t$ ) to population density considering two main biological variables, the mean group size (MGS) and daily range (DR: km/day) and two parameters of the camera, detection radius ( $r$ ) and angle ( $\theta$ ). In this case, the problem is to estimate DR. To avoid this problem an extension of REM was recently proposed: Random Encounter rate and Staying Time (REST). This method substitutes DR with resting time (RT) in the camera field of view but it is validated only in theoretical scenarios and need a testing phase in the field. In this case study, we used both REM and REST to assess the relative abundance and to estimate wild boar and roe deer density in a in a mountain area of southern Tuscany (Monte Amiata, Province of Siena) (an altitude range from 1116 to 1731 metres a.s.l.) of 600 hectares principally covered by beech (*Fagus sylvatica* L.) high forest (95.48%). The interest on studying the two target species in this forest, which is managed principally for timber production, is depending on the fact that they can affect both seeds availability and seedlings survival. Indeed, considering that this area is hunting restricted, the possibility of autumnal and wintering overabundance is concrete. During the summer, a first testing period (two weeks) of camera trapping was performed with the aim to acquire data useful for the eventual improvement of the approach and for evaluating seasonal abundance and density variations. This forest is rather homogeneous, and it was subdivided into three classes on the base of forest canopy cover and tree density. To choice camera traps (CTs) locations, we adopted a stratified sampling procedure. A fishnet of 1.5 x 1.5 km grids was generated overlapping the study area. The plot covering all the three principal forest types of the study area was chosen and divided into nine equal sections. We placed one camera into each 500 x 500 m nine sub-plots randomly. A 100 m buffer around grid's nodes was used to guide well-distributed locations. Finally, the number of CTs into each forest type was proportional to forest type availability. CTs were set to take a photo and a consecutive video of 1 minute. No time lapse was set between consecutive photos and videos. In this way, data potentially useful for both REM and REST were derived from each CT. Certain detection zone was

calculated by field test, resulting in 5.41 m<sup>2</sup>. The relative abundance index (CPUE) was computed as ratio between photographic events and total detection effort. Considering that, in particular during summer and in undisturbed areas, the target species could potentially move during both night and daytime, daily detection effort was equal to 24 hours (one day) for each CT. For wild boar, that is a social species, abundance and density estimations were corrected considering the mean group size. For REM estimations, the survey effort was the number of camera trapping days and DR was estimated by photo and video analysis considering distance references preliminary identified and highlighted directly in the field. Statistical analysis were performed with STATISTICA 7. Overall, 36 and 17 photographic events were recorded for roe deer and wild boar respectively. Observed roe deer (37 total) were solitary except in one occasion. Differently, wild boars (42 total) moved in groups of minimum two and maximum nine individuals. Roe deer DR and RT (mean  $\pm$  SE) were 6.43  $\pm$  1.39 km/day and 35.65  $\pm$  7.79 seconds respectively. CPUE was 0.38  $\pm$  0.13. REM and REST roe deer density estimations were 8.4  $\pm$  3.7 and 6.5  $\pm$  4.5 respectively. The variance analysis does not highlight significant differences between forest type and cover ( $F = 0.78$ ,  $p = 0.61$ ). Wild boar DR and RT (mean  $\pm$  SE) were 16.9  $\pm$  2.21 km/day and 23.00  $\pm$  4.43 seconds respectively. CPUE was 0.43  $\pm$  0.14. REM and REST wild boar density estimations were 5.80  $\pm$  2.26 and 1.76  $\pm$  0.80. Also in this case the effect of forest type and cover was not significant ( $F = 1.87$ ,  $p = 0.23$ ). As expected, a low trapping rate during the summer has been recorded because wild boars shift their distribution area in plain and hills areas searching for food without hunting disturbance, while roe deer minimize movements because engaged in territorial and parental phases. Preliminary results showed a very large variability in all the estimated parameters suggesting the opportunity of planning a longer camera-trapping period of one month at least. Indeed a minimum of 50 independent captures per species is suggested for reasonable REM density estimation. Furthermore, because the homogeneity of forest type and cover cannot explain a so larger data variability by itself, evidently we have to detect for other factor potentially affecting species abundance and distribution. We are waiting for data concerning the forest renovation status and of undergrowth density. On the base of this information, a resampling could be appropriate. As expected, roe deer showed both feeding and territorial behaviours, that resulted in an overall longer resting time in the camera field of view respect to wild boars that were only observed moving between habitat. This could be the reason of the very low REST wild boar density estimation.

*Acknowledgements:* We thanks Macchia Faggeta forest company for the financial support.

**Keywords:** camera trapping, REM, REST, wild boar, roe deer

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### Recent advances on Grey partridge (*Perdix perdix*) genetic

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**Abstract:** The grey partridge (*Perdix perdix*), a polytypic species having an Eurasian chorology, is included in the Least Concern (LC) category of the IUCN Red List of Threatened Species. Furthermore, it is listed among the Species of European Conservation Concern (SPEC 2) and it is known that small and isolated residual populations are concretely exposed to a risk of extinction vortex. The ecological and biological long-term success of artificial restocking, aimed to reinforce grey partridge populations, depends on breeder's reliability. Recently several studies based on

mitochondrial DNA (mtDNA) focused on grey partridge genetics. It is already known that mtDNA is more useful than nDNA for phylogenetic population studies and to evaluate gene flow and migration. Nevertheless, several researches showed exceptions to the unilateral inheritance of mtDNA and in particular heteroplasmy since the rarest gene recombination have been described in different taxa of animals, fungi and plants. Previous researches concluded that particular pattern in heteroplasmy may result in a reduction of the genetic drift or of the selection frequencies at the population level. Therefore, the presence of heteroplasmy at stable positions may be considered as a possibility to influence the viability and evolution of mitochondrial genome and should not be underestimated. Also considering the presence of heteroplasmy in several Galliformes, we investigated the occurrence of this phenomenon in grey partridges. For this purpose, we used two mitochondrial molecular markers (Cytochrome Oxidase Subunit I and the Control Region) and two nuclear markers specifically identified for this research. To better understand the obtained results and to exclude artefacts, some amplicons were cloned and sequenced; the sequences of each clone were then compared with the original ones. We evaluated the management implication of the obtained results. Further researches should focus to increase the knowledge on the possible hybridization scheme of *Perdix* species and on the existence of interfertile species, helping in the comprehension of its evolutionary history and management.

**Keyword:** Grey partridge, *Perdix perdix*, Heteroplasmy, Hybridization

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### **Recent advances on Rock partridge (*Alectoris graeca graeca*) genetic**

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**Abstract:** *Alectoris graeca graeca* Meisner, 1804, the nominal subspecies of the Rock Partridge, is a polytypic species already considered as vulnerable at global scale due to a trend of population decline and, for these reasons, included in the "IUCN Red List of Threatened Species". In Italy this decline is mainly referred to anthropogenic causes, above all the environmental changes and the release of the alien species *Alectoris chukar* (Gray, 1830). In 2017 our research group, in collaboration with other colleagues, demonstrated the presence of heteroplasmy in Rock Partridge, i.e. the coexistence within a single individual of genetically distinct mitochondrial genomes. This phenomenon underlined the possibility of a diffuse introgression and the presence, in nature, of vital hybrids of *A. graeca* X *A. chukar*. Such introgression could cause serious problems to natural populations, compromising the conservation of indigenous genomes. Considering the strictly matrilineal nature of mtDNA, at the light of heteroplasmy, is easy to understand why so frequently it is difficult to validate a correlation between mtDNA and morphological character. For these reasons we focused our attention on the development of a set of nuclear markers, with different aims: 1. Identification of hybrids between the two species; 2. Species-specific specimen attribution; 3. Correlation between hybrids and heteroplasmy; 4. Correlation between morphological and molecular characterization. The set of nuclear markers allowed to confirm the existence, in nature and in protected areas as well as in farms, of hybrids between *Alectoris graeca* and the introduced *Alectoris chukar*. This is a critic and important result, underlining a concrete situation of genetic pollution that acts compromising the autochthonous species, accentuating its decline. To date the frequency of hybrids is approximately 16.5%, that is in itself fairly alarming, also because the specimens were apparently perfectly viable. The management implication of these data is

certainly magnified also by the fact that, although the alien species has been present in Italy for relatively few years, it is already widespread in the territory. The correlation between morphological and molecular data is not simple because different morphological pattern emerged related to different molecular pattern and should be object of future investigation. In conclusion, the development of a set of biparental molecular protocol of species-specific attribution of the specimens, able to discriminate possible hybrids between the two species *A. graeca* (autochthonous and with three subspecies) and *A. chukar* (allochthonous), represent a powerful tool for the species management. In particular, allowed the identification of potential breeders to be used in reproduction programs for reintroduction purposes in protected areas, for a medium-long term conservation practice.

**Keyword:** Rock partridge, *Alectoris graeca*, *Alectoris chukar*, Heteroplasmy, Hybridization

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### **Status of rock partridge in Sibillini Mountains National Park: an update**

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**Abstract:** The rock partridge, a European distribution polytypic species (Madge and McGowan 2002), is present in Italy with three subspecies; in Sicily (*Alectoris graeca whitakeri*), in the Alps *A. g. saxatilis*, in the Apennines *A. g. graeca* (Randi *et al.* 2003). The Apennine rock partridge is genetically similar to the subspecies of the Balkans (*A. g. graeca*) (Randi *et al.* 2003), but due to prolonged isolation and demographic independence of the Apennine subsp. it is considered a Management Unit (MU - Moritz, 1994), and of specific conservation interest (Randi *et al.* 2003). According to the National Action Plan for the rock partridge Trocchi *et al.* (2016), the Apennine rock partridge is considered an Evolutionary Significant Unit (ESU - Ryder, 1986). In this regard, Trocchi *et al.* (2016) have highlighted, the need to implement all the Actions necessary to guarantee the conservation and genetic integrity of the Apennine population. The species, listed in Annexes I and II / A of the Birds Directive (147/2009 / EC), lives in an unfavourable European conservation status (SPEC 1) and its Italian populations have been classified as Vulnerable (VU) by the Italian committee IUCN (Rondinini *et al.* 2013). The results of four years of pre-reproductive and post-reproductive monitoring of rock Partridge in the National Park of Monti Sibillini are reported. The model of nesting site suitability (Amici *et al.* 2016) was used to estimate the pre-reproductive population parameters. The surface of the Park suitable for nesting was 10,072.36 ha, the pre-reproductive survey of 2019 involved 9.9% approx. of the total suitable surface. The following parameters were estimated, Territorial Males (MT), Abundance Mileage Indices (IKA) and Density of Territorial Males ( $D1 = MT / 100$  ha) detected in 2015 (Valbonesi *et al.* 2015), in 2016 (Amici *et al.* 2016), in 2017 (Amici *et al.* 2017), in 2018 (Amici *et al.*, 2019) and in 2019 (present survey). The figures for the density of territorial males referring to 2016, 2017, 2018 and 2019, have also been calculated considering only the area with a suitable vocation ( $D2 = MT / 100$  has a vocation). The density (average  $\pm$  95% confidence limit) of territorial males estimated in the spring of 2019 was  $1.17 \pm 0.6$  territorial males / Km<sup>2</sup> of total surface (D1) and to  $2.21 \pm 1.05$  males territorial / Km<sup>2</sup> of surface suitable for nesting. Overall, over the four years of observation, the tendency of the density of territorial males / couples, expressed on the total surface investigated is positive. However, the fluctuating trend described over the last three years suggests the opportunity to monitor population dynamics. Starting from the average density of territorial males

expressed on the nesting area the pre-reproductive abundance in the Park territory can be estimated as  $222.6 \pm 105.8$  pairs. This estimate should be increased by 10% and reduced by 20% if this proportion is assumed to be on average representative of the number of coupled units compared to the total number of males surveyed (Bernard - Laurent & Laurent, 1984; Pandolfi *et al.*, 2001; Sorace *et al.* 2011). In 2019 a capture plan was started in June and captured bird, named "First" was attributed to subspecies *A. g. graeca* H3 haplotype (as for Randi *et al.* 2003). The animal was fitted with a VHF tag. This activity has allowed, through triangulation and homing-in, to constantly monitor the movements of "First" to obtain both data on the movement ecology of the species in the reproductive period, and signals or changes in the ethogram such as to suggest the start of hatching. In this regard, it should be noted that the double nesting behaviour, which would also involve males in the hatching of a second nest, has been demonstrated, in the Alpine context, by Bernard Laurent *et al.* (2017). The surveys carried out during the observation period provided a total of 16 fixes resulting from both triangulations and homing-in. The monitored animal was found to be mobile, with a minimum convex polygon (MCP), which defines the vital area used by the animal during the observation period of 52 ha approx, showing a more widely used sector with a surface area of less than 1 ha (Kernell Density Estimator - KDE), on which the search for a possible nest were oriented. These preliminary results suggest that the disturbance and stress caused to the animal during capture and manipulation operations did not lead to dispersive behaviour and consequent substantial modifications of the territory used by the animal (Convesso Minimo Polygono) after the capture, which in fact has continued to include the station where he was captured.

**Keywords:** Sibillini National Park, *Alectoris graeca*, rock partridge, space use, VHF

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## Wild boar abundance distribution patterns as affected by the refuge effect

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**Abstract:** the wild boar (*Sus scrofa*) is a worldwide problematic species because of the impacts on human activities, ecosystems, public health and safety. In recent decades, the expansion of the species has led to an escalation of conflicts economically and socially unsustainable. At the national scale and in the Lazio Region, traditional hunting was the only demographic control action adopted, for three months per year, until recently. The application of a hunting effort limited in time and space, and the absence of a coordinated demographic control strategy allowed the species to get safe and undisturbed refuge areas also during the hunting season. According to the “*ecology of fear*”, animals try to avoid predation risk shifting their home range toward refuge areas. This phenomenon, also known as “*refuge effect*” (RE), induces animal movements from or to the refuge areas depending on the hunting disturbance and on the carrying capacity of the refuges and causes fluctuations in hunting performance. Previous works focused on wild boar agricultural impacts under the effects of hunting pressure and ban hunting areas. Differently, the aim of the present work is the evaluation of the RE on the wild boar abundance distribution pattern. For this purpose, we used the Catch Per Unit Effort (CPUE) as abundance index calculating, for each one of the 350 hunting zones identified in the province of Viterbo, the ratio between the number of hunted animals and the hunting effort (number of hunting actions) on a weekly basis. Data of three successive hunting seasons were used. At each hunting zone was associated the respective Euclidian distances from the nearest refuge area (159 ban hunting areas)



characterized by accessibility and availability of wooded resting places. Hunting zones were then grouped in five classes of distance including the same number of hunting zones (70). To evaluate, the existence of a negative tendency of the abundance respect to time (hunting weeks), as expected in the case of a closed populations without return movements from refuge areas, a correlation matrix was performed. Differences in abundance were tested by a full factorial ANOVA (GLM) selecting hunting season (year), week and class of distance as factors. The Fisher LSD test was used for pairwise comparisons. Statistical analysis was performed with Statistica 7 and for all statistics the significance level was fixed at  $p = 0,05$ . For all the classes of distance no significant correlations were detected between abundance and time (hunting week). This suggests that wild boar goes back to all the hunting zones, regardless the distance from known official refuge areas. The seven percent (7%) of the hunting zones is used only ones per hunting season suggesting that these are managed by hunters to maintain some refuge areas. A significant effect of the distance from refuges (class) on the wild boar abundance was detected ( $F = 6.74$ ,  $p = 0.00006$ ). Significantly higher CPUE ( $2.28 \pm 0.52$  mean  $\pm$  SD) were recorded in hunting zones that are in contact or close ( $<50$  m) from the refuge areas if compared to other distance classes. Abundance decreases in the successive three classes of distance with no significant differences between those (CPUEs  $1.92 \pm 0.57$ ,  $1.91 \pm 0.61$ ,  $1.90 \pm 0.49$  in class 2, class 3 and class 4 respectively). Significantly lower CPUE ( $1.56 \pm 0.70$ ) respect to the previous ones was detected in the fifth class of distance (distance  $\geq 2.148$  m). Our results proved that, at least during the hunting period, wild boar abundance resulted positively influenced by the proximity to refuge areas. As suggested by other authors, the spatial distribution of refuge areas defines a “*landscape of fear*” that affects the wild boar distribution with the risk of localized overabundance coherent with the higher agricultural and ecological damage recorded in the inner and neighbouring areas.

**Keywords:** refuge effect, relative abundance index, CPUE, distribution, landscape of fear

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## **Wolf (*Canis lupus*) predation sites are affected by land cover and territorial planning**

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**Abstract:** Wolf predations are increasing in the last years and are accompanied by a massive press campaign that creates alarm and distrust of the wolf. For this reason, a collection of information was carried out at the Territorial Hunting Association (Ambito Territoriale di Caccia - ATC) on reports of predation presented by breeders to obtain compensation. Data collection is not complete due to many reasons. The dataset of collected data includes the period from 2011 to 2015 and involves the provinces of Viterbo and Rieti where it was possible to carry out an analysis of the different cases of predation of wolf (*Canis lupus*), even if in some cases there is an incompleteness of the data provided. All data have been implemented in an Excel sheet containing the following information: Date, Position (province, municipality, location, predation site), Animal (species and *status*). Subsequently to carry out the georeferencing, the following data were collected: Territorial Hunting Area (ATC), neighboring Institute or Protected Areas, coordinates (Google Earth). The georeferencing was carried out with the use of ArcGis, version 9.8, using the topographic layer of the IGM 1: 25.000 tablets on which the toponyms of the locations are indicated. Since the wolf is a specialized predator, it prefers to feed mainly on wild ungulates such as wild boar and roe deer but also deer and chamois (Capitani *et*

*al.*, 2004), being a food opportunist, however, tends to feed on all that is most available, such as sheep and goats. For this reason, it has been analyzed the possible interaction between the areas of wild boar hunting and cases of damage to livestock. In the province of Rieti it was noticed that the maximum peak of events occurs between 0 and 1 km from urban areas (63%) with an average of about 800 m and a standard deviation of about 1.2. The Private Hunting Farms (AFV), having prevailing naturalistic and faunistic purposes, also affected predation. On average, events in the province of Rieti occurred between 0 to 6 km from AFV with a standard deviation of 4.7. These values are similar in Viterbo province. Many events were incomplete or not understandable, lackin date or species or hour. An important Large part of the predation events occurred within 5 km distance with a percentage of about 40%. It has been shown by Heilhecker *et al.* (2007) that wolves present in North-America with a high population density, accustomed to the presence of man, are the main culprits of attacks on livestock (Espuno *et al.*, 2004; Mech *et al.*, 2000; Russo *et al.*, 2014; Strbenac *et al.*, 2010). While Chavez & Gese (2006) claim that during the day the wolves avoid the pastures, frequenting them at night where there is a low human presence; agree Mattiello *et al.* (2012) that analyzed the risk factors in Tuscany.

**Keywords:** predation, *Canis lupus*, Wolf, management, land cover

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## Property rights patterns of wild large herbivores management in Europe

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**Abstract:** There are major concerns regarding the management of increasingly abundant wild large herbivores and their adverse effects on European forest ecosystems. While maintaining too high stocks, hunting generates negative externalities for forest landowners due to browsing, which may strongly affect the forest regeneration and the species composition. To address a lack of information on the institutional drivers of the hunting negative externalities, we have analysed the property rights structure in deer management using Ostrom's Institutional Analysis and Development framework. Our analysis on 18 countries and regions shows that private and res nullius regimes on game tend to be correlated with higher level of browsing than direct control of game populations by state owners. To compensate the game damage, the civil liability is the most commonly implemented, but the burden of charge is put on forest landowner. The main message of the paper is that the property rights structure and the liability rules for game-inflicted damage do not offer a proper institutional setting to cut with the current trend of increasing deer stocks. The recent acknowledgement of landowners' right to oppose hunting reverses the historical trend of prevailing hunting interests but have the potential to lead to even higher deer stocks.

**Keywords:** wild ungulates; hunting rights, wildlife property; deer

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## An update on the foraging ecology of *Testudo hermanni* in Italy

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**Abstract:** *Testudo hermanni* Gmelin, 1978, is a terrestrial tortoise distributed in the Mediterranean regions of Southern Europe. In Italy, autochthonous populations are mainly found along the coastal regions of the Peninsula and in Sardinia and Sicily. According to literature, the diet of the Hermann's Tortoise is mainly composed of vascular plants, but also includes fruits, mushrooms, mosses, invertebrates, carrion and faeces. In the present work we studied the composition of the diet of *T. hermanni* in nine Italian regions. All captured individuals were sexed and measured. Fecal samples were collected in different seasons (spring, summer and autumn) from 2012 to 2016, and a total of 67 fecal pellets were analyzed. The composition of the diet was determined using the micro-histological technique applied to fecal analysis. Ivlev's selectivity index was calculated for the sites where assessment of trophic availability was possible, i.e., some geographical areas of Apulia, Tuscany, and Sardinia. Our results confirm that the diet of *T. hermanni* is primarily herbivore. Sex does not seem to influence the diet, while year and sampling season, as well as sampling area, significantly affect diet composition. A great variety of plant families features the diet of the Hermann's Tortoise, primarily Fabaceae, Asteraceae, Plantaginaceae, Rubiaceae and, to a lesser extent, Poaceae, Caprifoliaceae, Ranunculaceae, Smilacaceae and Bryophyta. In a limited number of samples, mushrooms, mosses and gastropods' shell fragments, as well as fruits (*Knautia integrifolia* and *Phillyrea angustifolia*), were also found. Fabaceae and Rubiaceae were positively selected in all study areas where Ivlev's selectivity index was calculated, whereas Poaceae appear to be widespread but not selected. Ranunculaceae are selected in the Sardinian and Tuscan study areas, though not in Apulia. Other selected plants are Brassicaceae, Plantaginaceae and Smilacaceae (and, to a lesser extent, Oleaceae) in Apulia, Caprifoliaceae and Plantaginaceae in Tuscany, and Convolvulaceae and Lamiaceae in different areas of Sardinia. As expected, the botanical composition is less variable in areas partially influenced by human activity, where higher amounts of Poaceae and fruits are observed. Future research could highlight further aspects of *T. hermanni*'s foraging ecology and the complex relationships between animal behaviour and ecosystems.

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**Keywords:** *Testudo hermanni*; micro-histological analysis; diet selection; foraging strategy; tortoise; phytocoenosis

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## **Implementation of wild hunting: zoonosis associated with its use as food**

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**Abstract:** The consumption of wild animal's meat is increasingly demanded by consumers. The sanitary inspection of animals killed in hunting is an important part of veterinarians' work. In the conference we will review the main game meat and its culinary properties. We will see the importance of the industry associated with the consumption of game meat. Finally we will comment on the most dangerous zoonosis that can be detected in veterinary inspections: transmitted by bacteria, viruses, rickettsias, bedsonias and parasites.

**Keywords:** Zoonosis, wild hunting

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## **Reproduction and socialization of a litter of wolves in the Iberian Wolf Center of Castilla y León (Robledo – Zamora - Spain)**

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**Abstract:** The Iberian Wolf Center of Castilla y León “Félix Rodríguez de la Fuente”, opened in October 2015, is located in the small village of Robledo in the within the Hunting Reserve of “Sierra de la Culebra”. The Iberian Wolf Center is an educational and socio-economic resource based on the Iberian Wolf (*Canis lupus ssp. signatus*). The center covers an area of 20.8 hectares with a Visitor Center of 1,800 m<sup>2</sup> and two large fenced enclosures of more than six hectares that houses twelve wolves in conditions of semi-freedom. The Center is currently completing with three elevated observatories, more than 1.5 km of paths for visitors, a quarantine area and an auxiliary building for the management and veterinary care of the wolves. On April 2019 the Center housed eight adult wolves. Five of these wolves came from other centers, two came from the wild (unrecoverable) and the last one was born in the center itself in 2016. The objective for this year 2019 was to get a litter of wolves in order to rejuvenate the average age of the specimens and show them to the visitors. For this purpose a couple of wolves was chosen, Atila (male) and Oscura (female). They stayed together during the mating season. In the first days of May it was confirmed by the veterinary team that Oscura was pregnant. On May 29, five puppies were born, four alive and one dead. From that moment the veterinary management and control team carried out an exhaustive monitoring of the mother and the puppies. During the following weeks the puppies were monitored 24 hours a day and the process of socialization of the puppies with the management team began. The next stage consisted in the socialization of the puppies with other wolves of the Center. The idea is that visitors can observe from the observatories a pack of 7-8 wolves that includes puppies with adult wolves. This action is taking place during the month of September 2019.

**Keywords:** Iberian Wolf Center of Castilla y León, Sierra de la Culebra, puppies, veterinary management and control team, socialization

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## Research on the presence and fluctuation of the species mainly captured at the MonITRing station of Ripasottile from 2015 to 2017

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**Abstract:** The MonITRing ringing National project was started at Nature Reserve of Lakes Lungo e Ripasottile, with the aim of broadening ornithological knowledge in the Nature Reserve territory over the course of an entire year. This final elaboration studies the data gathered by means of the MonITRing project in the three-year period between 2015-2017. During this time 4606 birds, totaling 44 species, were netted of which 3076 were first captures and 1530 were re-captures. From the data it has been noted that of 44 species, 3 are to be found in Annex I of Directive 2009/147/CE on the conservation of Wild Birds and 3 species are found to be in Annex II. Furthermore the species were sub-divided according to the IUCN Red List (breeding in Italy and breeding in Lazio) in order to furnish a useful tool in the management of the Nature Reserve. Following this, a parameter was established for the evaluation of which species presented enough data to make an in-depth study possible. The species which filled those criteria were: Eurasian Reed Warbler, Eurasian Black Cap, Long-tailed Tit, Common Chiffchaff, European Robin and Cetti's Warbler. For these species it has been possible to compare the strategies used for dealing with seasonal changes as reported in the data with those which are to be found in the current literature. Finally it can be concluded that in order to fully understand and confront the strategies of all the species captured, apart from the 6 species which have been studied in detail, a wider spectrum of data is necessary which can be obtained through the continuation of the MonITRing project.

**Keywords:** Project MonITRing, Natural Reserve of Lakes Lungo e Ripasottile, Birds, Ornithology

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### 1. INTRODUCTION

The project was started at the Nature Reserve of Lakes Lungo and Ripasottile, a moist area with a high naturalistic value (for other information you can see Di Carlo *et al.*, 1960, 1981). The protected area has a SIC, ZPS and a ZSC zone (IT6020011). Specifically, the study was started in the MonITRing station of Ripasottile, which, since 2015, adhered to the National Scientific research Project, promoted and coordinated by ISPRA (Superior Institute for environmental protection and research), based on the ringing method for scientific purposes.

This project has the purpose to make a group of ringing station operate located in the territory, on the same period and using the same method to obtain series of snapshots of the ornithological Italian situation, comparable over time. Such project is developed on a national level. For the Nature Reserve the objective goal is to constantly monitor the local situation.

#### 1.1 Study area

The MonITRing station of Ripasottile is located on the shore of the lake Ripasottile, a humid environment. The place is composed mainly of reed thicket, hydrophilic Grove, cultivated and faluned fields (for other information Rampini E. 2018)

### 2. MATERIALS AND METHODS

#### 2.1 The ringing method for scientific purposes

The ringing method use the practice of “capture-marking-re-capture”. Such method allows to understand and, in some cases, to predict the trends of an ornithological population across the study of

the demographic rate, the reproductive success, the survival etc. For many reasons, like the ubiquitous presence of birds, it is used to monitor environmental and climatic changes. This research method is done through the use of capture nets Mist-net (fog-nets), which allows the capture of birds. Once released from the nest, the birds are taken in special bags to the place where the ringing operations will be carried out. Once the workplace is reached, the bird is extracted from the bag and the species is recognized. Afterwards, we proceed by marking the animal with the affixing on the tarsus a special ring, with a unique, serial and alphanumeric code that will allow, in case of a re-capture, the identification of the bird. Then, if possible, we recognize the sex of the animal and the age. Finally, we proceed with some specific morphological measures. Once we brought these processes to an end, the bird is freed (for other information Rampini E. 2018; Magnani A. *et al*, 2000). The Data collected are then transferred to a computer database and sent to ISPRA where they are stored together with those of all the ringing Italian station.

## 2.2 Discriminant parameter

Specifically, it was decided to investigate the most caught species based on the annual behaviors. In order to identify them, a discriminated parameter has been used, based on twice the average number of annual catches of individual species. This parameter was then compared with the number of annual catches of the individual species and those which exceeded it in at least two annuities were chosen.

## 3. RESULTS

With the help of the MonITRing station it was possible to capture 4606 birds, divided into 44 species and captured in 96 days. In the first year, 1784 birds were caught, in the second 1504, and in the third 1318. Considering the sampling effort, the situation does not change. The average day catches in the first year were 61,52 birds, the second year 44,24, and the third year 39,94. The species that exceeded the chosen minimum parameter were 6: Eurasian Reed Warbler (*Acrocephalus scirpaceus*), Eurasian Black Cap (*Sylvia atricapilla*), Long-tailed Tit (*Aegithalos caudatus*), Common Chiffchaff (*Phylloscopus collybita*), European Robin (*Erithacus rubecula*) and Cetti's Warbler (*Cettia cetti*). For these specieses it was possible to study their numerical fluctuations (significant), the strategies to face the seasonal changes and so compare them to the literature.

## 4. CONCLUSIONS

The captures at the Ripasottile MonITRing Station have been reduced. In addition, from the data it has resulted that 6 species, among those captured are present in the Bird Directive 2009/147/CE, are Red-Backed Shrike, Moustached Warbler, and Kingfisher are present in the Annex I. Differently, the Blackbird, Song Thrush and the Staling are present in the Annex II. For the Red List of Breeding Birds in Italy the species caught are classified in the following way: 2 species NA (Unenforceable), 31 species LC (At lower preoccupation), 5 species NT (Nearly threaten), 4 species VU (Susceptible) are the Red-Backed Shrike, Moustached Warbler, Tree Sparrow, and Penduline Tit, 1 species EN (At risk) the Torcicollo, and 1 species CR (In pericolo critico) the Warbler. For the Red List of Breeding Birds in Lazio 2011 the species are divided into this category 40 species NE (Unvalued), 1 species DD (Unvalued), 2 species VU (Susceptible) the Kingfisher, and the Goldcrest, 1 species EN (At risk) the Hawfinch. Meanwhile the Great Spotted Woodpecker, and the Green Woodpecker are mentioned in the list in the art. 2 of Law 157/92 which indicates the species to which particular attention and protection must be given. Analysing the 6 species in detail it has been observed that:

The Eurasian Reed Warbler (*Acrocephalus scirpaceus*), in literature, nests in the reeds in the months of June and July, and its migratory behavior has also been confirmed.

The Eurasian Black Cap (*Sylvia atricapilla*), in literature, is considered sedentary and migratory based on the population of the area, while from the catches it is absent during the wintering period, in December and January (in contraddition with the literature).

The Long-tailed Tit (*Aegithalos caudatus*) is absent in the months of May and July, but it is present sporadically in the months of June and August, to then have a numerical increase in the winter period. These data are in contrast with the literature which reports the species to be sedentary, nesting, regular and winter migrant. The discrepancy between the data could be the small number of individuals of the species that nest in the area of study, making the probability of capture in the period considered extremely low.

In the literature the Common Chiffchaff (*Phylloscopus collybita*) is considered as a nesting, short and long range migrant and wintering in Italy. From the results this species is present in the migratory period (autumn and winter) and is wintering. Differently, it is in small numbers in the rest of the year. It also appears that the species has suffered a strong decline in the autumn of 2017, information to reconfirm with future investigations.

The European Robin (*Erithacus rubecula*) is considered as a nesting species, regular migrant, wintering and partially sedentary in Italy. The data collected on this species coincide with the literature.

Cetti's Warbler (*Cettia cetti*) is a sedentary, migratory, wintering and nesting species. The data agree with the literature.

## 5. DISCUSSION

The study carried out at the MonITRing station of Ripasottile has demonstrated the naturalistic importance of the Lakes Lungo and Ripasottile Natural Reserve, which are characterized by a strong presence of migratory, wintering and nesting species. Furthermore, analyzing the data, for some species, a numerical decrease can be detected even if the period examined is too short to provide complete information on the case. Various hypotheses have been formulated to explain this negative trend, two of which, already formulated in Mirko Mariani final work, seem to be the most probable:

1 Variations in the climatic/meteorological conditions of the migratory direction and of the area in question; Changes in ecological conditions within the Protected Area.

The second hypothesis finds confirmation in the anthropic use of high-impact cropping systems. This situation was a studied object from ISPRA, who carried out some exams in the Nature Reserve territory. In fact, the studies have shown the presence of pesticide residues even one year apart. These products are widely use in the Piana Reatina's territory, which includes the territory of the Nature Reserve. This kind of substances can act as a limiting factor for the presence of wildlife. The study carried out three years of activity of the MonITRing project, producing useful information both for a greater knowledge of the ornithic fauna and for a better management of the protected Area.

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## Spatial overlap of wild and domestic herbivories in dry mountain grassland of Central Apennine, Italy

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**Abstract:** in the Mediterranean basin, mountain dry grasslands and rangelands are of major concern for biodiversity conservation, since they are put at risk by the progressive change of traditional agrosilvopastoral activities. The presence of wild and domestic grazing ruminants affects plant community diversity, by the way of complex mechanisms involving the grazers behaviour, the browsing strategies, the primary production, the intensity of defoliation, the grazing loads, etc. Also, ruminants' communities are shaped by interaction with forage resources, and in the case of dry pastures of Apennines, it would result in competitive interactions, because of spatiotemporal limited availability. Animal species should avoid competition through resource partitioning, although resource overlap can occur. As part of a larger research project in the Central Italian Apennine, we evaluated the distribution and the density of different wild and domestic herbivory species, and here we present a quantification of the spatial overlap among them. This study was conducted in the surrounding mountains of the Fucino basin, located in Abruzzo (Italy), 750-1,500 m a.s.l. Grassland plant communities mostly belong to *Festuco-Brometea* and *Nardetea strictae* phytosociological class. In July–October 2018, the extent of spatial overlap of livestock (cattle, horses, sheep, goats) and wild herbivores (red deer, roe deer and hares) was estimated through pellet group counts, faecal standing crop method. To assess spatial overlap, we use both the inverse distance weighting interpolation in a GIS environment, and the index of Jaccard (I). All species used a large part of the study area (85% red deer, 44% roe deer, 59% hare and bovine, 41% sheep and goats, and 35% horses). Spatial overlap between domestic free-ranging (bovine-equine) and wild species was (I) 0.10–0.67, while 0.11–0.90 was assessed between domestic guided by shepherd and wild. Bovine and small ruminant tend to be segregated. The effect of spatial overlap between domestic and wild ruminants directly affects the quantity and quality of trophic resources available to both groups, and must be considered in grazing and management plans, especially when vulnerable ungulate is also present. However, different grazing behaviour could be exploited to maintain or restore degraded rangeland habitats (i.e. encroached grasslands).

**Keywords:** grazing behaviour, competition, intraspecific interactions, ungulates, vegetation change

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## Preliminary Ecological Niche Factor Analysis for the diurnal rest of Eurasian woodcock (*Scolopax rusticola*) during wintering phase

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**Abstract:** Woodcock is a cryptic species that is difficult to contact and in Italy there are no data on abundance/density and on migration periods (Rondini *et al.*, 2013). The need to determine the current status of woodcock populations wintering in Italy has given away to some regional monitoring projects. In the Lazio Region, in addition to the ten-year monitoring carried out at the Castel Porziano estate (Aradis *et al.*, 2001), a monitoring project has been started in 2017 on the whole regional territory. In the present study, we used the Ecological Niche Factor Analysis (ENFA) to compute the woodcock wintering habitat suitability (HS) regarding diurnal rest. Overall, nineteen Eco Geographical Variables (EGV) were entered in the analysis. ENFA is a geo-stochastic multifactorial analysis able to compute a predictive suitability model based only on presence data starting from a set of ecological predictors (EGVs) potentially affecting habitat suitability. All the procedures were implemented in the software Biomapper (Hirzel *et al.*, 2002). A total of 410 presence points was geolocated in the Lazio Region study area (Fig. 1) mostly through interviews with hunters. About 40% of the information derived from skilled personnel. The first eight factors explained the 82% of the total variance and the 62% of the overall specialization. The overall Marginality (M) and Specialization (S) were respectively 0.466 and 1.215 suggesting the species tends to rest in sites characterized by ecological condition quite different from the global ones. In particular, the average altitude, the distance from confluence and waterways, and the slope showed the highest eigenvalues respect to first factor (M), suggesting the species prefers to rest in sites at 300 – 500 m a.m.s.l, slow slope and in proximity to wetlands. Otherwise, the low percentage of S that explained (9%) by this factor suggested low sensitivity to shifts away from the optimal values of these variables. Differently, the next seven factors (S) suggested sensibility respect distance from waterways topographic convergence index and degree of forest cover. On the total surface of the Lazio Region (1,720,627 ha), 620,496 ha resulted suitable for the diurnal rest of the species although only the 35,4 percent (219,768 ha) were classified at medium - high suitability. The Boyce index ( $0.735 \pm 0.161$ ), although improvable, confirmed a non-random distribution of Eurasian woodcock presence. The fact that a high number of factors was necessary to explain only the 62% of the total S, makes necessary the improvement of data quality and quantity. We are confident that the unavailability of data for protected areas, which occupy most of the mountain areas of the region, may have resulted in an overestimation of the effects of the variable altitude. Moreover, an analysis based on presence data provided by hunters could be affected by conscious distortions due to the desire to protect personal information. Further researches should be oriented to record presence data directly in the field and test the effect of variable transformation. Probably, some qualitative EGV, as the type of forest cover, should be entered as quantitative ones, as well as some continuous EGV, as distance or density variables, in categorical ones.

**Keywords:** ENFA, Eurasian woodcock, resting places, wintering, habitat suitability

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## Effect of different frying oils on fatty acids of wild boar meat

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**Abstract:** The high quality of wild boar meat was modified with cooking in particular frying fat transferred to wild boar meat specific characteristics. Thus, it is possible notice a deterioration in the nutritional quality if the meat is cooked with lard as they increase the saturated fatty acids ( $P < 0.001$ ), while cooking with vegetable fats produces an increase of polyunsaturated fatty acids, in particular C18:2n6, worsening n6/n3. The use of linseed oil rich in C18:3n3 improves the nutritional characteristics of wild boar meat, both in terms of higher polyunsaturated and low n6/n3 ratio ( $P < 0.001$ ), but on the other hand increases the peroxidation index, the total trans fatty acids and TBARS value, making the meat easily to oxidation and harmful to health. Olive and peanut oil protects the meat from oxidation thanks to their richness in oleic acid and enhances the excellent nutritional qualities of wild boar meat.

**Keywords:** wild boar, meat, fatty acids, vegetable oil.

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### 1. INTRODUCTION

The use of wild ungulates as an alternative source of meat has reached a remarkable value in some European regions. Wild boar (*Sus scrofa*) is widely available in Central Italy (Ramanzin *et al.*, 2010). The meat of wild animals is an excellent source of essential polyunsaturated fatty acids (Amici *et al.*, 2015), but cooking has a marked effect on their composition, particularly during frying. Fry is common cooking technique both for industry and homemade, producing food more palatable (Razmaitė and Šiukščius, 2004). Anyway, the composition of the oil can markedly change the fatty acid composition of meals. In addition, the cooking process causes an increase of lipid oxidation, particularly for venison rich in iron considered a pro-oxidant element (Cifuni *et al.*, 2014). The aim of this work was to estimate the effect of different oils used for frying on fatty acid composition and lipid oxidation.

### 2. MATERIALS AND METHODS

The wild boar were culled in the Province of Rome, hunted in wild conditions in compliance to Italian rules (Scillitani *et al.*, 2010). Meat samples from 8 wild boar males were collected between October and November, when the weather conditions were generally good. Each carcass was dissected and a portion of longissimus thoracis muscle, between the 6<sup>nd</sup> and the 13<sup>th</sup> rib, from left side was taken and frozen at  $-80^{\circ}\text{C}$ . The meat samples, after thawed were subdivided in seven portions of about 80-100g, for raw and fried meat analysis. For each portion six different frying oils were used (Table 1). Fried was performed at  $175^{\circ}\text{C}$  up to  $75^{\circ}\text{C}$  inside. At last the samples were dried from oil and minced for the fatty acids analysis. Percentage of fatty acid analysis and mg/kg of malondialdehyde (TBARS) were obtained as described in Amici *et al.* (2015) and Cifuni *et al.* (2014), atherogenic (AI), thrombogenic (TI), index according to Ulbricht and Southgate (1991) and peroxidation index (PI) according to Arakawa *et al.* (1986) were calculated. The ANOVA statistical analysis procedure by SAS was performed to estimate the difference between cooking methods.

Table 1. Fatty acids composition (% of total fatty acids) of raw, frying oils and lard

	C16:0	C16:1	C18:0	C18:1n9	C18:1n7	C18:2n6	C18:3n3
Rice	20.1	0.28	2.67	42.9	1.01	32.1	0.89
Corn	11.5	0.14	2.23	30.7	0.62	54.4	0.42
Olive	12.9	1.02	2.36	75.3	2.07	5.91	0.35
Peanut	8.37	0.09	2.98	69.2	3.60	14.5	1.42
Linseed	5.52	0.05	4.27	21.1	0.31	13.2	55.6
Lard	25.2	2.06	13.6	43.2	2.95	12.3	0.58

For each portion six different frying oils were used (Table 1). Fried was performed at 175°C up to 75 °C inside. At last the samples were dried from oil and minced for the fatty acids analysis. Percentage of fatty acid analysis and mg/kg of malondialdehyde (TBARS) were obtained as described in Amici *et al.* (2015) and Cifuni *et al.* (2014), atherogenic (AI), thrombogenic (TI), index according to Ulbricht and Southgate (1991) and peroxidation index (PI) according to Arakawa *et al.* (1986) were calculated. The ANOVA statistical analysis procedure by SAS was performed to estimate the difference between cooking methods.

### 3. RESULTS

The wild boar raw meat was rich in monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (Table 2) showing a good n6/n3 ratio, AI and TI index. The cooking losses were evident for saturated fatty acid (SFA) both for C16:0 and C18:0, with the exception of meat cooked in lard that is an animal product and therefore rich in saturated fatty acids. Frying fat effect was strongly evident during cooking also for the vegetable oils, in fact they imprinted to meat specific changes in relation to the fatty acids that characterize them. The meat cooked with rice and corn oil were significantly rich in 18:2n6 ( $p<0.001$ ), olive and peanut gave to meat a large amount of oleic fatty acid (+10%), while the meat cooked in linseed showed very high quantity of 18:3n3 (19.80 % vs 1.16 % average of all other).

Table 2. Fatty acids composition (% of total fatty acids) of meat after frying process

	Oils							RMSE
	Raw	Rice	Corn	Olive	Peanut	Linseed	Lard	
C16:0	20.8 ab	18.6 b	17.6 bc	17.0 bc	16.0 c	14.2 c	22.6 a	1.75
C18:0	10.6 ab	5.9 cd	6.2 cd	7.2 c	5.7 d	9.6 b	11.9 a	1.06
C18:1 n9	38.5 b	38.3 b	38.7 b	49.4 a	52.4 a	32.4 c	40.1 c	2.56
C18:2 n6	15.5 c	25.1 b	28.8 a	16.6 c	14.2 c	12.5 d	10.7 e	2.88
C18:3 n3	1.1 b	1.3 b	0.7 b	1.2 b	1.2 b	19.8 a	1.4 b	3.92
SFA	33.1 b	26.4 c	25.1 c	25.5 c	23.4 c	25.2 c	36.8 a	2.53
MUFA	43.3 c	42.1 c	42.6 c	53.5 a	56.0 a	36.9 d	47.8 b	2.89
PUFA	23.6 b	31.4 ab	32.3 a	21.0 b	20.6 b	37.9 a	15.4 c	5.15
ΣTrans	0.41d	0.73c	0.65c	0.51cd	0.29d	1.91a	1.25 b	0.22
P/S	0.7 bc	1.2 ab	1.3 ab	1.0 b	0.9 b	1.7 a	0.4 c	0.36
n6/n3	7.1 c	10.8 b	23.4 a	8.6 bc	6.3 c	0.9 d	4.7 c	1.90
PI	53.3 b	54.5 b	48.8 b	45.1 b	46.7 b	75.4 a	34.8 c	8.67
AI	0.37 b	0.29 c	0.27 c	0.26 cd	0.23 d	0.23 d	0.45 a	0.04
TI	0.80 b	0.59 cd	0.60 c	0.59 c	0.49 d	0.32 e	0.95 a	0.14

Different letters in row means significant differences for  $P<0.05$

$VLCFA = \sum C20:5n3, C22:5n3, C22:6n3$ ;  $P/S = PUFA/SFA$ ;  $\Sigma trans = C18:1 t9; C18:1 t10; C18:1 t11; C18:2 tt, C18:2 tc; C18:2 ct$ .

The high value of linolenic also if improved the n6/n3 ratio and TI index and determined a bad peroxidation index, showing the higher value compared to the others in particular for the frying with lard (73.4 vs 34.8), this one on the contrary showed high AI and TI index. The total trans isomers both

mono and polyunsaturated ( $\Sigma$  trans), with exception of peanut oil, are higher in cooked wild boar meat than raw ones ( $p < 0.001$ ), particularly the formation of trans is noticeable in meat cooked with linseed oil and lard. The P/S ratio was improved for all fries with oils, while it was lower to the others using lard. TBARS (Fig. 1) value is higher in meat cooked in linseed oil, particularly if compared with lard that did not show oxidation increase with cooking. Olive e peanut oil had good performances, while rice e corn were in intermediate position, because abundant percentage of C18:2n6.

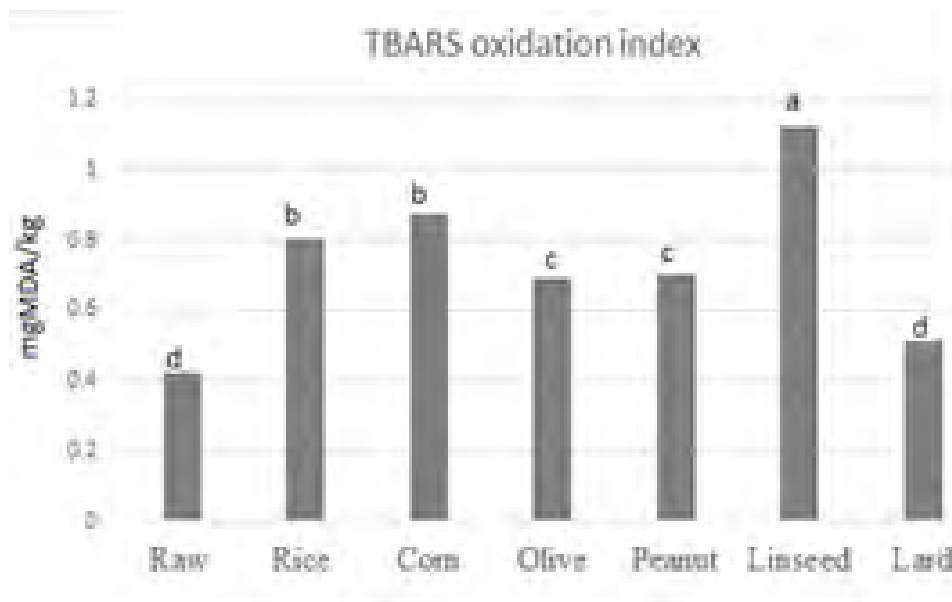


Fig. 1. TBARS oxidation index obtained with frying wild boar meat with different oils

#### 4. DISCUSSION

The composition in fatty acid and the calculated indices were similar to those reported in Razmaitè and Šiukšcius (2019), anyway showing a lower value of n6/n3 ratio similar to that reported in Amici *et al.* (2015). Cooking effect usually produce a loss of water and important constituent as protein and fat and produce lipid oxidation particularly if the meat is rich in polyunsaturated fatty acid (Amaral *et al.*, 2018). As reported in Saguy and Dana (2003) during frying in the presence of oxygen a variety of reactions cause a physical and chemical changes due a thermal alteration and formation of numerous polymerization products (hydro peroxides, conjugated dienes, carbonyls, trans isomers and malondialdehyde) very harmful to health. This compound are very higher if the raw meat is rich in free radicals and oxidation products due to the stress that the boar suffers during the hunt (Cifuni *et al.*, 2014). However, the present work showed a protective effect on fatty oxidation during the cooking of wild boar meat, using a good oil rich in MUFA like olive e peanut oil. From a nutritional point of view it is possible to conclude that frying modified the composition of meat lipids, which tended to be similar to that of the culinary frying fat used, significantly influencing the nutritional quality and safety and for this is necessary to use a good oil with limited pro-oxidant effect.

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## **Preliminary study on the presence of wild boar (*Sus scrofa*) and wolf (*Canis lupus*) in a peri-urban area with the camera-trapping's method**

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**Abstract:** The parallel expansion of anthropogenic environments and wild boar (*Sus scrofa*) populations has favoured the intrusion of the species into many European metropolitan areas, including Viterbo. The results have underlined that inside the study area, preys (ungulates) and predators (canine) follow the same temporal distribution. We have also studied how the increasing number of ungulates, especially wild boars have brought to our territory the largest canid of the Italian peninsula, the wolf, which now frequents the forests around the city of Viterbo. The study also showed that the distribution of the wild boar in the area is closely linked to hunting: through the photo-trapping method the different abundance of wild boar was highlighted, in relation to the surface used for the surveys; the CPUE (Catch per unit effort) was used as a parameter, which showed that in winter the abundance of wild boar reaches values of Cpue of 1.07 in 2018 and is almost doubled in 2019 (2.28), in fact in winter the species tends to be much more present inside the area where hunting activity is not permitted compared to the adjacent hunting areas (refuge-effect). The Cpue referred to the wolf shows in a temporal scale a trend very similar to wild boar and that showed how prey's presence is central in the reappearance after decades of the predator in a peri-urban environment near Viterbo's town.

**Keywords:** wild boar, wolf, Viterbo, peri-urban, CPUE, camera-trapping

### 1. INTRODUCTION

The parallel expansion of anthropogenic environments and wild boar *Sus scrofa* populations has favoured the intrusion of the species into many European metropolitan areas, including Viterbo (Amendolia *et al.*, 2019).

The purpose of the study was to use the camera trapping method to understand how the species moved into a peri-urban area during the seasons, and if hunting have effects to distribution of wild boar inside this environment.



Figure 1 Study area. It is possible to see on the left Viterbo and on the right Palanzana mount (circled in red)

## 2. MATERIALS AND METHODS

The study's area is represented by the mount Palanzana on whose slopes the Viterbo's town develops and reaches an altitude of 806 meters. The peri-urban area is characterised by a fairly wide heterogeneity with regard to land use and cover; in the north-west area, in fact, agricultural areas prevail, while in the southern and eastern there is a prevalence of natural areas and in particular of deciduous forests and limited conifer reforestation areas. In this study we used 7 camera-traps (3 multipir-12, 4 earthtree TC 600) for a total of 7195 hours (279-night and day trap) between July 2017 and February 2019. We have chosen 19 sites located in the 4 slopes of Monte Palanzana and in the areas adjacent to it close to the Natural reserve of "Valle dell'Arcionello" identified as suitable places for an analysis of the peri-urban wildlife. Other mammals present in the peri-urban environment, such as foxes, roe deer and mustelids, have been analysed as well as the wild boar and the wolf. The monitoring through camera-traps foresees the calculation of the number of photographs / videos that have been taken for each species in the chosen time unit (we choose the nights that photo-traps have been turned on; this parameter is called CPUE (Catch per unit effort) (according to Rowcliffe *et al.* (2008)).

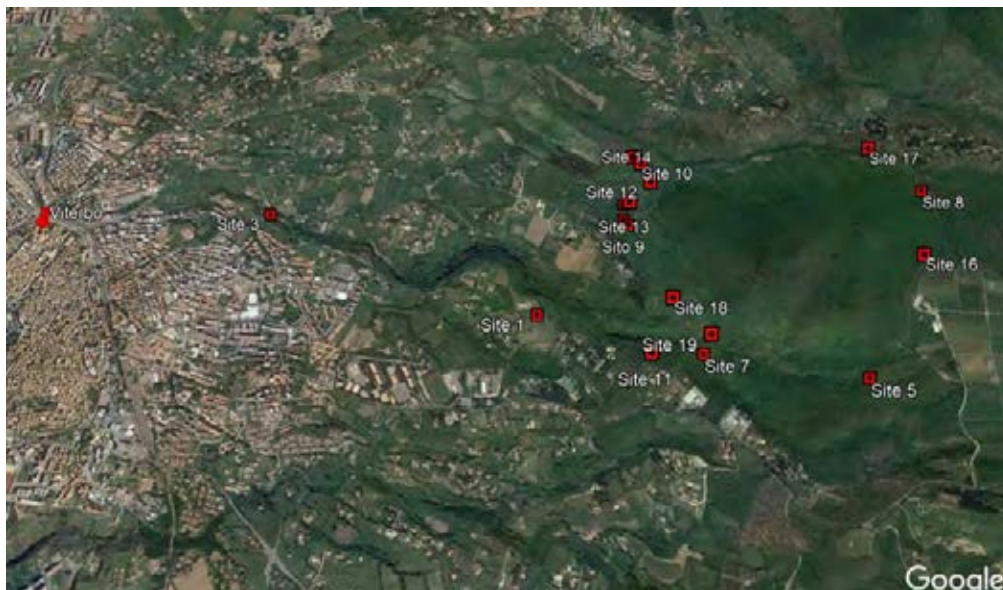


Figure 2 The 19 sites used during the study in Palanzana Mount

### 3. RESULTS

The results confirmed that the dominant species in the study area is the wild boar with a total of 312 individuals' observations. Analyzing the seasonal abundance of the species in the area we can see how it peaks in winter with a CPUE of 1.07 (2018) and 2.28 (2019) while it is minimal in late summer and early autumn (0.41) (tab. 1 and fig. 1).

Table 1. Trend of CPUE during the observation period

Season	Wild boar CPUE	Wolf CPUE
Winter 2018	1.07	0.073
Spring 2018	0.73	0.02
Summer-Autumn 2018	0.41	0
Winter 2019	2.28	0.047

Also, the wolf follows a similar trend as the wild boar (0.073 in winter 2018, 0.2 in summer/autumn 2018). It is showing a relation between the presence of the prey and predator. The differences between winter and summer are linked to the presence of hunting zones around the study's area that push into this, wild boar and sequentially wolves (refuge-effect). Analysis of the wolf's presence is critically important from the conservation point of view, in fact the species almost extinct in Italy in 1970, is undergoing a gradual growth of the individuals present in the peninsula. The presence of wolves even in the peri-urban environment also gives indications regarding the behavior of the species which, compared to the past, is less sensitive to human presence, in fact there aren't previous references with which to be able to compare the detected presence of the species in the peri-urban environment and thanks to the trapping system further research can be conducted using this technique, in collaboration with the radio-tracking for density estimates related to both wild boar and to the wolf (Rowcliffe *et al.*, 2008).

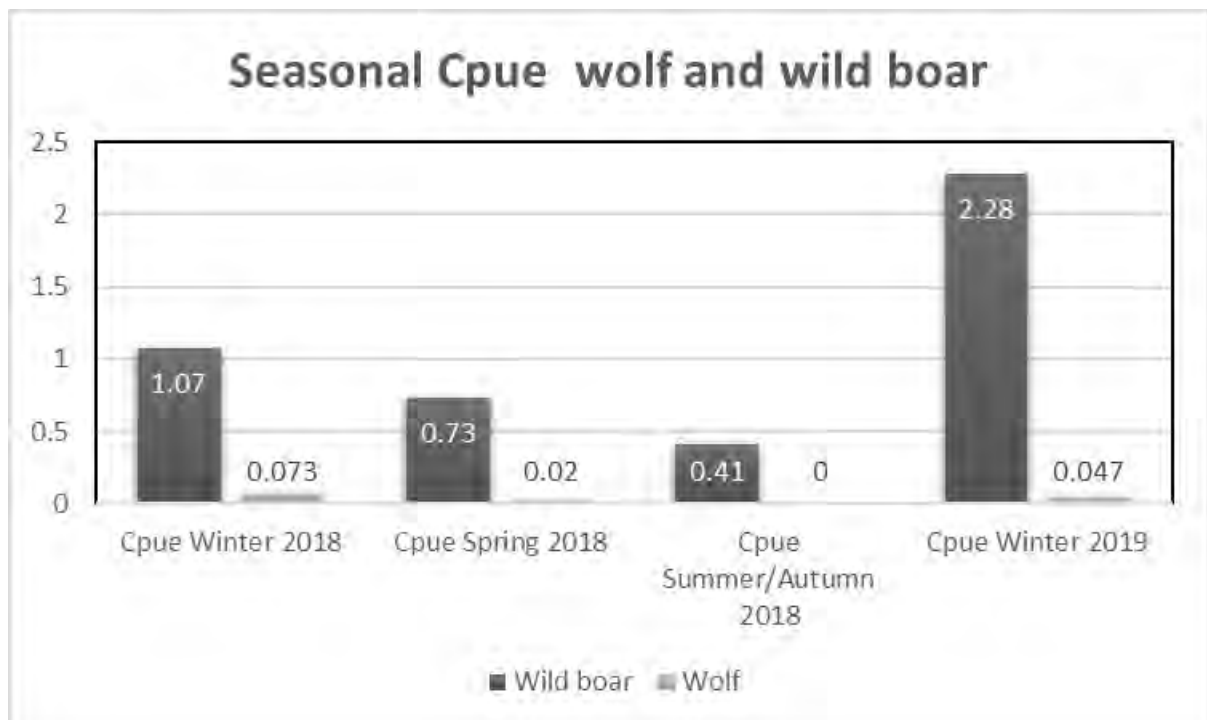


Fig. 3 Comparison between seasonal wild boar and wolf's Cpue.



Figure 4-5 Wolves captured by camera-trap during the study period.

## 5. CONCLUSIONS

In conclusion we can affirm that wild boars and wolves use peri-urban environment in winter to take refuge from hunting, preferring instead less populated areas during the summer and spring.

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## Poster

### Discovering mammal diversity by camera-trapping in the Khangai Nuruu National Park (Mongolia)

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**Abstract:** Mongolia still conserve some extremely important hot spot of biodiversity in the boreal belt. As a part of a wider multi-year project, a mammal community survey of Khangai Nuruu National Park, in the South of Arkhangai province was implemented by Green Initiative NGO, with the support of Mongolians and foreigners Institutions. The protected area, located in north-central Mongolia, host a variety of habitats dominated by Alpine landscapes and forest-steppe where forests, mainly of *Larix sibirica* and *Pinus sibirica*, are jeopardized with steppes of *Festuca* spp., *Artemisia* spp. and *Euphorbia* spp. The low human pressure and the extent of the areas left to natural evolution allows the survival of a complex and particularly well-structured animal community. From 5<sup>th</sup> to 25<sup>th</sup> August 2018, 45 camera traps were placed and left unattended until mid-October 2018, cumulating an effort of approximately 2000 camera nights-trap. The traps were positioned according to tracks and opportunity place thanks also to the help of the local rangers. The cameras were arrayed covering an altitudinal range from 2200 to 3000 m a.s.l. between valleys bottom and ridges. Twenty species for 285 independent detection events were recorded: *Capreolus pygargus* 55, *Vulpes vulpes* 43, *Ochotona alpina* 40, *Lepus tolai* 36, *Sciurus vulgaris* 26, *Mustela erminea* 17, *Canis lupus* 13, *Mustela sibirica* 13, *Spermophilus undulatus* 9, *Capra sibirica* 7, *Meles leucurus* 6, *Cervus elaphus* 4, *Gulo gulo* 6, *Tamias sibiricus* 4, *Martes foina* 3, *Otocolobus manul* 4, *Lynx lynx* 2, *Mustela eversmanii* 2, *Marmota sibirica* 1, *Mustela nivalis* 1. The results are underlining how the mosaic of the different landscapes are supporting a high differentiate large mammal diversity, with analysis for guild with 9 herbivores and 11 carnivores, as well as an impressive number of species for each taxon with 3 Artiodactyla, 4 Sciuridae, 2 Lagomorpha, 2 Felidae, 2 Canidae and 7 Mustelidae. The encounter rate is also high, especially considering the characteristic of the landscapes. The traditional lifestyle of local community enabling the survival of a such different mammal diversity, is also discussed

**Keywords:** phototrapping, checklist, mammals, occupancy, habitat richness, habitat density

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### Opportunities from remote recording of activity in wild ungulates: classification of behaviour in red deer (*Cervus elaphus*) using activity sensors in GPS collars

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**Abstract:** Recent advances in bio-telemetry allow to remotely record and infer behaviour in many wild species. For wild ungulates, collecting spatiotemporal data (i.e. location and time) could help understanding their habitat use, their responses to external cues and other aspects of their biology. The research aimed at evaluating the potentialities offered by GPS collars equipped with activity sensors

for inferring behaviour in red deer (*Cervus elaphus*). Four wild-born animals raised in captivity, 2 hinds and 2 stags, were equipped with GPS collars with built-in tri-axial accelerometers. The animals were equipped with the collars for a period ranging from 5 to 40 days, during the months of May, June, July, August, October and November. The device measured acceleration 4-8 times per second and automatically scaled raw acceleration data into levels ranging from 0 to 255, providing an average activity value every 300 seconds on two axes: the X-axis for forward/backward movements and the Y-axis for sideways and rotary movements. A set of 7 behaviours (bedded, bedded rumination, standing, grooming, feeding, walking, running) was directly observed in synchrony with collar measurements. Behaviours were classified with discriminant analysis (DA) using Gaussian Finite Mixture Models. Activity measurements on the X and Y-axes were predictors. Models were trained on a random subset and validated on the remaining data with cross-validation. We preliminarily tested models classifying both all observed behaviours and several behavioural classes obtained by merging similar behaviours (e.g. *inactive* behavioural class was obtained by merging bedded and bedded rumination). Overall, walking and running were misclassified in more than 90% of cases and were mainly assigned to feeding behaviour. Feeding activity was correctly classified in 84.1% of cases, while standing only in 35.5%. The model correctly classified bedded behaviour in 79.8% of cases while bedded rumination was mainly mistaken with bedded behaviour, with a rate of correct classification below 12%. Thus, bedded and bedded rumination were merged into an *inactive* behavioural class and the remaining behaviours into an *active* behavioural class. DA correctly classified respectively over 88% and 94% of active and inactive intervals. The use of activity sensors in GPS collars could thus represent an effective technique to track movements of wild ungulates and, at the same time, to monitor their activity levels as well as to infer with a reasonable accuracy at least their state of activity (active or inactive). Nevertheless, synchronous observation on sample animals is required to calibrate the predictive model. The scaling and averaging process of acceleration data over a predefined sampling interval probably represents one of the major criticalities that reduces the possibilities to infer a higher set of behaviours.

**Keywords:** tri-axial accelerometer, calibration, Discriminant Analysis, monitoring, biology

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## **Status of an American Mink (*Neovison vison*) population in Northern Italy**

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**Abstract:** The American mink *Neovison vison* (Schreber, 1777) have been introduced from North America in 28 European countries at least. This alien species, initially bred in strict captivity for fur production, is naturalized in many parts of Europe as result of accidental escapes or intentional releases. At the national scale, feral populations are present both in north and central Italy. In Italy, the oldest American mink population is reported in the province of Forlì, along the rivers Bidente and Ronco. This naturalized population originated, at the beginning of 1990, from animals accidental escaped, that found suitable habitats and progressively established along the river and its floodplain. Moreover, in the year 2001, 3000 individuals of this alien species were deliberately introduced by an animalist group in the same area. In the following decade others small releases were performed. At that time, most of the animal were captured by wildlife services guards and local hunters, but people who frequent the area affirm that many animals are still present. In the autumn of 2017, we attempted to

verify the presence of the species along the river looking for footprints, scats and food waste. The survey was performed along 30 linear transects of 500 m long and by camera-trapping (CT). 151 photos were taken during the night. 106 photos with animals in the camera revealed the presence of 16 different species. No minks were recorded although footprints, preys remain, and scats were found in the field along the river. Interviews with anglers also suggest a consistent presence of the species although significantly contracted compared to the past. In addition, sightings and specimens' records to witness an expansion towards the Savio valley and in areas of the plain, near the cities. Also in another farm in 2014 close to Ravenna 800 specimens were released in a location less than 20km from the northern part of Ronco river basin. At the moment, the population is in expansion and it is necessary to improve the control effort on the mink.

**Keywords:** *Neovison vison*, Northern Italy, alien species, population, management

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## Recent evidence on the presence of the Western Polecat (*Mustela putorius* L.) in the province of Rieti (Italy)

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**Abstract:** The European Polecat (*Mustela putorius* L.) is a species of the native Italian fauna belonging to the Mustelidae family. Currently, data available on the distribution of the polecat at the national scale are scarce and obsolete because it is an elusive species and its residual populations are decreasing and included in the least concern category in the IUCN Red List. In May 2017, a photo of an animal presenting all the external phenotypic characters of the western polecat was taken in the province of Rieti (Italy). This is a recent evidence on the presence of the species in the province of Rieti allowing to update the distribution of the species. On 13 May 2017, near the village of San Giovanni Reatino, in the Province of Rieti (coord. UTM 33T 323043E 4690550N), a photo hand of an animal presenting all the external phenotypic characters of the western polecat was taken.



The external phenotypic characters listed below (De Marinis, 2003), allow us to classify as western polecat (*Mustela putorius*) the detected animal: small and flattened head (very short splanchnocranium); short ears rounded and edged with white; white facial mask also surrounding the eyes and the mouth; colour of the blackish coat lighter on the sides (De Marinis, 2003). The species resembles the Ferret (*Mustela putorius furo* vel *M. furo*), which is considered its domestic form (Birks, 1999). It's characterized by a coat that can vary from the albino to the dark brown and can be

practically indistinguishable from the colouring of the wild form. The absence of the typical facial mask that extends to the throat excludes that the specimen of the photo is a Ferret or a European Polecat-Ferret hybrid. It is also excluded that it is American Mink, the only wild Mustelidae present in Italy similar to the European Polecat, because the coat of this species is dark brown spotted with white on the chin. This report represents an advancement in knowledge is due to the rare knowledge of the status of the species in Italy, to the reduction of sightings begun in the last decade of the last century (De Marinis *et al.*, 2002) to the absence of updated data on distribution (De Marinis, 2003). In relation to this knowledge, even the most recent publications on the distribution of European Polecat in the Lazio Region refer to obsolete and not always detailed bibliographic data.

**Keywords:** European Polecat, *Mustela putorius*, Mustelidae, Fauna, Rieti

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### **Wolves in a highly anthropic area in the Province of Pisa, Tuscany, Italy**

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**Abstract:** Recently in Italy the wolf population is extending its range to suburban areas of new colonization with an increase of conflicts between human activities and wolves. Since October 2018 the presence of wolf was investigated in a hilly area of 900 ha in Crespina-Lorenzana and Lari-Casciana Terme in the Province of Pisa. The area is characterized by a high environmental fragmentation with a dense network of ecological corridors and by an intensive presence of productive (i.e. sheep-farming, cutting of forest) and recreational activities (i.e. trekking, cycling, horse riding, bird-watching, hunting, mushroom and truffle search). The investigation was performed using non-invasive monitoring techniques: camera-trapping, search of signs of presence (faeces and tracks) on transects and genetic analysis on faecal samples. The genetic analysis were performed using the autosomal STRs to identify the individuals and the belonging population and the Amelogenin marker to sex the individuals. The camera-trapping records have shown the presence of a pack of 5 specimens. A total of 63 faecal samples were collected 10 of which were used for genetic analysis. The genetic analysis allowed us to identify 3 distinct individual, two female and one male. All identified individuals belong to Italian wolf population at 99%. The quality of DNA samples was not enough to make a comparison with the database of the identified wolf. However, a new sampling session is already underway in order to increase the quantity of pack information also related to predatory behaviour and diet. The investigated anthropic area is not yet included in the distribution map of the wolf drawn up in 2015 by ISPRA and the available data on Tuscany packs do not report the wolves in this area. However, some packs have been recorded in near areas (i.e Santa Luce and Livorno) and it is therefore important to understand if the investigated pack is really a new nucleus.

**Keywords:** *Canis lupus*, Human activities, Anthropic area, Humans-wolves coexistence, Non-invasive monitoring, Genetic analysis, Camera-trapping.

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## Evolution and distribution of wild boar damage in Campania, southern Italy

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**Abstract:** The increase in wild boar population has generated social, economic and managerial conflicts throughout its range. In Campania region this species is widespread although at different densities, with consequent damage to agriculture. The analysis of wild boar distribution and of damage events is fundamental for the planning of management actions to limit damage and problems. This study presents for the first time, the framework and the evolution of damage in Campania during six years, highlighting the critical areas (hot spots) for the presence of repeated damage over time. 2719 claims for boar damage from 2013 to 2018, were georeferenced with QGIS 3.6.3 and their distribution was analyzed by municipalities and provinces. Damage caused by wild boar in this region involved 311 municipalities equal to 56% of the total of Campania and 77% of the area of wild boar presence. Moreover, incidents are increasing in recent years, with 143 road accidents reported in 2018. The results suggest greater distribution and concentration of wild boar damage evolution in Campania and shows hot spots that require extraordinary management measures.

**Keywords:** *Sus scrofa*, Wild boar, wildlife damage, Campania, South Italy, wildlife management, geographic distribution

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## The role of drive hunting to manage wild boar population in Campania Region, Southern Italy

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**Abstract:** Wild boar population in Campania has exploded numerically in the last twenty years, colonizing all wooded areas and exploiting the protected areas. Its affirmation has increased the interest of the hunting sector by contributing to the development of a form of hunting that has so far been little practiced, but which in the 2018 hunting season involved 273 teams and 7,621 hunters. Currently in the Campania region the control of wild boar population is carried out on the regional territory exclusively through drive hunting, except for the National Park of Cilento, Vallo di Diano e Alburni where selective control is practiced. The present study analyzes data of seasonal game hunting effort from October 1<sup>st</sup> to December 31<sup>st</sup> 2018. Overall 5,574 hunting days were recorded with 89,321 participants and 40,374 comparing it to the results of the collection of the last three hunting seasons 2016, 2017, 2018 on the whole regional territory. The article focuses on:

- Hunting practice;
  - Hunting team effort and participation;
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- Hunting bags;
- Temporal trend of hunting bags;
- Hunting bag in relation to age;
- Female productivity rate.

The objective of this study is to identify game bags for future collection of data to plan large-scale wild boar management.

**Keywords:** *Sus scrofa*, Wild boar, wildlife management, drive hunt, planning bag, Campania, Italy

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## **A new strategy to manage ungulate populations: the results of the "Target Law" in the Tuscany Region**

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**Abstract:** The territory of the Tuscany Region is one of the richest in ungulates in Europe. There are considerable numbers of wild boar and roe deer, as well as deer, fallow deer and mouflon. The traditional crops of the territory, like the vine, which have made Tuscany famous throughout the world, have entered a crisis when the density of ungulates has increased considerably since the 2000s. Ordinary management tools have not proved effective. For this reason, the Tuscany Region, starting from the provisions of the "Guidelines for the management of ungulates" produced by ISPRA in 2012, approved a three-year "target law". This law is based on some simple indications, which could have a practical impact on the territory:

- subdivision of the territory between suitable areas and unused areas (agricultural) based on objective parameters and subdivision of the same into management units,
- concentrate the removal of ungulates in areas not suitable
- participate in the management of ungulates among the various management entities, without leaving anyone an exclusivity of action to increase competition
- management of the meat of hunted ungulates using a transparent procedure
- favor hunting compared to extraordinary control interventions provided for by national law 157/92 art. 19

After three years of applying the law, despite the resistance of a good part of the hunting and environmental world and animal-rights activist, the results were clear: the number of ungulate populations has unquestionably fallen while the damage to agriculture has decreased significantly

**Keywords:** Roe deer, wild boar, damage reduction, valuable crops, hunting strategies

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## **Digital innovations of the Tuscany Region Administration in wildlife hunting management**

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**Abstract:** The wildlife sector in Italy in recent years has been the subject of a contrasting phenomenon. On the one hand new scientific studies and university have been developed, increasing the number of specialists in this topic. On the other hand, with the development of social networks,

public opinion has become more influential in terms of management choices. In this framework, the Tuscany Region Administration has launched a new strategy for finding faunal data, taking advantage of the digital applications now made available to the technology. The first application, named "Toscaccia", is a digital hunting card, available through the app store in the latest generation smartphones. This app replaces the paper card, which is mandatory by law. In this way it is possible to know in real time the data related to the "hunting bags". The second application is the "Regional wildlife portal". On this website, data relating to the wildlife management of both ungulates and private hunting reserves (census, hunting plain), divided by management unit, are entered by over 100 authorized users. The third application is called "Gestofauna". It is an interface where farmers, freelancers and land management councils can interact to manage the problem of damage that wildlife causes to agricultural crops. The possibility of real data so complex in real time is fundamental to dialogue with the European Union, the Ministry of the Environment and ISPRA

**Keywords:** digital technology, hunting bags, crops damage, database

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## First data on the hunting of some species in the Ascoli Piceno province (Italy)

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**Abstract:** Even though hunting has historically been widely practiced in the Ascoli Piceno province, data statistically outlining the scale and impact on numbers of affected species have never been published. This study, which constitutes an essay on the large quantity of unpublished data collected during the last six hunting seasons, reports on the hunting of Hare, Pheasant, and Grey Partridge, also in relation to the extent of relative recurrent repopulation. In the Ascoli Piceno province hunting activity has historically been intense and widespread. The species most favoured by hunters are the Hare (*Lepus europaeus*), the Pheasant (*Phasianus colchicus*), the Grey Partridge (*Perdix perdix*), the Boar (*Sus scrofa*), the Rock Partridge (*Alectoris graeca orlandoi*), the Woodcock (*Scolopax rusticola*) and the Wood Pigeon (*Columba palumbus*). Despite this ancient hunting tradition and its widespread interest, and despite also the fact that hunting and wildlife management institutions have collected hunting and repopulation data, as yet this information has never been published. This paper is, therefore, the first time that hunting data and repopulation data are collectively submitted to the scientific world. Wild Boar, Rock Partridge, Woodcock and Wood Pigeon are not subject to repopulation activities, and therefore hunting is exclusively of natural populations. Hare, Pheasant and Grey Partridge, on the other hand, are annually subject to repopulation. These interventions, which current regulation deems transitory and which are as such limited to those years in which repopulation is necessary in order to render targeted populations self-sufficient, are actually carried out without any prediction of their definitive impact on numbers. If hunting levels were tailored to specific annual species increases, however, there would no longer be the need to intervene in repopulation. This study is aimed at providing managing bodies with the first quantitative hunting data, and where relevant a numerical comparison with repopulation figures. The data reported below is for the six hunting seasons from 2012/2013 to 2017/2018. Hunting figures for species not subject to repopulation: Wild boar 4219, Rock Partridge 39, Woodcock 6022, Wood Pigeon 26579. Total numbers of animals released in restocking activities against those hunted: Hare 3336/2591, Pheasant 14260/6002, Grey Partridge 11453 /1732. The available data clearly indicates that hunting of these latter species depends exclusively on repopulation. The possibility of hunting Grey Partridge, and to a lesser extent Pheasant,

would appear to be closely linked to the releases of these species. This problem appears less marked for the Hare. The institutions concerned should therefore initiate specific studies to understand what is causing a collapse in numbers of released species - a problem faced by the vast majority of institutions involved in repopulation - and to understand whether it might not be more appropriate to persevere in reducing hunting levels instead.

**Keywords:** Ascoli Piceno, hunting, Hare, Pheasant, Grey Partridge

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## **First data on containment culling of some opportunistic species in the Ascoli Piceno Province (Italy)**

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**Abstract:** As is the case in most Apennine environments, the fauna of the Ascoli Piceno Province includes, in addition to species of hunting interest, exuberant opportunistic species which have a negative impact on both the zoocenosis and agriculture of the area. Previously unpublished data for the abatement of these species gives clear indications as to the achievements of activities carried out over the five-year period from 2013 to 2017, aimed at restoring ecological equilibrium. Bibliographic data indicate a general national increase in numbers of exuberant opportunistic species such as the Fox (*Vulpes vulpes*), the Magpie (*Pica pica*) and the Hooded crow (*Corvus corone cornix*). This not only has a negative impact on the numbers of eggs, nestlings, and young of game birds. The re-composition of ecosystem equilibria, aimed at managing an appropriate productivity in game birds, and commensurate hunting levels, (where recurrent restocking is not required) is also dependent on an equally adequate control of exuberant opportunist species. The low appeal of these species to hunters means that numbers are only minimally reduced during hunting seasons. Analyses of hunting cards - in which hunters are required to declare their takings - indicate that the rate of withdrawal of these species is extremely reduced, if not negligible. As such, limiting numbers is dependent almost exclusively on special abatement plans. This study presents, for the first time, the abatement data in the Province of Ascoli Piceno (Italy) for the three species indicated above, resulting from specific abatement programs for the five-year period 2013-2017. The drive hunting technique was used for the Fox. The Magpie and the Hooded Crow were captured using a Larsen cage. In the five-year period in question, a total of 3089 magpies, 754 foxes and 306 hooded crows were captured. The yearly breakdown of these figures is as follows: 2013 - 367 magpies, 154 foxes, 12 hooded crows; 2014 - 481 magpies, 205 foxes, 82 hooded crows; 2015 - 1012 magpies, 166 foxes, 125 hooded crows; 2016 - 429 magpies, 78 foxes, 87 hooded crows; 2017 - 700 magpies, 151 foxes, 0 hooded crows. These unpublished data are interesting in that they provide information which can be directly related to the specific species densities estimated by the Ascoli Piceno Hunting Area during the 2013-2015 period, during the drafting of control plans. For the correct restoration of ecosystem balance, a cross-analysis of the results obtained is essential and of fundamental importance. In fact, only by following this procedure it is possible to assess whether the control plan and its degree of success have enabled the achievement of the specific species density targets set. In the case in question, densities of the Hooded Crow and the Magpie were estimated in relation to the number of nests detected per unit area (in winter, with a spring check of 20-30% of those previously detected), or according to the Kilometric Abundance Index. If the objective has not been achieved, new groups for reduction may be defined.

**Keywords:** Ascoli Piceno, hunting, Fox, Magpie, Crow

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## Forest fires and sites of wolf (*Canis Lupus*) rendez-vous: a case study in the Province of Rieti

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**Abstract:** The forest fires of 2017 created damage to the forest and the fauna that used the areas travelled by the fire. Between the damages caused by the fire to the macrofauna are widely known those of direct and indirect type. Those of the first type are usually addressed to subjects that are not very mobile, due to bio-ethological causes and / or to any contextual impediments (e.g.: fences, etc.) Indirect damages affect the fauna as a result of partial damage or complete destruction of the habitats used. In this study we investigated a case of forest fire that occurred in the summer of 2017 and that was extinguished about 2 km from a site of rendez-vous of Wolf. Differently from similar sites monitored in the province of Rieti, not close to the fire, the one studied was definitively abandoned by the herd that used it. In consideration of the fact that no other disturbance factors have been recorded; it is assumed that the abandonment of the site occurred precisely because of the disturbance produced by the fire. The hypothesis requires further specific insights.

**Keywords:** Wolf, Rieti, forest fire, rendez-vous

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## Invasive Alien Species: subspecies of chipmunks makes the difference

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**Abstract:** Wide-ranging species usually shows different spatiotemporal behavioural adaptations to contrasting environmental adaptations. These species are also those who usually shows the high invasive potential, mostly due to their wide physiological levels and ecological plasticity. Impacts of non-native species are currently considered the second most influential causes of the current global biodiversity crisis, after habitat loss and fragmentation. Therefore, assessing their invasive potential is crucial to prevent damages to native species and environments. Among invasive alien species, the Siberian chipmunk *Eutamias sibiricus* is a ground-dwelling squirrel native to north-eastern Asia, from Western Russia to Korean peninsula and Hokkaido island. Invasive alien populations of this squirrel currently occur in many European countries, as well as in other Japanese islands. We summarized differences in behavioural patterns between the Siberian (*Eutamias sibiricus sibiricus*) and the Korean (*Eutamias sibiricus barberi*) subspecies. It has been shown that in Europe, Korean subspecies finds suitable habitat and a significantly lower extension of area is suitable for the Siberian one. According to a review screening of 46 published studies, the Siberian taxon mostly lives in coniferous forests with *Pinus sibirica*. The staple of the diet is built up by pine cones and seeds, but small birds and invertebrates are also preyed upon. No debarking behaviour is reported to occur. Hibernation lasts usually from October to April. The Siberian subspecies is more strictly ground-dwelling than the Korean one, which, conversely, often climb on trees to search for food and shelter sites. Korean chipmunks mainly inhabit deciduous woodland with *Quercus* spp. Food resources mostly include acorns and other wild fruits; bird egg predation is reported as a very rare event. Occasional debarking behaviour has been described in some forested area in Korea. Hibernation lasts usually from

November to March. All these behavioural differences may have helped Korean chipmunks - as identified through previous molecular analyses - to well-adapt to European environments and to urban/periurban deciduous woodland where trophic sources provided by humans have prevented them from food search in the invaded areas.

**Keywords:** *Eutamias sibiricus*, invasive alien species, diet analysis, habitat use, hibernation

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## **Biodiversity and main biocenosis of the mid-Tyrrhenian Latium pre-Apennine ecosystem**

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**Abstract:** Biodiversity is one of the most important indicators of the natural and ecological status of a biotope. In the territory of the southern Lazio Region between the provinces of Rome, Latina and Frosinone, there are environments of common geological origin but with different morphological patterns: sandy coasts, high and rocky coasts, floodplains, interior valleys, hilly and mountainous areas. The mountain range of this area borders to the north-west with the valley between the Lepini Mountains and the Albani Valleys (Lat. 41.743355, Long. 12.833336 GD) and to the south-east with the Garigliano river (Lat. 41.28683, Long. 13.97905 GD); it is border to the north-east area by Latin Valley, while to the south-west, towards the Tyrrhenian sea, it descends into the Pontina Plain and Fondi Plain as well as into the high and rocky coast going from Sperlonga to Minturno. This mountain range has a total length of 100 kilometres, variable width (30-50 Km.) and an average altitude of 1,000 m.a.s.l., until 1,536 m. The climatic conditions are predominantly mediterranean and tempered in the higher northern areas. The anthropic activities and their settlements are more developed in the flat and coastal areas facing the Tyrrhenian Sea and the Latin Valley, as well as in the more internal and transversal valleys, such as the Amaseno Valley and the Ausona Valley. Historical settlements permeate the whole territory and the original natural environment has long been in contact with the human activities; they have often modified and altered the territory, whose modifications still produced today characterize the present landscape. The biocoenosis present in these environments are the result of the balances that have stabilised between nature, plant and animal species and the human activities exercised. The main historical modification have produced the anthropic grassland and pastures, garrigue and steppe, olive groves, orchards, chestnut woods and copse; while currently we have urbanization works, infrastructures, more advanced agricultural settlements and some livestock farming. The aim of this work is to represent the biotope of the mid-Tyrrhenian Latium preapennine ecosystem in relation to: geomorphological, geographical and hydrographic classification, land use, climate and climatic zones, division of altimeter bands for the description of flora, main forest and plant associations, main animal habitats (wild and farmed mammals, birds, reptiles and amphibians). The information reported derives from the consultation of studies and personal research, cartographies (Blasi C., 1993) and WebGIS software. This representation allows to deepen the knowledge about the ecosystem, in order to evaluate the opportunities for conservation and protection of natural biodiversity as well as human activities, about to proper integration.

**Keywords:** *Pre-Apennines*, *biodiversity*, *biocoenosis*

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## Changes in microscopic morphology of Corpus luteum and uterine body during pregnancy stages in the African buffalo (*Syncerus caffer*).

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**Abstract:** Although artificial reproductive techniques are considered to be a competitive tool for wild fauna preservation, often these techniques have limited results. This is frequently related to the lack of knowledge in reproduction of these species due to limited access to biological material. The objective of this study was to perform a morphological evaluation of the reproductive tract of female African buffaloes (*Syncerus caffer*) focusing on the changes at corpus luteum (CL) and uterine mucosa level during different pregnancy stages. Tissues of reproductive tract from 52 females of African buffalo at random stages of estrous cycle and pregnancy were collected at the Hluhluwe–Imfolozi nature reserve (South Africa) from buffaloes culled within a Tuberculosis monitoring program. Since the exact time of the mating is unknown in wild ranged animals, the stage of pregnancy was established based on the size of the reproductive system (uterine body and horns) and length of the fetus according to previous literature from the domestic buffalo (*Bubalus bubalis*). Within the pregnant animals, tissues from 6 females (n: 3 early pregnant and n: 3 middle-late pregnant) were analyzed. The tissues were fixed in neutral buffered-formalin 4%, embedded in paraffin, sectioned at 7 µm and stained with hematoxylin/eosin. For each sample a total of 10 microtome sections have been chosen and 3 random microscopic fields analyzed. Regarding the CL the luteal cells have been classified according to morphologic characteristics and counted; for the endometrium the glands number were counted, and the area occupied by them and height of the glandular epithelial cells were measured. In the CL, as reported for domestic buffalo, two different cytotypes were observed: one characterized by polyhedral shape, central nucleus and lipid droplets in the cytoplasm called large luteal cells (LLC) and one characterized by spindle shape and eccentric nucleus called small luteal cells (SLC). In early pregnancy animals a higher number of SLC, conversely in the middle-late a higher number of LLC were observed. In the endometrial mucosa different gland distribution was detected. In the early pregnancy a higher number of glands with a smaller area was observed and, in the middle-late the opposite. Furthermore, the epithelial cell height was greater in the middle-late than in the early stage. This preliminary study shows differences during the pregnancy stages in the microscopic morphology of CL and uterine mucosa in African buffalo, similar to domestic one. However, further investigations are necessary to accurately describe the changes that occur in the other parts of the genital apparatus during pregnancy.

**Keywords:** *Syncerus caffer*, Corpus luteum, uterus, glands, pregnancy stage, South Africa

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## Behavioural toxicity of environmental relevant concentrations of a glyphosate commercial formulation in zebrafish embryos

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**Abstract:** The use of herbicides with glyphosate as an active ingredient (a.i.) has increased dramatically in recent years, with its residues being often found in places close to its application site, either in soil or in water. Over the years, toxicological reports confirmed the negative effects on living organisms and concerns have arisen about its harmful side effects for both ecosystems and wildlife health. In this regard, toxicant-induced behavioral impairments are often used to study the underlying physiological deficits, in order to evaluate the ecological risk posed by a stressor. Therefore, the objective of this work was to assess the effects of a commercial formulation of glyphosate (Roundup Ultra Max®), at environmentally relevant concentrations, on zebrafish embryos through a set of behavioral paradigms. Zebrafish embryos were collected after fertilization, bleached and exposed to 0, 0.1, 1 and 5 µg a.i. mL<sup>-1</sup> concentrations of the glyphosate formulation for 72 h (from 3 to 75 hours post-fertilization (hpf)). After exposure, larvae were washed and maintained in system water until 120 hpf. At this point, the larvae behavior was evaluated using a battery of tests, to assess the general exploratory motility, escape-like responses, anxiety-related behaviors and social interactions. Overall, no significant changes were observed relative to the exploratory motility. The anxiety-related behaviors were also similar among groups and no social interference was observed following exposure to these glyphosate concentrations. On the other hand, the larvae exposed to 5 µg a.i. mL<sup>-1</sup> were not able to respond to an aversive stimulus, supporting the existence of glyphosate-induced changes in the sensory-motor coordination during development. In general, these results indicate a possible neurotoxic effect of this glyphosate-based formulation that should be further evaluated. Moreover, given the environmentally relevant concentrations used, the results obtained could impose a risk for wildlife sensitive species that should not be neglected.

*Acknowledgements:* Projects UID/AGR/04033/2019 and POCI-01-0145-FEDER-006958.

**Keywords:** Glyphosate, ecotoxicity, aquatic wildlife, zebrafish, larvae, behavior.

## Fish welfare: sedative effects of MS-222 and propofol under simulated transport conditions

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**Abstract:** The handling and transportation of fish commonly induces stress. A way to improve transport conditions and animal welfare includes the use of anesthetics, wherein MS-222 is the most commonly used one. However, due to its irritant and aversive properties, safety issues and residues, alternatives are needed. Therefore, the main objective of this work was to evaluate propofol as an

alternative agent to MS-222 for sedation during transport of live fish. All procedures were approved by the DGAV (Direção Geral de Alimentação e Veterinária) through the project license 014703/2017-06-16. Nile Tilapia (*Oreochromis niloticus*) (Til-Aqua International, Holand) under the following conditions:  $24 \pm 1^\circ\text{C}$ ,  $6.8 \pm 0.3 \text{ mg L}^{-1}$  of dissolved oxygen, and pH of  $7 \pm 0.2$  (14h light:10h dark) - were divided in four groups: (i) control in normal conditions (naive); (ii) control under simulated transport (UST); (iii) MS-222 ( $40 \text{ mg L}^{-1}$ ) UST and (iv) propofol ( $0.8 \text{ mg L}^{-1}$ ) UST. Fish UST were placed in a 8 L tank with the anesthetic (iii or iv) or with the system water (ii) for 6h and then transferred to recovery tanks with 200 L. Nitrate and nitrite levels,  $\text{O}_2$  and pH levels were controlled at 0, 3 and 6h. Sedation level was assessed by the evaluation of opercular movements and the response to different stimulus (object approximation, touch and tail pinch) at 0, 0.5, 1, 3 and 6h. At 3, 6 and 30h after the exposure beginning, animals (n=7) were sacrificed and blood parameters (hematocrit, hemoglobin and glucose) were measured. All fish were recovered. Environmental and water conditions remained constant throughout the experimental period with the exception of nitrate which increased in the MS-222 treated group within the limits allowed - recommend not exceeding concentrations of  $500 \text{ mg L}^{-1}$  in juvenile tilapia. The sedative stage of MS-222 and propofol was confirmed by the characterized slow operculum movement. Depth of sedation was higher in the propofol treated animals compared with MS-222, confirmed by the weak reaction to the different stimulus. A slight decrease of the hematocrit was observed in propofol group at 6h. Overall, propofol may be a good sedative for the transport of live fish. However, further studies may be important for the establishment of strategies that can be used to improve fish handling and transportation with the ultimate goal to preserve wildlife welfare.

*Acknowledgements:* Projects UID/AGR/04033/2019 and POCI-01-0145-FEDER-029542 (PTDC/CVT-CVT/29542/2017).

**Keywords:** sedation, fish, stress, transport, welfare

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## **Evaluation of fertility alteration in *A. m. Ligustica* drones caused by climate changes**

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**Abstract:** The climatic changes of recent years have allowed that in June 2019, in Italy, honey production has not yet begun. The primary functions of honey bees like productions and reproduction are based on food availability. The beekeepers select the drones for natural and artificial insemination of the queens observing some of their morphological and morphometric characteristics at the time of the presumed sexual maturity. Body structure, color, hydration and the size are compared to that of the others. The drone size is the main parameter to evaluate its reproductive potential. The sexual maturity of the drone starts around fifteen days old when it reaches a weight that, depending to subspecies, goes from 270 and 280 mg. On average, each drone produces about  $10 \times 10^6$  spermatozoa and about  $7.5 \times 10^6$  per microliter of sperm. Aim of this study is to assess how this climate change is affecting the reproductive potential of Italian *A. m. Ligustica*. To this purpose 10 drones bred in different areas of the Campania region, have been inspected, weighed and then used to collect semen. Body weight, concentration, motility and morphometry data of ejaculates of *A. m. Ligustica* drones of 2017 with those of 2019 have been compared, and different production over time and differences in the qualitative and quantitative parameters of the spermatozoa have been observed. The average weight of

the 2019 drones is of  $0.25 \pm 0.02$  g, lower than the optimal weights indicated in the literature. The average concentration of sperm/ $\mu$ l in 2018 was  $6.83 \pm 1.24$ , in May 2019 it was difficult to find drones ready for the mating flight, and in the first decade of June 2019 the average concentration recorded was  $2.58 \pm 1.00$  of sperm/ $\mu$ l.

**Keywords:** *A. m. Ligustica*, climate changes, fertility, honey bee, spermatozoa

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### **Training in health and hygiene for a higher level of the hunter**

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**Abstract:** The main reason for writing this work is to bring to the attention of the hunting and scientific world of the presence of specific regulations governing the handling of game and the safety of what is eaten. The Regulation "853/2004" of the European Parliament of 29 April 2004 "establishes specific rules on hygiene for food of animal origin", provides for the need to establish the figure of the trained hunter in order to certify the origin of the wild and the correctness of the first stages of processing that take place in the field in order to market game in the context of EC circuits. Trained hunter must be the figure that leads to the hunting world and therefore to all everyone who work in the hunting sector, the knowledge of basic hygiene and regulatory notions. It is the starting point of a journey that must be shared because the saying "what is cooked is all good" is no longer valid! The establishment of training courses in this subject must serve the hunter to become aware and acquire knowledge of the enormous heritage in terms of meat that is present in the territory, but only if managed correctly. In this work 186 tests carried out in 6 courses of "Format hunter" carried out in Tuscany were analysed; anonymous tests carried out before the course and were compared with the final exams were taken into consideration. This allowed to analyse the percentage of errors that were made before and after the course, especially with regard to the more difficult subjects to learn. We calculated the average of errors per hunter and percentage of wrong questions per topic. Evaluating the level of preparation before training helps to better understand the topics of the lessons.

**Keywords:** Hunter training, wild game meat, wild ungulates, Regulation (EC) No 853/2004, Italy

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### **The population of Mouflon (*Ovis aries musimon*) in Capraia Isle (Tuscan archipelago): structural and demographic characteristics**

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**Abstract:** In the last 40 years, due to hunting needs, new species of animals have been introduced on some islands of the Tuscan Archipelago as in other European countries. The island of Capraia is a territory partly managed by the Territorial hunting area n. 9 and partly from the Tuscan Archipelago National Park and from the mid-seventies the mouflon (*Ovis aries musimom*) spread there. The work focuses on the monitoring of this closed population through visual census sessions from 1999 to 2018. The density of the mouflon was ascertained with the method of direct observations from previously identified fixed points. The data show how, with a fluctuating trend, the mouflon population has

managed to colonize the entire island territory and find its balance, which has enabled the establishment of a Selection hunting district in 2008 for this ungulate (observing the data regarding the densities in the distribution area, we note how this increased, going from 4.9 animals / 100 Ha in 1999 to 17.8 / 100 Ha in 2007 and 2008). The ratio of small to female has been calculated over the years, the sex ratio remains, over the years, a value around the 1: 1 ratio. The ratio of young males and adult males remains constant over time, around values that are between 0.5 and 0.62.

**Keywords:** *Ovis aries musimon*, Mouflon, wildlife management, census, Tuscan archipelago, Italy

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## **Are hunting periods conditioning roe deer hunting bag? The experience of Tuscan Hunting District, Pisa, Italy**

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**Abstract:** Selective hunting has become common in Italy thanks to the numerical explosion of the roe deer (*Capreolus capreolus*) population, particularly in central-northern Italy. A fixed number of hunters is assigned annually to public hunting districts. Roe deer hunting is based on an assigned number of animals hunting subdivided for age and gender in four age classes: Fawn (0-1 year, Class 0) Male Yearling (1-2 years, Class I), Buck (> 2 years, Class II), Doe (F> 1 year, Class II). Culling season for buck, in Peninsular Italy, was until 2011 from August 1<sup>st</sup> to 30 September. Since summer 2012 it has been divided into two periods: from June 1<sup>st</sup> to July 15<sup>th</sup> and from August 15<sup>th</sup> to September 30<sup>th</sup>, before and after the mating phase. In this work we examine official game bag of buck in twelve hunting seasons in the districts of ATC Pisa 15 East in Tuscany, from 2007 to 2018. Age estimation to yearly age classes was performed basing on tooth eruption and wear characters in mandibles with temporary and permanent dentition. We comparing, for the first time in Italy, hunting times of yearling male and buck before and after the reproductive period of the species. In the reference period we evaluated annual size and composition (yearling or buck) of harvest of 2,239 males (26%) of 8,337 roe deer bags. Cull planning is determined before hunting season. The modification of hunting times from 2013, has increased the percentage of total males bag (+32%), but above all subtract bucks before the rut, preventing them from taking part in the reproduction. On average 76% of male of reproductive age are hunted between June 15 and July 15 when there are more visible. Hunting is generally a non-random process and when they can choose hunters take adult males. It is proposed to evaluate the opportunity to change for roe deer male hunting season after mating season.

**Keywords:** *Capreolus capreolus*, Roe deer, Selective hunt, Hunting period, Pisa, Italy

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## Seasonal movement patterns of a rock partridge population in the French Alps: implications for management

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**Abstract:** A radio-telemetry study of Rock partridge (*Alectoris graeca saxatilis*), a game bird whose IUCN status is Near-Threatened, was realized in the southern French Alps. Here we present the results from the analysis of the movements of 147 adult and 33 juvenile individuals tracked with VHF collar tags. Two main movement “strategies” coexist in the adult population studied: residency and migration. Residents were discriminated from migrants, on the basis of an overlap between spring-summer and autumn-winter ranges in residents *vs.* a spatial separation of both ranges distant more than 2 km apart in migrants. Most resident birds remain inside a home range whose diameter is below 2 km while some others, referred to as « excursionists », perform temporary long movements during spring outside their home range and return later on their usual home range. Migrant adults, monitored for at least one year, exhibited seasonal movements between breeding and autumn-winter grounds reaching up to 33 km. Some adults who moved more than 2 km during autumn but died during winter were considered as potential migrants. Among migrants and potential migrants, 38% of individuals exhibited short distance movements (2-5 km) and 62% long distance movements (between 5 and 66 km). Autumn movements (i.e. migrations from summer to winter) occurred from mid-September until the beginning of November and spring movements (i.e. migrations from winter to spring) occurred from the beginning of March to mid-April. Frequency in movements did not differ significantly between males and females (63 *vs.* 64% in spring, 62% *vs.* 45% in autumn). Timing, duration and distance of movements did not differ between the sexes. As a consequence of these various movement patterns, breeding grounds gather both migrant and resident birds who overwinter apart whereas winter grounds shelter birds who may breed apart. Directions of movements did not fit any particular pattern, which could be explained by the extent of mountain areas all around our study area. But interestingly, no "long distance" movement was observed towards the Northeast of our study area, across the Drac river, only 4 km wide, suggesting that this river would separate two demographic entities. Besides seasonal migrations, others types of individual long distance movements were observed and could be directly related to specific events such as: 1/heavy snowfalls during winter which may lead to the exploitation of two wintering sites 2/death of the partner or destruction of egg-laying in May-June which often starts excursionist movements to find a new mate and 3/ nesting failure or loss of the brood which may also cause some movements during summer. Movements of adult and juveniles during spring were similar in all respects. The detection of a partial migratory behaviour in a Rock partridge population is important because of its demographic, ecological or evolutionary consequences. At scales of more than 5 km, the movements of juveniles and adults play a role in redistributing birds within landscape units and affect the required size of protected areas. Therefore hunting and management policies should consider the spatial scale of a demographic entity which covers at least 1000 km<sup>2</sup>. Furthermore the great mobility and exploratory behaviour of some birds enable to compensate for high climatic vulnerability and allows to understand the high recolonisation potential of the species.

**Keywords:** movement, *Alectoris graeca saxatilis*, rock partridge, management

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## **CeRVEnE prevention systems and assistance plan to wildlife in case of fire disaster**

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**Abstract:** CeRVEnE (Regional Veterinary referral Center for non-epidemic emergencies) was established in 2017 in the Italian Region of Campania, to improving the timely management of veterinary non-epidemic emergencies in case of disaster. The “Fire Risk Assessment in Campania Region” (FRAC2018) aims at providing the Regional Government with a strategic tool able to gathering and supplying in short time all the necessary information to handle fire-related risks, and start safeguard actions and recovery of ecosystems, that involve wild and domestic biodiversity, based on the “Disaster Management Cycle” model principles. The present work intends to describe the conceptual framework and to introduce some preliminary results to be implemented to support the activities pursued by the FRAC Program. The main scope of the system are: (i) to monitor the wooded areas under risk of fire in the so-called “Vesuvius’ red zone”, and (ii) to determine the Optimal Evacuation Route for Animals (OPERA) in case of fire, for both domestic and wild animals. The necessary steps to accomplish the mentioned goals are: 1) the gathering of a set of specific information (e.g. Fuel parameters, wildlife population consistence, Digital Elevation Model Map, etc.); 2) an Evacuation Plan Model. The first step is requested to create a Fire Propagation Map, through which characteristics and dynamics of a fire episode can be analyzed and evaluated. The second step can be figured out by also adding data related to both the urbanization rate and the road system development surrounding Mount Vesuvius. To determine the OPERA such model has to be enriched by means of the mapping of the animal presence and the clear definition of type of vehicles requested to rescue the animal species involved in a fire episode. The development of the proposed integrated system becomes critical, as the fire risk has considerably increased in the last years in the whole Vesuvius’ surrounding area, which features a unique combination of both animal and anthropic elements within a very delicate natural ecosystem. The FRAC Program involved a big number of organizations that need to strictly interface with each other and, in case of fire, in a very short time. The implementation of a specific integrated system to support the FRAC Program is supposed to boost an improvement for CeRVEnE’s activities, pursuant the innovation perspectives coming with the effectual application of the Disaster Management principles.

**Keywords:** fire disaster, prevention scheme, animal assistance, burned wildlife, evacuation plan.

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## Foraging ecology of roe deer (*Capreolus capreolus* L.) in the Tuscan Apennines: diet composition and digestibility

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**Abstract:** The roe deer (*Capreolus capreolus* L.) is the most common deer in Italy. The aim of this study was to evaluate the botanical and chemical composition and the digestibility of the diet of a population of roe deer living in the province of Arezzo (Tuscany, Italy). A total of 45 samples, stratified by sex and age, were obtained from animals culled in 2010 and 2011 in three separate areas of the province, characterized by varying levels of anthropization and different ecosystems. Diet composition was assessed through micro-histological identification of plant species found in the rumen contents. Samples that could not be identified at species level were attributed to two macro-categories: dicotyledonous trees and shrubs, and dicotyledonous herbs. Ivlev's selectivity index was calculated for each study area. The digestibility and nutritional value of the diet were evaluated using acid-insoluble ash as an internal marker. Major diet components in the three study areas were trees and shrubs (57%), followed by herbaceous species (30%) and fruits and seeds (6.8%). The consumption of cultivated plants was only observed in Civitella, an area characterized by a high level of anthropization and agricultural areas (62%). Consumption of *Quercus* spp. was common in all study sites. A relatively high ingestion of Fabaceae was found in both Badia Tedalda (29%) and Stia (24%), both characterized by forest-covered areas (64 and 68%, respectively) and a low level of anthropization, but not in Civitella (0.4%). A high similarity index was found between the diets selected in Badia Tedalda and Stia. Ivlev's selectivity index generally confirmed a positive selection towards the species found in the diet. Chemical analysis of rumen and caecal contents showed no differences between sexes or age classes. Samples from Civitella showed higher levels of structural components and lower nitrogen levels ( $P < 0.05$ ). Digestibility coefficients were similar in all the study areas, in spite of the higher levels of structural components found in Civitella. In Civitella, where winter samples were available, a seasonal diet variation was detected, being graminaceous plants more abundant in winter and deciduous trees and shrubs in summer. In addition, a trend for a lower diet digestibility in summer was found for this area. In general, a remarkable adaptive capacity of this species to the available type of phytocenosis was observed. Data showed that, in spite of its traditional classification as a concentrate selector, roe deer can exhibit an opportunistic behaviour according to food availability.

*Research funded by Regione Toscana (Decree no. 1371 of 25/03/2010).*

**Keywords:** micro-histological identification, *Capreolus capreolus*, diet, feeding ecology, digestibility

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**Toxic and trace elements in body tissues of roe deer  
(*Capreolus capreolus* L.) in the Tuscan Apennines**

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**Abstract:** Anthropic activities are a source of environmental pollution by chemical substances, some of which may bioaccumulate in wild animals. The roe deer (*Capreolus capreolus* L.) represents the most common deer in Italy. This species has often been proposed as a valid bioindicator for environmental contamination. The aim of this study was to evaluate the relationship between anthropic activities and accumulation of environmental contaminants in body tissues of the roe deer population living in the province of Arezzo. A total of 80 samples were obtained from animals culled in 2010 and 2011 in three separate areas of the province. The three study areas were characterized by different ecosystems and varying degrees of human pressure: Civitella is distinguished by a significant agricultural and industrial presence as well as main traffic arteries, whereas there are low levels of anthropization and no industrial plants in both Stia and Badia Tedalda areas. Samples of diaphragm, liver, kidney, testis, bone and hair, stratified by sex, age and area of origin, were tested for chromium, nickel, copper, arsenic, selenium, cadmium, mercury and lead by inductively coupled plasma-mass spectrometry. The concentrations of all of the tested elements fell within the ranges indicated in literature. Sex did not influence the accumulation in any of the sampled tissues. Age affected the levels of cadmium in liver and kidney samples, with adult animals showing a higher ( $P < 0.01$ ) bioaccumulation in comparison to the younger ones. Except for mercury, the area characterized by more intense anthropic activities (Civitella) showed higher levels of most of the tested elements. The biological matrix was found to affect the level of bioaccumulation. There was a tendency for chromium, nickel, arsenic and lead to bioaccumulate in hair, while selenium and cadmium were more concentrated in kidney samples. Copper tended to bioaccumulate in liver, and mercury in both kidney and liver samples. These results confirm the possibility of using roe deer as a bioindicator for environmental contamination. Nevertheless, due to the individual variability observed in this study and the multiple factors influencing the bioaccumulation of the studied elements, further research based on a multi-disciplinary approach is needed to better assess contamination of the ecological niche of roe deer.

*Research funded by Regione Toscana (Decree no. 1371 of 25/03/2010).*

**Keywords:** *Capreolus capreolus*; bioindicator; environmental pollution; toxic elements; trace elements

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**Animals, seismic events and alert signs: literature and perceptions**

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**Abstract:** The potential capacity of animals to perceive seismic events in advance is commonly confined to the conjectures of the past. Yet there is a scientific bibliography that confirms, albeit only in part, these capabilities, and the CNR is conducting a specific research. This human dimension study investigated the issue with interviews in the crater of the 2016 Amatrice earthquake. 96.6% of those interviewed stated that they noticed non-normal behaviour to animals before the earthquake. This does

not attest that there is a proven correspondence between the two events. The study is of human dimension, therefore the datum quantifies the statistical position of the population with respect to the phenomenon investigated.

**Keywords:** earthquake, animals, Borbona, Amatrice, Rieti.

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## 1. INTRODUCTION


There is a bibliography dealing with the relationship between animals and seismic events, for which we refer to the specific paragraph, and the CNR is carrying out a specific research coordinated by Prof. Fedora Quattrocchi precisely on this topic. Based on the bibliography available on this topic I set the survey in the countries of Borbona, Amatrice and Montereale, areas affected by the earthquake of 24 August 2016 and 18 January 2017. The human dimension objective was to detect the relationships between animals and seismic events through interviews submitted to the local population. To this end, and in reference to the bibliographic indications, I have set up the data collection form. These interviews were administered to about 600 people living in the seismic crater and at different distances from the epicentre. This work aims to support the ongoing research of the CNR to which the results will merge.



Fig. 1. Amatrice earthquake 2016.

## 2. MATERIALS AND METHODS

The method used in conducting this study is the one described by Fidani *et al.* (2014) which refer to the earthquake in L'Aquila. The L'Aquila earthquake showed that the public is able to observe a large number of phenomena for which there were no instruments available at the time of the earthquake, including the unusual animal behaviour. The authors have verified that the Aquilans clearly remember their observations of previous earthquakes. On this basis they carried out a collection of data in relation to the Colfiorito earthquake that occurred before that of L'Aquila. Evidence of unusual behaviour of wild and domestic animals was collected in connection with the 1997 earthquake, testimonies collected years later. The questionnaire administered, after some time, ranged from the sighting of lights in the cosismic phase, up to the behaviour of animals in the presismic phase, describing places, times and conditions.



**UNIVERSITÀ  
DEGLI STUDI DELLA  
TUSCIA**

Raccolta dati per la stesura dell'elaborato finale di  
*Francesca Teofili (Borbona, Rieti)*

***Animali, eventi sismici e segni premonitori:  
letteratura e percezione***

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**Sulla capacità degli animali di avvertire in anticipo i fenomeni sismici.  
Una indagine di *human dimension* in Italia centrale.**

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Intervistatore: \_\_\_\_\_ Data: \_\_\_\_\_ (Referente prof. Settimio Adriani)

Intervistato:	Comune:	Età:
Titolo di studio:	Professione:	
Hai mai sentito dire che gli animali sono in grado di avvertire in anticipo gli eventi sismici?    SI    NO		
Se SI: secondo te è una cosa credibile:    SI    NO    NON SO    Secondo te ha basi scientifiche?    SI    NO    NON SO		
Sei a conoscenza di casi simili?    SI    NO    Si tratta di una esperienza:    diretta    raccontata da altri		
In quale anno si è verificato?		In quale località si è verificato?
In corrispondenza di quale evento sismico si è verificato?		
Quale specie animale ha riguardato?		
In che modo se ne è venuti a conoscenza?		
Puoi sintetizzare gli accadimenti?		

Fig. 2. Questionnaire administered.

### 3. RESULTS

The interviews administered at a variable distance from the epicentre, were 610, 510 were useful for the purposes of the study, 493 interviewees stated that they had noticed particular behavior by domestic and wild animals before the seismic events. These behaviours almost always manifested themselves with agitation of the animal before and after the tremors. The most sensitive animals were dogs, cats and sheep. Respondents therefore recognized the credibility and scientific basis of the study carried out.



Fig. 3. Epicenter.

### 4. DISCUSSION

Naturally the credibility of this study has been repeatedly questioned, as some subjects considered the anomalous behavior of the animals before the earthquake, random and not referring to what was about to happen.

### 5. CONCLUSIONS

The first objective set, that of administering the greatest possible number of interviews, has now been achieved, now all that remains is to provide for the transmission of the data collected to the CNR.

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## Update to 2018 of the checklist of SIC IT6020014: Mammals and Birds

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**Abstract:** Checklists of Sites of Community Interest (SIC IT6020014) were written in the early 2000s. In those years the cognitive documents and the management plans were realized, which since then have never been updated. The objective of this work is the revision of the faunistic checklist of the SIC after about fifteen years. The study concluded with the addition of three species of particular conservation interest and the cancellation of a species, which had been included even though it had never been present. Of the latter the scientific name was corrected, which had been wrongly written.

**Keywords:** Rascino, plateau, fauna, SIC, updating, management.

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### 1. INTRODUCTION

In the European Union, all the natural areas of particular value, reason being that they are characterized by the presence of habitats of Community interest or that host significant populations of animal and plant species of Community interest, constitute the "Natura 2000" ecological network. The

Natura 2000 network is provided for by the EU Habitats Directive: its activation and proper management are a legal obligation for all member countries. They are part of the "Natura 2000" network:

- the sites identified under the HABITAT DIRECTIVE, first called S.I.C. (Sites of Community Importance) and, once validated, Z.S.C. (Special Areas of Conservation);
- the Z.P.S. (Special Protection Areas), designated according to the BIRDS DIRECTIVE because they host significant populations of bird species of Community interest.

SICs and ZPSs have an obligation to provide themselves with technical tools aimed at safeguarding the important naturalistic elements that constitute them. These instruments are made up of two parts:

1. OVERVIEW - which describes the natural, environmental and socio-economic situation of the context under consideration.
2. MANAGEMENT PLAN - outlining eligible and ineligible actions in order to safeguard the environment.

## 2. MATERIALS AND METHODS

A phase in the implementation of the European project Natura 2000 Network has resulted in the drafting of management plans for Sites of Community Interest (SIC) and Special Protection Areas (SPA), a document that for the SIC IT 6020014 "Piana di Rascino" was drawn up between 2006 and 2007, and never updated. The Rascino Plain is located in the territory under the jurisdiction of the Municipality of Fiamignano, in the Rieti Province of Rieti, it is a Mediterranean biogeographical region. The Plain, located between 1160 and 1140 m altitude, boasts an extension of 244.8 ha. The pSIC cognitive framework drafting reports the following fauna community of the territory under study. SPECIES LISTED IN THE DIRECTIVE - MAMMALS: 1352 *Canis lupus*, 1354 *Ursus arctos*. BIRDS: A154 *Gallinago media*, A166 *Tringa glareola*, A338 *Lanius collurio*, A224 *Caprimulgus europaeus*, A412 *Alectoris graeca saxatilis*, A255 *Anthus campestris*, A246 *Lullula arborea*, A113 *Coturnix coturnix*, A280 *Monticola saxatilis*; Amphibians: 1175 *Salamandrina terdigitata*. OTHER RELEVANT SPECIES - FAUNA: *Martes martes*, *Coturnix coturnix conturbans*, *Emberiza cia*, *Emberiza citronella*. Considering that the faunistic communities have their own spatial/temporal dynamism, this work, partly bibliographical (publications of the faunistic observatory by the University of Tuscia, etc.) and partly experimental (deer census through bellowing; roe deer observation from fixed vantage points, etc.), is aimed at updating the checklist of the homeothermal faunistic community more than a decade after its first draft.

## 3. RESULTS

Bibliographic and field surveys have shown that the old checklist needs the following updates:

- 1) species to be added, because they have progressively colonized the investigated territory.

Deer (*Cervus elaphus*): the status of the species is constantly monitored with the bellowing technique (second half of September each year) with the scientific supervision of the DAPHNE of the University of Tuscia and the active participation of students of Mountain Sciences. In 2018, the presence in the vast area of the SIC is about 0.9 bellowing deer per 100 ha of investigated area (Adriani, 2018. Com. pers.). Therefore, it can be concluded that the species *Cervus elaphus* is to be included in the checklist of species belonging to the teriofauna present in the SIC.

Roe deer (*Capreolus capreolus*): In this case, monitoring takes place through observation from fixed vantage points, on sample areas (clearings) and occasionally through the drive counts census technique (forest). According to the latest survey data (2011-2014), it appears that in the vast area of the SIC, the roe deer is present with an estimated average density of 13.6 head/100 ha (Adriani, 2011, 2014).

Thanks to the presence of a distribution map of the species at the provincial level, it can be seen that in 2006 the roe deer was already present in the study area, but its presence in the overview has not been reported.

Griffon vulture (*Gyps fulvus*): Examination of the only scientific text found on the species shows that in 2013 the species regularly used the vast area of the SIC, although only as a feeding area (Bonanni *et al.*, 2013) and not for nesting, as the roosts were and are still concentrated exclusively in the Nature Reserve of Velino Sirente (Abruzzo), in areas adjacent to the site of reintroduction. The historical density of pastoral practices and wild grazing constitute the vast area of the SIC particularly suitable for the species, which being necrophagous finds in the territory abundant trophic resource. In addition to and in support of the data obtained from the consulted scientific work, a plan of direct investigation has been specifically activated, conducted using the technique of the interview given to a series of operators who for different reasons regularly attend the vast area of the SIC. Interviewed on the Griffon vulture sightings in the vast area of the SIC during the last three years, the following findings were obtained:

- a. 68% of the interviewees observed the species in the surveyed area at least once in the last three years;
- b. among these, 70.6% observed the species every year.

Therefore, it can be concluded that the species *Gyps fulvus* is to be included in the checklist of species belonging to the ornithological fauna that regularly uses the SIC territory.

2) species to be deleted from the list, because they have never used the inspected territory.

Rock Partridge (*Alectoris graeca orlandoi*): The texts examine shows that the study of the status of the Partridge, of the subspecies *Alectoris graeca orlandoi* (now classified as *A.g. graeca*), in the province of Rieti has been ongoing since 2004 (Amici *et al.*, 2004; Serrani *et al.*, 2005; Amici *et al.*, 2006). Since 2004, when the Environmental Assessment Model for the Partridge in the province of Rieti was developed (Amici *et al.*, 2004), it has been well known that the species was not present in the Rascino Plain SIC area at all, found in the presence sites always above 1500 m a.s.l., higher in the summer/reproduction areas and lower in the wintering areas, but in no case at 1150 m a.s.l., SIC altitude IT6020014. This situation is confirmed by all the studies conducted in the province of Rieti and by the ongoing monitoring, with the scientific supervision of the DAFNE of the University of Tuscia and the active participation of students of Mountain Sciences. Another important point to make is that in the pSIC management plan the species indicated as present is the *Alectoris graeca saxatilis*, and not the *Alectoris graeca orlandoi*. This being not a mistake of lesser significance if we consider that the first of the two subspecies is described as endemic to the Alpine arc. Ultimately, in the vast area of the SIC, the subspecies *Alectoris graeca orlandoi* is found only in the localities called Nuria, La Serra and Colle Alto, and absolutely not in Rascino. Therefore, it is safe to assume that the *Alectoris graeca orlandoi* species (and not *Alectoris graeca saxatilis*) is to be deleted from the checklist of species belonging to the hornitic fauna that uses the SCI territory.

#### 4. DISCUSSION AND CONCLUSIONS

In light of the above, it is proposed to make the following changes to the wildlife checklists contained in the Management Plan of the SIC Rascino Plain (IT6020014):

Avifauna checklist "Other relevant species":

1. insert the Griffon vulture (*Gyps fulvus*),
2. remove the Partridge (*Alectoris graeca orlandoi*).

Teriofauna Checklist "Other relevant species":



1. insert the Deer (*Cervus elaphus*),
2. insert the roe deer (*Capreolus capreolus*).

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### **Damage from wildlife in the province of Rieti (Italy). Which is the most responsible: the wild boar or the wolf?**

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**Abstract:** This study was conducted in the province of Rieti (Italy) and focused on a comparison between damages caused to farmers (crops and livestock activities) ascribed to two major wild species: wild boar and wolf. Specifically, the annual amount of damages (Euro / km<sup>2</sup> / year) ascribed to wild boar to agricultural crops was compared with damages to livestock ascribed to wolf. The lack of access to databases implied an investigation limited to two geographical areas only: the Regional Reserve named "Laghi Lungo e Ripasottile", where intensive agriculture is spread and damage from wild boar are regularly monitored; and the Cicolano, sub-region of the province of Rieti where sheep farming is still frequent and damages from wolves are historically recorded. During the period 2011-2013 the average annual density of damage in the study areas from wild boar and wolf species, was reported with a damage ratio of 11.29 (wild boar vs wolf). Should the latter figure be confirmed at national level too, then it might be difficult to understand the different management approach for the two species. Regarding the wolf, indeed, due to the allegedly excessive pressure of predation on livestock, it is now on the way to be approved an exception to the strict protection rules. Concerning wild boar, however, despite its harmfulness would seem to be largely superior (with respect to wolf), the numerical control was not introduced so far, as a compulsory activity to be carried out in synergy to hunting pressure that ordinarily fails in maintaining the species' population under control.

**Keywords:** Wolf, Wild Boar, damage, predation, Rieti

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#### 1. INTRODUCTION

Wildlife's damages to livestock and agricultural systems are at current an important issue in Italy, where two species create the majority of damages: the wolf and the wild boar. The wolf (*Canis lupus*) is a protected species, according to the EU Directive Habitats 92/43/EEC and national rules (Law

157/1992). Due to the damages that the wolf causes to agricultural activities, there is a procedure underway to allow selective hunting derogations to the rules of protection of that species (Boitani & Salvatori 2017). This created a raising debate between those who are in favour of such solution and those who strongly dislike or even show in public protesting against it. Environmental associations and public opinion have entered the discussion on that issue too, sometimes taking positions not grounded on technical and scientific data, but often driven by preconceived opinions. In Italy, the wild boar (*Sus scrofa*) is a hunting species (Law 157/1992); it has been showing an increasing abundance in recent years, which greatly affects agricultural systems. Despite the considerable hunting pressure on the species (about 600,000 units / year according to Boitani & Salvatori 2017), the reproductive capacity and the low natural mortality have determined constant replenishment, of the population, which is continuously increasing on the whole national territory. Boitani & Salvatori (2017) estimated that, at current, there are approx one million heads of wild boar in Italy. Such an amount represents a consistent source of risk affecting both farms and agricultural systems and environmental aspects such zoocoenosis of conservation interest (e.g., birds nesting on the ground). In the study the average annual density of damage (Euro / km<sup>2</sup> / year) of the two species are compared to better understand the extent of damages to agriculture created by wolf and wild boar. Unfortunately, the availability of data on damaged crops by wild boar and by wolf on livestock, is difficult to find with respect to a reliable time series. From 2010, indeed, the Local Hunting Associations (ATC) have been entrusted with the preliminary activity for evaluating the damages by wildlife occurred in non-protected areas to agricultural activities.

## 2. MATHERIAL AND METHOD

The analysis has focused on two areas in the Lazio region (Central Italy) in the territory of Rieti, where intensive agricultural areas are substantially separated from those in which the free-range animal husbandry is still abundant. Therefore, two different areas were found to study the damages to crops caused by wild boar and the loss of livestock caused by wolf.



Fig. 1. Wild boar damage to corn. (L. Cavagnuolo)



Fig. 2. Wolf predation on lambs. (S. Adriani)

Two distinct areas of study were chosen:

1. Reserve of Lakes Lungo and Ripasottile (~ 33 km<sup>2</sup>) for the damages to agricultural crops caused by wild boar;
2. The sub-region of the province of Rieti called Cicolano (including the municipalities of Borgorose, Concerviano, Fiamignano, Marcellino, Pescorocchiano, Petrella Salto and Varco Sabino. ~ 530 km<sup>2</sup>) for the damages to livestock caused by wolf.

The investigation covers the period 2011-2013. The economic assessment of the damages refers to the compensations paid to farmers and livestock breeders damaged.

### 3. RESULTS

With reference to the three-year period under consideration in the two areas of study, the results are the following:

1. Reserve of Lakes Lungo and Ripasottile: 53 damage events ascribed to wild boar were reported for a total of 29,568.00 Euro (annual average = 9,856.00; SD = 3,464.10) with an average damage of 557.89 Euro / event, and a density of annual damage of 300.67 Euro km<sup>-2</sup> year<sup>-1</sup>.
2. Cicolano area: 35 predation events by wolves (distributed depending on livestock species: 99 sheep, 21 deer, 13 horses, 3 cows), for a total of 42,349.00 Euro (average per event = 1,209.97 Euro event<sup>-1</sup>, S.D. = 2,366.63). The average annual density of the damage was 26.64 Euro km<sup>-2</sup> year<sup>-1</sup>.

The ratio between the average annual density of damage by wild boar and by wolf is then equal to 11.29 showing a total amount of damages caused by wild boar being more than ten times the damage ascribed to wolf. This occurred even if in the Reserve of the Lakes a prevention project was active against damages by wild boar during the early part of the period examined.

### 4. DISCUSSION

The damage ratio between the two species shows a stronger importance of the wild boar with respect to the damages caused to human activities in rural areas, in the area investigated. If the ratio between wild boar /wolf damages is confirmed at national level, then it would not make sense to still differentiate the management strategies at current adopted in Italy with respect to the two species. Regarding the wolf, indeed, due to the excessive predation pressure on livestock, at present it is on the way to be approved an exception to the strict protection rules (i.e. it would be allowed wolf withdrawal

in containment programs). Wild boar, on the contrary, despite being the most important cause of damages than the wolf (with respect the average damage per km<sup>2</sup>), both to agriculture and to environment (Amici *et al.* 2013a), at current, the hunting period is not deemed to be extended nor numerical control initiatives in synergy with hunting seem to be under discussion. Extension of hunting season, however, would be immediately feasible, thanks to the competent management bodies (e.g. ATC) that recently acquired the authority on this matter (e.g. in the Lazio Region see the Regional Executive Decision 847/2016) and to the specific knowledge (Garbuio & Friends 2013, Adriani 2014) and management instruments (Amici *et al.* 2013b) in their possession.

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## First data on the presence of the Wolf (*Canis lupus*) in the area of Mount Tancia (Rieti) Roncarà T.\*

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**Abstract:** After verging on extinction in the 1970s, in Italy, the Wolf (*Canis lupus*) has recorded an important numerical increase. It is currently present in most of the Apennine territory, preys on wild animals and interferes with livestock breeding. This jeopardizes the conservation process that has been undertaken at the national level. To start a correct management of the species it is necessary to know the total number of subjects living in the national territory. To achieve this result it is necessary to monitor a series of areas distributed in the area of presence with a standardized methodology. This survey provides for the first time the distribution data of the presence of the species in the vast area of Mount Tancia, in the province of Rieti.

**Keywords:** Wolf, *Canis lupus*, Wolf howling, Rieti, Tancia.

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## 1. INTRODUCTION

The presence of the wolf in Italy is to be considered historical and continuous, except for Sardinia where the species has always been absent (Cagnolaro *et al.*, 1974). As for the Apennine situation, the presence is to be considered stable until the middle of the last century; in the decades 1950-1970 the minimum historical density of the species in the Italian area was reached (Cagnolaro *et al.*, 1974). At the beginning of the 70s there were few separate nucleus along the Apennine ridge (Zimen & Boitani, 1975). In that period, the species was almost at risk of extinction. The main causes of the decline included: the road accidents, the never solved conflict with zootechnics that exasperated poaching against the species, the reduction of habitats and trophic resources, the conflict with the hunting

activities and genetic pollution due to hybridization with wild dogs (Genovesi & Duprè, 1999). Thanks to: i) increased awareness, favored by the work of some environmental associations, such as the WWF, that since the 1970s dealt with the difficult conflict between the carnivore and the breeders, ii) the rules of protection of the species and, iii) the numerous legal and illegal reintroductions of wild ungulates along the entire peninsula (Apollonio, 2004) a slow recolonization of the original area began.

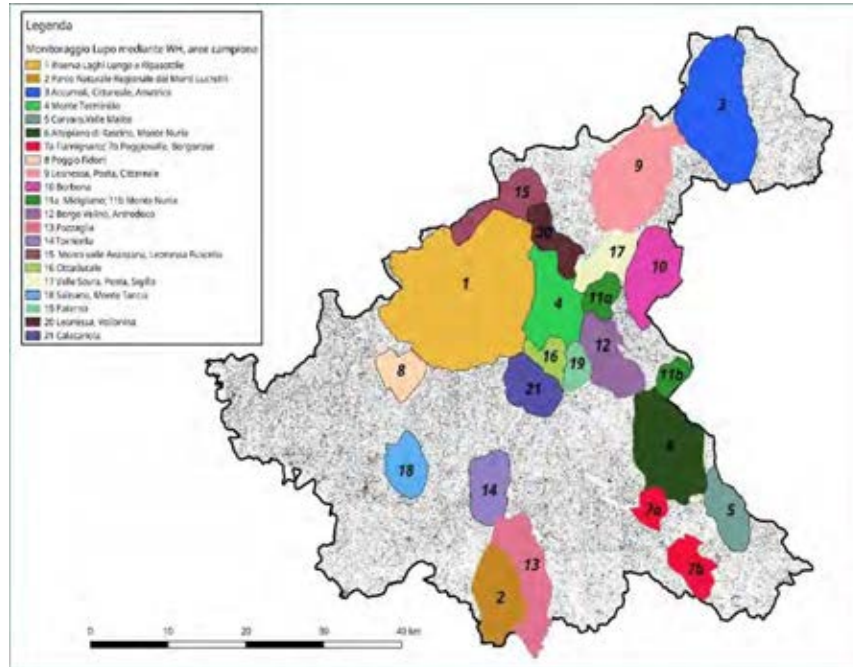


Fig. 1. Sub-areas of study.

In the province of Rieti four years ago the monitoring process started, supported by the Lazio Region, through the Riserva dei Laghi Lungo and Ripasottile and the DAFNE of the University of Tuscia, through professor Settimio Adriani and of professor Andrea Amici. It consists of 21 subareas. I was assigned the number 18 that falls in seven municipalities of the province of Rieti: Salisano, Monte San Giovanni, Roccantica, Poggio Catino, Montopoli di Sabina, Poggio Mirteto and Rieti with an extension of about 3,200 ha and an altitude that varies between 300 and 1280m, with a height variation of about 1000m.

## 2. MATERIALS AND METHODS

Normally, wolves respond to howls emitted by other individuals of the same species. On the base of this natural tendency, we used the Wolf-howling technique that is based on the reproduction of the howling through original amplified recording or human imitations (Nowak *et al.*, 2007), to ascertain, localize and estimate the composition of the wolves packs. This technique is widely used for territories of dimensions similar to those of the macro areas in which the territory of the province has been divided. In Italy, it was then applied to detect the presence and to estimate local density of the wolf. It was also used to perform large-scale monitoring programs and to locate and to monitor breeding sites (Mattioli *et al.*, 2014).

To implement this technique an appropriate equipment is necessary:

- Amplifier with max power of 25 W,
- 300-13000Hz emission frequency horn,
- MP3 player containing the track of the pre-recorded howl,
- Manual anemometer that measures the intensity and direction of the wind,

- Compass, essential for locating howls and rendez-vous,
- Stopwatch and GPS.

The Wolf-Howling technique involves a series of standard steps:

arrival on the spot by suitable vehicles, turning off of them and silent preparation to start the emission activity. From each previously established spot a series of 3 howls are emitted at two-minute intervals. Those emissions are followed by a 10-minute listening phase. The sessions are performed during the twilight hours or at night and in the absence of wind. The frequency of responses is highest in the mating season (February-March) and progressively decreases until the time of the deliveries (late spring-early summer) to grow again in late autumn. In addition, there are two Wolf howling sessions a year: one at the end of July for monitoring rendez-vous and wolf pups, and one at the end of November (Harrington & Mech, 1982).

The emission points have been chosen along roads / forest tracks that can be travelled by off-road vehicles to make the operation faster and also based on the morphology of the territory. In the investigated area 5 emission points were chosen, one of which falls within the SIC (Site of Community Interest) IT606020017 Tancia and Monte Pizzuto, for an extension of approximately 6821 ha.

### 3. RESULTS

In the following table, presence data collected with the wolf-howling technique were reported. In the reproductive period, in the two years of study, the presence of the species has always been positive; allowing to detect a pack. Thanks to this technique the identified group was always monitored in the same area, corresponding to the territory of the municipality of Monte San Giovanni and within the SIC. The identified area corresponds to the rendez-vous site for the pack, it falls on a dense herbaceous area, bordering on a deciduous forest.

Table 1. Results of Wolf Howling's activities.

Date	N° isolated individuals	N° packs
July 2017	0	1
July 2017	0	1
Autumn 2017	0	0
Autumn 2017	0	0
July 2018	0	1
July 2018	0	1
November 2018	0	0
November 2018	1 adult	0

Our results demonstrate the presence of a wolf pack in this area for the first time.

### 4. DISCUSSION

In order to know the dynamics of the pack and its relationship with zoocenosis, zootechnics and hunting, it would be appropriate to continue the research in the framework of the system adopted in this study.

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## Distribution of the red deer (*Cervus elaphus*) in Rieti Province: Update 2019

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**Abstract:** In the last years of the past century the red deer began the colonization of the Province of Rieti. The present population, which is in a clear phase of consolidation and expansion of the area of occupancy, derives from two distinct reintroductions which took place successfully in Abruzzo, in Velino Sirente Natural Reserve, in the Gran Sasso National Park and Monti della Laga. This work is aimed at updating the area of distribution of the existing species in the Province of Rieti. As for the Cicolano, the area of presence, which was limited to the right bank of the Salto River, is rapidly expanding on the orographic left of the same river and in the adjacent Turano Valley.

**Keywords:** Red deer, *Cervus elaphus*, Rieti, updating, distribution.

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### 1. INTRODUCTION

The noble deer is an artiodactyl mammal that belongs to the Cervidae family. In Italy this subspecies progressively reduced until it almost disappeared completely, remaining only in a small area in the province of Ferrara, the Mesola wood. Subsequently it was reintroduced and spontaneously extended to colonize a vast area. The presence of the red deer (*Cervus elaphus*) in the Province of Rieti dates back to the last decades of the 20<sup>th</sup> century. The present population derives from two distinct reintroductions, one in Abruzzo, in the Velino Sirente Natural Reserve and the other of minor importance, in Gran Sasso National Park and Monti della Laga. Within a few years, thanks to a rapid adaptation and the high environmental suitability of species-specific, these reintroduced groups quickly consolidated in the release area and subsequently began to colonize adjacent suitable areas spontaneously. The group released in Abruzzo has colonized the vast area of the Natural Reserve of the Duchessa Mountains (Rieti), where it has long been monitored by the Department of Agricultural and Forestry Sciences (DAFNE) of the University of Tuscia, internally and externally to the protected area. A specific survey showed that in some particularly suitable areas of the wintering areas of Lazio (in the Municipality of Borgorose, Rieti) (Adriani *et al.*, 2007, 2014), especially when the snow cover was high and lasting at high altitude, for long periods the density reached values of 25 head / 100ha (Adriani *et al.*, 2010). Given the high biogeographical and conservation value, this new presence on the one hand has enhanced the naturalistic level of the colonized areas (Amici *et al.*, 2007), on the other hand has produced a strong impact on crops and forest cover. Furthermore, the presence in the territory of numerous areas of wild boar hunting (*Sus scrofa*) conducted with the drive hunting

technique, has given rise to an intense poaching action (Bonanni *et al.*, 2015). The species has also gradually colonized the adjacent Valle del Salto, exclusively in the areas on the right bank of the river, and the areas of the plateau of Rascino and Monte Nuria, where it has created reproductive nuclei independent of the original one (Adriani & Giordani, 2009). The colonization continued North-West, on Mount Terminillo, up to the hills above the built-up area of Rivodutri, where the presence is still sporadic. The nucleus released in the Gran Sasso has consolidated with greater difficulty and has undertaken a slow process of colonization towards the South, which in the area identifiable with the common of Borbona (Rieti) is hindered by the presence of a consolidated nucleus of deer (*Dama dama*). This study aims to provide recent and unpublished information on the presence of the red deer.



Figure 1. Riserva Naturale Montagne della Duchessa. (Photo Valentina Fasciolo)

## 2. MATERIALS AND METHODS

The studies conducted (observation from fixed points of advantage, night counting of roaring males, Faecal Pellet Group Count, behavioral damages, etc.) (Adriani *et. al*, 2009; Fasciolo *et al.*, 2007, 2009) have highlighted a rapid increase in density. The ongoing investigations, conducted as part of the Wildlife Resource Management course, with the support of the Mountain Science students (Rieti), have allowed us to gather a considerable number of biological signs of presence (stages, excrements, hairs) and behavioral (debarking), even in areas outside the area of known presence. The data was initially transcribed into a form that contained all the necessary information and subsequently entered into a database.



Figure 2. Faecal Pellet Group Count (Photo Settimio Adriani)



### 3. RESULTS

This study aims to provide recent and unpublished information on the presence of the red deer.

This originally sporadic presence is rapidly consolidating, even if there are still no signs of reproductive events. The presence of the species is still sporadic in the reliefs above the urbanized area of Rivodutri, where the situation does not yet seem to have changed since the early 2000s, when the species was first reported.

### 4. DISCUSSION AND CONCLUSIONS

The interaction at the Red Deer, the Fallow Deer and the Roe Deer are factors that affect the correct evolution of the fauna community. Therefore, the monitoring must simultaneously be aimed at all three species. In this way it will be possible to correctly evaluate the specific dynamics in progress.

The hunting wildlife management institutes operating in the province of Rieti must necessarily pay more attention to the monitoring of the deer, because in a few years the impact of the specie on the agricultural crops will certainly be very intense. Contrary to what they should do, at the moment the Institutes themselves completely ignore the species, because despite being hutable (L. 157/1992) it is excluded from hunting.

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## The Wild Boar (*Sus scrofa*) in the province of Rieti (Italy): perception of presence and problems

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**Abstract:** The Wild Boar (*Sus scrofa*) populations present in Italy are the result of crossing with individuals of Central European origin introduced during the 1950s or naturally spreading from neighbouring countries with hunting purposes. These subjects have crossed with the autochthonous ones and not infrequently with the domestic pig giving rise to the form presently present in almost all of the national territory. The biological, ecological and ethological characteristics make the species particularly impactful with agroecosystems and human activities. Considering that the perception of current problems does not always correspond to the problem entity, this human dimension survey was conducted through interviews with a random sample of citizens of the province of Rieti, to understand, independently by the scientific reality, the perception on the presence of the species and on the problems that it generates in the territory.

**Keywords:** Wild Boar, *Sus scrofa*, hunting, perception, Rieti.

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### 1. INTRODUCTION

The wild boar (*Sus scrofa*) became extinct in almost all the national territory in the first half of the XVII century, in correspondence with the maximum population of the municipalities of the hilly and mountain rural area (Marsan, 2000). From the beginning of the fifties in the last century the species has again colonized most of the original area, also due to the release of subjects of Central European stock carried out for hunting purposes (Tosi & Toso, 1992). The first reports of presence in the province of Rieti date back to the 1960s, the first harvesting in the early 1970s (Adriani S., 2003). The biological and ecological characteristics have allowed the species to expand its range over the entire national territory. The substantial absence of limiting factors and the high reproductive potential have made possible to increase by 400% in the last 15 years. Despite the intense annual hunting, which is constantly 60% of the total, at present the presence of the species at national level is estimated at least 1 million head (Boitani & Salvatori, 2019). The deriving high density is one of the main causes of the enormous damage that is increasingly recorded in agricultural crops. The only natural predator of the Boar in Italy is the Wolf (*Canis lupus*), which is currently estimated to be worth around 1600 head (min. 1070, max. 2472) (Boitani & Salvatori, 2019). The numerical ratio of wolves to wild boars is about 1/500, therefore the action of the predator is not sufficient to effectively contain the ungulate. All this considered, the only real possibility of containment of the species, and of its impact on agriculture (for the province of Rieti: Adriani V. *et al.*, 2017), is represented by the calibrated planning and the correct management of withdrawals. These are activities which the current legislation assigns to the Local Hunters Association (ATC, public management institutes) and to the Private Hunting Farms (AFV, private management institutes). The same institutions are also entrusted with the collection and management of data on slaughtering and the damage that the species causes to agricultural crops. These data, which can help in optimizing picking and planning, are not completely transparent, and the only ones currently available are made accessible by protected areas. An example of transparency and effectiveness in data management in the Province of Rieti is represented by the Lungo and Ripasottile Lakes Natural Reserve, in which between 2001 and 2015 there were 533 boar damage events (average 35.53, SD±19.26) with an average damage of over 900 Euro/event (SD±837.17) (Adriani S., 2017).

The great managerial deficiencies of the species in the ATCs of the province of Rieti are evidenced by the intervention of the Prefecture of Rieti, which with a note on the subject «Problems connected with the presence of wild boar» (Prot. 19431 of 01.13.2018), convened all the stakeholders at a technical table, with the following reason: «[...] noted a conspicuous presence of ungulates, which causes a serious danger for road traffic as well as damage to agricultural areas and the environment» and the following objectives: «[...] to define, in a shared manner with all the interested subjects, suitable operational strategies that can put an end to the emergency in progress». With the note Prot. 1214 of 24.01.2019 the Prefect of Rieti also informed the ATC RI1 and ATC RI2 of the possibility of collaborating with the University of Tuscia of Viterbo (Department DAFNE, Management of faunal resources) in the drafting of «Control plans» specific species. Indications to which ATC have never followed. This work, of human dimension, was conducted through interviews administered to a sample of the resident population in the province of Rieti to understand what was the perception of the problems in progress.

## 2. MATERIALS AND METHODS

The interviews were conducted using appropriate forms containing a predefined set of 10 questions divided into two themes: the perception of the presence of the Boar, the problems connected to it and the effectiveness of the management in place. The interviews were collected by 45 operators in 38 municipalities in the province of Rieti. The administrators (students, professors and operators of the Agricultural Technical Institute of Rieti; students and graduates in the degree course of *Mountain Science*; guards of the reserves Laghi Lungo and Ripasottile, Monti Lucretili, Navegna and Cervia; officials of the Lazio Region; graduates in Free Forest Sciences professionals operating in the provincial territory) have been previously trained. To focus on any problems that might have occurred, they performed some simulations before starting the data collection campaign. To encourage complete and unconditional responses, the respondents were guaranteed the privacy and publication of the data collected exclusively in aggregate form and with exclusive statistical purposes. The data collected in the interviews were implemented in a spreadsheet. The processed data derive from a random sample of 932 citizens resident in the province of Rieti (average age 47.00, SD±16.1), divided into two components: A) hunters: 391 (average age 50.84, SD ± 16.96), B) non-hunters: 541 (average age 44.25, SD±14.82).

## 3. RESULTS

The perception of the issues examined is summarized in the following results:

- a) What are the origins of the Wild Boar that currently populate Italy?
  - 1) Natural 49.10%, Artificial 36.32%, Don't say 14.58%.
  - 2) Natural 52.50%, Artificial 41.22%, Don't say 6.28%.
- b) How do you consider the current numerical consistency of the Boar?
  - 1) Low 23.53%, Adequate 38.87%, Excessive 33.50%, Don't say 4.09%.
  - 2) Low 11.83%, Adequate 31.42%, Excessive 51.94%, Don't say 4.81%.
- c) Is the Boar a resource or a disadvantage?
  - 1) Resource 39.13%, Disadvantage 46.80%, Don't say 14.07%.
  - 2) Resource 31.79%, Disadvantage 45.66%, Don't say 22.55%.
- d) At national level, is the Wild Boar a problem?
  - 1) Yes 61.64%, No 28.64%, Don't say 9.72%.
  - 2) Yes 70.24%, No 18.85%, Don't say 10.91%.

e) In the last five years have you suffered damage from the Wild Boar?

1) Yes 42.97%, No 57.03%.

2) Yes 33.27%, No 64.51%, Other 2.22%.



Fig. 1. Left: hybrid Wild Boar (Photo S. Adriani). Right: Photo E. Morelli.

#### 4. DISCUSSION

Regardless of the group to which they belong, from the sample examined, it emerges the prevalent idea that the origin of the Boar present today in the vast majority of the national territory is natural, contrasting perception with the results of scientific investigations (Amici & Serrani, 2004). The perception of the current status of the species is quite varied, and clearly contrasts with the most recent and accredited estimates (Boitani & Salvatori, 2019). Most of the members of both investigated categories (hunters and non-hunters) consider the presence of the Boar to be a handicap, and believe that at national level the species is a problem. Despite this, most of the respondents in the last five years have not suffered direct damage from the species.

#### 5. CONCLUSIONS

If misinformation on faunal issues can be acceptable to non-hunters, it is absolutely serious and unacceptable for ATC and AFV members. These institutions, in fact, have among their institutional objectives the formation and information of the associates. The ATCs, in particular, for this purpose have, as per the by-laws, "Technical Directors with proven expertise in the faunal field", paid with the annual dues of the same members. Despite this, the fundamental formative action is not exercised.

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## **The Wild Boar (*Sus scrofa*) in the province of Rieti (Italy): is the management perceived as effective?**

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**Abstract:** the Wild Boar (*Sus scrofa*) is a ubiquitous species that in the last fifteen years has increased by 400% and has colonized almost all of the national territory. According to the latest estimates, the current size is at least 1 million individuals. The gregarious life, the high density, the strong *eurifagia* and the extreme mobility make the species particularly impacting on the agroecosystems and the anthropic activities. All this makes absolutely necessary an adequate management of the populations, which the current legislation attributes to the Local Hunters Association (public management of the species) and to the Hunting Wildlife Companies (private management of the species). This study of human dimension, based on interviews with a random sample of the population, tends to verify what is the perception on the quantitative aspects of the presence of the Wild Boar in the territory, on the effectiveness of the species management plans and on the skills of those designated to management.

**Keywords:** Wild Boar, *Sus scrofa*, perception, management, Rieti.

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### 1. INTRODUCTION

According to the most accredited bibliographic sources, the Wild Boar (*Sus scrofa*) has disappeared from most of the national territory in the first half of the seventeenth century, coinciding with the maximum population of the municipalities of the hilly and mountain rural area (Marsan, 2000). Following some releases with hunting purposes of subjects of Central European origin, in the 1950s the species recorded a vast recolonization of the national territory (Tosi & Toso, 1992).

At a provincial level, the first reports and the first harvest date back to the period between the 1960s and the early 1970s (Adriani S., 2003). The extreme adaptability and the ubiquitous nature have allowed the species to colonize substantially all the national territory. It is estimated that in the last 15 years its numerical increase has been 400%. The annual hunting is constantly around 60%. Nevertheless, at national level the species is currently estimated to be at least 1 million head (Boitani & Salvatori, 2019). In Italy the only natural predator of the Wild Boar is the Wolf (*Canis lupus*), which is currently estimated at around 1600 heads (min. 1070, max. 2472) (Boitani & Salvatori, 2019). Therefore, the Wolf/Wild Boar ratio is about 1/500. All this considered, the only real possibility of containment of the species, and of the damage it produces to agriculture (for the province of Rieti: Adriani V. *et al.*, 2017), is represented by a technically adequate management. In Italy the wildlife management is entrusted to the Local Hunters Association (ATC, public management Institutes) and to the Hunting Faunistic Companies (AFV, private management institutes). The data on slaughter and damage that these two institutions acquire are not absolutely transparent, the only ones available are provided by protected areas. On this regard, an important reference for the Province of Rieti is

represented by the Natural Reserve of the Lungo and Ripasottile Lakes, which extends for about 3000 ha. In this territory between 2001 and 2015 there were 533 damage from Wild Boar (average 35.53,  $SD\pm 19.26$ ), with an average damage of over 900 Euro/event ( $SD\pm 837.17$ ) (Adriani S., 2017). The gravity of the situation at provincial level is evidenced by the intervention of the Prefecture of Rieti. With a note on the subject «Problems connected with the presence of Wild Boar» (Prot. 19431 of 23.1.2018), the Prefect has called all the stakeholders to a technical table, with the following motivation: «[...] detected a conspicuous presence of ungulates, which determines a serious danger for road traffic as well as damage to agricultural areas and the environment» and the following objectives: «[...] define, in a shared manner with all interested subjects, suitable operational strategies that can put an end to the emergency in progress». With the note Prot. 1214 of 24.01.2019 the Prefect of Rieti also informed the ATC R11 and ATC R12 of the possibility of collaborating with the University of Tuscia of Viterbo (Department DAFNE, Management of faunal resources) in the drafting of «Control plans» specific species, in relation to the skills and long experience that the Department has gained. Information that has never been actively followed up. A specific human dimension survey was launched on this situation, conducted through interviews and tending to assess the perception that there is among the population residing in the province of Rieti on issues related to the presence of the Wild Boar.

## 2. MATERIALS AND METHODS

To make the collected data comparable, the interviews were conducted using a specific form. In addition to the personal data and the profession of the interviewee, the form contained a predefined set of 10 questions divided into two themes, both relating to the territory of the interviewee's residence: A) the perception of the presence of the Wild Boar; B) the problems connected to it and the effectiveness of the management in place. The interviews were collected by 45 operators in 38 municipalities in the province of Rieti. The givers (students, professors and operators of the Agricultural Technical Institute of Rieti; students and graduates in the degree course of Mountain Sciences; guards of the Laghi Lungo and Ripasottile reserves, Monti Lucretili, Navegna and Cervia; officials of the Lazio Region; graduates in the degree course of Forest Sciences, freelancers operating in the province of Rieti) have been previously trained. Before starting the collection campaign they performed some simulations, aimed at focusing on any problems that might have occurred. The interview forms were nominative. Respondents were guaranteed the privacy and publication of data collected exclusively in aggregate form and for the only statistics purpose. This condition has favored complete and unconditional answers. The data recorded in the paper forms have been implemented in a spreadsheet. The results of this work derive from a random sample of 932 citizens residing in the province of Rieti (average age 47.00,  $SD\pm 16.1$ ). In the analytical phase the sample was divided into two components: 1) hunters: 391 (average age 50.84,  $SD\pm 16.96$ ), 2) non-hunters: 541 (average age 44.25,  $SD\pm 14.82$ ).

## 3. RESULTS

The perception of the studied problems is schematized with the following results:

a) Is the Wild Boar managed in your territory?

1) Yes 26.08%, No 56.27%, Don't say 17.65%.

2) Yes 20.33%, No 55.08%, Don't say 24.58%.

b) Are the training and skills of the Wild Boar managers adequate?

1) Yes 16.62%, No 47.57%, Don't say 35.81%.

2) Yes 13.68%, No 41.77%, Don't say 44.55%.

c) Is hunting sufficient to contain the Wild Boar?

1) Yes 26.08%, No 59.59%, Don't say 14.32%.

2) Yes 26.80%, No 54.53%, Don't say 18.67%.

d) In addition to agriculture, does the Wild Boar create damage to?

1) Viabilities 68.03%, Other animals 6.65%, Other/Don't say 25.32%.

2) Viabilities 77.63%, Other animals 3.33%, Other/Don't say 19.04%.

e) What do you propose to improve the management of the Wild Boar?

1) Selective withdrawal/Numerical control 20.72%, Hunting season extension 21.23%, Other 26.60%, Don't say 31.46%.

2) Selective withdrawal / Numerical control 16.64%, Hunting season extension 7.02%, Other 40.48%, Don't say 36.86%.

#### 4. DISCUSSION

The complexity of the results emerged in this survey would deserve a wider space of analysis and discussion. An absolutely significant percentage of the entire sample (hunters and non-hunters) considers the training and skills of the technicians responsible for the management of the Wild Boar to be inadequate. This is not a new indication for the province of Rieti (Adriani *et al.*, 2015). It is equally clear the perception that ordinary hunting is perceived as insufficient to numerically contain the suide; while the proposed solutions to achieve this goal are rather varied. In addition to the damage to agriculture, for which there are also solutions with innovative practical facilities (Amici *et al.*, 2011), the impact on traffic is the most frequently reported problem (Amici *et al.*, 2013).



Fig. 1. *Left*: capture of Wild Boar. *Right*: crop damage by Wild Boars.

#### 5. CONCLUSIONS

The erroneous perception of the faunistic dynamics and problems in progress is accentuated by two main factors, both attributable to the wildlife management bodies, which are delegated to manage an unavailable patrimony of the State (estimates of the status of the species of interest, hunting, repopulation, short and long term management objectives, etc.). The first factor is represented by the non-transparency of the acquired data; the second from the missed training/information actions that the institutes themselves should institutionally carry out. All this happens in the total absence of control by the policy and the institutions in charge.

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### **Three-year monitoring of the reproductive couples of Little Bittern *Ixobrychus minutus* in the territory of the Regional Nature Reserve of Lakes Lungo e Ripasottile**

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**Abstract:** This study has interested the territory of the Nature Reserve of Lakes Lungo e Ripasottile, specifically the areas that surround the moist. The nesting pairs of Little Bittern (*Ixobrychus minutus*) have been counted by using the listening stations and the Playback method. From the data collected, they were identified from a minimum of 12 to a maximum of 18 pairs for an average density of one pair every 2.05 ha.

**Keywords:** *Ixobrychus minutus*, density, pairs, playback, ornithology

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#### 1. INTRODUCTION

The Little Bittern (*Ixobrychus minutus*) is a polytypical species. In Italy, this species has migratory, summering and regular breeding behaviors (Brichetti e Massa, 1998). Nationwide, its population is estimated to be 1300-2300 pairs (Brichetti e Fracasso, 2003). Differently, the European population of the species is estimated to be 9400-15000 pairs (BirdLife International, 2004). In the Regional Nature Reserve of Lakes Lungo and Ripasottile (RNRLLR) (RI), the Little Bittern is considered to be a regular migrant and nidifying (Rossi *et al.*, 2006; Brunelli *et al.*, 2011). From 2014, a data collection on the reproduction of the species was started yearly, and for the following three years, investigating around the area of the lakes of Ripasottile, Lungo, and Pozzo.



### 1.1 Study Area

The study area is the territory of Nature Reserve and devolves in a ZSC (Special Area of Conservation), a ZPS (Special Protection Area), and in a SIC zone (Sites of Community Importance) IT6020011. These areas are located in a field between the Apennine's mountains, the pre-Apennine zone, Reatini's mountains, and Sabini's mountains. Such areas reach an altitude of roughly 376 m above the sea-level. The territory includes two major lakes, Lungo and Ripasottile with an extension of respectively 60 ha and 80 ha, and a medium depth of respectively 2,5 e 3,8 (Di Carlo, 1960; Di Carlo & Castiglia, 1981). Also, in the plain there are some smaller lakes basins, and rivers, which with the major lakes form a water system within an environment that hosts a high biodiversity of considerable naturalistic interests among which a multi-specific oasis (Angelici *et al.*, 2013).

## 2. MATERIALS AND METHODS

For this type of study, it was used the playback method that lures, during the reproductive period, the response of the territorial male of the species. 15 listening stations have been positioned on the territory, respectively 9 in Lake Ripasottile, 5 in Lake Lungo, and one in Lake Pozzo. Every listening station has been positioned with the help of the QGIS software at a distance of about 500m from each other. In so doing, it was possible to obtain a homogeneous coverage and without unnecessary overlapping of the investigated territory. The field trips, during the three years of study, were a total of 21, of which 9 at Lake Ripasottile, 9 at Lake Lungo and 3 at Lake Pozzo, for a total of 42 hours.

Once the data have been collected, they have been translated on a map with the use of the software (QGIS), obtaining the estimated number of the surveyed couples and the extension of their reproductive sites (Pugliesi *et al.*, 1995).

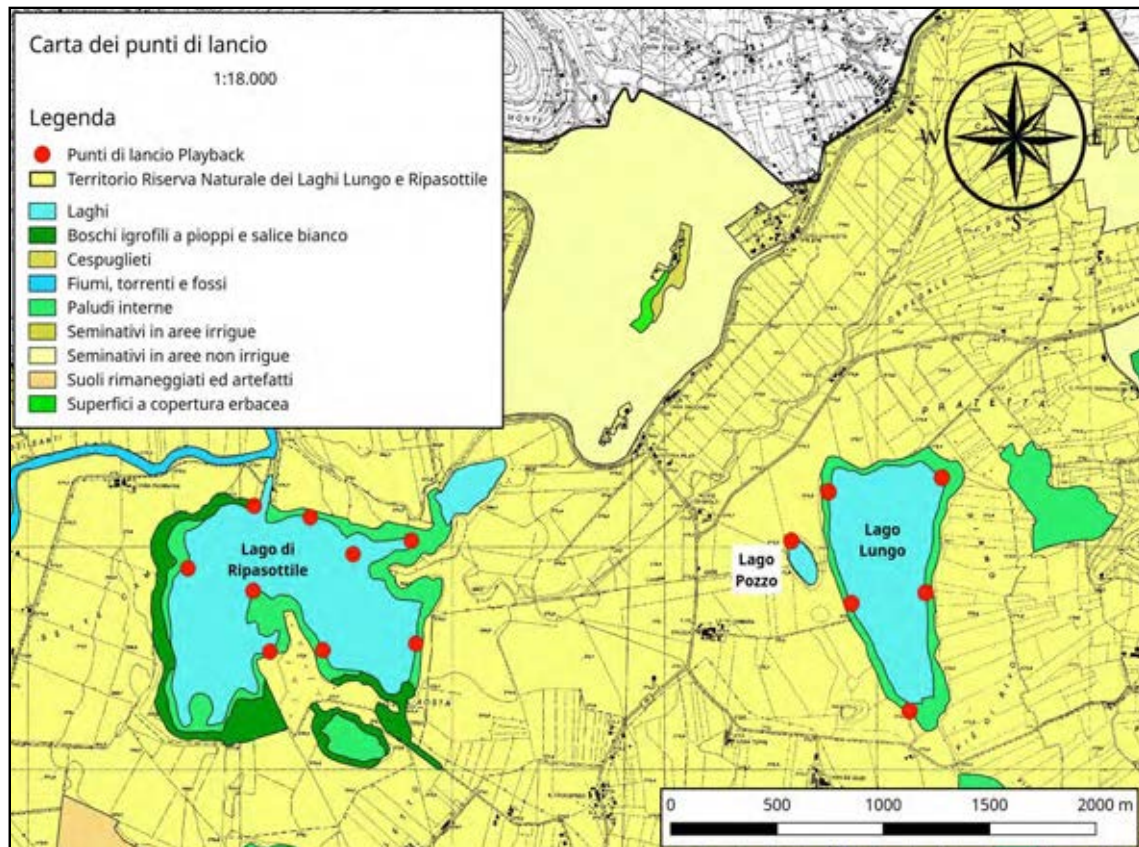


Fig. 1. Map points of launch of playback.

### 3. RESULTS

According to the data collected during the three years of study, in the Lake Ripasottile there is a presence of from 7 up to 10 couples of Little Bittern. On the Lake Lungo such presence goes from 4 up to 6 pairs, while in the Lake Pozzo from 1 up to 2 couples. Collectively, it has been estimated a maximum of 18 couples and a minimum of 12 in the whole analysed area. The area of vegetation suitable for hosting couples near the lakes turns out to be of 37 ha, with a maximum density of a pair every 2,05 ha and minimum of one pair every 3,08 ha.

**Chart 2.**

Years	Lake Ripasottile	Lake Lungo	Lake Pozzo
2014	7	4	2
2015	10	6	1
2016	9	5	2
Total couples	26	15	5
Total of couples in the three years			46

According to chart n°1, in the three years of study, 46 couples were surveyed in the protected area, of which 26 at Lake Ripasottile, 15 at Lake Lungo and 5 at Lake Pozzo. The Lake of Ripasottile is, therefore, the one with the highest number of presences of such species, due to its greater extension. In addition, having an area closed to the public provides a place free - or almost- from the anthropic disturbance.

### 4. DISCUSSION and CONCLUSIONS

The average density recorded this study is 1 couple every 2,56 ha. This data results to be lower than those obtained in previous studies (Pardo-Cerbara F., 2010). The cause of this low density can be linked to the kind of the lake's shores, which decline rapidly. Furthermore, the presence of the species could be limited by the latitude where the study area is located. Finally, considering that the Little Bittern is a kind of Union interest species, as inserted in the Annex I of the Bird Directive (2009/147/CE ex 79/409/CEE), this work represents a starting point on the knowledge of the species in the Province of Rieti and will be useful for its conservation.

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## Observation of the reproductive couples of Nightjars (*Caprimulgus europaeus*) in a area of the Piana Reatina

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**Abstract:** Starting from 2014 until 2016, we started monitoring the reproductive couples of the Nightjar species (*Caprimulgus europaeus*). The analysed area covers 4.200 ha, and it comprehends the Natural Reserve of the lakes Lungo and Ripasottile. The whole Study's area has been subdivided into UTM squares, obtaining 42 particles, from which the unsuitable ones have been excluded. The listening stations have been placed in the suitable particles. For the whole duration of the Study (three years), all 5 couples have been surveyed in the marginal areas and in the uncultivated lands with presence of gravel.

**Keywords:** *Caprimulgus europaeus*, reproductive couples, Natural Reserve.

### 1. INTRODUCTION

The Nightjar (*Caprimulgus europaeus*) it's a species with nocturnal habits, of which we lack of complete and full knowledge (Pedrini *et al.*, 2003; Bon *et al.*, 2004; Gagliardi *et al.*, 2007). The research was based on the reproductive couples of the Nightjar species present in a limited area including the Natural Reserve of the lakes Lungo and Ripasottile, the SIC (Sites of Community Importance), and the APS (Special Protection Area) IT6020011. Such portion of territory have been designed as ASC (Special Area of Conservation).

#### 1.1 Study Area

The analysed area covers 4.200 ha, and it comprehends a territory which is mainly used for agriculture. The presence of headwaters, lakes, rivers, and canals create a remarkable hydrographic scheme. In addition, in the research area there is also a presence of forests, shrubs, and uncultivated meadows. For additional information refer to (Rampini E. 2018), (Mariani M. 2018), (Di Carlo *et al.*, 1960, 1981).

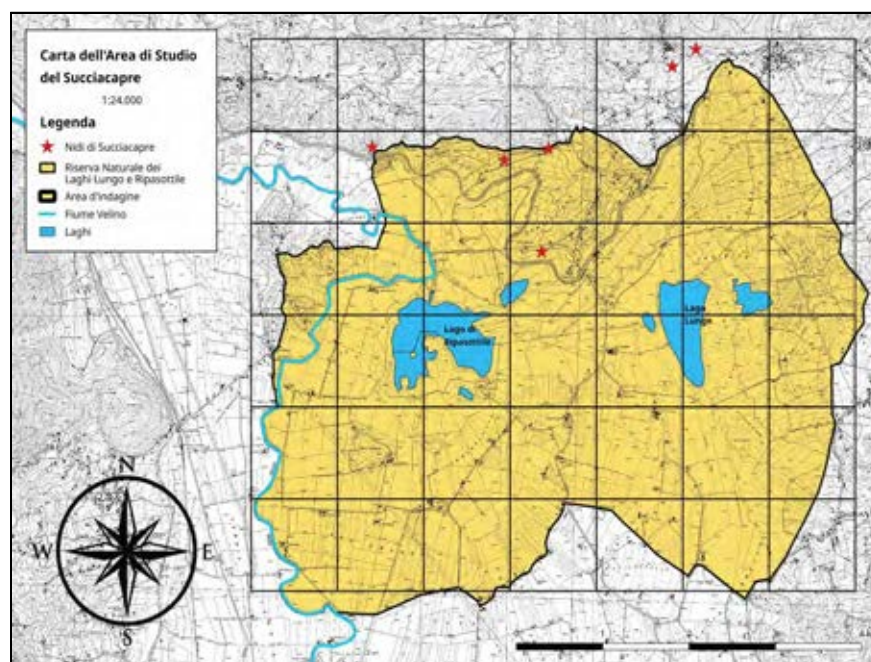


Fig. 1. Research area with grid and the 42 squares (1 km for each side).

## 2. MATERIALS AND METHODS

Thanks to the usage of the cartographic software QGIS, the studied territory was divided in square shaped survey units. Such survey units have been obtained through the overlapping of the UTM grid with square mesh of 1x1 km. 42 particles were collectively obtained. From these particles, all those units that did not meet the ambient eligibility requirements to host the Nightjar couples during the reproductive phase, were excluded. Then a listening station was positioned in each suitable unit. Once the research started, the operator in charge stayed in the station for 20 minutes. The same particle has been checked three times during the whole reproductive period, coming to a total of 9 times during the three years of research (2014-2015-2016).

## 3. RESULTS

For the whole three years of research (2014-2015-2016), it was taken a census on 5 couples of Nightjar (*Caprimulgus europaeus*) during the reproductive period in the analysed area. All the couples were positioned in the stretch of territory with presence of gravel, at the border of the stretch of territory itself, or at the border of some wooded areas. In such wooded areas, the agricultural activities do not disturb the presence and the reproduction of the Nightjar species.

Table 1. Reproductive couples.

Year	Couple 1	Couple 2	Couple 3	Couple 4	Couple 5
2014	*	*	*	*	
2015		*	*	*	*
2016	*	*	*	*	*

## 4. DISCUSSION

The meagre number of couples of Nightjars encountered during this research, has surely to be attributed to the peculiarities of the analysed territory. In fact, the territory involves, within different areas, lands intensively exploited in agriculture, sustained by an intense usage of pesticides. In addition, when it comes to the reproduction of such species, the Nightjars generally avoids mountains, forests, highlands dense of vegetation, mature plantations, cultivations, reeds, and high-altitude meadows. In fact, the Nightjars, to reproduce its species, prefers dry, open and well-drained habitats, which are hard to be found in the territory of the Protected Area, exception made for hilly areas. Indeed, all the discovered nests, were found in hilly areas.

## 5. CONCLUSIONS

The Nightjar species can be found in the Attached I of the Birds Directive (2009/147/CE ex 79/409/CEE). By being a matter of public interest, it is appropriate to start some measures for the conservation of the environments used and needed during the reproductive period. It also needs to be taken in consideration the reduction of the usage of pesticides, which can reduce the trophic resource. In the Lista Rossa of nestlings' birds in Italy, the species is considered to be "of Minor Concern" (Peronace *et al.*, 2012). In addition, the species itself results to have insufficient data (DD) (Brunelli *et al.*, 2011), and in Italy the population is suffering of a general decrease (LIPU, 2009) and (Calvario *et al.*, 2010). This research represents a starting point on the knowledge of the species of the Nightjar within Rieti's Province, and it will be handful to the preservation of the species itself.

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## Reproductive success of some couples of Long-Eared Owl (*Asio otus*) found in the Piana Reatina area

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**Abstract:** Study of reproductive success of some couples of Long-Eared Owl (*Asio Otus*) in a part of the Piana Reatina area. From the five couples studied has resulted that the reproductive success it stands at 2,4 nestlings involed for each nest.

**Keywords:** *Asio otus*, Reproductive success, Piana reatina

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### 1. INTRODUCTION

The study has interested the reproductive success of some couples of Long-Eared Owl (*Asio otus*), nesting on the area of Piana Reatina, Lazio (IT). This area includes the Nature Reserve of Lakes Lungo e Ripasottile it is a zone signed such as SIC (Site of Community Interest), ZPS (Special Protection Area) and ZSC (Special Area of Conservation) IT6020011.

#### 1.1 Study area

The study area has an extension of about 9.607 ha, with an average altitude of 370 m s.l.m. inside the area there are: two major lakes (Lungo e Ripasottile), Velino's river, minor lakes and a series of canals. The vegetation includes: 69,94% cultivated fields, 3% wetlands, 14,3% woodland and 8,2% area with ruins.

The Long-Eared Owl's nests were found on woodland or in groups of trees.

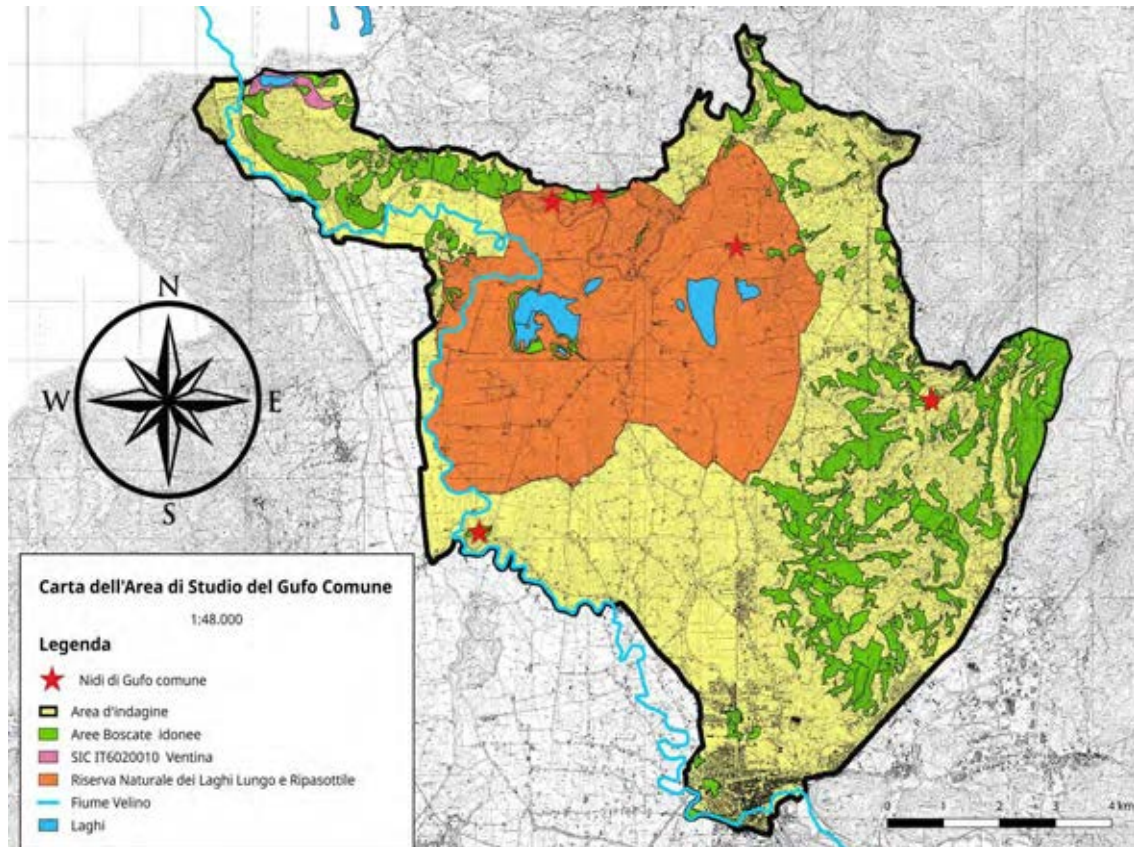


Fig. 1. Study area map.

## 2. MATERIALS AND METHODS

### 2.1 Creation of fitness map

For this study has been used the Quantum Gis software, that helped the analysis of the studied area. With this software it was possible to create a fitness map to study only the areas eligible to host the Long-Eared Owl nests. The requirements for an eligible area for this species are: broadleaved trees or coniferous trees surrounded by open areas, parks and gardens (Chiavetta M., 1992; Bricchetti P., Fracasso G., 2006).

### 2.2 Calculation of the reproductive success

The calculation of the reproductive success was made by dividing the number of nestlings involved by the number of breeding pairs (surveyed) from 2014 to 2016. To this purpose was made a count of the nestlings involved using their ethology. In fact after they have involved, the nestlings move for some time in the area surrounding the nest by emitting a typical lure to attract parents. Using this behavioural trait was possible survey the nestlings involved.

## 3. RESULTS

The outputs in the studied area have occurred regularly and allowed the identification of 5 nests belonging to the species investigated. The nestlings involved from the five nests are in totally 12, by dividing this last for the nests we get a reproductive success of 2.4.

Table 1. Nestlings involved for each nest surveyed.

	Nest 1	Nest 2	Nest 3	Nest 4	Nest 5
Nestlings involved	3	3	2	2	2

#### 4. DISCUSSION

The study of the nesting of the couples of Long-Eared Owl, discovered in the Piana Reatina territory, has highlighted a reproductive success of 2,4 nestlings for each nest, this value is far above the 0,88 obtained from David E. Glue (1977) and superior to 0,89 obtained from Davorin Tome (2011), while it turns out to be much lower to the 3,4 obtained from Jeffrey S. Marks' (1986). This data discrepancy it is certainly attributable to the different territorial morphologies Study environments and the different trophic availability. Indeed, this species studied is influenced considerably for its reproduction, from the trophic resources available in the territory (Davorin Tome 2011). Besides the Long-Eared Owl's nests can be object of pillaging eggs, both by predators (birds and mammals) also from poachers and collectors (David E. Glue 1977).

#### 5. CONCLUSIONS

In the studied area was found the presence of the nests of the studied species in a few portions of fragmented territory; it would be advisable to preserve these differences by safeguarding both the plants that house the nests and those that surround them (Denver W. Holt 1997; F. Henrioux 2002).

Will then be necessary pursue the study to the all Piana Reatina's area for the survey of all nesting sites of Long-Eared Owl in order to create a paper useful for the management of the territory that reports the information collected.

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### **First data on the hierarchy of waterbirds for the use of roosts in the breeding colony of herons (garzaia) of Ripasottile.**

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**Abstract:** The aim of this study is to verify the existence of an interspecific and intraspecific hierarchy for the use of perches among the species of waterbird in the area of heronry located in the Ripasottile lake. The final analysis identified: the existence of an interspecific hierarchy for the use of perches; the hierarchical order for the use of perches; the existence of an intraspecific hierarchy for the use of the

perches; the presence of interspecific cohabitations for the use of the perches. However, since this is an experimental study, these results cannot be considered absolute but related to the area of investigation. Therefore, this work represents a basis for further studies on the hierarchical order also in other environments.

**Keywords:** waterbird, hierarchy, rivalry, cohabitation, intraspecific, interspecific.

## 1. INTRODUCTION

This study was done in the Wildlife Reserve of Lungo and Ripasottile lakes with the purpose of verifying the existence of an interspecific and intraspecific hierarchy between the waterbirds in the Reserve for using the two roosts situated in the heart of the garzaia of Ripasottile lake.

### 1.1. Objective of the study

The purpose of the study is to verify:

- The existence of an interspecific hierarchy for using the roosts;
- The possible hierarchical order for using the roosts;
- The existence of an intraspecific hierarchy for using roosts;
- The existence or not of interspecific cohabitation for using the roosts.

### 1.2 Area of study



Figure 3: Area of study.

The area of the study was chosen for the position of the roosts, in the heart of the garzaia, which, thanks to its expansion and the high number of hosts, and availability of food resource, dispatched for the newborns, makes these two roosts very coveted. For additional information regarding the area of the study, refer to: Fermani 2019; Di Carlo & Castiglia 1981.

## 2. MATERIALS AND METODS

The study was realized thanks to the photo trapping technique, that allowed to monitor the two roosts for five consecutive months, specifically from July to December 2016, the period in which it is possible to supervise the situation with both resident and migratory species. Therefore, two photo traps have been installed in proximity of the only two roosts present in the part of area of study occupied



from the lake. The batteries and the memory cards that permitted the photo traps to work, have been replaced weekly, in order to keep a constant supervision on the roosts. The use of a motorboat and PPE (Personal Protective Equipment) was necessary to reach the above-mentioned roosts.

In a second phase, the multimedia files recorded have been displayed on a computer, then filtered and divided in:

1. Interspecific Interactions:

- Rivalry;
- Cohabitation.

2. Intraspecific Interactions:

- Rivalry;
- Cohabitation.

This operation revealed to be indispensable to data analysis.

### 3. RISULTS

In a period of 252 days of survey, have been studied ten ornithic species:

Mallard (*Anas platyrhynchos* L.), Pochard (*Aythya ferina* L.), Grey Heron (*Ardea cinerea* L.); Cattle Egret (*Bubulcus ibis* L.); Little Egret (*Egretta garzetta* L.); Night Heron (*Nycticorax nycticorax* L.), Glossy Ibis (*Plegadis falcinellus* L.), Coot (*Fulica atra* L.), Hooded Crow (*Corvus corone cornix* L.), Cormorant (*Phalacrocorax carbo* L.).

Such species were protagonists of 71 events:

- 12 cohabitation events (9 between different species);
- 60 rivalry events due to the use of roosts:
  - 35 intraspecific;
  - 25 interspecific.

### 4. DISCUSSION

The study satisfied the initial objectives since the existence of an intraspecific and interspecific hierarchy for using roosts was confirmed, as the same as an existence of an interspecific cohabitation. Moreover, was possible to establish a hierarchic order, which is reported in the underlying Figure n. 2:

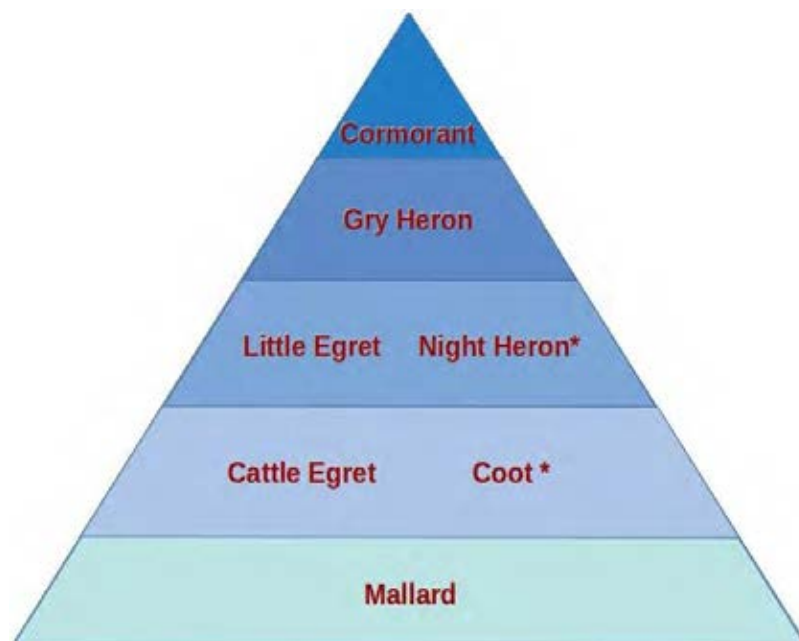


Figure 4: Pyramid scheme of the hierarchical order.

## 5. CONCLUSIONS

In the pyramidal graphic reported in the Figure n. 2 were excluded 3 of 10 studied species (Pochard, Hooded Crow and Glossy Ibis) because of a lack of information. The classification of the Coot and of the Night Heron is temporary, since they only “confronted” to other species positioned under them. Given that it is an experimental study, these results can’t be considered absolute, but connected to the research area. Then this survey is a base to further detailed studies that can verify, in this way, if the found hierarchic order results unchanged in other places.

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## The faunal community of the colony of Lake Ripasottile: a figure of the last decade

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**Abstract:** The goal of the study is to verify and to justify possible numeric and behavioral changes of the five avian species, constituting a (plurispecific) colony and resident in the colony, inside the Reserve of Lakes Lungo and Ripasottile, that is: Night heron, Grey heron, Squacco heron, Little egret, Cattle egret. For this reason, in order to have a global picture of the births and the growth of the colony, we have considered and examined data collected by the bird-watching post of the Reserve from 2009 to 2018, to be able to ascertain the density of the studies species as well as the frequency and the modality of nest-building and possible problems related to that. Therefore, this paper is the result of a research divided into two sections, bibliographical and purely scientific. In fact, initially we analyzed, studied and compared data from 2009 to 2017, after we concentrated on the data related to 2017 and 2018 to obtain new data in order to have an outline of the last decade. The final analysis revealed a progressive increase of nests and birds and the Grey Heron set the record of the colony at Regional Level.

**Keywords:** Colony, plurispecific colony, ornithology, numeric increase, last decade, monitoring, record.

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### 1. INTRODUCTION

The term "garzaia" identifies the habitat that hosts the nesting of different species of Ardeidae, which tend to prefer to build very close nests on the same tree or shrub, creating multi-species colonies. That of Ripasottile, specifically, hosts the nesting of five species of Herons, such as the Night heron (*Nycticorax nycticorax*), Grey heron (*Ardea cinerea*), Squacco heron (*Egretta garzetta*), Little egret (*Ardeola ralloides*) and Cattle egret (*Bubulcus ibis*). To this date, it appears to be the largest in the Lazio Region and one of the largest in central Italy, for the species Gray Heron. What also makes it completely original is the location of the nests on the reeds, a very rare event, usually nesting on trees or bushes. Basically, the heronry is born as a single species: the settlement in a specific area happens, in fact, by a single species, the reproductive success of which, will work as an attraction for other species. The preference to nest in the same site, creating multispecific colonies is, in fact, probably related to the greater degree of security that this condition confers.

#### 1.2 OBJECTIVE

The objective of the study is to verify and justify any numerical and behavioral variations in the five bird species living the Garzaia inside the reserve.

Therefore we decided to:

- compare and analyze previous investigations;
- monitor the presence of the species in question;
- monitor any behavioral changes;
- count the number of nests;
- identify any problems and consequent strategies, able to solve them.

The hope is to be able to apply in the future all the measures necessary for the protection of this particular habitat.

## 1.2 STUDY AREA

The study area includes a part of the territory of the Laghi Lungo and Ripasottile Natural Reserve, that includes four nesting points:

- the island of Ripasottile (0.37 ha);
- willows and poplars, respectively to the east and south of the island;
- the edge of the reed land beneath the trees;
- an isolated plant, near by.

However, all these sectors fall into the IT6020011 SAC "Long and Ripasottile Lakes" (Special Areas of Conservation, ex SPA and SCI ") of the Natura 2000 Network.

Finally, the protected area contains two trout breeding facilities, located in the Municipalities of Colli sul Velino and Rivodutri, which probably are an attraction for the Herons, primarily promoting their feeding and, consequently, their winter break.

## 2. MATERIALS AND METHODS

The conduct of the study essentially involved a bibliographic and a purely scientific phase. As for the latter, the work was divided into two further phases, namely an organizational one (F1), at first and an operational one (F2), subsequently, including both, various steps:

- analyze the study area;
  - identify vantage points for observation;
  - create the field card and the survey card;
  - constitute the cartography;
  - get the necessary equipment;
  - go to the place of investigation;
  - position yourself at a safe distance from the nesting point;
  - observe and identify as much useful information as possible;
  - complete the field card;
  - take photographs;
  - analyze and process raw data, outlining the final results.
- 
- ```
graph LR; subgraph F1; T1[analyze the study area]; T2[identify vantage points for observation]; T3[create the field card and the survey card]; T4[constitute the cartography]; T5[get the necessary equipment]; end; subgraph F2; T6[go to the place of investigation]; T7[position yourself at a safe distance from the nesting point]; T8[observe and identify as much useful information as possible]; T9[complete the field card]; T10[take photographs]; T11[analyze and process raw data, outlining the final results]; end;
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The materials used were: Suzuki Jimny car of the Nature Reserve, Nikon Monarch 8x42 binoculars, Swaroski telescope with 20-60 zoom, Nikon D810 camera.

## 3. RESULTS

From the moment of birth till the present, was monitored a gradual increase in nests, individuals and species within the Ripasottile heronry. In the last year dedicated to this study, we recorded a further increase, according to the progressive trend found during the entire decade of the observation period. Indeed, it has been ascertained that:

- with regard to the nesting of the Gray Herons, always the most numerous, it has gone from a total of 202 nests to more than 206;
- as for the Night heron, the 50 nests of the previous year have become 70;
- the nests of the Little egret have increased up to 10;
- on the contrary, the Cattle egret is the only one to have remained stable;
- finally, Squacco heron, absent for three years, it's still absent.

#### 4. CONCLUSIONS and DISCUSSION

The study allowed:

- to determine that Long and Ripasottile Lakes Reserve is capable of hosting the nesting of Ardeids;
- to ascertain that the site was probably chosen by the environmental characteristics of the Nature Reserve, such as the presence of wetlands, marsh vegetation, poplar groves and willows, lake areas with not very deep water;
- to assume that, in all probability, it was of fundamental importance the availability of trophic substance, in this specific case, because of the fish plants present in the reserve;
- to hypothesize that another fundamental aspect was the safety of the nesting point;
- to ascertain the tendency to prefer willows, specifically, which previously represented only a hypothesis.

In this regard, it was decided to create a PSR project, already approved, aimed at protecting and guaranteeing, according to whether it already exists or not, the presence of the latter plants. Finally, the loyalty of the Ardeids in relation to the reproductive sites and the customary formation of multi-species colonies, not only invite new monitoring, but hint the possibility that such colonies can accommodate the nesting of species so far present only during the summer, as well as the probability that the numerical increase continues.

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## Status of the Fox (*Vulpes vulpes*) and burrows distribution within the lakes of Lungo and Ripasottile Nature Reserve

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**Abstract:** The work consider the species Fox (*Vulpes vulpes*), and was carried out in order to estimate the number inside the Reserve and identify the within problems associated with it. The results derive from the analysis of the data obtained in two distinct types of census, reproductive sites (census) and the spot light census (count of individuals). The field survey made it possible to estimate the presence of 298 individuals in the entire territory of the Reserve, a consistency that corresponds to the density of about 1 individual every 20-ha. The excessive presence of this small predator can cause damage to both the wild and the farms of low court animals. In addition to the predatory impact, which can be a limiting factor for zoocoenosis (eggs, nestlings, small of different species, etc.), the Fox can be a vehicle for the transmission of various diseases. This species is subjected to hunting as per law 157/92, but it is not a prey much sought by hunters and this allows a strong numerical increase, highlighted even more conspicuously within the Nature Reserve, where hunting is prohibited (L. 394/1991) and the trophic availability is quite high.

**Keywords:** Nature Reserve, Red fox, *Vulpes vulpes*, burrow, census.

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### 1. INTRODUCTION

This study was carried out thanks to the support of the Riserva Naturale dei Laghi Lungo e Ripasottile, and thanks to the cooperation with UNITUS. It derives from a period of my training carried out within the natural area and subsequently continued with the studies that led to the preparation of this final paper. The work concerns the species Fox (*Vulpes vulpes*), and was carried out with the aim of estimating the consistency of fox within the territory of the Nature Reserve and detecting the problems connected with its management.

#### 1.3 Study Area

The study area is represented with the entire territory of the Nature Reserve, which is located in the Piana Reatina and covers about 3,300 hectares, including 500 hectares of lakes, smaller parts of water and low-lying areas that are erratically flooded, the rest of the territory it is mainly represented of flat and irrigated arable lands. Furthermore, there is an area designated as SIC (Sites of Community Importance) SPA (Special Protection Area) today SAC (Special Area of Conservation) IT6020011. For further information, see (Di Carlo, 1960; Di Carlo & Castiglia, 1981).

### 2. MATERIALS AND METHODS

The results derive from the analysis of data obtained in two different types of census: reproductive sites and the spot light census (count of individuals).

#### 2.1 Reproductive sites

In the first case, the work teams, consisting of young undergraduates, trainees, volunteers and staff of the Nature Reserve, proceeded to cover the transects, searching for dens and / or signs of presence, signalling their possible finding and reporting the data on the field card. The latter specifically concerned the following parameters: the width and height of the "mouth", the slope of the first entrance section of the den, the direction of exposure in degrees of the mouth, the slope of the adjacent land, the possible use of the den, the type of surrounding vegetation, correlated with an estimate in %

of the self-sown vegetation coverage, and the type of soil. A photograph of the den and subsequently georeferenced and numbered it finished the study.

## 2.2 Spot light

In the second case, special lights were used to send out a beam of light of about 70 meters, and which were installed on board the off-roads and / or convertibles vehicles, one on each side, in order to analyze about 140 meters of territory at the same time, counting only the members encountered of the species under examination inside the beam of light. These vehicles, with the two operators on board, travelled at low speed along some pre-determined transects, guaranteeing a minimum observation coverage of 10% and a maximum of 20%, of the entire study area.

## 3. RESULTS

The spot light census allowed us to calculate an average presence of 17.87 individuals in the 362 hectares investigated, corresponding to 13% of the 2800 hectares (in the total territory minus the flooded areas) of the Nature Reserve. This data has led to a population estimated of about 275 individuals in the entire Nature Reserve area. The estimated density for the species *Vulpes vulpes* is about 1 individual every 20 hectares.

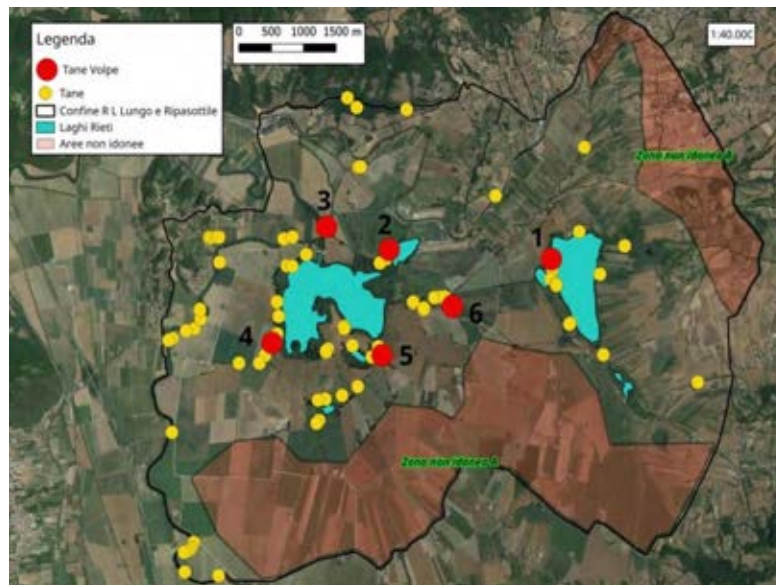


Fig. 1 Burrow card present in the study area.

The census of the reproductive sites led to the survey of 90 dens, and with the use of camera traps we were able to certify the presence of 6 foxes. Moreover, as can be seen from the map, the distribution of the burrows is not uniform within the protected area but is influenced by anthropic interventions.

## 4. DISCUSSION AND CONCLUSIONS

The presence, in the protected area, of an individual Fox every 20-ha was higher than the average found in the literature, of one every 245-ha (Provincia di Rovigo 2011). This small predator can cause damages both to the wild and to the farms that have poultry. In addition to the predatory impact, which can be a restricting factor for zoocenosis (eggs, nestlings, babies of different species, etc.), the Fox can carry various pathologies that can be transmitted. This species is subject to hunting in accordance with Law 157/92, however it is not considered a interesting prey for italian hunters and this allows a great increase in numbers, even more inside the Nature Reserve, where hunting is not allowed (L. 394/1991) and trophic availability is quite high.

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### *Sitography*

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- <http://www.oasicannevie.com/schede/Volpe.pdf>
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- <http://www.soveratometeo.it/index.php/i-venti/classificazione-del-vento-e-scala-beaufort>
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## **Are the official data on wild boar hunting bags reliable?**

### **The case study of Rieti province**

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**Abstract:** In the province of Rieti, boar hunting is the main hunting activity, both for the number of hunters (more than 4000) and the number of animals hunted. Some of the investigations, conducted by cross-referencing data related to hunted animals with the number of carcasses visited by veterinarians of the Local Health Authority (ASL), show that only a part of the hunted animals is reported. There are different reasons for this phenomenon, and these are the most significant: limiting the costs of the removal of slaughter waste and veterinarian visits and making the hunting area less desirable by simulating a shortage of animals to the local authority. On the other hand, the adequate number of animals to be hunted cannot be planned, a huge quantity of meat (not certified from a health perspective) is circulating, and a big quantity of slaughter waste is illegally and dangerously abandoned in the territory, at the expense of the agriculture and the ecological balance the containment of the species is often ineffective. On the basis of analogous investigations conducted in the past, this study will estimate the percentage of hunted animals reported compared with the actual number of animals hunted. This objective was reached by monitoring circa 11% of the hunting teams operating in the province. For each of them, the number of hunted animals reported compared with the number of animals hunted was recorded during a three-year period.

**Keywords:** hunting, Wild Boar, Rieti, hunting bag, reliability

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## 1. INTRODUCTION

In the first half of 1600 the wild boar became extinct in the almost totality of the Italian territory. This was done in the historical period in which it was registered the highest human presence in areas hilly



and mountainous. At the beginning of the first half of the last century this species has suffered as a result of intense anthropogenic activities, a process of consolidation and the recolonization (Tosi & Toso, 1992) also as a function of environmental changes (Pelorosso *et al.*, 2007), the management of forest land (Friends *et. al.*, 2008) and of the policies for the protection of the territory taken (Friends & Esposito, 2009). In the past in Italian territory was present the subspecies *Sus scrofa majori* (Perco, 1987). Is difficult to define the subspecies currently present (Amici *et al.*, 2003) for different causes: the entries made with hunting purposes, the gradual junction with subjects belonging to the populations French and Yugoslav, initially present only in northern Italy which then have expanded their initial Distribution Ghariyal Distribution (Tosi & Toso, 1992) and the subsequent coupling with the domestic pig where was traditionally held in wild breeding (Apollonio *et al.*, 1988). All this has caused a "genetic pollution" generating problems about the exact identification of the suide currently present.

## 2. MATERIALS AND METHODS

In the province of Rieti, hunters practising hunting for wild boar with the technique of the hunted are accredited in about 120 teams. \*Starting from the assumption that no type of rational management may be assumed if you do not know the status of the target population, to properly handle an animal population it is necessary to have certain data, homogeneous and arising from scientific investigations are planned and applicants. Reaffirming that for the province of Rieti is known that official data relating to hunting are not in keeping with the reality, this fault is caused by the fact that a substantial fraction of hunters declares only a part of the animals actually slaughtered (Adriani *et al.*, 2008). \*The data acquired and processed in this survey, tending to update the quantitative aspects of the phenomenon already denounced by Amici *et al.* (2008) come from the monitoring of 15 teams regularly credited and operating with the technique of the hunted in the province of Rieti, who voluntarily and on a relationship of trust and confidentiality shall cooperate with the DAFNE of the University of Tuscia periodically providing the data of the entity of the levy reported to the competent authority and those of the levy actually carried out.

The sample of the teams of hunting collaborators is constituted from the 11.5% of those credited and operating in the provincial territory.

## 3. RESULTS

For reasons of privacy, and in respect of the agreements concluded with the teams collaborators, the results of the survey are given only in aggregated form.

Three-year period 2015/2018

- Heads reported 664 (Average = 14,76/team; D.S.±10.18).
- Heads really taken 1934(Average = 42,98/team; D.S.±23.50).
- The average difference between the real levy and that denounced is 28,22 heads (D.S.±26.76).
- Fraction of heads reported with respect to those actually taken ~33%.

The estimation of the heads annually killed in the Province of Rieti, levy estimated real ~5600 heads/year; in the face of ~1900 reported. Only 4/15 of the teams monitored in each hunting season regularly denounce the number of animals actually taken.

#### 4. DISCUSSION

For a correct management of wildlife and wildlife hunting is necessary to arrive as quickly as possible to the transparency and accessibility of databases of bodies that manage the hunting. This would facilitate the monitoring of the correctness of the data declared, it is also necessary to have made to comply with applicable legislation and, in particular, that it is urged the writing of three-year plans by the ATC, with clear indications on the objectives of density specific species set.

#### 5. CONCLUSIONS

The proper management of wildlife and hunting, once again, is characterized to be severely hampered by the missed or wrong political decisions and by the interference of the policy itself in the scientific and technical issues.

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### Historical analysis and current situation of wolf (*Canis lupus*) populations in Europe

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**Abstract:** As a result of the historical decline, which led it to almost extinction, the Gray Wolf is returned to occupy a large part of Europe. Today it is estimated 17.000 specimens and 9 populations. In order to maintain its favourable conservation status, it is necessary to analyze the threats that could represent an obstacle, as the poaching, livestock predations and hybridation with dogs, and the specific actions to be implemented. Between those who approve and justify any conflict between man and wolf, and those who reject even the slightest possibility of coexistence, the truth is this: the wolf plays a fundamental ecological role, and its conservation has considerable economic and cultural importance. After centuries of almost uninterrupted hunting by man, the wolf, in Europe, has regained much of its historical area, from which it had been historically persecuted. Today the wolf is back, and it is estimated that about 17,000 specimens and 9 populations, almost always cross-border, and therefore shared by two or more countries, and distributed in different areas and territories. In order to favour the maintenance of a satisfactory conservation status, it is therefore necessary to consider every possible obstacle. The conservation of the wolf depends on the conservation of the prey species, especially the populations of wild ungulates, which see in the wild boar and roe deer, the basis for the

right balance. One of the possible obstacles is hybridization, and cross-breeding with stray dogs, a potential source of scaremongering that threatens the genetic identity of the species. Another aspect to consider are the contrasts with man, especially with regard to the livestock sector and the predation of livestock, especially to the detriment of sheep farms, but also goats, cattle, horses and reindeers. These conflicts worsen, unintentionally, the unjust and excessively exaggerated fame, which has always pursued it, but are still interconnected to the low or insufficient density of prey populations, present in the same area, and on which depend the increase or reduction of wolf populations. The predation of the livestock, however, can be solved. The reintroduction of traditional prevention measures, such as the guard dog or the adaptation of innovative or updated measures, such as the fladry or the electrified fences, are undoubtedly the most recommended and advantageous solution, and may lead to a total or partial reduction of the cases of aggression, not only by the wolf, but also by other large carnivores, such as the brown bear, the lynx and the wolverine, which share a similar epilogue, which has led from a phase of decline to a phase of recovery. Another possibility is compensation, with ex ante (preventive) and ex post (compensatory) compensation. All this, in relation to the different management regimes, between member and non-member countries of the European Union, and as expressed in the Bern Convention, for the conservation of wildlife and its biotopes in Europe, and the Habitats Directive No 92/43/EEC

**Keywords:** Wolf, populations, subspecies, hybridation, livestock, prevention, Europe

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## ***Main lectures***

### **CeMedForVet: Forensic Veterinary Medicine against poaching**

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**Abstract:** Veterinary forensics is a medical discipline, which aims to provide technical and scientific support to the judicial authority and police, to bring to justice those responsible for criminal acts against animals. A growing request for support in investigations by the judicial authority, and a new and marked thoughtfulness of public opinion towards the protection of animals, has led to the need of identifying a specific scientific technical body. CeMedForVet (Centro di Referenza Nazionale per la Medicina Forense Veterinaria), was established by decree of the Italian Ministry of Health in 2009 (G.U. n° 225, 28/09/2009), at the Istituto Zooprofilattico Sperimentale of Lazio e Toscana, Grosseto. Main tasks assigned to CeMedForVet concern the development and standardization of laboratory techniques and forensic toxicology for the detection of toxic substances used for malicious purposes; comparative analysis of hairs or other organic samples, using molecular biology techniques, for the suppression of poaching; individual identification (DNA typing), attribution of paternity, identification of sex, and recognition of intraspecific hybrids using forensic genetics techniques. CeMedForVet provides technical and scientific support to the judicial authority and police, to investigate criminal acts against wild and domestic animals, including illegal killings, poaching, poisoning, or illegal trades. The role of the forensic veterinarian in poaching cases is to look for elements that could connect the three actors of poaching, namely the crime scene, the animal victim and the offender. In this process, some major steps can be identified where application of best practice is essential to achieve accurate and fair criminal justice. The final technical report can answer questions about cause of death, manner of death, post-mortem interval, and any other non-accidental injury. Compliance with code of criminal procedure, chain of custody and trace integrity are to be assured at any time during the forensic process. Forensic necropsy should be performed according to published national guidelines, including photographic documentation with metric reference, skinning of the carcass, and careful inspection of gastrointestinal contents (both macro- and micro-scopic). During the necropsy, the pathologist may acquire new evidence and/or assign evidence to other labs (toxicology, histology, entomology, ballistics ...). In about 9 years of activity, CeMedForVet provided support in 930 poisoning cases, performed 863 forensic necropsies and 4250 forensic genetic analyses, provided 182 qualified expert testimonies and technical consultancies, 134 training events, 28 peer-reviewed publications on international journals, more than 70 participation in national and international conferences, and published 7 informative manuals.

**Keywords:** forensic genetics, necropsy, poaching, poisoning, veterinary forensics

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## Meat-borne parasites in wild boar

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**Abstract:** Wild boar is one of the species of large game most hunted in Europe. As a wild animal, it may be exposed to numerous pathogens, some of them zoonotic, like meat-borne parasites. These parasites include *Trichinella* spp. and *Toxoplasma gondii*, which are the most commonly reported, as well as *Cysticercus cellulosae*, *Sarcocystis suihominis* and *Alaria alata*. In 2014, FAO/WHO presented the global ranking of food-borne parasites by “importance” and their primary food vehicle. From those, *Taenia solium/Cysticercus cellulosae* (1<sup>st</sup>), *Toxoplasma gondii* (4<sup>th</sup>) and *Trichinella spiralis* (7<sup>th</sup>), were listed in the top ten parasites with more importance. Those will be the focus of this presentation. In fact, wild boar meat can represent a source of these parasitic zoonotic hazards but, the question that must be addressed it is: What can be done to mitigate the risk to humans derived from consumption of this meat? For all of these three parasites, we should say that, in general, if meat it is cooked under 60 –75°C (Core temperature) during 15–30 minutes, it should be sufficient to inactivate these hazards. This means that the presence of disease in humans is related to consumer habits like consumption of undercooked meat (e.g. Spit roasted-cooking method) or raw meat products (dry, fermented sausages) not submitted to sanitary inspection. Sanitary inspection performed in a game meat establishment it is mandatory if wild boar meat is used for placing on the market (Regulation EU 853/2004). However, this EU regulation is not mandatory for private consumption, which may represent a considerable number of events in some European countries. Regarding wild boar inspection it is important to understand that, for these three parasites, this official control can be effective to detect *Trichinella* spp. through artificial digestion analyses (Regulation (EU) 2015/1375). To detect cysts of *Cysticercus cellulosae*, incisions procedures may be useful, if present in a visually way. But, regarding *Toxoplasma gondii* detection, sanitary inspection it is not effective. Under this epidemiological scenario, some strategies should be implemented in order to control/prevent these diseases. Those can range from hunters’ training/education to monitoring programs. Some will be highlighted during the presentation.

**Keywords:** *Trichinella* spp., *Toxoplasma gondii*, Meat, Wild boar, Sanitary inspection

## Applying epidemiological concepts to infectious diseases management in wildlife

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**Abstract:** Infectious diseases have been one of the main causes of human and also animal deaths for years. But due to several reasons, during the second half of the last century, since 1950, the relevance of these infectious diseases in both human and animal populations decreased significantly. However, in the last 40-50 years, the emergence of an important number of novel infectious diseases have brought back microorganisms as an important and very relevant global health problem. When we focus on infectious diseases in wildlife management, there are three main reasons for their relevance; (i) most of these emergent or re-emergent diseases affecting humans are zoonotic diseases originated from wildlife, (ii) some infectious diseases have been linked to mass mortality events in wildlife, (iii) wild animals could become the source of infection for domestic animals. In order to understand the role of wildlife in the epidemiology of infectious diseases and to develop rational control measures, it is

necessary to understand some epidemiological concepts. A natural reservoir, disease reservoir or reservoir of infection is any person, animal, plant, soil or substance in which an infectious disease etiological agent normally lives or multiplies and it will always play a relevant role in the survival of an infectious etiological agents. The basic reproduction number or R<sub>0</sub> is the number of secondary cases that each infected animal can generate on average over the course of its infectious period. This number has been estimated for a number of infectious diseases and depends on three main factors; how many contacts a sick animal is likely to make, which is the probability of transmission after a contact and how long infected animals shed the microorganism. Finally, a spillover infection, pathogen spillover or spillover event occurs when a pathogen is able to jump from its natural reservoir to another population. This epidemiological knowledge together with wildlife ecology should be used in order to manage wildlife infectious diseases. Current methods for disease management in wildlife would include: (i) improvements in biosecurity to prevent spill-over or even spill-back of diseases from or into wildlife populations and (ii) decrease in the basic reproduction number to limit the number of secondary cases generated in the wildlife populations. Lethal control or culling reduces the population and then decrease contacts by reducing overall host density. It also reduces the basic reproduction number by limiting the duration of the infectious period and therefore the number of newly infected animals. On the other hand, vaccination can also reduce the basic reproduction number by decreasing the number of susceptible animals in the population.

**Keywords:** wildlife infectious diseases management, epidemiology, basic reproduction number, spillover event, natural reservoir

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### **Veterinary aspects of wild life management**

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**Abstract:** Authors are presenting actual results of projects and clinical veterinary works in aspect of free living wild life as well as game farming management in Slovakia. Recently large scale projects were introduced in which veterinary aspect played key role for successful results. It was telemetry monitoring of brown bears in aspect of home range determination, interaction of large carnivores as wolf, bear and lynx with red dears. At present time Slovakia reached the culmination of main game species as well as large predators. Conflicts with bears are highest in Europe. Veterinarians play significant role in development of functional chain of rehabilitation stations for injured wild life with special attention to raptor species of European importance like saker falcon or imperial eagle. Disease prevention and monitoring of pathogens affecting game farms is essential in combating infectious disease like fasciolosis, tuberculosis, paratuberculosis in ruminants, or west nile virus infection in bird species. Application of antihelmintics and their resistance is frequently discussed. Effective and safe immobilization protocols are presented for European bison, chamois (*Rupicapra rupicapra*), brown bear or feral wild pigs and red deer significantly pricing the work of wild life veterinarians. Authors are also presenting veterinary measures of prevention and eradication of African swine fever in Slovakia.

**Keywords:** Immobilization, game farming, large carnivores management, rehabilitation

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Recently the number of free living game species like red deer, wild boar, fellow deer and muflon are culminating in Slovakia. Same times the nature and environment is passing through dramatic, even drastic ecological changes like deforestation, environment fragmentation and habitat loss. This negatively effects the behaviour of large carnivores which reached the highest number and abundance in Europa. This results in frequent human-wild life conflicts. Veterinarians are frequently part of fast intervention teams together with environment protection authorities. Their tasks is assess damage, observe killed game, road kills and removing conflict individuals. Veterinarians are also part of projects studying the migratory routs and are offering practical help with clinical services to public in touristic areas. In Slovakia are more the 500 registered game meat farms and over 200 game-trophy hunting farms. Hunting is very popular and hunting within fenced areas is challenging veterinarians for special services like sampling, darting or transporting valuable game species. Free living european bisons-visons (*Bison bonasus*) were introduced in north-east located Poloniny national park facing wild life veterinarians with translocation of such a large ruminants. In opposite intensive large scale agriculture and pesticides dramatically reduced small game species like partridge, pheasants and brown hares. Wild rabbits almost extinct. Loss of habitat effected mainly capercaillie (*Tetrao urogallus*) and great european bustard (*Otis tarda*) population. Special breeding and rehabilitation centers were built for their protection and reintroduction. Veterinary management is crucial for their success. For injured protected species there was developed a chain of rehabilitation centers which offers first hand care as well as long term rehabilitation in fly pans. Practical example is Life energia project focused on electrocution of birds in power lines. Veterinarians can find their importance in following field of game or wild life management:

1. Research
2. Infection disease monitoring
3. Immobilization
4. Rehabilitation
5. Education

The main issues effecting public opinion in context of wild life management are the questions related to frequent bear-human conflicts, large number of road kills and recently spreading of African swine fever from Hungary to Slovakia. To fulfil the demand of public and hunting community or scientists in field of wild life management a special annual training courses have been implemented. Their were organized by Slovak Veterinary Chamber as well as by the University of Veterinary Sciences and Pharmacy in Kosice, which modified education syllabus, in which veterinary aspects of wild life management is a compulsory part for each student. Institution has its own game farm and in cooperation with Bird Life Slovakia is running a wild life rehabilitation center.



Fig. 1. Transportation of breeding stag –red deer in game farm.



Fig.2. Immobilization of wild feral vison in Poloniny NP.

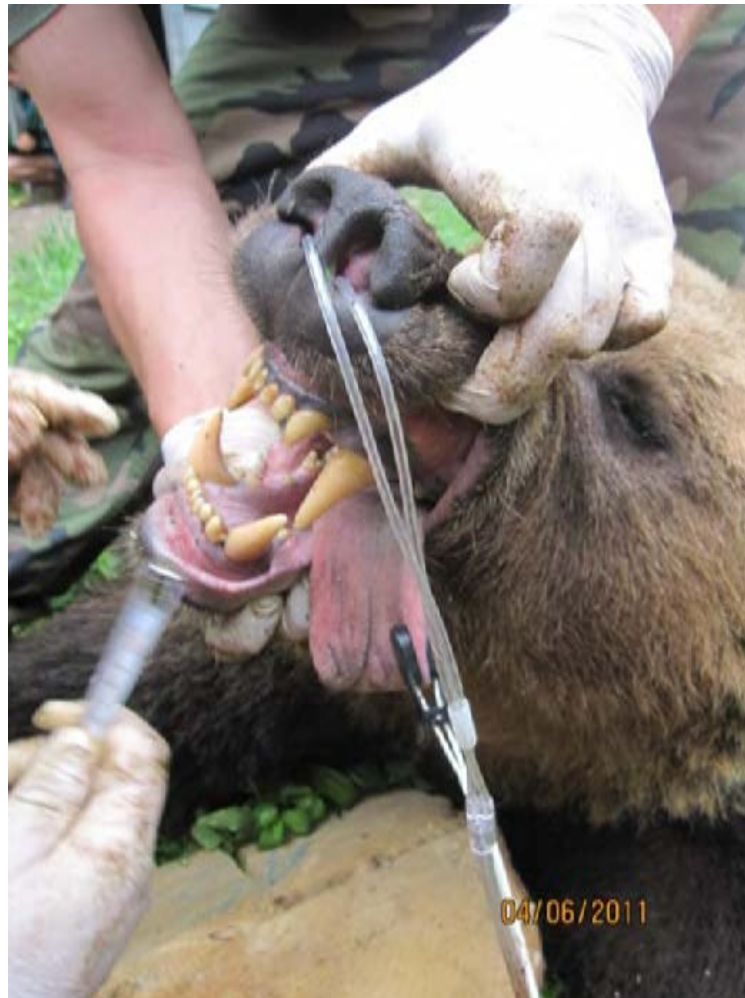


Fig. 3. One of 25 brown beard immobilized for telemetry monitoring.



## **The ecological drivers of avian influenza outbreaks in Europe**

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**Abstract:** Avian influenza viruses (AIV) are not only a threat to global poultry production, but they represent a public health concern, given their potential to cause severe disease in humans. The natural reservoir of AIV is the waterbird ecosystem, where virtually all of the possible AIV subtypes exist in the low-pathogenicity (LP) form. In particular, the main orders involved in the AIV transmission and maintenance are Anseriformes and Charadriiformes. In this system, infections are characterized by low virulence and high survival rates and the circulation of viruses is regulated by a complex interplay between immunity, host preference and the environment. On the other hand, the direct or indirect contact between an AIV infected wild bird and poultry might lead to the adaptation of a virus to one or two poultry species (usually chicken and turkey) and its rapid spread across a naïve population of animals. Under these conditions and in particular, under intensive farming regimes, viruses of the H5/H7 subtypes that are introduced as LP can acquire specific mutations resulting in the emergence of highly pathogenic AI (HPAI) viruses. In 1996, this paradigm was challenged by the emergence of a HPAI H5N1 in geese, in China, where the largest population of domestic ducks and geese resides. The virus caused little sign of infection in these species and this feature together with other factors, such as the growing trade of live poultry and poultry products, the presence of live bird markets and poor biosecurity standards contributed to its spread from China across Eurasia and Africa, and to its current state of endemicity after 2 decades in many of these regions. One of the most intriguing features of this virus was its ability to cause illness and death in wild birds and yet spread via migratory birds, as demonstrated by the 2005/6 European wave of HPAI H5N1 introduced from birds flying from the Qinghai lake in China. H5N1 proved to be unusually promiscuous in forming novel genotypes through recombination with other LPAI viruses. From this ancestor, many H5NX HPAI viruses, have been circulating in poultry and occasionally among waterfowls, subsequently disseminating to different countries, causing multiple epidemic waves. Two years ago, Europe experienced an unprecedented epizootic, counting more than two thousand outbreaks ignited in large part by multiple introductions of H5N8 HPAI from migratory birds and resident populations of wild birds. In this new epidemiological perspective, understanding the role of wild birds in avian influenza ecology has become strategic to prevent and control future epidemics. New technologies are now available to translate mapping and sampling of wild birds in Europe and Asia into prediction tools of HPAI introduction. Realtime tracking of migratory birds, bird and virus identification through biomolecular methods, as well as climatological monitoring represent some of the tools that can feed advanced mathematical modelling to predict the spread of complex diseases such as HPAI. Risk-based surveillance and the implementation of preventive measures will highly benefit from a deeper understanding of AI in its wild reservoir. AI viruses and their epidemiology are showing an impressive ability to adapt to a rapidly changing environment. Virologists, health authorities and the poultry industry are now called to face this challenge with a novel ecological approach.

**Keywords:** avian influenza, ecology, wild birds

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## Oral communications

### Studies on the ectoparasitic flies of Genus *Lipoptena* in Central Italy: the case of an adventive species, *Lipoptena fortisetosa* (Diptera: Hippoboscidae)

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**Abstract:** Hippoboscids are hematophagous flies that parasitize various species of domestic animals and wildlife. The representatives of the genus *Lipoptena* live on cervids although can accidentally infest other species. In the last decades the presence of ungulates in Italy has greatly increased causing several management problems and a consequent expansion of their parasites (ISPRA, 2013). Even if the knowledge about these insects remains sparse, they are important under a sanitary point of view because can occasionally attack humans. These affect hosts causing sickness and stress and maintaining the transmission of pathogens and zoonoses (i.e. Borreliosis, Anaplasmosis, Tripanosomiasis) (Dehio *et al.*, 2004, Härkönen *et al.*, 2009, Lee *et al.*, 2016). Until 2017, *Lipoptena cervi* (Linnaeus, 1758) was considered the only species of the genus *Lipoptena* present in Italy, but during a research conducted in Tuscany, *Lipoptena fortisetosa* (Maa, 1965) has been recorded in the Country for the first time (Andreani *et al.*, 2018). This species is native to Japan and its presence could be due to the introduction in Italy of *Cervus nippon*, the original host, recently identified in some Italian provinces (Ferri *et al.*, 2016). Based on this new report, a survey was planned in order to determine the spread of *L. fortisetosa* and to increase the knowledge of this species that has never been studied in detail. In particular, the research focuses on the presence of the parasite in some areas of Central Italy, with the aim of evaluating its preference in parasitizing different ungulate species. Specimens of *Cervus elaphus*, *Capreolus capreolus* and *Dama dama* hunted from 2017 to 2019 in some provinces of Tuscany and Emilia-Romagna were analyzed and Hippoboscids were collected from their fur. The ungulates were homogeneously grouped into females and fawns, subadults males and adult males, in order to highlight any possible difference in the process of the host selection. The examined subjects hosted a great number of ectoparasites, most of which were *L. fortisetosa*. Each analyzed species carried parasites, but specimens of Red deer (*C. elaphus*) were particularly infested, suggesting the preference of the parasite for this ungulate. Results have been discussed on the basis of parasite distribution and its interaction with the hosts. The study shows that *L. fortisetosa* seems to strongly compete with the native *L. cervi* and it can be assumed that the species is currently spreading into a new area adapting to other hosts. This research has revealed the need to intensify parasite control by monitoring wildlife. The finding of a new parasite in Italy is particularly worthy of attention as *L. fortisetosa* is potentially harmful for human health, indeed, further investigations are ongoing to study this aspect.

**Keywords:** Deer keds, ectoparasitic diptera, wildlife, parasitology, allochthonous species, Hippoboscidae

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## **Tuberculosis in wild boar (*Sus scrofa*) in Calabria Region (Southern Italy)**

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**Abstract:** Tuberculosis (TB) caused by *Mycobacterium tuberculosis* complex (MTC) that infects different domestic and wild species, is one of the most critical zoonoses in the world. Wild boars have mainly been infected by *M. Bovis*, *M. caprae*, and *M. microti*. In Italy, national surveillance programs are running based on organ/meat inspection and an intradermal tuberculosis test (IDT), the last carried out *in vivo*. It is now established that wild boar (*Sus scrofa*) are responsible for the propagation of various infection diseases both in farm animals and in humans. The data of TB distribution in wild animals in Vibo Valentia (VV) province (Calabria region, Southern Italy) are very few and underestimated. This work aimed to carry out a study on the prevalence of TB in wild boar regularly killed on VV province during the 2017-2018 and 2018-2019 hunting season. The VV province extends for 1150.64 km<sup>2</sup> and borders with provinces of Catanzaro and Reggio Calabria. In this area, 955 wild boars were inspected in the 2017-2018 hunting season and 1926 in the 2018-2019 hunting season. According to the World Guide for Animal Health (OIE), the detection of TB was performed by a detailed *post-mortem* examination in order to assess the presence or absence of TB compatible lesions followed by Polymerase Chain Reaction molecular tests and bacteriological culture and isolation (reference standard test) performed by Istituto Zooprofilattico Sperimentale del Mezzogiorno in Catanzaro. Results showed a TB prevalence of 2.51% (24/955) in the 2017-2018 hunting season and 0.42 % (8/1926) in the 2018-2019 hunting season. Over the last few decades the number of wild boar in Calabria has increased enormously. The relationship between the persistence of infection diseases and the constant-increasing number of wild animals has been documented. The assessment of TB in wild animal species is an event that must take into account the possibility of contagion between wild and domestic animals that coexist in the same area. Although not high, the prevalence of tuberculosis in wild boar, highlighted in this study, can pose a danger for cattle, making ineffective the plan for the eradication of bovine tuberculosis in Calabria provided for by the Decree n. 118 of 25 November 2011.

**Keywords:** wild boar, tuberculosis, Calabria region, zoonotic infection, bovine pathogen

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## **Epidemiological and molecular survey on *Toxoplasma gondii* in wild boars from Campania region (southern Italy)**

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**Abstract:** *Toxoplasma gondii* is a widespread protozoan parasite belonging to the phylum Apicomplexa that causes a zoonotic parasitic disease known as Toxoplasmosis. In Europe the presence of this protozoa has been reported in wild boars however, in Italy epidemiological data regarding this parasite in this ungulate are scant. The aim of this study was to evaluate the prevalence of *T. gondii* in

wild boar population in southern Italy and to assess the infection risk for the consumer. A total of 338 wild boar carcasses, collected from different boar hunting areas, were inspected during the hunting season 2018 by 51 veterinary involved in the regional project “Piano Emergenza Cinghiali Campania”. Boar origin (hunting area), gender and age were recorded for each animal. Hundred seventy-six (52.1%) were males while 162 (47.9%) were females. Boars’ age was estimated by teeth examination and animals were classified in three age classes: piglets (<1 year) (n° 17 – 5%); yearlings (1-2 years) (n° 178 – 52.7%) and adult (>2 years) (n° 143 – 42.3%) with a mean age of 2.6 years. A specific molecular diagnosis was performed by real-time PCR on 884 different tissues (263 brains, 310 hearts and 311 masseter muscles). PCR analysis showed the presence of *T. gondii* in 134 (72 males and 62 females) out of a total 338 wild boars (39.6%). The rate of infection was of 47.1%, 39.3% and 39.2% in piglets, yearlings and adults, respectively. No significant statistical difference between the three age classes was found ( $\chi^2=0.41$ ;  $P=0.814$ ). The highest prevalence of *T. gondii* was found in masseter muscles (n° 74/311 – 23.8%) followed by heart (n° 70/310 – 22.6%) and brain (n° 58/263 – 22.0%). No significant statistical difference between the tissues were found ( $\chi^2=0.26$ ;  $P=0.876$ ). This study confirmed the presence of *T. gondii* in wild boar population in Campania region. The high prevalence found in the masseter muscle underlines the necessity of veterinary controls of wild fauna, considering that this muscle is used to prepare a raw meat product (guanciale) which may represent an important source of infection of *T. gondii* in humans. Elucidations on the role of wild boars in the epidemiology of *T. gondii* and its zoonotic potential requires further investigations.

**Keywords:** *Toxoplasma gondii*, Wild Boar, Real-time PCR, Italy, Epidemiology, Zoonosis, Hunting.

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### ***Taenia hydatigena* cysticercosis in wild boars (*Sus scrofa*) from southern Italy: epidemiological survey**

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**Abstract:** *Taenia hydatigena* cysticercosis, due to *Cysticercus tenuicollis*, is a widespread parasitic disease infecting domestic and wild animals. Cysticercosis leads economic and productive losses in industrialized and developing countries. Several studies have investigated cysticercosis in domestic pigs, however, data regarding wild boars are scant. In the last years, wild boar populations in Europe, as in Italy, were increased and this ungulate could play an important role in the spreading of cysticercosis in hunting dogs and wildlife. The present survey aimed to determine prevalence and diffusion of *Taenia hydatigena* cysticercosis in wild boar population of Campania region, southern Italy. Post-mortem exams were performed by 51 veterinary practitioners involved within the regional project named “Piano Emergenza Cinghiali in Campania”. A detailed form was collected including data on hunting area, gender, and age of the boar obtained. Boar’s age was estimated by the examination of the teeth. All viscera were examined to determine number and location of the cysts, which were morphologically identified. Out of a total of 3363 wild boars examined, 229 (6.8%) harboured *C. tenuicollis* cysts. Positive boars were 120 males and 109 females, respectively. The age of positive boars ranged from 1 to 9 years (average 1.3). Boars harbouring single cysts were 188 (82.1%), whereas 41 (17.9%) more than one. Most of the positive boars, 187 (81.7%), had cysts on

liver; 14 (6.1%) on omentum, 9 (3.3%) on spleen, 5 (2.2%) on lungs, 3 (1.3%) on diaphragm, and 1 (0.4%) on large intestine. We also found 7 (3.1%) and 3 (1.3%) wild boars with liver-lungs and liver-omentum coinfections, respectively. The total number of cysts collected from positive boars was 301. Most of the cysts were large, with an average size of 5 cm in length and a long neck. Linear measurement of the larval hooks was conducted for 100 liver cysts, showing a mean of length of the large and small hooks as 200.3µm and 136.8µm, respectively. Our findings suggest that the wild boar could be involved in the epidemiology of *T. hydatigena*, due to the significative amount of boar raw offal available to hunting dogs, foxes and wolves, during the hunting seasons. Hunting dogs may spread several parasites and pathogens that have a high economic impact on farmers and pose a significant health risk to domestic animals. Elucidations on the role of wild boars in the epidemiology of *Taenia hydatigena* cysticercosis in Italy requires future molecular updates.

**Keywords:** Cysticercosis, *Taenia hydatigena*, *Cysticercus tenuicollis*, boar, Italy.

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### **Evaluation of intraocular pressure in healthy Land Turtles (*Testudo hermanni*) during hibernation and at the awakening**

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**Abstract:** The so called “erroneous-management diseases” are the most frequent diseases associated to a not sufficient specie-specific knowledge of turtles and other kind of reptile. In recent process of taming of turtles, the eyes suffering rates seems to be increased due to the wrong owners management. The climate changes, the habitat’s segmentation and the defective trade put some species of reptiles, in a grave danger of extinction and studies involving endangered species as *Testudo hermanni*, can contribute to the surviving of them. This study is focused on the evaluation of intraocular pressure (IOP) in healthy land turtles (*Testudo hermanni*) during hibernation and at the awakening, in order to determinate the physiological values of IOP in this species, using the Rebound Tonometry technique. The background of our investigation was the study of Zouache MA, Eames I. and Samsud A. (2016) who described the physiological mean values of the IOP and Aqueous Humor Flow Rate in the five Classes of Vertebrate Eyes, using TonoVet®. Reptiles showed an average IOP value of 10.07 mm Hg. Selleri P. (2012) describes the average IOP values in the *Testudo hermanni* in the period before the lethargy. The average values obtained through TonoVet®, using the P setting for undefined species, was  $15.74 \pm 0.20$  mm Hg for this species, with a range from 9 to 22 mm Hg. Twenty-one Hermann tortoises were tested (11 females and 10 males) between the last week of February and the first week of March 2019. The subjects were legally held by private individuals and housed in a *terrarium* (two) or were free in the gardens (19). Measurements were made during hibernation (14) or a few days after awakening (7). The average of IOP values, using the D (dog) setting, (42 eyes) was  $16.56 \pm 3.63$  mm Hg (right eye 16.10 mm Hg; left eye 17.00 mm Hg). In the subjects examined during hibernation, no significant difference was found between the values of IOP right vs left ( $p = 0.71$ ). Likewise, in the subjects examined on awakening ( $p = 0.16$ ). Furthermore, the differences between the results obtained in the awake and in the lethargic subjects ( $p = 0.74$ ) and between males and females were not significant ( $p = 0.24$ ). Compared to the study carried out by Selleri (2012) in the pre-hibernation period ( $15.74 \pm 0.20$  mm Hg) and with P setting, our results show slightly higher values ( $16.56 \pm 3.63$  mm Hg) and this could indicate differences with the setting of tonometer or with the phase of hibernation or awakening.

**Keywords:** reptile eyes, Tonometry, IOP, *Testudo hermanni*, physiological parameters, hibernation parameters.

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## Epidemiological and molecular survey on cystic echinococcosis in wild boars (*Sus scrofa*) from southern Italy

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**Abstract:** Cystic echinococcosis (CE), is one of the most important parasitic zoonotic diseases in the world and also represent an important public health and socio-economic concern. In the Mediterranean basin, CE is widespread and in Italy it is endemic, with a major prevalence in the south and islands. In the last few decades, the wild boar population in Italy has increased thus promoting the spread of CE in the wild. Here we report an epidemiological survey on *Echinococcus granulosus* sensu lato (s.l.) in wild boars obtained during hunting seasons 2016-2017 in the Campania region of southern Italy. The carcasses of wild boars, collected from different boars hunting areas, were examined during two hunting seasons by 51 veterinary practitioners involved in the regional project “Piano Emergenza Cinghiali Campania”. Wild boar origin, gender and age were recorded per each animal. When cysts were found, their number, morphology and fertility were determined by visual and microscopic examination. Protoscoleces and germinal layers were collected from individual cysts and DNA was extracted. A specific molecular diagnosis was obtained by sequencing PCR-amplified mitochondrial genes encoding for the NADH dehydrogenase subunit, ATPase subunit 6, NADH dehydrogenase subunit 2 and partial cytochrome c oxidase subunit 1. An overall 4.4% prevalence of *E. granulosus* s.l. was found in the 2,108 wild boars examined, with a fertility rate of 1.0% (CI 95% 0.5-1.3). CE was found in 48.4% of males and in 51.6% of females, aged between 1 to 8 years. The average number of hydatid cysts per infected wild boar was 1.3 (min 1 - max 13). The total number of cysts collected was 123, of which 118 (95.9%) were located in the liver, 4 (3.3%) in the lungs, and 1 (0.8%) in the spleen. Of all the cysts analyzed, 70 (56.9%) were fertile and 53 (43.1%) were sterile/acephalous. Molecular characterization revealed the presence of *E. granulosus* sensu stricto (20.7%) and genotype G7 (65.5%) in the wild boars harboring fertile cysts examined ( $\chi^2 = 10.12$ ;  $P = 0.0014$ ). In contrast with previous studies, more frequently showing a tropism of G7 for pulmonary parenchyma, the molecular characterization carried out in the present survey showed G7 only in hepatic cysts (19/29). No animal was found to be infected with both parasites (*E. granulosus* s.s. and G7). These results document the prevalence of *E. granulosus* infection and reveal, for the first time, the presence of the pig strain (G7) in wild boars from Italy. The role of G7 in the epidemiology of CE in Italy and its zoonotic potential requires further investigations.

**Keywords:** Echinococcosis, Hydatid, genotyping, boar, Italy.

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## **Livestock and wildlife: study about the presence of *Dichelobacter nodosus* in wild ungulates in Central Italy**

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**Abstract:** Wild ungulates population is impressively increasing in Europe and Italy mainly due to a marked forest expansion and a subsequent increase of trophic resources and habitat. The relationships between domestic and wild ungulates have been progressively investigated as infectious diseases at the wildlife–livestock interface represent a threat to health and well-being of wildlife, domestic animals, and human populations. Footrot is a highly contagious disease of domestic and wild ungulates, caused by *Dichelobacter (D.) nodosus*, an anaerobic, Gram negative rod. The disease, endemic in many countries worldwide, represents a major cause of economic losses and welfare concern for the sheep industry. Clinical lesions range from a mild interdigital dermatitis (benign footrot) to a complete separation of the horn from the underlying tissues (virulent footrot). Clinical observations of epidemic pododermatitis have been reported in European mouflon (*Ovis musimon*) and Alpine ibex (*Capra ibex*) populations in different European countries including, but *D. nodosus* has rarely been demonstrated. The epidemiology of the disease has not been thoroughly investigated yet, but it is reasonable to assume that a transmission between domestic and wild ruminants can occur. The aim of this study was to investigate the presence of *D. nodosus* in wild ungulates (*Capreolus capreolus* and *Dama dama*) in Umbria region (Italy). During 2018 a total of 48 roe deers and 3 fallow deers, hosted in a Wildlife Rescue Center, were sampled using a one 4-feet model of the same animal and analysed using duplex Real-Time PCR. Of the 51 foot swabs sampled, none yielded evidence for *D. nodosus* strain and no lesions were found at feet examination. The present study represents the first preliminary investigation about the presence of *D. nodosus* on wild ungulates in Italy and their potential role in the dynamics of the infection. The presence of *D. nodosus* in wild ungulates in Europe and closer in Switzerland has been demonstrated but there is still a gap of knowledge in the epidemiology which need further and deepened investigations. Virulent footrot is a notifiable disease in some states and eradication and control programs can vary. The identification of transmission routes and bacterial reservoirs in the environment are a critical point for the control of the disease. The transmission of footrot between wild and domestic ungulates may result in severe consequences on both populations. For these reasons, authors strongly encourage an active sampling protocol throughout a multi-actor approach involving health organizations, wildlife and hunting associations to sample culled and rescued animals to totally understand the epidemiology of footrot and the reservoir species.

**Keywords:** *Dichelobacter nodosus*; *Capreolus capreolus*; *Dama dama*; Italy

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## **Preliminary results on the study of field isolates detected between 2017-2019 of canine distemper virus circulating in wild animals of the Regions Latium and Tuscany, Italy**

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**Abstract:** Canine distemper virus (CDV) is a frequent cause of disease, often fatal in domestic and wild carnivore and non-carnivore species, causing respiratory, enteric and neurological clinical forms. In Italy, different CDV strains were described by various authors (Martella *et al.* 2002, Balboni *et al.* 2014, Di Sabatino *et al.* 2016). From 2017 to March 2019, the Istituto Zooprofilattico Sperimentale for Latium and Tuscany collected samples of 79 wild animals belonging to different CDV susceptible species (47 foxes, 22 wolves, 5 badgers, 2 raccoons, 1 ferret, 1 mongoose, 1 marmot) found dead or killed to define which strains were circulating in these Regions, as limited relative data is available. This study was conducted within the framework of a research project funded by the Italian Ministry of Health (IZS LT 13/15 RC). When present, brain, lung, urine and nasal, conjunctival, rectal and bladder swab, and intracardiac clot were collected from each animal. The viral RNA was extracted using the QIAamp Cador Pathogen Mini Kit (QIAGEN) and used as template for the PCR real time method as described by A. Scagliarini *et al.* (2007), which amplifies a fragment of the phosphoprotein gene, using AgiPath Universal Master Mix (Applied Biosystems™, ThermoFisher Scientific). Of the 79 subjects examined, 11 (nine foxes and two wolves) were detected as positive, for at least one of the organs examined. For the phylogenetic analysis, the extracted RNA was reverse transcribed in cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems™, ThermoFisher Scientific). The amplicon was subsequently sequenced for the characterization of the viral strain detected, based on the nucleotide sequence of the 287 bp fragment, present at the amino-terminal region of the nucleoprotein gene (Verna *et al.*, 2018). All the amplified sequences showed a sequence identity between 99.48% and 100% and a 100% query cover with the sequence KX545421.1, corresponding to strain CDV599/2016, detected already in February 2016 in Abruzzo Region (Di Sabatino *et al.* 2016). In the present study, this strain was detected from wild animals of the provinces of Arezzo for Tuscany and of Rome and of Rieti for Latium.

**Keywords:** Canine distemper virus; circulating strains; wild animals; phylogenetic analysis; Tuscany and Latium regions

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## ***Hepatozoon canis* in hunting dogs in Campania Region**

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**Abstract:** *Hepatozoon canis* is the most widespread haemoprotozoan organism of the genus *Hepatozoon* that infects domestic and wild carnivores in Europe. Climate change and urbanization of rural areas have enhanced contact between wildlife and domestic animals, increasing the spread of pathogens and the diffusion of vector borne disease (VBD). However, health monitoring of wildlife is difficult, so hunting dogs play an important role as sentinel in control of circulating diseases in wild carnivores and the real risk of infection for domestic dogs. The main vector of *H. canis* is represented by the brown dog tick *Rhipicephalus sanguineus* sensu lato and the transmission to dogs occurs through the ingestion of the infected arthropod vectors. The aim of the present survey was to determine the prevalence of *H. canis* in hunting dogs living in Southern Italy and assess the related risk factors. Whole blood samples were collected from 1,433 healthy dogs in the Napoli, Avellino and Salerno provinces of Campania region (Southern Italy). Samples were tested by quantitative real-time polymerase chain reaction (qPCR) assays to amplify the DNA of *H. canis*. PCR results showed 200 positives, with an overall prevalence of 14.0% (CI 12.2–15.9). Hound breed dogs (P <.0001), medium (OR 1.73; 95% CI 1.15-2.61) and long hair coat (OR 1.89; 95% CI 1.00-3.55), *Ehrlichia canis* PCR positivity (OR 12.21; 95% CI 4.73-31.49) and living in Salerno province (OR 5.49; 95% CI 3.55-8.38) were identified as risk factors. In contrast, dogs living in the urban area of Naples showed the lowest risk for *H. canis* infection (OR 0.03; 95% CI 0.002-0.055). In our study population most of the dogs (98.4%) were treated with ectoparasiticide molecules; however, compared to previous studies on different tick-borne diseases (TBDs) in hunting dog population living in the same area, *H. canis* was the pathogen agent with the highest prevalence. This higher prevalence can be probably due to the high abundance of *R. sanguineus* s.l. and the different route of transmission. In fact, the dog could ingest an infected tick before it has the chance to make a blood meal and then transmit other pathogens and the close contact with wildlife could be associated also to the acquisition of *H. canis* infection through carnivorism. In conclusion, hunting dogs in Southern Italy are exposed to *H. canis* infection and the prevalence of *H. canis* infection is greater than other TBDs. Further studies are necessary to understand the epidemiological and clinical aspects of the protozoan infection in this specific canine population and clarify the parasite transmission modalities related to relationship between hunting dogs and sympatric wildlife populations.

**Keywords:** *Hepatozoon canis*; Hunting dogs; Wildlife; Tick borne disease

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## FLOTAC for copromicroscopic diagnosis in reptiles

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**Abstract:** The FLOTAC techniques are a series of multivalent copromicroscopic techniques initially developed for diagnosis of parasitic diseases of livestock, but more recently extended to traditional and unconventional pets, including reptiles. Active and passive surveillance is ongoing at CREMOPAR to monitor the parasitological scenario in snakes, lizards and tortoise. The FLOTAC Dual technique with analytic sensitivity of 2 eggs/larvae/(oo)cysts per gram (EPG) of faeces is routinely used to investigate about helminths and protozoa. An interesting study was conducted on 150 reptiles (lizards and snakes). All the reptiles were asymptomatic and hadn't received any antiparasitic treatment. Snakes diet included rodents, chickens and rabbits; lizards were fed mainly with vegetables and arthropods. Out of the 150 samples examined, 66 (44.0%) showed the presence of parasitic elements (eggs, oocysts, larvae), specifically 16 (64%) out of the 25 lizard examined and 50 (40%) of the snakes examined. Among the positive samples, multiple parasitic infections were found in 5 (31.2%) of the 16 lizards and 10 (20%) of the 50 snakes tested. The most frequent parasites were: Oxyurids, *Rhabdias*, *Kalicephalus*, *Capillaria* and Eimeriidae. Pseudoparasites, specifically, eggs of *Myocoptes musculus*, *Trichuris muris*, *Hymenolepis nana*, *Aspiculuris tetraptera* and *Syphacia obvelta* were found in 49 (39.2%) of the 125 snakes tested and eggs of mites were found in 3 (12%) of the 25 lizard examined. Another study was conducted on 24 Italian wall lizards, 20 belonging to the subspecies *Podarcis sicula klemmer* (insular population), and 4 individuals belonging to the species *Podarcis sicula* (mainland population) confined to a small, 1 km<sup>2</sup> large islet, Licosa, off the western coast of Italy. This study highlighted the presence of various parasites in particular eggs of pinworms (20/24; 83.3%), *O. natricis* (12/24; 50%), and Dicrocoelidae (6/24; 25%) as well as oocysts of coccidia (11/24; 45.8%). Moreover a survey of the prevalence of oxyurids in 53 tortoises, belonging to five species (30 *Testudo hermanni*, 13 *Testudo marginata*, 7 *Testudo graeca*, 2 *Testudo horsfieldii*, and 1 *Geochelone carbonaria*), was conducted in southern Italy. Eggs of oxyurids were recorded in 43 out of the 53 tortoises examined (81.1%). The use of FLOTAC for diagnosis of parasitic infections in reptiles has demonstrated to be a rapid and sensitive test to improve diagnosis and acquire information on the parasitological fauna of reptiles in order to plan efficient control strategies.

**Keywords:** FLOTAC, reptiles, parasitological fauna, lizard, Italy

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## Forensic approach on the livestock predations

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**Abstract:** In recent years, and particularly in Italy, the conflict between livestock breeders and wolves has intensified to such an extent that the Italian Government has proposed the killing of dangerous animals. However, the state of protection in force in Europe for *Canis lupus* and the difficulties in distinguishing the predations operated by the wolf or by other canids has, for the moment, stopped the possibility of shoot to predators. In this context, the need to establish a certain death's cause of the

preyed livestock has stimulated the present work. At the moment we are still far from having a viable solution to establish a relationship of collaboration between the shepherds and the territorial management Entities. In a period ranged between 2010 and 2016, 4,679 reports of livestock predation, were classified and studied in three Italian protected areas (Aurunci, PNCVDeA, PNALM) and one Spanish hunting reserve (Vidriales). The results obtained show that 82% of predations can be attributed generically to canids while, the remaining 18% was classified as doubtful, unverifiable or not attributable to canids. The reported attacks involved, in decreasing order, sheep (41%), goats (30%), cattle (15%), horses (14%). The attacks attributed to the canids were 87% respectively in Vidriales; 70% in Aurunci; 62% in the PNALM; 51% in the PNCVDeA. Out of 270 certain reports of attacks due to canids, the wolf was recognized as the responsible predator in 72.4% of cases and the dog in 13.8%. In 13.8% it was not possible to go beyond the judgment of an undetermined classification of canids. The periods of attack on the herds were different for species: between February and June in horses; between January and April in the goats; between April/May and September/October in the sheep; between May-September/October (pasture, mountain pasture) in the cows. The period of greatest concentration of attacks (80%) was that of late spring/summer (May-September). The choice of prey is different between wolf and dog ( $P < 0.01$ ), and respectively sheep (68% vs 95%); goats (28% vs 34%); cattle (3% vs 1%). With regard to the time band, the attacks occurred at night in 59% in the case of the wolf and in 81% of the dog ( $P < 0.05$ ). At the time of the attack, 90% of the animals were free, 2.5% in hurdling and 7.3% in pens ( $P < 0.01$ ). In conclusion, although it appears necessary to make a death's diagnosis of the preyed livestock, the latter is not always possible. In fact, the verification of predations may be too late and the cadavers may be deteriorated (putrefaction) or consumed in stages following predation by necrophagous opportunists. The confusion of the anatomo-pathological situation can be resolved by the veterinarian specialist who uses recognized intervention protocols. Ultimately, only the determination of the real death's causes can contribute to the protection of *Canis lupus* even in the Italian territories.

**Keywords:** livestock predation, wildlife management, necropsy, dead diagnosis, wolf.

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### **Circulation of Hepatitis E virus in wild boar population in Central Italy**

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**Abstract:** Hepatitis E virus (HEV) is an RNA virus causing an acute generally self-limited disease in humans. Over the last 10 years in Europe an increasing number of autochthonous cases linked to foodborne transmission of HEV genotype 3 have been reported. Pigs and wild boars are considered the main reservoirs. Human cases have been frequently linked to the consumption of raw or undercooked pork products and wild boar meat. In this study, we sampled 92 and 116 wild boar (*Sus scrofa*) livers during the hunting seasons 2016-2017 and 2017-2018, respectively. HEV RNA was detected in 62 livers with prevalence ranging from 0.0% to 65.7% depending on the hunting area. Phylogenetic

analysis showed that strains clustered within the different subtypes HEV-3c, HEV-3f and other clusters not assignable to any subtypes described so far. The latter could be considered novel subtype. The main result of sequencing was the wide heterogeneity of circulating strains, reporting the result to the confined area investigated ( $\approx 720$  km<sup>2</sup>). Animals possibly belonging to the same family group hunted by the same team were infected with a unique strain (100% nucleotide identity). Since wild animals are a proven source of HEV transmission to humans, results obtained underlined the risk of consuming raw or undercooked wild boar meat and thus this subject deserves further investigations. Furthermore, the occurrence of such variable HEV strains in the wild boar population investigated highlights the importance of the wild animals as reservoir of the zoonotic HEV genotype.

**Keywords:** hepatitis E virus, wild boar, genotype 3, foodborne, zoonoses

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### **Influence of the drought in the wild boar Tuberculosis-like lesions incidence: a pilot-study in Idanha-a-Nova (Portugal)**

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**Abstract:** Animal Tuberculosis (TB) is a chronic zoonotic disease caused by Mycobacteria species that belongs to the Mycobacterium tuberculosis complex (MTC). In this Peninsula there are different prevalence of TB for each region, being the region of Mediterranean climate the most affected. The Mediterranean Iberic regions have a favorable ecosystem to MCT bacteria development, where habitat, population dynamic and climate are important factors to the high prevalence of TB in the wild boar. Our objective was studying the effect of food source shortage, as occurs in dry season, on Tuberculosis-like lesions (TBL) dynamic in Idanha-a-Nova wild boar population, during 9 hunting seasons (2008/09 to 2016/17). The risk factor "previous hunting season with periods of drought" revealed a statistically significant association ( $p < 0.05$ ) with the prevalence of TBL in wild boar in Idanha-a-Nova county during the 9 hunting seasons; being found that in hunting season preceded with by periods of drought, the probability of TBL prevalence in wild boars was 1,2 (OR = 1,2) times higher than when no have periods of drought. The climate influences some environmental characteristics, which in turn influences the TB dynamic in the wild boar population. It is evident an influence on the *M. bovis* persistence in the environment, amount of food available, animal body condition and on the anthropogenic actions (for example, the manager of artificial food supplementation to game differ seasonally). Our study contributes to pointing out the importance of periods of drought in the increase the TBL occurrence in wild boars inspected in field in the sequent season. This result allows to advice, in time, hunting managers to adopt extra protective measures every time they are aware of the presence of periods of drought. This climate trait can become an alert sign for the increase of TBL occurrence in the next season allowing the implementation of feasible an effective measure in time to control TB in wild boar population.

**Keywords:** wild boar, *M. bovis*, Tuberculosis, drought, Portugal, Mediterranean climate

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**An overview on Wildlife Rescue and rehabilitation Centre “CRAS Federico II”**

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**Abstract:** The aim of this study was to describe the main activities of the wildlife rescue and rehabilitation center “CRAS Federico II” of Naples during year 2018. A total of 1912 animals were admitted in the centre; n=91 were mammals, n=44 reptiles, n=309 raptors, n=691 passerines, n=46 waterfowl (Anatidae, shorebirds, ardeids), n=470 synanthropes (laridae, corvids, columbidae) and n=261 animals including coraciformes, apodiformes, galliformes, bucerotiformes, psittaciformes, ciconiiformes, cuculiformes, caprimulgiformes and piciformes. The main causes of hospitalization are: traumas (25%) including fractures, luxation, and wounds; medical causes (15%) including shock, starvation, infections etc; orphaned juveniles (15%); legal seizures (36%), and other causes as plumage injuries, metabolic disease etc. (9%). The hospitalized animals come from all the provinces of the Campania region. Specifically, 1402 animals come from Napoli, 198 from Caserta, 136 from Avellino, 140 from Salerno and 36 from Benevento area. The average length of permanence is related to the causes of hospitalization and includes: first aid, intensive care and rehabilitation units. In case of trauma, hospitalized animal stay from 7 to 60 days; the orphaned juveniles, from 15 to 50 days; medical causes, from 48 hours to 15 days; seizures, from 48 hours to 180 days; other causes, from 48 hours to 180 days. Excluding the seized animals (n. 685) the total number of hospitalized animals is 1227, of which 56% were reintroduced into the wild, 41% are dead due to serious complications, 1% are long-term patients (more than 180 days) and the 2% are exotic or hybrid subjects whose reintroduction in nature is not possible; these animals were entrusted to the care and detention of private and/or public institutions. In line with the trend of the past years, the admissions are higher from May to August with 936 animals. This trend is due to the reproduction and breeding season with a higher percentage of juvenile subject admitted. The CRAS Federico II is also a center of wildlife epidemiological monitoring for zoonotic diseases (i.e. Influenza virus, West Nile virus, Brucellosis, Tuberculosis) in the Campania Region. Its epidemiological role is represented by collection of several samples derived from carcasses of dead animals and cloacal/oropharyngeal swabs in live animals. Our results could represent a reference for wildlife rehabilitation process in order to develop, with further studies, clinical guidelines and recommendations that will lead to an improvement of the knowledge of this complex process.

**Keywords:** wildlife; rescue and rehabilitation center; hospitalization causes; animal care; public health

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## **Benzodiazepine delorazepam interferes with the early developmental stages of sea urchin, a model marine invertebrate**

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**Abstract:** Sea urchins are benthic organisms typical of coastal marine areas. Here they form extensive beds that provide food and shelter to a rich vertebrate and invertebrate community. The coastal waters, however, are those suffering the strongest anthropic impact: hence it is fundamental to know the effects that various contaminants have on coastal communities. In this study the potential impact of a benzodiazepine, delorazepam, on early developmental stages of the common sea urchin *Paracentrotus lividus* was addressed. Four experimental treatments were carried out: delorazepam was added at a concentration of 1 µg/L to the sperms or the eggs before being used for fertilization, to the sperms and eggs at fertilization or to the zygotes soon after fertilization. Development was followed up to the pluteus stage, and the percentage of delayed and/or degenerated embryos were recorded. Results indicate that spermatozoa are a target for delorazepam and that the action takes a few minutes to be completed. According to the literature the effect would be exerted via interference with mitochondria, probably by reducing sperm motility. However, interference with mitotic spindle and/or GABA receptors at the moment cannot be excluded.

**Keywords:** water contamination, sea urchin sperms, sea urchin eggs, sea urchin zygotes

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## **Rescue protocols for wild animal in case of a fire alert**

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**Abstract:** In the year 2017, the Campania Region (Dept. Veterinary Public Health and Prevention) establishes the Regional Reference Center for Veterinary non-Epidemic Emergencies (CeRVEnE) with the intention of providing the territory of an organizational structure able to furnish the essential levels of veterinary assistance (LEA), in case of disasters. The Directorate for "Training, Information and Applied Research" starts working (2018) in the "Vesuvio area" with the aim of carrying out a program of "Good practices of the services of the Prevention Department for the animals, in case of fire risk". During its first year, the Program named "FRAC1" provided the Dept. Veterinary Public Health and Prevention with a first "context study", starting from the "Vesuvio area", really affected by the fire disaster in 2017. The results described in this work offer an up-to-date picture of early intervention approaches and methodologies, to deal with the risks of exceptional events such as fires; highlight the critical territorial and logistic points during an evacuation consequent to a forest fire (including animals) and indicate the best response capacities of the Campania Region for the return to normality

and the restoration of ecosystems. The protocols proposed tend, in fact, to indicate the best practices for the animals involved in a fire, ensuring their interventions and assistance in compliance with the rules on animal welfare but also to protect human health (food supply chain management and food safety). Compared to the expected results during the first year, the following were achieved:

- The retrospective analysis of the causes, locations and damage of fires in the "red area of Vesuvius", relative to the incendiary events recorded in the year 2017.
- A methodological proposal to be completed before standardization for data collection.
- A first analysis of the fire and animals (wild and domestic) risk that could be involved.
- A first approach to reach an interface between the bodies involved in the surveillance and monitoring system of fire risk factors.
- the start of information and training days dedicated to the disastrous forest fire event in areas of high human concentration and high urbanization.
- A structure suitable for the assessment of the "Risk assessment" sufficiently valid to organize the second phase of the FRAC Program which will be dedicated to the "business impact analysis".

**Keywords:** Fire risk; Animal rescue; basic health care levels (LEA); disaster; wildlife assistance; Regional Centre for non-epidemic veterinary emergency (CeRVEnE)

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### **Leishmaniasis in Iberian wolves (*Canis lupus signatus*) in Northwest Spain**

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**Abstract:** Canine leishmaniasis (CanL) is a disease with nonspecific clinical signs, precise diagnostic methods are necessary for the identification of the protozoan parasite *Leishmania* spp. The methods most used for diagnosis are based on immunodiagnostic techniques; however, these can give rise to false negatives, in case of seroconversion of antibodies after an infection, or to false positives, when the antibodies remain elevated after successful treatment. The objective of the present work was to develop a quantitative PCR (qPCR) for the non-invasive detection of *Leishmania infantum* in wolf mouth scraping samples. We analyzed samples of wolves from different areas of Zamora (Spain). The technique described here has a high sensitivity, 35 parasites in a buccal swab, and specificity against the parasite. The results were expressed as number of parasites/50,000 cells. Ten samples from wild wolves from Northwest Spain were analyzed. We also evaluated dog prevalence in Castilla y León, where the initial results indicate 33%. Therefore, through this qPCR it is possible to make an early diagnosis of the infection, determine the effectiveness of a treatment or the evolution of a disease, painlessly for the animal. These results allow to show the health of the wolves of Zamora.

**Keywords:** Leishmaniasis; *Leishmania infantum*; wolves; qPCR; diagnosis

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## 1. INTRODUCTION

Canine leishmaniasis (CanL) is a disease caused by a protozoan parasite *Leishmania infantum* and is transmitted by insect sandflies. In some cases, dogs and other wild animals are the main species

affected, while in others – mostly dogs – are considered reservoirs of the disease and are vectors for human transmission (1). The usual practice is the clinical diagnosis of the disease, supported by laboratory tests, which is based on the presence of clinical signs and epidemiological information. However, leishmaniasis is a systemic disease that can affect any organ or tissue and therefore manifests with non-specific clinical signs. Diagnosis of CanL is based on the detection of amastigotes with Giemsa or Diff Quick stains, which involves a swab of lymph node aspirate or examination of skin biopsies, with the complications involved (1, 2). That is why the method most used nowadays is the detection of antibodies by indirect immunofluorescence (IFI) or by the ELISA technique. However, today more sensitive methods based on molecular biology techniques, such as PCR, are being developed. In this sense, quantitative PCR (qPCR) techniques have recently been described in order to compare the parasitic loads of non-invasive (urine and mouth scrapes, conjunctival and vaginal) and invasive samples (bone marrow and blood).

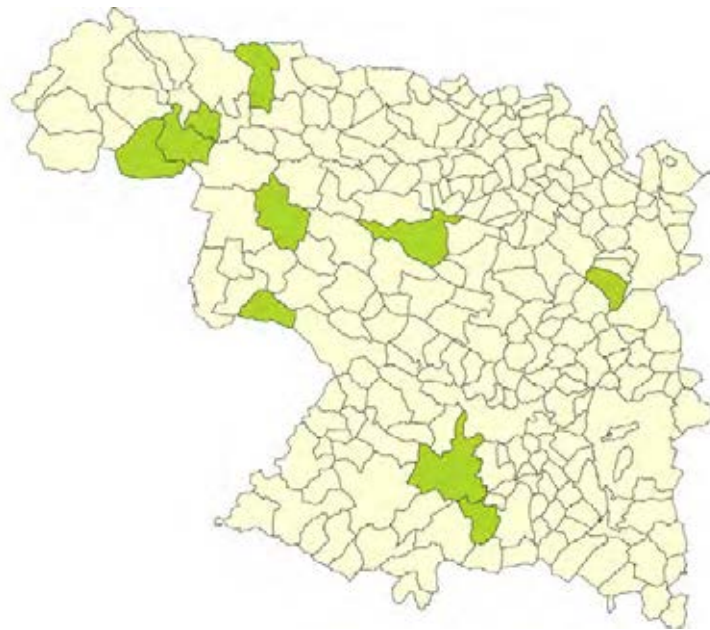


Fig. 1. Geographic origin of wolves. Zamora map.

The Iberian wolf (*Canis lupus signatus*) shows a growing expansion in the Northwest of Spain, colonizing areas in which it had disappeared 50 years ago. The search for food in peri-urban areas, in which the phlebotomine vector abounds, adds a growing social demand in terms of health and safety on the transmissible zoonoses shared with wildlife. A recent study on the prevalence of CanL in Zamora, offers alarming data regarding the existence of the parasite in domestic dogs and its possibility that it can be transmitted to wild species. The Game Reserve of “Sierra de La Culebra”, Castilla y León (Spain) has the largest number of wolves (*Canis lupus signatus*) all over Western Europe. The objective of this work has been to develop a non-invasive qPCR for the easy detection of *L. infantum* in wolf mouth and ear scrapings. In addition, thanks to the use of this technique, epidemiological evaluation of wolf samples from different areas of Zamora has been carried out.

## 2. MATERIALS AND METHODS

### 2.1 Animals in study

During the course of the “Management Plan for Iberian Wolves” of Castilla y León, we had access to samples of live and dead wolves from the Game Reserve of Sierra de La Culebra located in Castilla y León, Zamora (Spain). A total of 11 wolves were selected from the wildlife of Zamora (Figure 1),



collected by veterinary professionals, within a prevalence study for the detection of *L. infantum* started in the Community of Castilla y León.

## 2.2 Sampling

The samples were collected by passing a sterile swab over the internal surface of the buccal and ear epithelia, trying to collect as many cells as possible inside the cheek of the animal by scraping for 1 min (Figure 2). The samples are then kept in the cold chain during transport from the collection area to the laboratory. Once there they were stored at -20°C until the extraction of the DNA.



Fig. 2. Collecting sample.

## 2.3 Development of qPCR technique

For the development of the qPCR method, a pair of primers (Forward: CCCAAACTTTTCTGGTCCTC; Reverse: TTACACCAACCCCCAGTTTC) designed to amplify a fragment of 131 base pairs (bp) corresponding to a conserved region of the minicircle of the kinetoplast of *L. infantum* was used. As an internal control, the number of wolf cells is also quantified by means of the implementation of a fragment of 303 bp of the gene coding for the sodium-calcium exchanger of wolves or wolf  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX1). The qPCR was performed using the 7500 Real Time PCR System (Applied Biosystems) in 96-well plates. The sensitivity of the technique was calculated as the smallest number of parasites that could be detected in a sample. The specificity of the technique was also determined using DNA from samples from dogs not infected or infected by other agents such as *Ehrlichia canis*, which, like *L. infantum*, also infects white blood cells.

## 2.4 Quantification of parasitic load

For the quantification of the parasitic load, as well as the number of wolf cells present per sample, the quantified DNA of a known number of promastigotes was used as a standard sample, for the amplification of the DNA of *L. infantum*, and plasmid DNA of a clone that contained the band that amplified a 303 bp fragment of the NCX1 gene. Serial decimal dilutions were made with each of the samples of the standard DNA to construct a standard line and thus perform the quantification of the parasitic load in each sample. The results are expressed as number of parasites/number of wolf cells.

## 3. RESULTS

The technique presented a limit of detection of 35 parasites for each buccal swab, regardless of the number of wolf cells that were collected in the sample. The specificity of the technique was determined by checking that there were no cross-reactions with either wolf cells or DNA from animals infected with *E. canis*. The qPCR was set up to express the results as number of parasites/50,000 wolf cells. For the interpretation of parasitic loads, it must be borne in mind that a result between 1 and 100 parasites / 50,000 dog cells represents a very low load, probably due to contact with the vector, although without the development of disease. However, from 5,000 parasites/50,000 dog cells in some study clinical signs related to the disease have been observed (Table 1).

Table 1: qPCR results of *Leishmania infantum* infections in wolf samples used in the present study expressed as number of parasites/50,000 wolf cells.

| ID  | Geographic origin                 | Sampling date | qPCR           |
|-----|-----------------------------------|---------------|----------------|
| 203 | Palacios de Sanabria (mouth swap) | 10/1/2018     | Negative       |
|     | Palacios de Sanabria (ear swap)   | 10/1/2018     | Negative       |
| 199 | Puebla de Sanabria (mouth swap)   | 2/3/2018      | Negative       |
|     | Puebla de Sanabria (ear swap)     | 2/3/2018      | Negative       |
| 198 | Lobeznos (mouth swap)             | 17/3/2018     | Negative       |
|     | Lobeznos (ear swap)               | 17/3/2018     | Negative       |
| 225 | Tábara (mouth swap)               | 18/10/2018    | Doubtful (<35) |
|     | Tábara (ear swap)                 | 18/10/2018    | Negative       |
| 229 | Cabañas de Sayago (mouth swap)    | 22/10/2018    | Negative       |
|     | Cabañas de Sayago (ear swap)      | 22/10/2018    | Negative       |
| 233 | Brevanes (mouth swap)             | 22/10/2018    | Doubtful (<35) |
| 227 | Cañizo (mouth swap)               | 23/10/2018    | Negative       |
|     | Cañizo (ear swap)                 | 23/10/2018    | Negative       |
| 194 | Robledo de Sanabria (mouth swap)  | 1/11/2018     | Positive (85)  |
|     | Robledo de Sanabria (ear swap)    | 1/11/2018     | Positive (8)   |
| 234 | Pererueta (mouth swap)            | 4/12/2018     | Negative       |
|     | Pererueta (ear swap)              | 4/12/2018     | Negative       |
| 238 | Mahide (mouth swap)               | 10/12/2018    | Negative       |
|     | Mahide (mouth swap)               | 10/12/2018    | Negative       |
| 232 | Vega del Castillo (mouth swap)    | 10/3/2019     | Negative       |
|     | Vega del Castillo (ear swap)      | 10/3/2019     | Negative       |

## 4. DISCUSSION

Across the area of Mediterranean influence canine Leishmaniasis represents a current issue. Not only because it is an endemic area, but also to the possible contagion to the man from the disease, since it is a zoonosis. The method that is usually used in veterinary clinics is rapid kits by immunochromatography, or the titration of the samples is carried out by means of IFI or ELISA. In all cases, the diagnosis is made by detecting antibodies in the blood. However, immunological assays can lead to false negatives in the first months after infection, because until the seroconversion takes place, the antibodies have not risen sufficiently for detection; or they can also give rise to false positives, when the antibody titer is maintained for a long time after a successful treatment. To avoid this problem, qualitative and quantitative PCRs have been developed that determine the presence of the parasite in different organic fluids. In the present work we give results from the development of a non-

invasive test, which supposes a greater facility in the collection of the sample, and at the same time quantitative, that allows to determine the parasitic load. Despite the little number of wolves analysed, 1 up to 11 was clearly positive after analysing the two samples collected (mouth and ear), representing 9 % prevalence. Taking into account the two doubted sample the prevalence raises until 27%. These results are in accordance with the study performed by Oleaga *et al.* (2018) in Asturias region (North of Spain), who reported that the prevalence of this infection in wolves was 33% by PCR.

## 5. CONCLUSIONS

The use of qPCR for the detection of *Leishmania* DNA in wild carnivores, including 11 wolves, sampled on 2018-2019 provided valuable information about the prevalence and geographic distribution of *Leishmania* in wild carnivores in Zamora (Castilla y León; Spain), showing the presence of the infection in the region.

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## Distemper in otter (*Lutra lutra*), a case report

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**Abstract:** We describe a clinical case of distemper in an Otter. Viral disease is increasingly present in wild and domestic mustelids. This diagnosis has served to know a new weakness of wildlife. The otter is an animal considered biomarker of the good state of our rivers. The work of the Wildlife Reception Centers (CRF) and the Wild Animal Recovery Centers (CRAS) is essential for knowledge of the Environment and the health of the ecosystems that surround us.

**Keywords:** Distemper, Otter, Mustelid

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## 1. INTRODUCTION

In the Iberian Peninsula its distribution is very irregular. It is relegated to mountainous areas frequently with heavy rains. The presence of the otter in a river is the best indication we have about the good state of a riverbed. Unfortunately the pollution of the rivers, the uncontrolled garbage dumps, the cutting of

trees and vegetation of the riverbanks, the bleach and sulfate used by poachers in trout fishing, and other aggressions suffered by our rivers, are causing the decrease of its population. It is included in the List of Species included in the List of Wild Species under Special Protection Regime (R.D. 139/2011). This species is protected by the CITES International Convention, in which it is cataloged as of imminent extinction due to trade, which is explicitly prohibited. It is also included in Annex II of the Berne Convention, among animals whose danger of extinction is serious and requires special care for recovery.



Fig. 1. Otter (*Lutra lutra*).

The Moquillo, also known as Distemper or Carré's Disease, is a pathology that mainly affects domestic dogs. The disease is caused by a virus belonging to the genus Morbillivirus and family Paramyxoviridae, and it is a single-stranded RNA virus. Recently it has become a very common disease of mustelids, especially ferrets. Normally attends with respiratory, digestive and nervous symptoms.

## 2. MATERIALS AND METHODS

In October 2017, a specimen was collected by an Environmental Agent in Manganeses de la Lampreana, a small village in the region of Tierra del Pan, Zamora (Spain). The municipality belongs to the Natural Reserve of the Lagunas de Villafáfila. The otter was transferred to the Wild Animals Reception Center (CRF) of Villaralbo where the action protocol for cataloged species was applied. Due to its general poor health condition, it was immediately explored.



Fig. 2. Reception the Otter (*Lutra lutra*) and take an intravenous line.

### 3. RESULTS

#### Clinical signs

Ataxia, incoordination, dehydration and extreme weakness were observed. The fur lacked without shine and was in poor condition. The animal had incipient alopecia in various areas, without apparent injuries. Other symptoms were hypothermia, 37 ° C, slight tachycardia and dyspnea, as well as the anal area spotted by watery diarrhea. The body weight was 3,350 gr. An intravenous line was placed to improve hydration and Ringer lactate (150 ml) was applied. Broad spectrum antibiotic treatment was also applied. Fig. 2. Blood and biochemical analysis did not show significant alteration of the parameters analyzed. The observed nervous signs were ataxia, incoordination, along with the lack of strength and weakness. All together the animal suffered of starvation and a pathological process that affected the central nervous system. Unfortunately, four days after admission and after a relative and apparent improvement, with food intake and improvement in the strength of the animal, appeared dead in its confinement. Regulated necropsy was performed and samples were sent to the Pathology Department of the University of León, where they make the diagnosis as Distemper.

#### Histological results

The lung presented areas with thickening of the interalveolar septa due to muscle fibre hyperplasia and the presence of lymphocytes and macrophages, alterations compatible with interstitial pneumonia, Fig 3. By means of the immunohistochemical technique with a monoclonal antibody against morbillivirus, intense positivity was detected against the viral antigen in bronchiolar and macrophage epithelial cells. For the diagnosis, a positive control of distemper (dog lung) was used. Other alterations observed were the following: lymphoid depletion and extracellular spleen hyalinosis, Fig 4, nephrosis and disseminated intravascular coagulation (DIC) Fig 5. In addition to the distemper related lesions, pathology results described in the kidney: Intense vacuoming of the cytoplasm of the epithelial cells of the proximal contoured tubules. In addition to the presence of eosinophilic material in tubular lights (hyaline cylinders), it was compatible with protein nephrosis, Fig 6. Neutrophil infiltrates in renal pelvis (acute pyelonephritis?). In the heart: An intracellular cyst with bradyzoites compatible with *Sarcocystis* spp. Fig. 7. In liver samples: Mild liver degeneration. Some lymphocyte portal infiltrates. The stomach, pancreas and intestine were not altered.

#### 4. PHOTOGRAPHIC ANNEX

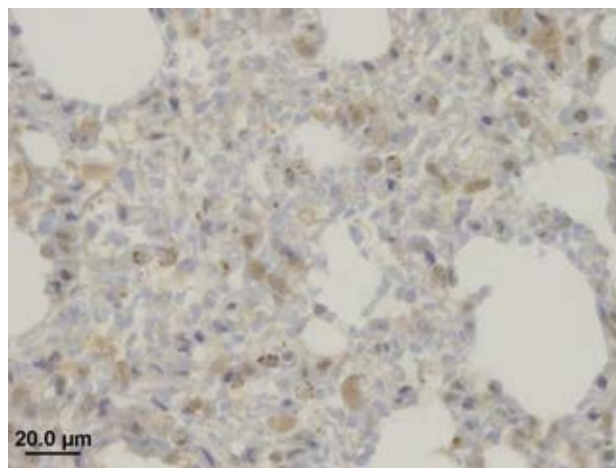


Fig. 3. Affected distemper lung. A characteristic lesion of the distemper is interstitial pneumonia, and the presence of viral antigen in macrophages.

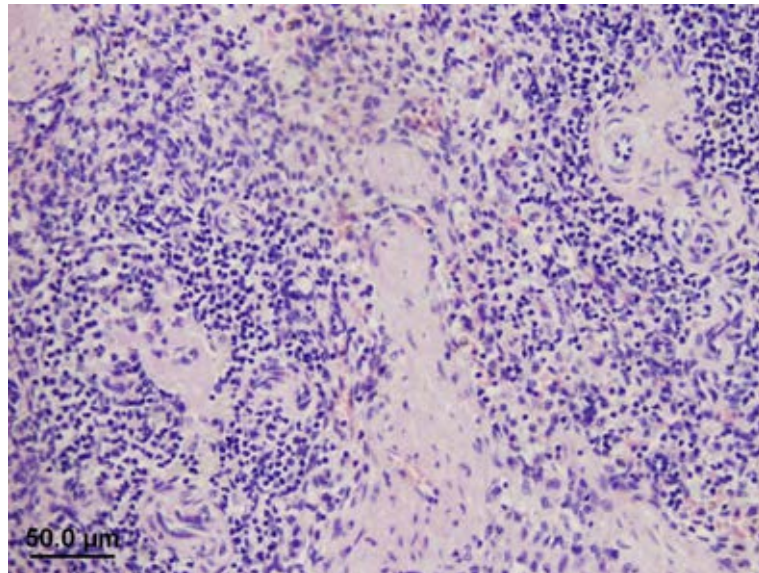


Fig. 4. Lymphoid depletion and extracellular hyalinosis in spleen.

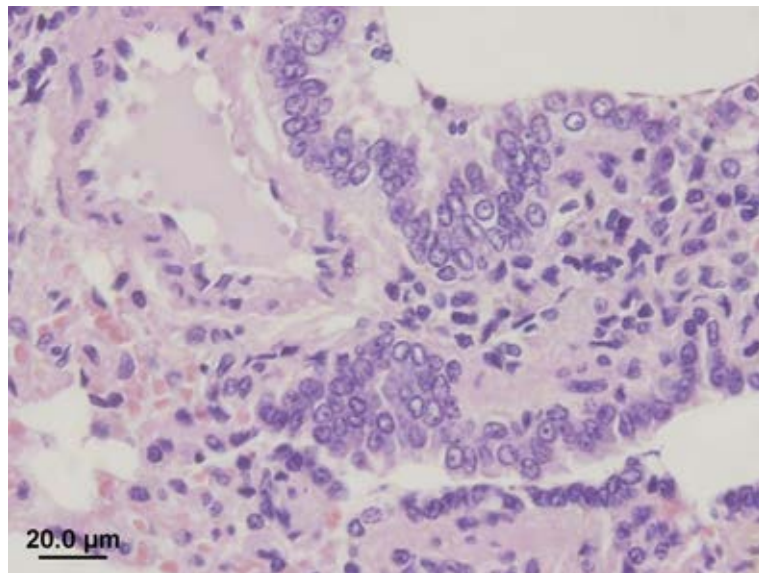


Fig. 5. Disseminated intravascular coagulation (DIC).

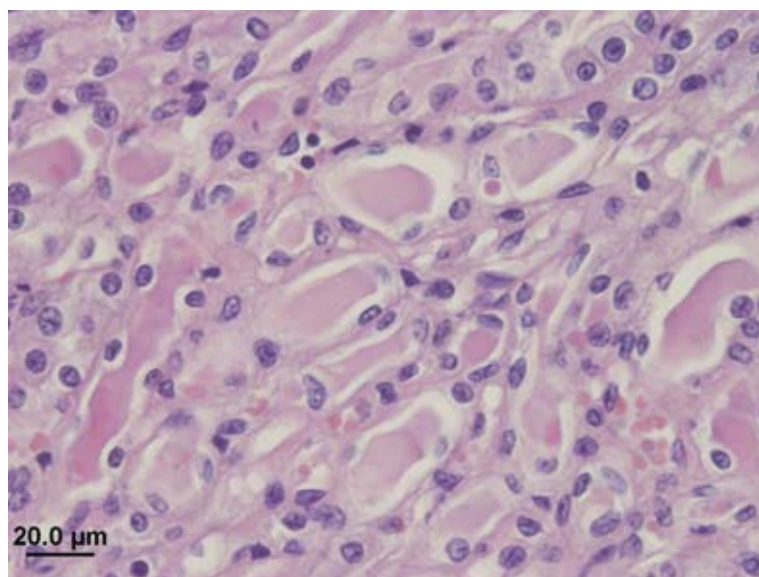


Fig. 6. Presence of eosinophilic material in tubular lights (hyaline cylinders), compatible with protein nephrosis.

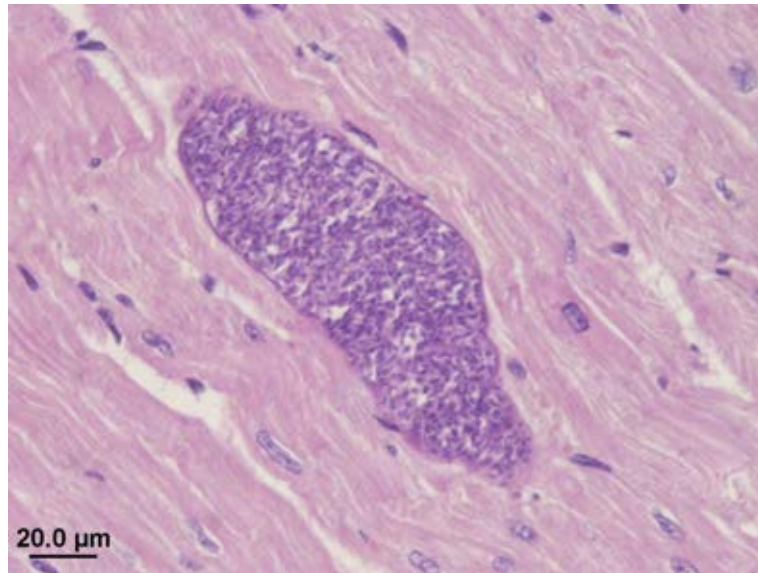


Fig. 7. Intracellular cyst with bradyzoites compatible with *Sarcocystis* spp.

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**Poster**

**Helminths of Fallow deer (*Dama dama*) bred in Southern Italy**

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**Abstract:** In Calabria region of Southern Italy, there are several Cervidae farms and in the Sila National Park, there are the largest bred of fallow deer. Among the health problems found in Cervidae farms, parasitic diseases are the most widespread and underestimated, though parasite infections have a very large impact on the health and productivity of animals. Therefore, it is necessary to perform parasitological studies, for the development of appropriate prevention programs to control parasitic infections in these animals. Since in Calabria region the studies concerning the diffusion of helminths in fallow deer farms are very poor, the aim of this research was the acquisition of recent data on the prevalence and intensity of helminths in this species. The study was conducted between September and December 2016 in all fallow deer farms (5 in total) of the Sila National Park. In these farms all animals, 35 males and 45 females, of different estimated ages (mean age of 6yr) were subjected to an individual fecal sample. Feces samples were collected from a pasture area, immediately after animals defecated, with care to take the feces from the apex of fecal heap. Fecal microscopic exams were performed using the FLOTAC *dual technique*, and two flotation solutions, sodium chloride-based solution with a specific gravity (SG) of 1.200, and a zinc sulfate-based solution (SG 1.350) to identify parasitic elements (eggs, larvae) at a different specific gravity. The analytic sensitivity of the FLOTAC *dual technique* was 2 eggs/larvae per gram (epg/lpg) of feces. Our research described the presence of several helminths in fallow deer bred in the Sila National Park and showed an average prevalence and intensity of infection with gastrointestinal nematodes at 85% and 32 epg respectively; *Paramphistomum* spp at 17.5% and 16 epg; *Capillaria* spp at 15% and 7 epg; *Strongyloides* spp at 12.5% and 1 epg; lungworms at 10% and 1 lpg; *Nematodirus* spp at 8.7% and 3 epg; *Trichuris* spp at 3.7% and 1 epg. All data were analyzed by T test and no significant correlations among parasitic prevalence with the sex and age of the examined animals were found. These results confirm that gastrointestinal nematode infections are very common in fallow deer and, with the exception of *Paramphistomum* spp, are in line with other surveys conducted on national territory. The latter parasites were found only in 2 farms where fallow deer are reared in promiscuity with domestic goats, which are the natural *reservoir* for this parasite and the contact between the two species could have favored the infection. Even if the underlined parasitic picture is not a serious entity, over time it could have possible consequences on the animal welfare, so it is clear that the qualitative and quantitative copro-microscopic exams are indispensable tool in order to perform an appropriate therapy for the control of parasitic infections in fallow deer bred in wild-state.

**Keywords:** Helminths, Fallow deer, farms, FLOTAC technique, Southern Italy



## **Management of meat from ungulates hunted in the Tuscany Region: an advanced model in peninsular Italy**

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**Abstract:** The management of ungulate meat in Italy has long affected the Alpine Region. From the second post-war period in peninsular Italy, and in particular in Tuscany, there was a strong development of wild ungulates: wild boar, roe deer, red deer, fallow deer and mouflon. Consequently, hunting of these species has also developed, with the use of hunting techniques and weapons not previously known to Tuscan hunters. The hunting of these species provides the hunter with a considerable amount of meat, which must be treated correctly, from an organoleptic and hygienic-sanitary point of view. The ATC (Ambito Territoriale di Caccia) Pistoia 11, a public agency of hunting management, first in Tuscany, has invested in technology and knowledge meat processing. Thanks to its own and public funding, it has built four "ungulates control centers" and has signed agreements with meat processing companies. The example of Pistoia has been followed in recent years by the other hunting management units of the Tuscany Region, after the approval of the regional law 10/2016, which indicated the lines of exploitation of game meat. In three years, a growing number of animals have passed through the meat processing centers. The enhancement of the hunted game meat has been an incentive to increase the legal game and health checks.

**Keywords:** wild boar, roe deer, hygiene and health control, transparency, legality

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## **Persistent organic pollutants (POPs) in Eurasian otter (*Lutra lutra*) from Southern Italy**

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**Abstract:** Persistent organic pollutants (POPs) are a group of chemicals with a potential for long range transport, bio-magnification, persistency into the environment. Some are generally used as pesticides due to their toxicity, while others are products and by-products of industrial processes. Because of their toxic effects, many POPs were banned after the Stockholm Convention in 2004. Despite of the ban and several other legal restrictions, POPs have continued to persist in nature, associated with a wide range of deleterious health effects in wildlife and humans. The Eurasian otter (*Lutra lutra*) is widely recognised as an emblem for nature conservation being a top predator and an important biological indicator of the health of rivers and lands. We determined, for the first time, concentrations of twenty OCPs (organochlorine pesticides) and six ndl-PCBs (non dioxin like polychlorinated biphenyls) in otters from Southern Italy. The study was performed on 10 specimens of river otter, collected during the IZSM (Istituto Zooprofilattico Sperimentale del Mezzogiorno) systematic surveys on infectious diseases of wildlife. Carcasses were sexed and classified as juveniles (<12 months old) or adults (>12 months old), with body weight between 4.2 and 6.2 kg. Liver and muscle tissue were removed from the carcasses and sent to the IZSM Department of Chemistry, where analytic processes

have been performed using a Perkin Elmer Autosystem gas chromatograph with an electron capture detector (ECD). While these contaminants have been banned for over 30 years, we detected four out of 20 OCPs and three out of 6 ndl-PCBs (polychlorinated biphenyls) only in liver samples. Endrin, Heptachlor, p,p-DDD (dichlorodipenyldichloroethylene) and o,p-DDE were present at the highest concentrations (min 0.010 mg/kg- max 0.235 mg/kg), that were greater than the mean values detected in Illinois river otters. As regard the ndl-PCB, concentrations found out were significantly higher in males (5) (range: 0.009–10.11 mg/kg) than females (2) (range: 0.179–0.479 mg/kg). The ranges and means of POPs concentrations we detected in the Eurasian river otters highlight the need to understand the exposure of wildlife and humans to these at the watershed level in Southern Italy. In particular, exposure to the organochlorine Endrin and Heptachlor remains as a concern in Italy.

**Keywords:** river otter, wildlife, environment, organic pollutants, Southern Italy

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### **Last stop for the wolf killers: forensic analysis puzzles out a fierce crime against wildlife**

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**Abstract:** The recent trend towards an increase in the number of wolves in Italy has led to a resumption of conflicts with human activities and to possible retaliatory killings. A carcass of a male wolf that was poisoned, tortured, killed and hung on a bus shelter was found in an early morning of November 2017 near a small Italian town. Law authorities acquired images of a surveillance camera that had captured a van circulating during that night close to the hanging wolf carcass. The van, owned by two workers of a farm, was confiscated and inspected. A pitchfork, trousers and a suit were found inside the van and seized. Biological evidence (blood traces, hairs) were also found and subjected to forensic analysis to assess the source animal species. DNA was extracted from all the samples, amplified and analysed at mitochondrial loci and with species-specific STR (Short Tandem Repeats) panels, according to the procedures currently in use in our Forensic Genetic Laboratory. Hairs turned out to come from *Ovis aries* (sheep), while a blood trace found inside the van was identified as belonging to the *Canis lupus* species. A complete STR-based genotype was obtained from the blood sample and then compared with that from the wolf carcass. The blood trace and the carcass showed the same STR multilocus profile, revealing that possibly they belonged to the same wolf individual. Statistical support for DNA match probability between the two samples was calculated using our in-house database built on hundreds canine genotypes from Italian unrelated dogs and wolves. A conservative estimate of the overall probability of identity was obtained. The result indicated that the estimated probability of any two highly inbred individuals sharing the same multilocus genotype by chance alone was approximately 1 in 700,000. Given that the wolf population in Italy is estimated in less than 2,000 individuals, we concluded that the blood trace on the van belonged to the animal cruelly killed. The trial is currently pending and is having a considerable media coverage.

**Keywords:** animal genetics; *Canis lupus*; DNA match; microsatellites; wildlife forensics

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### ***Sarcocystis* spp. in wild boars in Campania Region**

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**Abstract:** *Sarcocystis* spp. (Apicomplexa: Sarcocystidae) are coccidian parasites of animals, including humans, that usually have a strict two-host cycle with herbivores as intermediate hosts and carnivores as definitive hosts. Domestic swine and wild boar are intermediate hosts of three *Sarcocystis* species as *Sarcocystis miescheriana*, *Sarcocystis sui hominis*, and *Sarcocystis porcifelis*. *Sarcocystis sui hominis* is the only specie of zoonotic concern with public health significance. Human infection occurs by the ingestion of raw and/or undercooked meat containing cysts with bradizoytes, reminding that the humans are the definitive host of this parasite. Few data are available about the spreading and human risk of this protozoa. Considering that wild boars are widespread in Italy, as well as the consumption of game meat, the aim of this study was to perform a survey on *Sarcocystis* spp. infection and related pathological changes in wild boar population. Samples of muscle tissues (N°71 heart, N°19 diaphragm and N°14 heart/diaphragm) were collected from 113 wild boars shot during hunting season 2017 and investigated for *Sarcocystis* spp. infection within the regional project “Piano Emergenza Cinghiali Campania”. For histopathological investigations each sample was formalin-fixed, dehydrated embedded in paraffin, sectioned (2–4 µm) and stained with hematoxylin and eosin for microscopic identification and evaluation of cysts presence. 104 out 113 wild boars were positive to *Sarcocystis* spp. with an overall prevalence of 92%. The muscle showed heterogeneous pattern of lesions and in a few cases inflammatory reaction with characteristics diffuse lymphocytic infiltration and rare presence of eosinophils. Others lesions were: vascular congestion, necrosis and degeneration of muscle fibers. In some cases the muscle infected did not show any type of histopathological alteration due to the presence of the parasite. The absence of macroscopic lesions underlines the importance of the *Sarcocystis* research in routine analysis, in order to clarify its real spread in wild boars’ population. The results of this study are the first report of presence of *Sarcocystis* spp. in wild boar in Campania Region and further studies are necessary to investigate the most widespread species and the risk for human health.

**Keywords:** *Sarcocystis* spp., hunting season, Wild boar

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### **Evaluation of lead pellets ingestion in pheasant (*Phasianus colchicus*) living in a Wildlife Hunting Company in Central Italy**

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**Abstract:** Lead is a metallic element potentially affecting all vertebrate species. Lead and its compounds have been used for several human activities for a long time. In the late 19th century, lead's toxicity was recognized, and its use was banned for many applications. However, many countries still allow the production and the sale of lead based products, including some types of paints and hunting ammunitions. Many studies have been conducted in different fields (biomedical, veterinary,

ecotoxicology), and often they concluded that traditional hunting ammunitions could represent a significant source in lead environmental pollution. In particular, birds could be exposed to lead through direct ingestion of lead pellets (primary exposure) or by scavenging of animals with lead pellets or fragments embed inside their tissues (secondary exposure). Ingestion of lead ammunitions was reported in more than 130 avian species. Many studies focused on the ingestion of lead pellets in water birds, especially before the use of lead based ammunitions was banned for waterfowl hunting. Differently, scarce information are available on the ingestion of lead pellets by terrestrial birds. This study is aimed at verifying the eventual ingestion of hunting lead pellets by free-living pheasants (*Phasianus colchicus*) in a Wildlife Hunting Company in the province of Pisa (Italy), evaluating also the quantity of pellets ingested. We examined 81 gizzards of ring-necked pheasants shot by hunters during the 2017/2018 hunting season. We carried out RX examination on these gizzards in order to highlight the presence of radiopaque bodies referable to hunting pellets; subsequently, on a radiographic evidence, we performed a necroscopic examination on positive samples. Through the necroscopic examination, we found that 18 out of 81 (22.2%) samples contained lead pellets. The number of lead pellets found in each sample ranged between 1 and 3. Among these, 14 pellets were intraluminal (56%), presumably due to ingestion, and 11 were intramural (44%), presumably due to gunshot wound. On the base of these evidences, our study can be considered as a preliminary study concerning lead intoxication in terrestrial game birds. It should be noted that cases of poisoning due to the ingestion of lead pellets does not concern only aquatic species, which are actually protected by restrictive laws, but also terrestrial species (e.g. pheasant) which represent a serious risk for food safety.

**Keywords:** Lead, pheasant, pollution, hunting pellets, food safety

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### **Endoparasites in some wild animals in Eastern Slovakia**

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**Abstract:** Man has more and more opportunities to meet wildlife. Many of these wild animals may be an essential element of the life cycle of endoparasites, causing parasitic zoonoses such as giardiasis, toxoplasmosis, toxocarosis, echinococcosis, dirofilariosis and trichinellosis. The aim of this study was to determine gastro-intestinal endoparasites of model animal species from the family Canidae (red fox, raccoon dog), Felidae (European wild cat) and Mustelidae (European badger). Between 2016 and 2018 a total of 8 adult European badgers, 2 one-year old individuals of European wild cat and 2 young individuals of raccoon dog by complete helminthological necropsy were examined. In addition, 21 faecal samples and 9 blood samples from red foxes were obtained. All samples came from the districts Košice-okolie and Vranov nad Topľou. Tapeworms (f. Taeniidae) were found in the small intestine of 3 European badgers and 2 European wild cats. In both cases of wild cats, also roundworms (*Toxocara cati*) were present. The coprological examination of 21 faecal samples from red foxes showed, that 90.48 % of samples were positive for the presence of endoparasites (*Coccidia* oocysts 33.33 %; *Trichuris vulpis* 47.62 %; f. Ancylostomatidae 14.29 %; *Capillaria* spp. 4.76%; *Toxocara canis* 9.52 %; *Toxascaris leonina* 28.57 %, f. Taeniidae 14.29 %). Faecal samples from 4 European badgers were positive for the presence of endoparasites (*Coccidia* oocysts 12.5 %; *Capillaria* spp. 25 %; f. Ancylostomatidae 50 %). Both raccoon dog faecal samples were negative. *Trichinella* larvae were not

detected from muscle samples of examined animals. PCR examination of 21 blood samples or spleen for the presence of *Dirofilaria* spp. was negative. Population density of wild animals in urban and suburban areas can have a significant impact on parasitic loads and the diversity of parasitic species. As some species are increasingly observed in close proximity to human dwellings, the risk of parasite transmission may increase.

**Keywords:** endoparasites, wild animals, Eastern Slovakia, Canidae, Felidae, Mustelidae

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### Parasite load of cobitid fish from Slovakian rivers

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**Abstract:** During the period 2016-2018 a total of 457 spined loaches (*C. elongatoides* Bacescu and Mayer, 1969) were examined for endohelminths. Samples came from 3 different rivers (Okna, Turňa and Bodrog) and from 2 localities of Okna river (Okna white locality, Okna green locality, before and after waterfall in green locality). We obtained samples during different seasons (in the year 2016 just one sample during September; in 2017 - 3 samples – June, July and November; in 2018 – 8 samples – June, July, August, September and October). For sampling we used electrofishing, induces electroanaesthesia. The method used to catch the fish was the “point sampling method”. The main parasitological indices were evaluated; prevalence (P), intensity of infection (i.i.) and mean intensity of infection (m.i.) by Margolis *et al.* (1982) and Bush *et al.* (1997). For evaluation of the fish condition we used Clark condition coefficient. The dominant parasite species we found in loaches were trematodes - *Clinostomum complanatum* larv. localised subcutaneous or intramuscular in different part of the body (mainly around the mouth opening) and *Allocreadium transversale* in intestine. We found a much higher intensity of infection of *C. complanatum* compared to *A. transversale* in all samples. In the majority of samples we found that females had a higher parasitic burden of both parasite species compared to males, probably due to their bigger size (a greater number of consumed feed). We observed higher prevalence of parasites in diploid specimens, which is contradictory to literature which suggests that polyploids are more likely to have higher parasite burden. It is difficult to interpret the reason behind these findings as little is known about the life cycle and development of *A. transversale*. We compared the coefficient of condition in infected and uninfected specimens (both sexes) with parasite load of dominant parasites. There were no important differences in Clark’s coefficient of condition – from year 2017 in infected (0,71) and uninfected (0,7) and from year 2018 in infected (0,75) and uninfected (0,77).

**Keywords:** Clark’s coefficient of condition, cobitidae, trematodes

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## **Regional monitoring surveillance zoonosis program in wildlife in Campania Region: evolution in the last three-years period (2016-2018)**

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**Abstract:** In Campania, the Wildlife Health Surveillance Program was approved by Executive Decree 147/2012, moreover the Executive Decree 112/2016 has been adopted procedures related to the "Regional Monitoring Program on the surveillance of zoonoses in wildlife", this Program is updated every year by the CRIUV (Regional Reference Center for Urban Hygiene). Aims of the Program are the increasing active and passive surveillance on zoonoses, the development of guidelines and standardization of procedures to coordinate activities, the collection of results in a regional database, the processing of risk maps based on results, promoting interest to Wildlife. Surveillance activities are carried out on samples from wild species sensitive to Tuberculosis, Brucellosis, Avian flu, West Nile Disease, Rabies. Particular attention was given to studies on Tuberculosis and Brucellosis, based on the epidemiological situation in Campania. Over the three years, samples were collected by staff of Veterinary Public Service and private veterinarians under the supervision of the Regional Center CRIUV. Over the last 3 years 10000 animals were analysed in 2016, 10300 in 2017, 11700 in 2018. Animals, originated from all the provinces of the regional territory, were analysed at Zooprofilaxis Institute of South Italy. Part of the samples was reserved for Early Detection of emerging diseases, in 2019 African Swine Fever was chosen: up to June samples from three wild boars were analyzed by PCR, there were not positivity. The sampling activity will increase in the next hunting period.

**Keywords:** Surveillance, Brucellosis, Tuberculosis, Data Base

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## **Contribution of gastric content analysis to investigation of poaching cases: a case study**

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**Abstract:** Investigations into crimes against wildlife are challenging. Often, only the Secondary Crime Scene (where the animal eventually died, or its body was dumped) is available for the investigators to process. Localization of the Primary Crime Scene (where the crime actually occurred), is crucial to retrieve key evidence (especially ballistic evidence) and to identify offenders. Veterinary forensics can contribute to the identification of the Primary Scene, e.g. through the analysis of the gastrointestinal content of the victim. On September 2014, a male adult marsican brown bear was found dead on a trail in the Pettorano municipality (AQ). A forensic necropsy was undertaken at the Centro di Referenza Nazionale per la Medicina Forense Veterinaria (Grosseto, Italy), including complete shaving and skinning of the carcass, and inspection of gastrointestinal content. From the necropsy, cause of death was acute septic peritonitis, secondary to intestinal perforations caused by gunshot from a smooth-

barreled firearm to the back of the animal. Since death could have occurred in a few hours, during which the animal likely wandered around the area, the place of discovery of the carcass could represent a Secondary Crime Scene, and not the point where the animal had been shot in the first place (Primary Crime Scene). The stomach of the bear was full and its content consisted of parts of animal origin and to a lesser extent of a vegetable component. Vegetables were figs, fruits of the genus *Prunus*, a jujube, and cereal seeds. The animal component consisted of muscles and entrails of avian species (gizzards containing cereal seeds, a heart and a cloaca), bone fragments, white and black feathers, and a beetle (*Cetonia aurata*). Inside the esophagus there was a hollow organ, consistent with the caecum of a poultry species. This likely represented the last bite ingested by the bear just before being shot. The discovery in the digestive tract of elements pertaining to domestic poultry, made it possible to link the firearm shot to the aggression by the bear to a chicken coop, whose location was already known from the local press prior to bear's death. Within four hours from the start of the necropsy, investigators were able to receive this information and localize the Primary Crime Scene, where the suspect pledged guilty of shooting the bear. The trial led to a first acquittal for self-defense, the sentence of appeal is awaited.

**Keywords:** brown bear, crime scene, gastric content, poaching, veterinary forensic

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### Parasitological surveillance in birds of prey in southern Italy

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**Abstract:** The Federico II Wildlife Rescue and Rehabilitation Center (CRAS) of Naples (Italy) is a non-profit Center established in 2010 to reintroduce in nature, whenever possible, wildlife that has undergone medical care and rehabilitation in the Center. The aim of this study was to perform a one-year parasitological surveillance in birds of prey hospitalized at the CRAS Federico II. A total of 133 birds of prey, belonged to ten different species, was examined: *Accipiter nisus* (2 individuals), *Asio otus* (5 individuals), *Athene noctua* (15 individuals), *Buteo buteo* (34 individuals), *Circaetus gallicus* (1 individual), *Falco peregrinus* (6 individuals), *Falco tinnunculus* (56 individuals), *Otus scops* (7 individuals), *Strix aluco* (5 individuals), *Tyto alba* (2 individuals). Trauma was the most frequent cause of hospitalization in these animals and occurred in 38% of the cases, with 49% of animals presenting one or more fractures; 22% of animals were admitted because immature; 21% were sequestered animals; 10% were medical cases and 9% were admitted for "other causes". With respect to the final outcome: 58% of animals were released, 11% and 25% were euthanized or died, respectively, 4% is still hospitalized and 2% was entrusted. In the CRAS, in addition to high specialty healthcare, the personnel perform epidemiological monitoring and investigation activities on infectious and parasitological diseases of wildlife, especially those with a possible zoonotic impact. For this purpose, faecal samples were collected both from hospitalized and deceased animals and sent to CREMOPAR (Department of Veterinary Medicine and Animal Production, University of Naples Federico II) where they were analysed by the FLOTAC Dual technique with an analytic sensitivity of 2 eggs per gram (EPG) of faeces. The birds of prey hospitalized were all infected by at least one endoparasite with the only exception of *Tyto alba*. The 67% (89/133) of the birds of the prey were found positive to the

following genera of parasites: *Capillaria* spp (55.1%), *Serratospiculum* spp (15.7%), *Centrorhynchus* spp (6.7%), *Caryospora* spp. (4.5%), *Porracaecum* spp. (3.4%), *Echinostoma* spp. and *Amidostomum* spp. (1.1%). These results show that the FLOTAC represents an effective copromicroscopic technique for the detection of endoparasites in wild birds of prey.

**Keywords:** FLOTAC, wildlife, birds of prey, endoparasites, Italy

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### **Tuberculosis in wild boar, epidemiology and control perspectives.**

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**Abstract:** Tuberculosis in wild boar is caused by several species of *Micobacteria*. Within this *Genera* the infection caused by *Mycobacterium bovis* is mostly considerable because relevant for zoonosis and animal health. In Marche Region (IT), tuberculosis is documented since year 2002; a single genotype (SB0120; VNTR 33533) is shared by cattle and wild boar, in a eco-pathological interface located in San Vicino mountain (1450 km<sup>2</sup>; 1480m asl) in the Apennine district between the provinces of Macerata and Ancona. *M. bovis* was isolated from specific lesions of wild boar viscera brought to the Veterinary Inspection Service (mandatory in Marche Region since 1999). Despite a quite similar amount of hunted wild boars per year, cases of tuberculosis rose from 35 in the period 2002-2016 to 77 cases in the last hunting seasons 2016/17 and 2017/18. This increased trend suggests the needs for a selective culling in the infected area with the goal of the disease control. This aim should take in account several eco-pathological variables as: the site of the lesion and its characteristics, the age and sex of the affected animals. The matriarchal groups within the population should be the object of the selective culling. For the control of the tuberculosis, an integrated wildlife-livestock surveillance should be organized in the area, also for consumers protection in respect of wild meat consumptions.

**Keywords:** wild boar; bovine tuberculosis; wildlife surveillance; zoonosis; eco-pathological interface.

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### **Thermotolerant *Campylobacter* in living game birds**

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**Abstract:** Human campylobacteriosis has increased in both developed and developing countries over the last 10 year. It represents one of the main food-borne gastrointestinal disease world-wide (WHO, 2015), where thermotolerant *Campylobacter jejuni* and *Campylobacter coli*, are the most commonly reported bacteria (for over 95%) in enteric infections in humans (Dipineto *et al.*, 2017). Chicken meat has been considered the main source of human infection but *C. jejuni* and *C. coli* have also been reported in a range of livestock and wildlife species (Seguino *et al.*, 2018). Current scientific knowledge on the presence of *Campylobacter* spp. in game bird is scarce and fragmentary and to address this lack of information, we evaluate, in this study, the prevalence of thermotolerant



*Campylobacter* spp. in commercially reared partridges, pheasants and quails in southern Italy. To achieve this goal, a total of 550 living game birds (partridges n=240; pheasants n=240 and quails n=70), equally shared between females and males, were examined by cloacal swabs. Bacterial isolation methods, DNA extraction and multiplex polymerase chain reaction (PCR) were performed as described by Dipineto *et al.* (2008). Birds data (sex, and age) were analysed by univariate statistical analysis (Pearson's chi-square test for independence) using the *Campylobacter* spp. status (positive/negative) as the dependent variable. Statistical analysis was performed using SPSS 13 for Windows. Thermotolerant *Campylobacter* spp. was isolated in 237 out of 550 (43.1%) living game birds analysed. *C. coli* (100%) and *C. jejuni* (14.8%) were identified by PCR. In particular, 104/240 (43.3%; 95% IC = 37,0% - 49,9%) pheasants were positive to *Campylobacter* spp, of these all were positive to *C. coli* (104/104), and 14/104 (13,5%) were also positive to *C. jejuni*; 118/240 (49,2%; 95% IC = 42,7–55,7%) partridges were positive to *Campylobacter* spp, of these 15/118 (12,7%) were also positive to *C. jejuni*; 15/70 (21,4%; 95% IC = 12,9 – 33,2%) quails were positive to *Campylobacter* spp. and of these, 6/15 (40,0%) were also positive to *C. jejuni*. Adult game birds showed a significantly higher prevalence value (P<0.001) than younger individuals because *Campylobacter* spp. colonization in birds is typically observed from the second to the fourth weeks of life). In contrast, in line with other studies, no significant difference related to sex were observed. These results reinforce the assumption that *Campylobacter* spp. may be found in the intestines of apparently healthy game birds that may be considered as potential carriers of this bacteria, for humans and other animal species since this birds are frequently used for repopulation of protected areas (e.g. National Park, Natural Reserve) as well as game reserves.

**Keywords:** *Campylobacter* spp.; game birds; southern Italy; Food-borne infections; Zoonoses

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## Diversity and intensity of environmental contamination with gastrointestinal parasites of wolves in Northern Spain

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**Abstract:** Parasitic infections severely affect wildlife populations in general and wolves in particular. From the environmental health perspective, wild animals, namely wolves, may spread eggs and oocysts of gastrointestinal parasites on the environment, contaminating other wild animals as well as some domestic animals who share the same environment. The aim of this study was to assess the diversity and intensity of environmental contamination with gastrointestinal parasites of wolves in northern Spain. For this purpose, faecal samples (n=101) were collected from the environment in predetermined transects between May and August 2012. These samples were previously analysed regarding their form and constitution, namely because of wolves feeding habits, and even if genetic analyses were not systematically performed, this process assures us their wolf origin. Samples were analysed using Mini-FLOTAC. The overall prevalence was 27.7%. At least six different parasites have been identified with prevalence ranging between 0.99% to 13.86%: *Capillaria* spp. (13.86%), Taeniidae (5.94%), *Toxocara* spp. (4.95%), Ancylostomatidae (1.98%), *Spirocerca* sp. and *Toxascaris leonina* (both, 0.99%). The intensity was very low, ranging between 5 to 140 eggs/g. Despite the

diversity of parasites found, especially regarding the environmental origin of the samples, the intensity was very low, so the potential spreading seems to be residual. Nevertheless, it should not be neglected that some of them have zoonotic potential: Ancylostomatidae, *Toxocara* and Taeniidae. Taeniidae eggs may be *Taenia* spp. or *Echinococcus* spp, as we did not carry out species determination. Nevertheless Taeniidae eggs are immediately infectious underlying the need to think from a One Health perspective.

**Keywords:** *Capillaria*, *Toxocara*, Taeniidae, Mini-FLOTAC

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## Malicious poisoning of Wildlife in Lazio and Tuscany Regions between 2005 and 2018

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**Abstract:** The phenomenon of malicious animal poisoning is widespread throughout the Italian territory. Until 1976, the use of poisoned baits was legally permitted for the control of wild "pest" animals (wolf, mustelids, fox and diurnal birds of prey). In 1976, the Decreto Ministeriale of 22 November set the precedent for the protection of wolf and the ban of poisoned baits. Later on with the law concerning the regulation of hunting activity (Legge n. 968, 27 December 1977) in Italy; the use of toxic substances and poisons for the control of wild populations was forbidden. Nevertheless, due to the widespread occurrence of the intentional poisoning of animals, some Regions such as Tuscany, Umbria and Puglia, promulgated regional laws that prohibited the use and possession of poisoned baits (L.R. Toscana 16 agosto 2001 n. 39; L.R. Umbria 22 ottobre 2001 n. 27 e L.R. Puglia 4 dicembre 2003 n. 27). Despite this, the concurrent death of several Marsican bears due to poisoning in the territory of the National Park of Abruzzo, Lazio and Molise, occurred in December 2008. This motivated the Ministry of health to establish a Ministerial Ordinance (MO) on the prohibition of the use and possession of baits or poisoned baits. This Ordinance defined in detail the role of public authorities, veterinarians and Istituti Zooprofilattici Sperimentali from the reporting of animal death due to suspected fraudulent poisoning to confirmation of the diagnosis and notifications to the Authorities. Since 2008 the MO has been renewed annually. This paper presents the data on animal malicious poisonings occurring in Lazio and Tuscany regions from 2005 to 2018. The data cover the four years before the MO (2005-2008) and the ten years after the MO (2009- 2018). Between 2005 and 2018, 4,702 samples ( $\cong$  335/year) were analysed in Lazio and 6,268 ( $\cong$  447/year) in Tuscany. In Lazio, among the 2874 samples referring to animals, 1,736 were positive; of these 116 (7%) were wildlife: 42% (49) birds, the remaining mammals. In Tuscany, 2,014 out of 3,642 animal samples tested positive; of these 346 (17%) were wildlife: 62% (215) of these samples consisted of birds while the rest were mammals. The wild mammals mainly killed by poisoned baits were fox and wolf. Surely, the number of wildlife poisoned is bigger than that recorded but the finding of wildlife carcasses is difficult and therefore we can only refer to it as a "minimum number proven". For this reason, it is also important to investigate malicious poisoning in domestic animals; this may serve as an indicator to monitor this criminal phenomenon in the territory.

**Keywords:** malicious poisoning, wild mammals, wild birds, veterinary forensic medicine, poisoned baits

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## Antimicrobial resistance in lactic acid bacteria isolated from Iberian wolf in Castilla y León (Spain)

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**Abstract:** Antimicrobial resistance (AMR) is nowadays a major worldwide threat to public health with serious implications for human and animal health. Multidrug resistant (MDR) bacteria or “superbugs” exist across the animal, human, and environment triangle and there is a continuous interchange within this triad (Carroll *et al.*, 2014). Hence, wild animals which do not naturally come into contact with antimicrobials are also exposed to resistant bacteria or genetic elements containing antimicrobial resistance genes within their ecological niches. Accordingly, several wild birds and mammals have been proposed as reservoirs and potential sources of AMR (Jones *et al.*, 2008; Goncalves *et al.*, 2011; Carroll *et al.*, 2015). Commensal gastrointestinal bacteria play a major role in disseminating bacterial resistance. Among them, *E. coli* and *Enterococcus* spp. are frequently used as indicators of antimicrobial resistance, although lactic acid bacteria have also recently pointed as a potential reservoir of transmissible resistance genes (Devirgiliis *et al.*, 2013). The aim of this research was to determine the prevalence of AMR in *Lactobacillus* spp. isolates recovered from Iberian wolf faecal samples collected in Castilla y León (Spain). Wolf faecal samples were collected from two natural reserves in the province of León, Spain (39) and from the Iberian Wolf Center in Robledo de Sanabria, Zamora, Spain (6). A total of 53 *Lactobacillus* spp. isolates were recovered by microbiological culture in De Man, Rogosa and Sharpe (MRS) agar plates. Antimicrobial resistance was determined using a microdilution test on 96 wells commercial plates (VetMIC™ Lact-1 and VetMIC™ Lact-2, SVA) according to previous reports (Huys *et al.*, 2010) and the recommendations of the EFSA (EFSA, 2018). Minimal inhibitory concentration (MIC) was determined for 16 antibiotics: gentamycin, kanamycin, streptomycin, tetracycline, erythromycin, clindamycin, chloramphenicol, ampicillin, neomycin, penicillin, vancomycin, quinupristin-dalfopristin, linezolid, trimethoprim, ciprofloxacin and rifampicin. Moreover, the available microbiological cut-off values proposed by the FEEDAP Panel for *Lactobacillus* species were used to classified isolates as resistant or susceptible. The proportion of resistant isolates among isolates recovered from wild wolves in the two natural reserves varied among 50% of resistant isolates from chloramphenicol, 21.9% for kanamycin, 15.6% for streptomycin, tetracycline and ampicillin, 9.4% for clindamycin and 6.2% for gentamycin and erythromycin. High MIC values were also found for vancomycin and trimethoprim. A higher proportion of AMR was reported among managed wolves from the Iberian Wolf Center as compared with free-range Iberian wolf ( $\text{Chi}^2= 73.54$ ,  $p<0.001$ ); the risk (Odds Ratio) of being resistant to any of the antimicrobials was 6,7 higher (CI 95% 4.2-10.3) among “humanized” managed wolves as compared with free-range ones. Our results confirm that wolves are potential vectors of resistant bacteria and genetic determinants of AMR.

**Acknowledgments:** the authors would like to thank all guards from natural reserves involved in the sampling for their invaluable contribution.

**Keywords:** Iberian wolf, *Lactobacillus* spp., antimicrobial resistance, Castilla y León

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## Cardiac magnetic resonance in *Trachemys scripta elegans* turtles

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**Abstract:** Currently, magnetic resonance is the gold standard for the functional and anatomical cardiac evaluation in human medicine. It provides precise and repeatable images and measurements allowing the study of the same patient along disease evolution. Nowadays, magnetic resonance has become a common approach in small animal cardiology and the availability of equipment have allowed its use in wild animals of particular value. Two cardiac magnetic resonances were carried out in red-eared slider (*Trachemys scripta elegans*) using a Signa HDx 3.0 T device. In both cases, FIESTA sequences (GE®) in the specific planes (two, three and four chambers and the short axis) were performed for the anatomical and functional evaluation of the heart. Blood flow into the aorta and pulmonary arteries was measured with phase contrast sequences. At the same time, gradient Eco sequences were carried out for the evaluation of the myocardial perfusion. All the sequences had enough quality for quantification although animal prone position and electrocardiography electrodes paramedial lineal positioning made easier to get evaluable images. From a cardiovascular point of view, the anesthetic protocol turned out to be safe. The use of two consecutive rounds of scout sequences allowed us to get, in both animals, the correct two, three and four chambers and short axis planes. A total of 46 anatomical and functional values were obtained, some of them not previously reported. However, the limited size of our sample with only two animals do not allow us to propose standard values for this animal species. Cardiovascular magnetic resonance could provide broad and in-depth assessment of cardiac function and structure in wild animals such as *Trachemys scripta elegans* turtles.

**Keywords:** Cardiac, magnetic resonance, turtles.

## Assessing Chronic Wasting Disease risk in Portugal

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**Abstract:** The identification of Chronic Wasting Disease (CWD) in Norway guides the possibility that cervids population in Europe could be at risk for Transmissible Spongiform Encephalopathies (TSEs) and can represent a potential prion reservoir, as it occurs in other diseases, menacing the livestock and public health. A collaborative project (Project 029947IC&T 02/SAICT/2017-SAICT) was established between the University of Trás-os-Montes e Alto Douro (UTAD), the National Institute for Agricultural and Veterinary Research (INIAV) and the Polytechnic Institute of Castelo Branco (IPCB) with the aim of evaluating the risk of a potential occurrence of CWD in Portugal. This is a synergistic collaboration as both UTAD and IPCB are located in areas with a closer contact with the cervid population and INIAV is the national reference laboratory for animal TSEs. The objectives defined in October 2018 at the beginning of the project were: i) To determine the *PRNP* gene variability in the cervids population; ii) To define if there is genetic susceptibility / resistance to CWD; iii) To evaluate the risk of exposure of the cervids population in Portugal to prions (BSE, Classical and atypical

scrapie); iv) To increase CWD awareness among stakeholders. By now, a total of 250 samples were obtained. Fifty tissue samples from Portuguese red deer, fallow deer and roe deer were already used for DNA extraction. Specific primers designed to amplify *PRNP* gene were used in order to amplify a 1416 bp amplicon corresponding to the entire coding region and containing regulatory sequences. All the PCR products were already obtained with success. The sequencing analysis of this region in all the animals will allow us to find and characterize polymorphic sites and to genotype them and compare with the data from other countries. The study of susceptibility/ resistance of cervids to CWD is essential to define its risk of dissemination/development as well as its potential as prion reservoir. The susceptibility/resistance to prion diseases are influenced by polymorphisms in the *PRNP* gene, so, both genotypic characterization in cervids and PrPres survey will contribute to delineate the dissemination risk of CWD in Portugal.

**Keywords:** CWD, Cervid, Prion, Prionic disease, Transmissible Spongiform Encephalopathies, TSE

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### **Computed Tomography in two traumatized Red Foxes (*Vulpes vulpes*)**

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**Abstract:** Computed Tomography (CT) is a diagnostic imaging technique with a higher sensitivity and contrast resolution compared to Radiography having also a higher spatial resolution and a shorter time of execution compared to Magnetic Resonance Imaging. Aim of this study is the description of two cases of traumatized Red Foxes in which CT had a crucial role for diagnosis and prognosis and to address the right therapy. Case description. Two young red foxes (*Vulpes vulpes*), a male weighing 3.5 and a female weighing 3 kg, both with an estimated age of about 1 year, were recovered at the Wild Animals Rescue Center. The male subject was rescued in the Benevento province and showed dysuria and ataxia of the hind limbs. The female was rescued in the Salerno province and was affected by a severe vestibular syndrome. Both foxes were referred at Veterinary Radiology Center to undergo a CT study. On the female fox, it was performed a scan of the skull, before and after contrast administration (iopamidol, Iopamiro©, Bracco, Milan), 2 ml/kg e.v. On the male fox, a total body study was performed. Both subjects were anesthetized using a bolus of propofol (PropoVet©, Esteve, Milan) 4 mg/kg and, after tracheal intubation, maintained with isoflurane (Isoflurane Vet©, Merial Italia, Milan) 2.5% in oxygen. The CT studies were performed with a helical multislice device (Brightspeed, General Electric Italia, Milan). No lesions on the brain but a fracture not dislocated of the left palatine and nasal bones were found on the female fox. On the male fox, multiple pelvic fractures, left sacro-iliac diastasis and ventral subluxation of S3 were visible; a moderate mineralized periosteal reaction along the profiles of the fractures attested a chronic condition. In both cases, CT was able to visualize the main lesions. In the female fox, the fracture of the palatine bones was better assessed using the Multiplanar reformation (MPR) images obtained along the dorsal plane. In the male, in addition to the MPR in the sagittal plane, useful to assess the sacral fracture, the 3 Dimensional Volume Rending (3D-VR) better demonstrated the severity of the condition. A good prognosis was emitted for the female

fox while an uncertain prognosis was done for the male fox. The female fox completely recovered from the neurological symptoms. The male fox recovered the urinary bladder function in a longer period but completely.

**Keywords:** Red Fox, Computed Tomography, traumatic lesion, skull, pelvis

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### **A serological survey of Aujeszky disease in Eurasian Wild Boar (*Sus scrofa*) in Campania Region**

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**Abstract:** Aujeszky's disease (AD) is a viral disease of suids caused by Suid Herpesvirus 1 (SHV-1) also referred as Aujeszky's disease virus (ADV). The virus belongs to the Genus *Varicellovirus*, subfamily *Alphaherpesvirinae*, Family *Herpesviridae*. Wild boar (*Sus scrofa*) are the natural host, but a wide range of species can be infected with SHV-1. In this study we evaluated the presence of antibodies against Aujeszky's disease in wild boar hunted in the Campania Region during the hunting season 2016-2017. Serum samples collected from 503 wild boars (*Sus scrofa*) were tested for antibody against Aujeszky's disease virus (ADV) using an AD, blocking ELISA assay performed according to the manufacturer's instructions. For each sample, we recorded the location, the date of collection, the sex and the age of the animal. Of 503 serum samples, 120 sera (23.85%, 95% Confidence Interval CI: 20.15 - 27.55) tested using Blocking ELISA assay, were positive to ADV. Multivariate analysis indicated that the sero-positivity to ADV was not associated with gender and province but is statistically associated with age (>36 month p<0.05). Our data show that wild boar in the Campania region are highly exposed to Aujeszky's disease virus infection.

**Keywords:** Aujeszky's disease virus, Pseudorabies, wild boars, blocking ELISA

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### **Molecular investigation on *Echinococcus granulosus* and other *Taenia* spp. in grey wolves (*canis lupus*) in Umbria region**

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**Abstract:** The aim of this study was to find out the presence of *Echinococcus granulosus*, *Echinococcus multilocularis* and other *Taenia* spp. in Grey wolf (*Canis lupus italicus*) faeces in Umbria region. Fecal samples were analyzed by a multiplex PCR using primers targeting mitochondrial genes. Positive samples were submitted to sequencing, in order to identify the parasites. This approach proved to be useful for the identification of taeniid eggs directly from fecal samples of wild animals and laid the groundwork for more large epidemiological studies.

**Keywords:** *Echinococcus granulosus*, *Echinococcus multilocularis*, *Taenia* spp., *Canis lupus*, multiplex-PCR, sequencing

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## Serological investigation on wild ruminants diseases in Central Italy

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**Abstract:** In the last ten years there has been an increasing interest regarding the sanitary surveillance of the wild fauna. The role of diffusers/reservoirs of infectious agents that some wild animals can play, with negative repercussions in the zootechnical context and in public health, is absolutely certainly. Bibliographic data related to health surveys in wild animals are still rather small. In health monitoring programs on wildlife disease, a difficulty that is often encountered is the retrieval of samples. In view of the above, the collaboration between the Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati" (IZSUM) and the Regional Wildlife Rescue Center WildUmbria (WU) has led health monitoring protocol on cervid population: roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*), with the aim of examining the presence of some livestock-related pathogens, of which some of them were zoonotic. The protocol was applied to all animals deceased or euthanized during the rescue operations in 2018. We analyzed 57 roe deers (34 males and 23 females) and 4 fallow deers (3 males and 1 female) of different ages and physiological status. During the necropsy of these animals, samples of intracardiac blood clot were submitted to the lab where serum was extracted and subjected to different serological tests (ELISA, SAR, MAT) for Bovine Herpesvirus tipe 1 (BHV1), Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza virus tipe 3 (PI3), Bluetongue virus (BTV), Visna Maedi virus (VMV), *Brucella spp*, *Leptospira Interrogans*, *Mycobacterium avium spp. paratuberculosis*, *Chlamydia spp.*, *Coxiella burnetii*, *Neospora caninum*, *Toxoplasma gondii*. Sera tested were positive for the presence of antibodies against *C. burnetii* in 5 samples out of 51 tested; One sample of 28 tested was positive for *L. Interrogans*; finally, *T. gondii* 8 samples out of 53 tested. The differences between number of samples analyzed is due to the high degree of hemolysis of some of them. However, these difficulties led to a new approach on 2019: from January, to by-pass this technical difficulty, the serum was directly withdrawn on live animals during the rescue operation. To date, the following results have been obtained from 9 sera: a positive one for the presence of anti PI3 antibodies; one positive VMV sample; one *T. gondii* positive sample; one doubtful result on a sample for BHV1; and finally, a doubtful sample for *Chlamydia spp*. The new sampling method seems to be more sensible and more effective, and will allow a greater knowledge of our territorial reality and the drafting of more effective surveillance plans for the protection of livestock and all the operators in the sector.

**Keywords:** Roe deer, Fallow deer, wildlife monitoring plan, serum, infectious diseases

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## Drought as a risk factor to Tuberculosis infection in free-hunted red deer in Southeast of central Portugal

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**Abstract:** In the Mediterranean ecosystem, one of risk factor described, in the literature, to the Tuberculosis (TB) infection in wild species is the climate, specifically the occurrence of drought periods. The red deer is one of the most abundant wild species in Portugal and reveals a high prevalence of TB (7-10%). Our study contributes to define that periods of drought increase the TB incidence in free-hunted red deer inspected *post mortem* in the southeast region of central Portugal during 8 hunting seasons (2009/2010 to 2016/2017); inferring an positive effect of periods of drought in the transmission of TB.

**Keywords:** drought, Tuberculosis, red deer, *Cervus elaphus*

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### 1. INTRODUCTION

Wild animals are susceptible to infection by most of the same pathogenic agents that affect domestic animals. From those, Tuberculosis (TB) is one of the infectious diseases which causes more concern worldwide. TB is a multi-host disease caused by mycobacteria of Mycobacterium tuberculosis complex (MCT). About TB in the wildlife in the world, the literature refers that one of the most affected class of animals are the cervid, including the red deer (*Cervus elaphus*) and white-tailed deer (*Odocoileus virginianus*). In the Europe, specifically in the Iberian Peninsula, the most abundant and which presents a higher occurrence of TB infection is the red deer (Palmer, 2013). Areas with Mediterranean climate, with large number of extensive livestock farms and with large number of wildlife hosts are the regions with a higher prevalence of TB in wild and domestic animals (Vicente *et al.*, 2013). In Portugal, studies in the southeast of central region (area with the mentioned risk characteristics), reveals prevalence of TB in red deer between 7 and 10% based on inspection *post mortem* data (Vieira-Pinto *et al.*, 2011). In one Mediterranean ecosystem, one-third of TB positive red deer, with laboratorial confirmation, at necropsy have generalized tuberculosis-like lesions (TBL) (Vicente *et al.*, 2013). The main route of infection is the alimentary, once the gastrointestinal lesions are in greater number comparatively to respiratory lesions (Nugent *et al.*, 2015). Literature refers some potential risk factors to the TB infection in the red deer: low genetic variability, high population density, the habitat (mountain areas with forest with Mediterranean climate), spatial aggregation and permanence of fenced areas (Vicente *et al.*, 2013; Queirós *et al.*, 2016). The existence of a hunting industry in some parts of Iberian Peninsula (the southeast of central Portugal, for example) where the hunting species, such as red deer, are restricted to a certain area enhance all these risk factors (Vicente *et al.*, 2013). The Mediterranean climate is characterized by periods of alternating drought and rainfall. This characteristic can influence the transmission of TB between the red deer and another cohabitant species. Drought can promote a direct or indirect contact between intra and inter-species due to the considerable decrease of food and water supplies during this time (Vicente *et al.*, 2013). Our objective in this study was studying the influence of the drought in the TB infection in free-hunted red deer in the southeast of central Portugal, during 8 hunting seasons (2009/2010 to 2016/2017).



## 2. MATERIALS AND METHODS

### 2.1 Area of study

The study was developed in the southeast region of central Portugal, more specifically in the Idanha-a-Nova county (lat 39° 55'N; long 7° 14'W; 1416,0 km<sup>2</sup>). Geographically, Idanha-a-Nova is a county with a temperate Mediterranean climate (dry summer and rainy winter), but with marked drought periods. In relation of the landscapes, Idanha-a-Nova have a marked territory of mountains mixed with large plateaus. Par excellence, it is a region for hunting activities in Portugal, mainly large game.

### 2.2 Data collection

In this study, the data used for the statistical analysis were the number of free-hunted red deer TBL positive at inspection *post mortem*. The data collection occurs in 8 hunting seasons (2009/2010 to 2016/2017) in Idanha-a-Nova. The sample represents a total of 1289 free-hunted red deer presented to sanitary inspection in the spot after hunting journey. It was excluded of the sample the hunted red deer in fenced areas and subject to artificial supplementation. Data about severe drought periods in Portugal was obtained by means of public records published by Instituto Português do Mar e da Atmosfera, I.P. (IPMA, I.P.). The IPMA graphic (Figure 1) allow us to determine which drought periods in Portugal have influence in our sample of hunted red deer.

In this study, was analysed the influence of a drought period before the beginning of the hunting season in the possible increase of TBL prevalence in red deer in the moment of the data collection.

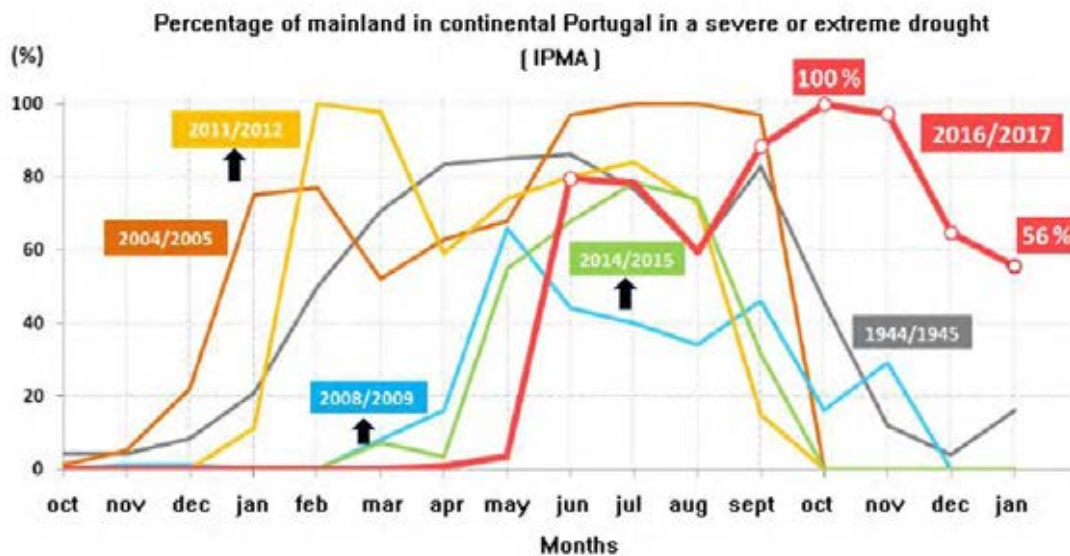


Figure 1. Graphic with indication of main drought periods in continental Portugal in last decade. With black mark is ticked the drought periods considered to our study sample (2008/2009; 2011/2012; 2014/2015). (Adapted from IPMA).

### 2.3 Statistical analysis

The data was organized in a two-by-two table in order to analyse “previous drought period before the beginning of the hunting season” as a risk factor (exposure) to increase the prevalence of TBL in red deer (outcome). To the analysis of variables were used: Fisher's exact Test to evaluate the significance

of the association between variables and Odds ratio to measure the strength of association between an exposure and a outcome) and. The probability value (p) <0.05 was considered as statistically significant. Both were carried out through the program of data statistical analysis called EpiTool version 0.5-6.

### 3. RESULTS

Of the 8 hunting seasons analysed, 3 (2009/2010; 2012/2013; 2015/2106) were considered risk factors, since the previous period was considered with severe drought. During these 3 risk seasons, a total of 360 animals exposed to the risk were evaluated during sanitary inspection at the spot. From those 32 were TBL positive (8,89%). During the 5 non-risk hunting seasons, a total of 929 no exposed to the risk were analysed of being the TBL prevalence of 4,74% (44/929). According to the statistical analysis, the drought period before the hunting season revealed a statistically significant association (p <0.05; IC 95%) with the prevalence of TB-like lesions in free-hunted red deer. After one drought period, the probability of TBL prevalence in red deer in the next hunting season was 1,96 [*Odd ratio*=1,96; (1,22; 3,15)] times higher than when are not exposed to a drought period in the previous season. A previous drought period had a relative risk of 1,88 and a population attributable risk of 0,01.

### 4. DISCUSSION

The TB infection in red deer was deeply related with habitat characteristics. In a Mediterranean ecosystem, to the red deer, the summer was the limitative season. The dry conditions in some months in this ecosystem affects the body condition of the red deer, making them more susceptible to TB infection or to generalization of the TB-like lesions pre-existent (Vicente *et al.*, 2013) In addition, extensive drought periods, usually in Mediterranean climate, after an hot and dry summer in the previous season, promotes an aggregation of the species. In natural conditions is extremely difficult to find a wild population with only one specie and the natural coexistence of several species can be a factor of promotion of the TB transmission, especially in a multi-host system such as Portugal (Queirós *et al.*, 2016). The aggregation, especially in specific points rich in resources, facilitate an intra and inter-species contact and the transmission of infectious agents. Our study confirms a positive influence of drought periods in the increase of the TB prevalence in the red deer, included a duplication of the probability of TB positive red deer in a hunting season after one period of severe or extreme drought. Collect information of the sanitary inspection *post mortem* in the hunting season is an affordable and cheap method of determine the prevalence of TB in red deer and stablish an TB-like lesions pattern to the specie (Vicente *et al.*, 2013). Specifically, for the red deer, studies have identified that only a small proportion of lesions are in respiratory tract, which proof that the main infection entryway is not by contaminate aerosol, but by the alimentary way (Nugent *et al.*, 2015). The oral infection was linked with the contamination of the water and food. In the red deer, most of TB infection occur by indirect contact in risk points, where the aggregation of TB susceptible species is frequent and exist a share of food and water resources (Vicente *et al.*, 2013).

### 5. CONCLUSIONS

This pilot-study should be extended to another climate or anthropogenic factors described in the literature like risk factors. The study of these habitat characteristics in the Iberian Peninsula, where the Mediterranean ecosystem has a marked influence in the dynamic of the red deer population, it is important to find feasible and effective solutions in the control of the TB.

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