

**First Iberian Congress of Applied Science on Game
Resources (CICARC)**

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ABSTRACT BOOK



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Analysis of red deer and roe deer antlers

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Key words: Ungulates, antlers, cortical bone, minerals, trophy.

The trophies of hunted ungulates, such as antlers, are a main secondary sexual character that animals can use as a weapon for defence; their expression can inform about the quality and *fitness* of the animal that bears them. For antlers to be effective during fights, they need an adequate mineral composition and mechanical properties to avoid the risk of breaking. Here we study the mechanical properties, internal structure and mineral profile of the cortical bone of red deer (*Cervus elaphus*) antlers in order to better understand the quality of trophies. Thus, it was hypothesized that antler composition could be used as a tool to monitor the quality of an animal or population, or to study the effects of environmental factors and differences in diet. Samples from various positions of the trophy were used: cross sections are obtained to analyse the internal structure, and cortical bars were extracted from cylinders to perform mechanical tests and analyse mineral content. Recently similar research has been conducted on other game species, such as roe deer (*Capreolus capreolus*). Promising results have been obtained for populations characterized by different habitats and management clearly, which are reflected by differences in the trophies of roe deer. This method is a promising tool for the study of antlers quality and to estimate if a sustainable use of wildlife has been achieved as a natural resource. In this study, the external characteristics of the antlers, their structure, mechanical properties and mineral content showed similar patterns to those previously published for red deer raised with different quality of their diet.

Managing hunters' satisfaction

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Key words: hunters' satisfaction, game management, large game species.

This work shows the key elements determining the satisfaction of large game species hunters during hunting chases. No similar studies have been conducted so far in the Iberian Peninsula. The study will contribute to evaluate whether current game management models try to ensure hunters' satisfaction as final customers and whether the necessities of hunters act as modulating factors of hunting markets. Furthermore, this type of studies is essential to provide specific data about hunters' motivations to practice hunting activities in the framework of the current socio-political debate. Field work consisted of making questionnaires to hunters after the chase, collecting biometrical data from hunted specimens, and compiling data on chase organization and on the estate management system. The questionnaire had a first block to classify hunters according to age, expertise and expectations. A second block questioned about relevant events happening during the activity. Finally, a last block includes 11 items to which hunters must assign a satisfaction value according to a Likert scale from 1 (very low) to 5 (very high). The results show that hunters' satisfaction is highly correlated with the satisfaction regarding the hunting estate. Non-selective chases result in higher satisfaction values. The number of shooting stations and the number of casts with the opportunity to shoot are tightly related to overall satisfaction. It is noteworthy that intangible elements like enjoying the countryside, spent a day with friends or sharing the results of the activity with other mates obtained high values in the satisfaction scale.

Evaluation of the economic and social impact of hunting in Spain

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Key words: employment, Gross Domestic Product, hunting, tax return rate.

Hunting is considered as a key source of income in the Spanish countryside, with an added social value owing to the employment generated in areas where farming and livestock are not very productive. However, there is a lack of large scale studies assessing the contribution of hunting to the Spanish economy and society, which was the aim of this study. We evaluated the expenditures of different stakeholders using input-output tables published by the National Statistics Institute, and the specific multipliers for each sector together with macroeconomic aggregates. In this study, we chose the regions of Andalucía, Aragón, Castilla-La Mancha, Castilla y León and Extremadura, as they cover approximately 70% of hunting ground and 65% of hunters registered in Spain. On-line and targeted surveys were addressed to hunters, landowners, dog-pack owners and shooting providers, conducting a total of 4500 surveys. We also evaluated the expenditure of the game meat industry, estates agencies and national and regional administrations. In 2016, the value to the Spanish economy of the shooting sports was 5470 million euros, equivalent to 6475 million euros when calculated as Gross Domestic Product (this amount constitutes a 0.3% of the total Spanish GDP). Additionally, the rate of return generated by hunting was 614 million euros. Shooting supported a total of 186,758 full-time equivalent jobs (FTE), which represents 1% of the Spanish work force, being 141,261 indirect jobs and 45,497 direct jobs (employed by shooting estates and providers). Eighty percent of shooting estates organized awareness-raising activities and 33% took part in programs for wildlife conservation, investing a total of 287 million euros in management targeting wildlife. Our research shows higher values of expenditure compared to previous studies, confirming that shooting is a key economic sector for the countryside.

The tip of the iceberg of illegally shot raptors in Castilla-La Mancha: importance of the effect of the partially closed season

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Key words: protected birds, illegal hunting, shot, prosecution by humans, raptors.

Raptor birds have been traditionally prosecuted in Europe because they are considered a threat to game species. Shooting raptors was banned several decades ago, although unfortunately, it still happens in many European countries, which contributes to population decreases of these emblematic species. In this work, we assess the admissions of shot diurnal raptors in Wildlife Recovery Centres (WRC) in Castilla-La Mancha. A 4% (619/14895) of all avian admissions in WRC are because of direct shot to protected species, and an 88.2% of these admissions (546/619) are raptors. Among raptors, the percentage of admissions related to shot animals is 23.1% (126) during the closed season, 22.9% (125) during the partially closed season, and 54.0% during the open hunting season in autumn and winter. Given that the former period lasts for 124 days, and that the partially closed season is only one fourth of the open season (30 days), the weekly admission rate of raptors is significantly higher during the partially closed season ($p=0.002$). We discuss several reasons to explain this large difference. The partially closed season coincides with a period of post-nuptial migration and post-generative dispersal of raptors, which become especially vulnerable to illegal hunting. Because of the increased frequency of illegal acts against raptors happening during this period, and because of the scarcity of the two species involved in the partially closed season (European turtle dove and common quail), we propose: (1) an elimination of the partially closed hunting season; (2) raising awareness of hunters; and (3) an increase of surveillance and punishment tightening by administrations. It should be highlighted that the admission of shot raptors in WRC is just the tip of a large-dimension iceberg suggesting that the real number of crimes against protected birds could be much higher than what we present here.

Distribution and abundance of wildlife in Europe: towards harmonization in data collection and use

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Palabras clave: distribution, abundance, wildlife, spatial modelling, risk assessment.

The consortium ENETWILD runs a project (www.enetwild.com) for the European Food and Safety Authority (EFSA) which aims at improving the European capabilities to monitor wildlife populations, developing standards for data collection and validation and, finally, creating and promoting a data repository in order to analyze the risks of shared diseases among wildlife, livestock and humans. This information is also essential for conservation and management of wildlife in Europe. ENETWILD has focused so far on the collection of wild boar (*Sus scrofa*) abundance and distribution data, which will be completed during the coming years with other groups of species: migratory birds relevant to avian influenza, as well as ungulates and carnivores. In addition, we are conducting a parallel project, also funded by EFSA, to assess the usefulness of citizen science to collect information on the distribution and abundance of wild mammals in order to improve the availability of information throughout Europe, and complete gap areas where there is no data. In this communication we present (i) the differences between the hunting data collection frameworks (exemplified in ungulates) across Europe, (ii) the state of data collection by the project, and (iii) the last wild boar spatial distribution and abundance models based on them. Finally, there is a need for a common basis for wildlife management in Europe, and we exemplify the response to the emergence created by African swine fever in wild boar, not always based on technical knowledge.

Systems for collecting wild boar hunting statistics in Spain (European context): first approach

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Key words: ungulate, hunting bag, wild boar, Spain, trends analysis.

Hunting statistics at regional, national and international levels are useful for assessing current population trends, and how populations react to variations in extraction. However if each region of Spain collects hunting data using its own specific methods, a common use of these data will not be possible. This report analyses the differences between wild boar hunting data collection systems in Spain and contextualises them in the rest of Europe. We used questionnaires and retrieved data to describe the main strengths and weaknesses, suggest realistic and short-term improvements to make the data comparable between territories, and propose a hunting data collection framework for wild boar, which could turn useful to other large game species. Based on hunting statistics, we make an approximation on the current state of wild boar population dynamics at national and European level in order to define trends of species throughout their distribution. We have shown that, at the European level, Spain has one of the best data collection systems, although it is heterogeneous depending on the region. In order to harmonize the collection of hunting data in Spain, we propose to prioritize the collection of data at a better spatial and temporal resolution in each region: hunting event level, at least in part of the regions. This will allow us to characterize the effort and effectiveness of hunting. With the purpose of monitoring population trends and develop practical and reliable monitoring systems, it is recommended that a national hunting observatory is established. In this network of controlled sites, census protocols would be established to determine the abundance of populations, and long-term hunting and abundance statistics would be collected.

Use of ecological change indicators to assess red deer (*Cervus elaphus*) management in Quintos de Mora

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Key words: management, ecological change indicator, *Cervus elaphus*, habitat, density.

The red deer (*Cervus elaphus*) is the most important game species in Europe and one of the most emblematic ones in the Iberian Peninsula. Management of this ungulate raises great ecologic and socioeconomic relevance. We assessed red deer management at the Quintos de Mora estate, where comprehensive interventions on hunting and habitat have taken place over the years. For the 2006-2017 period, variations in ecological change indicators (namely, demographic pattern, breeding success, development, physiological and sanitary status) were analyzed after considering environmental variations. Analyses revealed that population density tended to remain stable ($r = -0,35$, $p = 0,23$, $n = 13$) throughout the study period, with average densities by time intervals being 0,36, 0,26, and 0,32 individuals/hectare for 2006-2010, 2011-2014, and 2015-2017, respectively. Reproductive success remained constant across the study period. Development indicators, such as the kidney fat index (KFI), have been increasing both in the adult ($n = 546$) and juvenile ($n = 209$) populations, consistent with the results from parasitic burden studies. However, the trophy quality and health condition remained unchanged. Results indicate that the red deer population management, based mainly on culling quotas and supplementary feeding (by creating grass- and legume-based pastures) was appropriate to maintain red deer populations at levels which do not result in unacceptable damages in terms of most of the ecological change indicators that have been evaluated.

Critical analysis of three decades of game species monitoring in Navarra. A proposal for methodological standardization

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Key words: methodology, standardisation, census, hunting activities, Navarra.

Since the beginning of the 90s, the Government of Navarra has implemented a population monitoring system for the main game species. The small game species analysed are the red-legged partridge (*Alectoris rufa*), the European hare (*Lepus europaeus*), the Iberian hare (*Lepus granatensis*), the rabbit (*Oryctolagus cuniculus*), the fox (*Vulpes vulpes*), the magpie (*Pica pica*) and the turtle dove (*Streptopelia turtur*). As for big game species, the ones that are monitored are roe deer (*Capreolus capreolus*), deer (*Cervus elaphus*) and wild boar (*Sus scrofa*). The methodology used is the Kilometric Abundance Index (KAI), either in its nocturnal or diurnal version depending on the behaviour of the monitored species. A total of 210 routes throughout Navarra have been monitored uninterruptedly by the environmental wardens of the Game and Wildlife Department. We present the results of these three decades of monitoring of the main game species in Navarra, describing the advantages and limitations of the methodology used, in case it would be useful in terms of methodological standardisation of game species monitoring protocols.

Population status of the Iberian hare (*Lepus granatensis*) after the mixomatosis outbreak in Castilla-La Mancha

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Key words: density, lagomorphs, management, Poxvirus, survey.

The Iberian hare (*Lepus granatensis*) is an endemic species widely distributed across the Iberian Peninsula. Although hares were once abundant, their populations have declined during the last decades, and a mixomatosis outbreak affecting hares was detected for the first time in 2018. This study aimed to evaluate the population status of hares and assess the possible impact of mixomatosis. We conducted a national survey targeting shooting estates to get information about hare management and impact of mixomatosis. Additionally, we conducted 54 nocturnal hare counts in Castilla-La Mancha region with the help of spotlights to assess hare densities, using line transects with variable strip-width, and testing stratification by habitat type and province. A total of 242 shooting estates took part in the national survey, from eight regions and 31 provinces. Hares with mixomatosis were observed in 45% of the estates, mainly from Andalucía, Castilla-La Mancha, Extremadura and Madrid. In Castilla-La Mancha, the impact of mixomatosis (observed dead/sick hares) varied significantly among provinces, ($F_{(4,51)} = 6,58 P < 0,001$), with two areas being clearly differentiated according to the estimated impact: Albacete, Ciudad Real and Toledo, with a higher impact, and Cuenca and Guadalajara, with a lower/null impact. The impact of mixomatosis was negatively correlated with altitude ($F_{(1,42)} = 7,44 P < 0,01$). A total of 550 hares were counted, with an average density of 7.6 hares/100 ha, and differences were observed between Cuenca province (3.25 hares/100 ha) and Albacete/C. Real (8.6 hares/100 ha). An increased impact of mixomatosis was found in arable when compared to shrubland and forest, though marginal differences were found ($F_{(1,42)} = 3,32 P = 0,07$). Hare densities from this study are similar to available data before the mixomatosis outbreak, which suggests that hares have not suffered a strong population decline.

Monitoring of woodcock (*Scolopax rusticola*) in mainland Portugal

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Key words: monitoring, Portugal, relative abundance, demography.

The woodcock (*Scolopax rusticola*) is an important migratory game species in Europe that cannot be monitored by common census techniques. In mainland Portugal, where it is only present during autumn-winter, the Portuguese Woodcock Hunting Association (*Associação Nacional de Caçadores de Galinhas*, ANCG) collects information during the hunting season (November to February), that can be used to monitor this species. For each hunting trip, the hunters report the date, location, duration, number of observed and captured woodcock, and the number of hunters and dogs that participated. For captured birds, they are asked to determine the sex (by gonad observation), record the location and date of capture and send a wing for age determination by plumage analysis. We used generalized additive mixed models (GAMMs) to investigate the variation in the number of woodcock observed per hunting trip, during and between hunting seasons (2009-2010 to 2018-2019). Demographic parameters (sex ratio females:males, and age ratio juveniles:adults) among the captured birds were also analysed. The data collected confirm the occurrence of the woodcock in the 18 districts. The variation of relative abundance during each hunting period shows inter-annual differences in migratory phenology. Relative abundance tended to be higher in the South, regardless of the hunting season. Despite the variation observed between hunting seasons, relative abundance remained stable. Sex ratio remained close to one. Until 2016-2017, the age ratio varied between 1.0 and 2.2, but fell to 0.5 in 2017-2018. This work is an example of the effective use of data collected through citizen science, which aims to contribute to maintaining a favourable conservation status of the woodcock, while making a rational use of their populations through sustainable and controlled hunting.

Reliability or effort economy? A new proposal for common Quail surveys

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Key words: *Coturnix coturnix*, survey, reliability, costs.

Field surveys are useful to estimate the abundance, distribution or population trends of species, and are essential to determine their conservation status. Therefore, it is important that surveys are as reliable as possible. Most common Quail (*Coturnix coturnix*) surveys (SOCC and SACRE in Spain) are passive (i.e. the number of calling males along a transect at the given survey time is recorded). These passive methods have several advantages like their remarkable economy of effort (only two fieldtrips per year), which allows for a broad geographical scale implementation. However, their reliability has been questioned by recent studies because they have been shown to be density dependent, which can bias population trend estimates, and almost incapable of detecting animals in areas of low population density. The most reliable alternative would be the application of an active method, which consists of a digital female decoy that actively stimulates the calls of the males. However, this method requires an increased effort investment (a weekly sampling day during the whole breeding season), which causes their practical implementation to happen on a local scale only. In the present study, we conducted an analysis with the aim to reduce the effort necessary to implement the active method without involving a significant reduction in its reliability. This will allow this method to be implemented on a broad territorial scale. The results showed that in areas of good Quail habitats, the reliability of surveys is maintained by performing either four annual visits to 10 listening points, or seven annual visits to five points. In contrast, in areas with unfavourable habitats, seven visits to nine listening points or eight visits to seven points are needed.

The grey partridge (*Perdix perdix hispaniensis*) in Castilla y León: monitoring of this previously hunted species in the region and reflection on its status in Spain

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Key words: *Perdix*, grey partridge, Spain, population.

Grey partridge populations were monitored in several Zones of Special Protection for Birds (ZEPA) during the period 2017-2018, as part of a larger monitoring scheme of Natura 2000 areas of Castilla y León. After designing the census, fieldwork was carried out in 2017 (Sierra de la Demanda and Sierra de Urbión) and 2018 (Sierra de los Ancares, Montes Aquilanos, Picos de Europa and Fuentes Carrionas-Montaña Palentina). The methodology used consisted of walk-up transects with dogs conducted during spring (May-June) and autumn (September-October). Plots were previously selected with the objective of determining the area of occupation, detecting breeding pairs in the first period and successful in the reproduction (brood sizes) at the end of summer-beginning of autumn. A total of 124 plots were sampled (72 in spring and 52 in autumn), obtaining 237 contacts with the species: 122 bird sightings, 103 contacts with dropping presence, 6 contacts with feather presence, 3 responses to playbacks and 3 others types of contacts. With the 122 sightings, a total of 379 partridges were seen: 84 contacts with 152 partridges in spring (1,81 partridges / contact) and 38 contacts with 227 partridges in autumn (5,97 partridges / contact). The presence of the species was confirmed in 35 out of 46 surveyed UTM 10x10 squares. The fieldwork was carried out with the close collaboration of mountain hunters, especially the Hunting Federation of Castilla y León. Hunting associations have a great interest in the study of this species, which may require protection, temporarily or permanently. The population, conservation and hunting status of the species in Spain are discussed.

Deciphering the anthropogenic impacts on the genetic background of the red deer in the Iberian Peninsula

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Key words: *Cervus elaphus*, population management, genetic diversity, hybridization.

The evolutionary history of the red deer (*Cervus elaphus*) has long been shaped by large-scale biogeographic phenomena and anthropogenic impacts. In the Iberian Peninsula, although current phylogeographic pattern is deeply marked by past natural events (i.e. glacial cycles), human-mediated factors seem to modulate the genetic pool of red deer populations, namely by the introduction of exotic genes into natural populations. In this study, we aim to i) quantify the extent of hybridization and introgression among Iberian red deer populations; ii) evaluate the influence of management regimes in current patterns of hybridization and introgression; and finally iii) understand how hybridization and introgression shape current patterns of genetic diversity across Iberian populations. To achieve these goals, forty-seven populations were sampled throughout the Iberian Peninsula, including populations from fenced, free-ranging and protected management regimes. In addition, nine free-ranging red deer populations were sampled across Europe and used as reference European populations. In total, 1307 samples were genotyped for a set of 11 highly polymorphic microsatellite markers and sequenced for a fragment of mitochondrial control region gene. A Bayesian clustering approach was used to classify individuals as native or native x exotic hybrids. The proportions of hybridization and/or introgression with exotic alleles of the Iberian red deer populations were compared among management regimes, and their impact on genetic diversity estimates was also assessed. In addition, we show how the common practice of introducing exotic red deer to improve hunting trophies may impact on genetic background of Iberian red deer populations and disrupt well-adapted genes to native ecosystems.

The risk of genetic introgression in the red-legged partridge populations of the Sierra de Guadarrama National Park

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Key words: partridge, *Alectoris*, introgression, genetics, Guadarrama.

The red-legged partridge (*Alectoris rufa*) has suffered a remarkable regression in the last decades that has been counteracted by the hunting industry with massive releases of farm-reared hybrid partridges, producing alternations in wild populations that have homogenized and contaminated their natural gene pool with a strange domestic genome. The Sierra de Guadarrama maintains high mountain populations that have remained oblivious to these problems suffered by the species. The objective of the study is to confirm the genetic purity of the populations of the National Park, evaluating the risk of introgression and comparing its genetic structure with that of populations at the foot of the mountain range. We used two types of samples for the genetic analyses: on the one hand, tissue of specimens captured either live (with the use of a decoy) or shot in hunting grounds, and, on the other, non-invasive samples collected in roosts (feathers and faeces). Faecal samples accounted for approximately 22% of all analysed samples. From a total of 65 samples, good quality DNA was obtained in 51 tissue samples (tongue, blood, finger and muscle), although in the case of feathers, quantity and quality of the extracted DNA varied greatly depending on its conservation state. The DNA extracted from faeces (n = 14) was always degraded and scarce, although the performance improved as the extraction protocol was perfected. To study genetic introgression, 24 diagnostic markers of hybridization between red-legged and chukar partridge were used. In the DNA obtained from faeces, at least four markers were genotyped in all samples. The base study on hybridization in Guadarrama has led to a more ambitious study on phylogeography and population genetics, the first results of which will be presented at this congress.

***Blastocystis* spp. in partridge, quail and steppe birds. Possible effect of stress and zoonotic subtype in farmed partridges**

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Key words: metabarcoding, parasites, stress, zoonoses.

In this study, we use metabarcoding analysis, with broad-spectrum 18S primers, to detect *Blastocystis* parasite in game species and in protected steppe bird species, and determine which subtypes (ST) appear in these birds as well as their importance. We analysed fresh faecal samples of six species grouped by flocks or origin; six flocks of great bustard (*Otis tarda*), 15 flocks of little bustard (*Tetrax tetrax*), 10 flocks of pin-tailed sandgrouse (*Pterocles alchata*), two flocks of black-bellied sandgrouse (*Pterocles orientalis*), seven flocks of red-legged partridge (*Alectoris rufa*), five farms of red-legged partridge and one group of quails (*Coturnix coturnix*). All samples were collected in Spain except for one flock of little bustard that was sampled in the Republic of Kazakhstan. We found *Blastocystis* on all of studied samples, but the parasitic loads were very different among species. The percent of *Blastocystis* sequences in great bustard (3.62% of all sequences, 95.6% of protist sequences) and little bustard (10.82% of all sequences, 90.6% of protist sequences) were particularly high. Although we do not know the effect of *Blastocystis* on the gut microbiota of these hosts, proliferation of this parasite in humans and other species has been associated to immunocompromised or stressed individuals. This could indicate that the sampled individuals were under severe stress or suggest a pathogenic potential of *Blastocystis* for these two species. *Blastocystis* sequences were also detected in the other species; in farmed red-legged partridges, percent of *Blastocystis* sequences was of 0.24% and in the rest of species, it was lower than 0.1%. On the other hand, we found one *Blastocystis* sequence of a zoonotic subtype. This was the most common sequence in farmed partridges, but was rare in wild partridges as well as in the other studied wild birds.

Effectiveness and biases of the battue and nocturnal single hunt as methods in the control of wild boar populations

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Key words: selective hunting, population control, methods, population structure.

Conservation measures and urbanistic planning in the massif of Collserola (Barcelona, Spain), have caused new environmental conditions where wild boar live adapted. In periurban areas of some cities as Barcelona, wild boar populations growing up to 15 individuals/100 ha triggering security conflicts and social alarm. From hunting season 2014-15 to 2018-19 the hunting bags of 94 battues (39.5 hunters on average) and 859 nocturnal single hunt (years 2014 to 2018) were analysed. The battue with dogs is the traditional hunting method in Collserola, and is similar to drive hunts conducted elsewhere in Europe. Battues are carried out during the hunting season (October to February) in 21.1% of Collserola area. Single hunt method involves the use of bait to attract wild boar to a specific point where only one hunter stands with artificial light. This is used as alternative method when and where the hunting is forbidden (72.7% of the Collserola area). The hunting efficacy was similar for both methods (0.36 wild boar harvested/observed in single hunt and 0.35 in battue), but the efficiency was greater in single hunt (0.42 wild boar hunted/hunter) than in battues (0.11). Although the single hunt allows slightly selective harvesting regimes, it was biased toward adult-male (37.4%, respect 28.7% in battues). The results reported here could help to manage wild boar populations, mainly in areas where the traditional hunting is forbidden.

Is a double wild rabbit management necessary? Updated review of the differences between *Oryctolagus cuniculus cuniculus* and *O. cuniculus algirus*

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Key words: behaviour, growth, management, population trends, reproduction.

The wild rabbit (*Oryctolagus cuniculus*) plays at the same time important ecological, hunting and economic roles in its native range, the Iberian Peninsula (IP). As a direct result of this complex setting, the interests of the stakeholders sometimes come into conflict, making necessary the establishment of clear and accurate management measures for each framework. For this, it is essential to rely on rigorous scientific and technical information on the rabbit's ecology and biology. Until recently, such information has been mostly obtained from areas where the species was introduced, to further extrapolate them directly to management in the IP. Furthermore, it is precisely at the IP where both rabbit subspecies, *O.c. cuniculus* and *O.c. algirus*, coexist naturally as a result of 2 million years of isolation; both subspecies still keep a quite allopatric geographical distribution. This work presents an updated revision of the existing scientific knowledge about rabbit subspecies differentiation, with a special focus on its repercussions on the current rabbit management model. For this, it will be shown not only some of the best known differences, such as some biological, genetic or morphological aspects, but also other less known differences between both subspecies, including reproductive and growing parameters, behavior or populations. Finally, it will be discussed the necessity or potential benefit of setting different management frameworks for each subspecies.

Advances in predation reduction by conditioned aversion

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Key words: non-lethal predation control, wildlife management, fox, partridge, rabbit.

In the last decades, the populations of the two main species of small game of the Iberian Peninsula, the European wild rabbit (*Oryctolagus cuniculus*) and the red-legged partridge (*Alectoris rufa*), have experienced generalized declines. To improve these populations, predator control and translocations are two of the most used hunting management tools. The conditioned aversion (CA) allows reducing the consumption of a prey and, therefore, its predation rate, by adding a chemical substance that causes discomfort (vomiting) to the consumer. The predator will avoid the consumption of that prey in subsequent encounters, even in the absence of the substance that generated the discomfort. We have experimentally tested the application of CA on red foxes (*Vulpes vulpes*) to: 1) reduce predation of partridge nests and test the effect of this reduction on the productivity and density of wild partridges; 2) reduce the initial predation during rabbit translocations, to increase rabbit survival and improve population recovery after the translocation. The application of CA reduced predation on artificial nests by 27-50%, increasing productivity by 132-677% and subsequent density of partridges by 28-107%, compared with control areas. Furthermore, CA reduced rabbit predation, improved survival in the first days after the translocation, and significantly increased occupation rate in the warrens of release, and changed population trends. The abundance of rabbits tripled in the treatment areas, but decreased slightly in control areas. These results indicate that CA can be a non-lethal tool to reduce predation by wild canids, with a clear application in the management of hunting resources.

The distribution of the Egyptian mongoose (*Herpestes ichneumon*) in Castilla-La Mancha. First results from MELOCAM project

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Key words: Egyptian mongoose, environmental warden, expansion, surveys, Castilla-La Mancha

The Egyptian mongoose (*Herpestes ichneumon*) is the only mongoose species (Herpestidae family) occurring in Europe since historic times. It is currently expanding in the Iberian Peninsula from south-western areas. Hunters from certain areas of Castilla-La Mancha complain about the supposed impact of the Egyptian mongoose on some small game species. However, it is unclear whether the mongoose is actually spreading and if the hunters' complaints are rather due to a false perception of high abundance explained by its diurnal habits and frequent movements in groups. Knowing the species situation and possible impacts over potential prey are consequently of great interest. These are the main aims of the MELOCAM project. We employed two methodologies to determine the distribution and possible expansion of the Egyptian mongoose in Castilla-La Mancha. On one hand, we distributed an online questionnaire to environmental wardens about observations of the species. On the other hand, we gathered data of Egyptian mongoose occurrence supplied by technicians and researchers. The information obtained through the two methods is complementary. The questionnaires to environmental wardens provided systematic information of most part of the region, covering 90% of municipalities. Meanwhile, technicians and researchers provided occurrence data for specific points from some areas. The Egyptian mongoose is distributed throughout most of the Toledo and Ciudad Real provinces and the most western area of Albacete province. Its presence is occasional in Cuenca and Guadalajara provinces. The Egyptian mongoose in Castilla-La Mancha has spread to new areas from the southern nucleus of Ciudad Real and Toledo in the last decade. These results suggest that the application of this kind of methodology, based in questionnaires to experts, could be useful for assessing the distribution of many wildlife species, especially of those for which the monitoring through traditional methods is costly or complicated.

Migration of teal *Anas crecca* wintering in Portugal. The use of metal rings vs. nasal saddles vs. PTT vs. GPS/GSM

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Key words: Anatidae, movements, tagging, efficiency, costs.

The teal is the most abundant duck during the winter, is a game specie and is exclusively a wintering migrator in Portugal. More than 5000 teal were captured and ringed in Portugal since 1993. From those, more than 4300 were nasal-saddled and produced more than 10,300 resightings (www.pt-ducks.com). The recoveries, recaptures and resightings, modeled by geographic information systems, allowed the definition of migratory routes from the breeding grounds to the wintering sites in Portugal. Most of the teal used the East Atlantic migratory route, with breeding grounds from Iceland to Siberia (Russia). The nasal saddles allowed a 70% increase on the data obtained internationally, with several individuals responsible for multiple observations in different sites. The use of seven satellite transmitters based on the technology Argos "Platform Transmitter Terminal" (PTT), since February 2017, and of 10 GPS/GSM transmitters, since February 2018, already produced new information, some unexpected, and will allow a better future modelling with geographic information systems. The teal migrates essentially during the night and can fly more than 1050 km during one night or more than 1650 km during two consecutive nights. The present advantages of PTTs in relation to GPSs/GSM, on this species, are only more reduced weights and position sending in remote areas (without GSM coverage). The GPSs/GSM cost about 1/3 of the PTTs, are much more precise, can work all day, can be remotely programmed, can have no external antenna and are reaching the weights of PTTs.

Effectiveness of the hunting regulatory measures for a species in decline: What data indicate about the European turtle dove (*Streptopelia turtur*)

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Key words: sustainable hunting, *Streptopelia turtur*, management, conservation, decline.

Halting the decline of the European turtle dove is one of the great current challenges of sustainable hunting and conservation. In declining game species it is essential to have a good knowledge about their population parameters and the hunting pressure they are being subjected to. Recent analyses suggest that the current levels of European turtle dove hunting are unsustainable. For this reason, implementing measures to regulate hunting in Spain to avoid overexploitation of the species is especially relevant. Evaluating the effectiveness of hunting regulatory tools is fundamental to be able to estimate their impact and therefore their validity to achieve the proposed objectives. We analysed the effectiveness of the measures implemented in some autonomous communities to reduce the number of turtle doves hunted, such as the application of quotas (number of turtle doves / hunter / day, including a more restrictive one in 2017 generalized to all regions), the reduction in the total number of hunting days or the number of hunting weekends in the season. We used mixed generalized linear models to compare catches at the provincial level between 2007 and 2017 with each regulation variable. Our results show no decrease in the number of turtle doves hunted at the provincial level associated with any of the hunting regulation measures. This may be due to the ineffectiveness of the measures themselves, to them not being adequately applied, or to official hunting capture data not being reliable. These results emphasize the importance of monitoring the management measures, as well as the urgency of finding appropriate measures to control hunting and conservation of the European turtle dove.

Quantification of the community of tuberculosis hosts in the Iberian Peninsula

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Key words: animal tuberculosis, multi-host disease, Iberian Peninsula, quantitative epidemiology.

Animal tuberculosis is a disease of economic importance for the livestock industry, subject to eradication programs in cattle. Despite the success of tuberculosis control in cattle, the trend in recent years has been a slight increase in prevalence. Epidemiological evidence points to the importance of wildlife and domestic species other than cattle in the transmission of tuberculosis to cattle. Observational and experimental studies support the maintenance of animal tuberculosis in the Iberian Peninsula in a multi-host system. The objective of this work is to quantitatively characterize the host community in the Iberian Peninsula, estimating the number of wild animals infected by tuberculosis. The actual prevalence was estimated based on the apparent prevalence, sensitivity and specificity of the diagnostic tests and combined in a Bayesian context with host abundance data to estimate the posterior distribution of the number of infected hosts. A geographically structured approach was used for wildlife because of the large regional differences in prevalence or abundance previously described in these species. The estimated number of animals infected by tuberculosis in the Iberian Peninsula is 225,760 - 1,295,162. Estimates of infected non - bovine species exceed that of infected cattle, with a ratio of 92.8 (IC₉₅ 22.1 - 955). These results support that in the Iberian Peninsula animal tuberculosis is a disease maintained by a community of domestic and wild hosts. The search for innovative control tools and the combination of multiple approaches to decrease the prevalence of infection in the main host species will need to be strengthened, in line with the strategy envisaged in PATUBES.

Mortality associated with serotype 4 of the blue tongue virus in populations of Iberian ibex (*Capra pyrenaica hispanica*) in Andalusia

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Key words: Andalusia, bluetongue, serotype 4, Iberian ibex.

The bluetongue (BT) is an infectious disease caused by the BT virus (BTV) (genus *Orbivirus*). This virus is transmitted by vector bites of the genus *Culicoides*, affecting different species of domestic and wild ruminants. Between October and November of 2018, 23 specimens of dead Iberian ibex (*Capra pyrenaica hispanica*) were detected in nine municipalities of the province of Málaga, which activated the emergency sanitary plan of the wildlife epidemiological surveillance program by the Department of Agriculture, Livestock, Fisheries and Sustainable Development of the Junta de Andalucía (JA). The necropsies and the histopathological analyses, carried out in the JA's Wildlife Analysis and Diagnosis Centre, of three animals revealed the presence of generalized congestion, oedema (especially subcutaneous in cervical and alveolar area), presence of sero-haemorrhagic exudate in various cavities, haemorrhages in serous of different organs and adrenal medulla, as well as fibrotic lesions in the lung, pericardium and meninges. Likewise, from blood samples from 10 of the 23 specimens obtained as part of the emergency sanitary plan were sent to the JA's Animal Production and Health Laboratory of Seville, where RNA of BTV was detected by molecular techniques (RT-PCR). Subsequently, using serotype-specific RT-PCRs at the Central Veterinary Laboratory of Algete (Spanish Ministry of Agriculture, Fishing and Food), it was confirmed that the serotype BTV-4 was involved in the outbreak. The results confirm, for the first time, the susceptibility of the ibex to the blue tongue. This outbreak coincides with the declared outbreaks of BTV-4 in seven livestock farms of domestic ruminants in the same province and period. Future studies are needed to evaluate the impact of the outbreak on the ibex populations and determine the origin of the BTV-4 involved in it.

Spatial-temporal analysis of the worldwide spread of the novel rabbit haemorrhagic disease virus (*Lagovirus europaeus*/GI.2)

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Key words: epidemiological outbreak, globalization, Lagovirus, Lagomorphs, RHDV2.

The virus that has traditionally caused the viral haemorrhagic disease of the rabbit (RHD), *Lagovirus europaeus* / GI.1, was first identified in China in 1984. RHD is a viral hepatitis that is usually lethal for both wild and domestic rabbits (*Oryctolagus cuniculus*), negatively affecting the dynamics of their populations. In 2010, a new variant of the RHD virus was identified in France (*Lagovirus europaeus* / GI.2 or RHDV2 or RHDVb). Since then, this variant has rapidly spread around the world, causing a large negative impact on wild rabbit populations. In this study, we carried out a thorough bibliographic review of scientific literature published since the appearance of the first GI.2 outbreak in May 2010 to November 2018, with the aim of evaluating the dispersion of this new lagovirus worldwide. In addition, we compared the dispersion of GI.2 with that of the original strain GI.1 during the first years after its first outbreak. The results reveal that at European level no differences in the rate of dispersion of both viruses are detected. Considering the detections worldwide, it seems that GI.1 reached its maximum distance of dispersion faster (4 years) than GI.2 (8 years). However, this difference could be explained by the action of man since, while there is no evidence to date of the deliberate release of GI.2, GI.1 was deliberately introduced in Oceania as a measure of biological control. Overall, our study confirms the very rapid expansion that both viruses have had worldwide. Finally, we discuss the implications of these results for the future management of other viruses, especially those associated with wildlife and species of hunting interest.

Shared food, shared pathogens. Avian pathogenic *Escherichia coli* (APEC) and antimicrobial resistance in faecal bacteria from red-legged partridges and other avian species using partridge feeders and drinking troughs

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Key words: red-legged partridge, feeders, release of farm-bred gamebirds, enterobacteria, antibiotic resistance.

The release of farm-bred birds, and the disposition of feeders and drinking troughs are the most important tools employed in red-legged partridges (*Alectoris rufa*) management on hunting estates. The feeders also benefit other species and, in estates with farm-bred partridge releases, may act as a contact and aggregation point between these and other birds. The need to control mortality and diarrhea in farm settings frequently require the use of antibiotics that may lead to the selection of populations of enteric bacteria that carry mechanisms of resistance against antibiotics or certain virulence factors that they then may excrete into the environment after release. We used camera traps to document the use of red-legged partridge feeders in two different hunting estates, one with partridge releases one without, in both, a pre-release (July) and a post-release period. We daily collected the feces of birds and other animals that had been using the feeders and used these to isolate enterobacteria and more specifically *Escherichia coli* carrying antibiotic resistance mechanisms and virulence factors marking them as potentially pathogenic for birds (avian pathogenic *E. coli* APEC). The prevalence of APEC and *E. coli* resistant to enrofloxacin was significantly higher (20.9% and 53.5%) in the estate with regular partridge releases than in the estate without partridge releases (0 and 1% respectively) ($F=7.682$, $df=1$, $p=0.003$ and $F=15.720$, $df=1$, $p<0.001$). The prevalence of APEC increased significantly after partridge releases, both in feces of red-legged partridges (0 to 60%), and of magpies (*Pica pica*, 5 to 20%) and other birds (0 to 50%). These results demonstrate that virulence factors and antibiotic resistance mechanisms may be transmitted into enteric bacteria of free-living birds by farm-bred, released partridges, and underline the need for thorough sanitary management on gamebird farms.

First approach to the gut microbiome of wild rabbit (*Oryctolagus cuniculus*)

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Key words: intestinal bacteria, food efficiency, rRNA 16S gene, Iberian Peninsula, rabbit subspecies.

The composition of the group of microorganisms present in the intestine (gut microbiome) is usually related to the physiological and immunological status of the animals. Most studies on the microbiome have focused on domestic animals, while there is a scarcity of studies on wild individuals and particularly on game species, despite the potential implications of this aspect on the management of their populations. In this work, we study the gut microbiome composition in European wild rabbit (*Oryctolagus cuniculus*) populations from central-south Iberian Peninsula and its variation as a function of the type of habitat, age or rabbit subspecies. With this purpose, we used genetic analysis to identify intestinal bacteria in 43 faecal samples corresponding to 25 wild populations and in more than 50 samples obtained from rabbits maintained in semi-captivity under controlled experimental conditions. Our results show a high variability among wild rabbit populations in microbiome composition. Those populations from the southwestern part of the study area, which corresponds to the distribution area of the subspecies *O.c. algirus*, showed the most heterogeneous microbiome. Such differences between subspecies disappeared when analysing the microbiome of captive individuals. In addition, the variability in microbiome composition was lower in captive individuals compared to the wild ones, which could affect the success of restocking events commonly performed with hunting purposes. We also observed a negative relationship between the overall microbiome heterogeneity and the age of the animals. Microbiome composition may affect the efficacy of rabbits to exploit their food resources, and hence their physical condition. Therefore, a detailed study of those factors that can modulate the microbiome composition could be a key element to improve game species management.

Testosterone and reproductive effort: effect on parasite burden in male Iberian red deer

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Key words: parasitic burden, testosterone, dark ventral patch, reproductive effort, *Cervus elaphus hispanicus*.

Testosterone is a decisive hormone in the regulation of many aspects of the reproductive physiology of males of many species. The secretion of testosterone is correlated with a large number of behaviors involved in intrasexual competition, such as the defense of the territory or the couple. Testosterone increases aggression and sperm quality, and is crucial during the development of secondary sexual characteristics. However, its secretion can involve costs at the immunological and metabolic levels, but evidence on this is still scarce in wild populations. In this study, we analyse whether the parasitic burden depends on faecal testosterone levels and the size of the dark ventral patch in males of Iberian red deer (*Cervus elaphus hispanicus*), a sexual trait that indicates reproductive effort in males. For this purpose, samples were collected from a total of 217 individuals from 14 different populations during two seasons in hunting actions in the southeast of the Iberian Peninsula. Coprological analysis was carried out to estimate the parasitic load of both gastrointestinal and bronchopulmonary nematodes, as well as protozoa of different genera. Testosterone faecal metabolite levels were determined by competitive enzyme immunoassay using a commercial kit. We found a positive effect of faecal testosterone levels and the size of the dark ventral patch on the parasitic load of male Iberian red deer. These results are discussed under the hypothesis of the immunocompetence handicap of testosterone, suggesting a cost at the immunological level, and therefore greater susceptibility to parasitization of males who make a greater reproductive effort.

Climate change and Iberian red deer rutting behaviour: less roaring but higher opportunity for sexual selection

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Key words: red deer, climate change, sexual selection, rutting intensity, mating system.

In the last decades, climate change has caused a reduction in average rainfall in southern Europe, which is expected to reduce resource availability for herbivores. Resource availability can in turn influence animals' physical condition and population growth, but much less is known on its possible effects on sexual selection. In this study, we assessed the impact of drought events on several red deer (*Cervus elaphus hispanicus*) rutting features. We measured the intensity of male sexual behaviour, mean female crowding, percentage of territorial males, and the opportunity for sexual selection in Doñana Biological Reserve (Southwest Spain). We used data from daily observations collected during the rut over a period of 22 years. We found an increasing trend for less rain and poorer associated environmental conditions, as well as trends for a decreased rutting intensity (less roaring), less territory-defence mating strategies, and an increased female crowding. This favoured higher degree of polygyny and opportunity for sexual selection, although all these relationships were modulated by population density and sex ratio. This study highlights how climate change (rainfall reduction) can alter the conditions for mating and the opportunity for sexual selection in a large terrestrial mammal.

Complementarity between technologies registering interactions at the wildlife-livestock interface: a methodological approach using social network analysis

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Key words: GPS, proximity loggers, social network analysis, wild ungulates, cattle.

The correct management of shared diseases between wildlife and livestock requires a reliable estimate of the pathogen transmission rate. The calculation of this parameter is a challenge for epidemiologists, since transmission can occur through multiple pathways. Among the possible methodologies used to detect interactions, GPS technology and proximity loggers are the most common nowadays. Despite the advantages that they offer, both have specific limitations. In the present work we studied the complementarity between methodologies registering different types of interactions at the wildlife-livestock interface. During 2015, nine red deer, seven fallow deer, six wild boar and nine cattle were monitored using GPS-GSM-Proximity collars in Doñana National Park. In addition, 16 proximity loggers were set in aggregation points. Using social network analysis, we studied the network of interactions between individuals of different species and the potential transmission of pathogens within this network. The results showed that GPS technology described most of the interaction that occurred between the targeted animals, however, the proximity loggers in aggregation points provided new information not recorded by the GPS. Within the network, we differentiated four communities that included individuals of all the species. Regarding the transmission of pathogens, we could observe that the infection was greater when the first individual infected was a fallow deer. In addition, we saw that there were communities within the network with a higher probability of pathogen transmission. The present work shows the need to consider different types of methodologies in order to understand the complete functioning of the network of interactions at the wildlife-livestock interface. It also provides a methodological approach applicable to the management of shared diseases.

Nutritional and microbiological quality of Iberian red deer (*Cervus elaphus hispanicus*) meat

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Key words: deer meat, nutrients, microorganism, hunting, stalking.

Consumption of wild game meat is increasing in recent times, as consumers consider as 'natural' the consumption of animals are not farmed and feed on predominantly on natural vegetation. Spain is one of the top game meat producers in the world, exporting the majority of the meat. However, a lack of studies on game meat quality may hamper its consolidation in both internal and external markets. In order to improve knowledge on deer meat quality, we evaluated meat quality in 90 wild deer (76 through driven hunting, and 14 through stalking) hunted in different shooting estates from Central Spain during the 2017-18 hunting season. Samples were taken from the cranial portion of the loin (*Longissimus dorsi* muscle) and belly to evaluate bacteria loads as sanitary indicators (counts of total microorganisms, *Escherichia coli*, *Clostridium* sulphite reductases and *Staphylococcus*, together with the identification of *Salmonella* and *Listeria monocytogenes*). In addition, we evaluated meat macronutrients (fat, protein, minerals, vitamins and calorie content). Bacteria counts were significantly higher in deer meat coming from driven hunting compared with stalking. Also, meat quality was worse when deer were harvested during milder months (early autumn) and when the animal was shot in the abdomen. Deer loin meat comply with current European Union legislation and regulation related to nutrition and health claims (EC No 1924/2006 of the European Parliament) and was characterized by a high content of proteins, zinc, vitamin B12 (cyanocobalamin), low salt and fat content, and was a source of phosphorous, iron, copper and vitamins B2 (riboflavin) and B3 (niacin). Our results confirm that deer meat can be labelled to show appropriate nutrition and health qualities related to minerals and vitamins (EC No 432/2012 of the European Parliament).

Residues of lead ammunition in game meat products: estimation of food safety risks

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Key words: lead, meat products, game, risk assessment, public health.

The use of lead ammunition leaves in hunted animals lead fragments that can be ingested by consumers, with the consequent health risk. There is hardly any information about the presence of these lead residues in meat products that are elaborated from game specimens. We have conducted a study on 86 pieces of red sausage (*chorizo*), white sausage (*salchicón*) and ragout made from red deer and wild boar meat from different manufacturers across Spain. **Objectives:** the specific objectives of the study were: 1) to detect with x-ray the presence of lead ammunition fragment in processed products; 2) to quantify lead concentrations; 3) to compare results with the maximum residue levels (MRL) established by the European Commission (EC) for consumer safety. **Material and Methods:** after a visual inspection of X-rays, parts of each product where lead fragments were found were removed. Then, those parts free from x-ray-detectable lead fragments was homogenized to obtain a sample where we analyzed the percentage of water content and the concentration of lead (through Anodic Stripping Voltammetric-ASV polarography). **Results:** Almost half of the analyzed pieces had radiopaque fragments. These fragments were more frequent in wild boar pieces, although lead concentrations were higher in red deer meat. 47% of the analyzed pieces had lead concentrations above 0.1 mg/kg (dry weight), and 12.5% of them had concentrations more than 10 times above the EC's MRL. **Conclusion:** The estimation of exposure safety margins for extreme and intermediate consumers suggests a potential health risk associated with metallic lead consumption. Conflict of interest: The study was funded by WWF-Spain throughout a contract signed with the Veterinary and Forensic Toxicology Service of the University of Murcia.

Effect of agricultural management on testicular and spermatoc variables in the Iberian hare (*Lepus granatensis*)

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Key words: agroecosystem, wildlife, pesticide, spermatoc parameters, testicular parameters.

The intensification of the agricultural sector during the 20th century has caused an important homogenisation of the landscape due to land ownership consolidation and introduction of pesticides and chemical fertilisers to the environment. This intensification has been related to the loss of flora and wildlife biodiversity. Concerning pesticides, there are important shortcomings in the knowledge of their secondary or sublethal effects on wildlife. In order to clarify if pesticides are responsible for wildlife reproductive impairment in the Iberian agroecosystems, several male reproductive parameters of the Iberian hare were compared from areas with different agricultural management. Specifically, testes were collected from animals shortly after hunting in areas where pesticides are used (treated area, n=14) and others in pesticide-free areas (control area, n=9). At the laboratory, after biometrical measurements of testes, epididymal spermatozoa were collected to evaluate different quantitative and qualitative spermatoc variables. The results obtained showed significant differences between animals from control and treated areas in the following parameters (control vs treated, average values): total motility (83.89% vs 47.79%); morphological abnormalities (2.56% vs 10.18%); viability (99.25% vs 98.29%) and membrane functional integrity (78.39% vs 50.71%). Moreover, marginally significant differences were observed in testicular weight (8.30 g vs 6.86 g). Hares from pesticide-free areas showed better sperm quality compared to those from pesticide-treated areas. This study is part of a more extensive project which will enable us to establish if the observed effects are due to a direct or indirect influence of pesticides caused by, among other factors, nutritional differences related to the decrease of flora diversity in treated areas.

Biogeographical approach to the historical distribution of the subspecies of European rabbit (*Oryctolagus cuniculus*) in the Iberian Peninsula

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Key words: favourability, fuzzy logic, management, subspecies, trend surface analysis.

The European rabbit *Oryctolagus cuniculus* is native to the Iberian peninsula (IP), where two subspecies occur naturally. Previous studies have suggested that *O.c. cuniculus* distribution extends towards the north-east of the IP, while *O.c. algirus* is restricted to south-western regions; a transition area crosses the IP from the north-west to the south-east. The aims of our study are to define with greater precision the historical distribution areas of both rabbit subspecies, to evaluate the spatial interaction between their expansion waves and to identify the cores from where both subspecies spread. We used molecular tools to determine the subspecies of 4500 rabbits from 197 populations distributed throughout the IP. The spatial structure of both subspecies was obtained by combining a Trend Surface Analysis and the favourability function. Only populations in which all individuals belonged to a subspecies independently depending on the genetic marker (mitochondrial or nuclear) were used. We combined the different spatial structures using fuzzy logic operations. Our results identify two areas located in the Guadalquivir and Ebro valleys as respective expansion cores. In comparison to previous studies, we found an increase in the distribution range of *O.c. algirus* to the northwest and a shift of the transition zone eastwards in its northern area. Furthermore, competitive exclusion may have had a greater role than expected shaping the expansion of both subspecies. The combination of suitable tools, such as molecular analysis and fuzzy logic, contributes to improve knowledge about the biogeography of the European rabbit in its area of origin, which can help to improve its management and conservation.

Favorability analysis of the presence of rabbit warrens on highway slopes

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Key words: corridor, CyberTraker, *Oryctolagus cuniculus*, outbreaks of terrestrial vertebrates, road ecology.

Terrestrial vertebrate species considered as pests cause significant ecological, social and economic impacts. Road slopes or railways are often used by these pests as dispersal corridors, but little is known about the specific features of these infrastructures that facilitate the dispersal of these species. This is particularly evident in the case of outbreaks of native agricultural vertebrates such as the European wild rabbit (*Oryctolagus cuniculus*) in the Iberian Peninsula. Rabbit damages to agricultural production in the Iberian Peninsula have increased remarkably during the last years. In this study a 'favorability function' is used to model, to what extent, how the favorable macroecological conditions for rabbits, and different variables measured at fine scale, including environmental, anthropogenic and agricultural factors, favor the presence of rabbit warrens in the slopes of a highway network in southern Iberian Peninsula (i.e. Andalusia). With this aim, we conducted vehicle surveys at a constant speed of 80 km/h to detect the locations of rabbit warrens along 787 km of highway slopes. Our results show that the presence of rabbit warrens was correlated positively with the presence of olive groves and with the presence of favorable macroecological conditions for rabbits. Our results provide strong evidence of rabbits' widespread use of highway slopes, and that they are likely to act as corridors that facilitate the dispersal of the species. In addition, our innovative approach, based on an index of presence of rabbit warrens, the favorability function, and a combination of local and macroecological predictors, could be used in many other areas where rabbits cause crop damage to make large-scale predictions to detect highway or railway sections, with potential risk to favor the presence of rabbit warrens and, therefore, to allow the application of preventive mitigation measures.

GECISO® technical standard for certification of sustainable game management

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Key words: technical standard, certification, sustainability, management.

During the last decades of 20th century, several political, socio-economic and ecological changes affected the hunting sector, motivating the replacement of traditional game management models by new, intensive models focused on the commercial exploitation of game resources. Many studies have demonstrated how this intensification of game management models has an impact on conservation status of both habitats and populations of game species. In order to deal with this issue, several autonomous regions in Spain created, at the beginning of the present century, their own certifications to proof and enhance a sustainable game management. Some regions established that hunting estate owners wishing to obtain their certification should be subject of an external audit conducted by certifying entities accredited by the Spanish National Accreditation Entity (ENAC). However, none of the regions has established a viable system allowing owners to certify their management through the right of use of such accreditation. In this work, we propose a technical standard guaranteed by ENAC that establishes a conformity assessment useful to develop, implement and certify game management systems in the Iberian Peninsula. The technical standard sets up the accreditation protocols for the certifying entities to operate within the scheme, and established the conditions for integration of those regions already with their own certifications on place. The GECISO® technical standard is valid for all regional certification systems that want to adhere and allows any hunting estate manager from the Iberian Peninsula to certify its management system and to obtain the right of using the GECISO® trademark.

Project "PARETCI"

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Key words: management, hunting, sustainability, valuation, trophy.

From an objective point of view, hunting is necessary to maintain a balance in many of our ecosystems. This statement is not contradictory with the fact that over-hunting or a bad hunting management provoke negative effects on the conservation of certain species of flora and fauna. This project intends to carry out an analysis of a wide sample of trophy collections already measured or to be measured, evaluating the influence of different factors in the measurement result, as well as proposing which of those would be susceptible to be evaluated "in situ" prior to the hunt. This will allow the possibility of assessing "a priori", before hunting, those individuals of better quality, which, having the option of becoming trophies once killed, would imply a revaluation of the hunting stock of the hunting grounds. In addition, the analysis and characterization of antlers can give a very valuable and up-to-date information on the quality of the habitat, densities, pathologies, etc. of the populations, by species, within each hunting ground.

Challenges for hunting and conservation in Asturias and Spain

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Key words: hunting, conservation, Asturias, challenges.

Pest species are those that negatively affect human health or food safety. The wild boar is a wild suid from which the domestic pig originated. Its natural distribution ranges from the Iberian Peninsula to Japan. In recent decades, wild boar populations have multiplied, causing damage to agriculture, traffic accidents, public health risks, problems arising from the presence of urban wild boar, and especially, increasing conflicts with livestock and animal health. Wild boar has become a problematic or pest species throughout Europe. In Asturias, hunting contributes significantly to mitigating the demographic growth of wild boar. However, hunting activity faces challenges such as the lack of generational replacement of hunters, associated precarious employment, poor social image of hunting activities confronted with an increasingly virulent animalist movement, as well as a lack of empathy with the rural world. Hunting activities in Spain and the Principado de Asturias are immersed in a crossroad that could lead to important legislative and economic changes, and facilitate a lack of control of wildlife species such as wild boar. This would have consequences on animal health and agriculture, affecting the rural world, the primary sector and the users of the natural environment. We will present the situation of wild boar hunting in Asturias as an example of the management of a pest species in an environment with hardly any artificial intervention, where there is no food supplementation or fencing. We will analyse the trends considering the above challenges and the need for collaboration of all involved sectors: urban civil society, farmers and ranchers, hunters, conservationists and administrations.

Correction factors for estimating red fox food consumption from scats

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Key words: diet estimation, ingested biomass, predation, *Vulpes vulpes*.

An assessment of the diet of predators is required to estimate their impact on game species. The analysis of food remains in scats is probably the method most often used for this objective. The proportion of consumed biomass may provide an accurate approximation of the true diet. The use of correction factors (CFs) allows the transformation of mass of dry remains from scats into consumed biomass. CFs have been frequently used to estimate the diet of the red fox (*Vulpes vulpes*), a widespread generalist carnivore with a diverse diet and a potential key role as regulator of game species populations. We performed controlled feeding trials with red foxes to derive CFs for eight food categories, of both animal and vegetal origin. Red foxes consumed daily 615 ± 19 g (mean \pm standard error) of food and produced $6,4 \pm 0,3$ scats weighing $31 \pm 1,2$ g. The CFs varied significantly among food types, with the lowest values for red-legged partridges (*Alectoris rufa*) and the highest for red deer (*Cervus elaphus*), increasing linearly and significantly with the average body mass for prey. The precision of the estimated CFs was low for red deer, fruits and hares (*Lepus granatensis*), and median for the remaining foods. Values of red fox CFs previously reported vary among studies due to the experimental setup of feeding trials and to laboratory methods, mainly the sieve mesh size employed for washing the scats. Regardless of these variations, ungulates and lagomorphs consistently showed the largest CF values across studies, while small birds, small rodents and fruits showed the smallest values. We recommend considering intra-food variability when using CFs for estimating proportions of ingested biomass from weight of scats in studies of the diet of red foxes, and other generalist carnivores, as a better support for management decisions.

Factors affecting the persistence of fox scats: implications for ecological studies of terrestrial carnivores

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Key words: disappearance, mammalogy, predation, *Vulpes vulpes*

The red fox (*Vulpes vulpes*) is among the most abundant terrestrial carnivores and could regulate the abundance of small game species. Some of the most commonly used methods to study red fox ecology (e.g. diet) or to estimate its abundance are based on the use of scats. However, scat persistence may depend on different factors, such as food type or external factors like weather conditions or abundance of coprophagous invertebrates. Our objective was to estimate the persistence of fox scats and determine the factors influencing it. Between November 2006 and January 2009, we carried out an experiment to monitor fox scats in seven localities in central-southern Spain. On a seasonal basis, we placed scats from foxes that were fed in captivity with only one of eight food types: chicken, deer, grapes, hare, lamb, partridge, rabbit or rat. We defined the seasonal persistence as the percentage of scat dry weight that remained after three months, in relation to the initial dry weight. The variables considered to explain the persistence were the food type, the exposure time, the initial weight of the scat, the number of coprophagous invertebrates in the scat, the accumulated rainfall, the season and the locality. The factors most affecting fox scat persistence were the exposure time, the season and the food type. Scat persistence was shorter after a longer exposure time and during spring and autumn. The scats with rabbit showed the longest persistence and those with grapes the shortest, although the differences were only marginally significant. Therefore, we recommend taking into account the sources of variation in the scat persistence (exposure time, season, location, food consumed, etc.) to improve the reliability of estimates in studies of diet or population abundance, based on scat analysis and counting.

Improving the REM method by applying a correction factor to estimate carnivore densities

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Key words: camera traps, carnivores, density estimation, Random Encounter Model.

A reliable population estimate is essential to make an appropriate management of hunting species. The use of camera traps and subsequent capture-recapture analysis is a widespread method of estimating population sizes of individually recognizable animals. However, this method is of limited use in the case of species that do not naturally have identifiable markings. Over the past decade, a method – the Random Encounter Model (REM) – for estimating animal density using camera traps without the need for individual recognition based on modelling random encounters between animals and cameras has been developed. A key assumption of this model is that cameras are placed randomly, which means that most carnivores, more likely to be detected along trails, will be detected too infrequently for density estimates to be calculated. The aim of this study is to define a correction factor that allows the REM method to be applied in carnivore photo-trapping surveys in which cameras are placed along tracks to maximize the capture probability. We use lynx as a case of study. First, we estimated Iberian lynx densities using two independent survey methods: a traditional Iberian lynx camera-trapping design followed by SECR analyses, and telemetry-based range data. We estimated the differential use rate for tracks and the subsequent correction factor using GPS-GSM collar data. As expected, density estimates calculated with REM overestimated densities. However, the application of the correction factor to the REM model clearly improves the estimates making it more accurate. The corrected REM model shows great potential for density estimation of non-individually recognizable carnivore species, which could be of great interest to the management of red fox populations.

Spatial distribution and abundance models of European wildlife: data and analysis harmonization

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Key words: wild boar, abundance, models, citizen science.

Knowledge about distribution and abundance of European wild mammals is essential for their conservation and management. The ENETWILD consortium (www.enetwild.com) has been collecting presence and abundance data of wild boar, which will be completed with data from other wild mammals in the next years. Using the wild boar as an example, we develop a general framework to create spatially explicit abundance models from different data sources: occasional occurrences, hunting yields, density, etc. First, we review the available models to predict spatial distribution and abundance of wild boars at different scales (global, European, national and regional). Second, we present the approach and models developed by the ENETWILD consortium from different data sources, and evaluate their complementarity and the interactions needed to maintain the flexibility in order to include all available data. From our results, we identify those regions which require more and better data to improve the abundance predictions. In the next years, we expect to increase the amount and the quality of data, to allow us to produce more accurate and reliable predictions.

Experimental study of carbon and nitrogen stable isotopes in wild boar hair

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Key words: stable isotopes, hair, diet, wild boars, hunting estates.

The analysis of stable isotope ratios has been widely used to obtain information about the ecology and nutritional quality of wildlife. The isotopic ratios of carbon and nitrogen stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) analyzed in the different tissues are directly related to the diet of the animal and also inform about the environment where the individual has lived. Such analysis in hair samples has acquired great relevance since it provides information on the quality and composition of the diet in the long term, during the growth of this tissue. The main objective of this study is to clarify the differences in the diet composition and quality of wild boars within the same population, considering morphological variables such as body size, as well as age and sex. In addition, differences between populations were investigated, according to the open or closed nature (fenced perimeter) of a hunting estate depending on management strategies that affect population structure (sex and age). For this work, samples of boar hair were collected in 7 different hunting estates in Andalusia (5 fenced and 2 open estates). During the hunting season, animals are usually fed with corn to attract them to the hunting place. For each animal, duplicate hair samples were analyzed (hair split into two parts of equal size, each analyzed separately). With these analyzes it was possible to reconstruct a chronology of the isotopic signatures in the hair for each animal. Our results show differences between individuals from different farms.

Can we apply citizen's science in the monitoring of mammals on a European scale?

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Key words: collaborative science, citizen science, distribution, Europe, mammals.

The European Food Safety Authority (EFSA), through the MammalNet initiative (formed by seven European research groups and their network) aims to improve European wildlife monitoring capacities by assessing the feasibility of the application of citizen science to mammal monitoring at European level, and providing information on possible limitations, advantages and added values, as well as on best practices promoting data quality and citizen participation. The specific objectives are: (i) to seek different communication strategies differentiated by countries, languages and cultures to involve citizens in collecting data on the geographical distribution and abundance of European mammals using web platforms and/or mobile devices (such as telephones and tablets); (ii) to propose and implement citizen communication and participation strategies to encourage the collection of high quality data; (iii) to propose and implement a method to assess the quality of data collected by citizens and compare them with professionally collected data; (iv) to assess the feasibility of applying citizen science to wildlife monitoring at European level, and provide information on potential limitations, benefits and added values, as well as on best practices promoting data quality and participation. The two-year project is developed in two phases: in the first one, pilot strategies are implemented in four countries (Germany, Croatia, Spain and Poland). Then, with the learned lessons, it will be expanded to the rest of Europe, mainly through communication tools and use of social networks.

Effect of the aggregation in the precision of density estimates obtained by distance sampling. Red deer as model species

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Key words: abundance, *Cervus elaphus*, group size, monitoring, simulation.

Distance sampling (DS) is a widely used method to estimate the population density of wild species. However, the precision of the estimates must be reduced to be effective in a monitoring program. This precision depends on the variability in group size, encounter rate and detection function. In gregarious species it is recommended to carry out samplings in the period of least aggregation, since it increases the number of groups and decreases the variability in group size. In this work, with the objective of evaluating the effect of group size on the precision of the estimates, and considering the deer (*Cervus elaphus*) as a model species: i) we have sampled simulated populations with densities of 7, 15 and 30 individuals/km² in high (autumn) and low (spring) aggregation periods, and ii) we have sampled a natural population with a density around 30 ind/km² at the moments maximum and minimum aggregation. The results obtained in the simulation show that the precision increases an 8% on average in the three populations during the spring samplings. However, in the natural population, the precision decreases by around 5% during the spring sampling. This decrease could be due to a more elusive behaviour of the species in spring than in autumn, which results in a reduced probability of detection. Thus, the number of groups detected in the natural population in spring can be a limiting factor when adjusting precise detection functions. We can conclude that, although a smaller aggregation increases the precision in the simulated samples, these results can be compromised due to a lower detectability that influences the precision associated with the encounter rate and the detection function. Therefore, carrying out samplings in the spring may require a greater effort in order to enhance all sources of precision.

A quantitative estimate of annual quotas for hunting Iberian ibex (*Capra pyrenaica*) in the Iberian Peninsula

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Key words: *Capra pyrenaica*, hunting, annual quotas, Iberian Peninsula.

The Iberian ibex, *Capra pyrenaica*, is a highly valued big game species in the Iberian Peninsula. Despite many studies have focused on its biology and ecology, very few have addressed its exploitation through hunting, generally focusing on the temporal evolution of trophy size only. The main goal of our study is to estimate the annual collection quotas for the species and their evolution over the last decades. Additionally, we analysed the strategy adopted in each region (e.g., trophy collection, control of density). For this purpose, we accessed the official databases of hunted animals of those regions where the species is hunted. During the last decades, the number of hunted specimens significantly increased to surpass the 8500 animals in the 2015-2016 season. Aragón, Andalucía and Castilla y León are the regions with highest quotas (together, more than 60% of the national total). Finally, we discuss the impact of this activity on the ibex conservation, as well as the usefulness of the hunting programs.

Optimizing non-invasive genetic tools to study and manage wild boar (*Sus scrofa*) populations

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Key words: population monitoring, individual ID, spatially explicit statistical models, faeces, diet preference, DNA metabarcoding.

Elusive species like the wild boar (*Sus scrofa*) are difficult to study and monitor using traditional ecological approaches, which rise concerns about the accurate management of their wild populations. In the Iberian Peninsula, wild boar is widely distributed, inhabiting both the Mediterranean and Atlantic/Alpine regions, with a steep increasing trend of population abundance over the last decades. This increase poses several threats, among others, to animal health (i.e. due to the infectious diseases), human safety (i.e. due to car-collision and city incursions) and biodiversity (e.g. excess predation pressure on other wildlife species). To better evaluate the wild boar population abundance and its impact on ecosystems, we sequenced mitochondrial and nuclear markers to infer species and sex from non-invasive samples (i.e. faeces) and genotyped a set of 13 microsatellites to infer individual ID, in order to estimate population abundance through spatially explicit statistical models. In addition, we optimize a methodology to characterize the diet of wild boar through DNA metabarcoding analysis of faeces, which can determine the range of species predated and/or consumed by wild boars. So far, we analysed 69 faecal samples from Castilla-la-Mancha region and Doñana National Park, from which 41 amplified for mitochondrial markers and allowed for species identification. Twenty-nine out of 32 faeces identified as wild boar were amplified for nuclear markers. The probability to identify and the sibling identity were calculated for the 13 microsatellites using our reference dataset of more than 400 genotyped wild boar and domestic pig in the Iberian Peninsula. Finally, the diet of the 29 identified individuals was characterized using the same faecal samples and two pairs of primers: i) the trnl primer to amplify plants; and ii) the COI primer to amplify vertebrates. The possible role played by wild boars on Iberian ecosystems, and the role of non-invasive genetic tools to study and manage this species, are discussed. Overall, our results support the inclusion of these genetic tools within the tool-box for population monitoring of this relevant species.

Wild or domestic? *algiurus* or *cuniculus*? A novel genetic approach to infer the genetic integrity of European rabbit subspecies

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Key words: *Oryctolagus cuniculus cuniculus*, *Oryctolagus cuniculus algiurus*, Iberian Peninsula, SNPs, hybrid index.

The European rabbit (*Oryctolagus cuniculus*) is a keystone species in Mediterranean ecosystems and also one of the most appreciated small game species in the Iberian Peninsula. Over the last decades, however, natural populations have encompassed steep fluctuations in numbers and distribution as a consequence of habitat loss, infectious diseases, high predation and overexploitation by hunting. In an attempt to overcome the continuous decline of wild populations, restocking operations have been conducted across Iberia with rabbits reared in intensive farms, without any concerns regarding the preservation of the genetic makeup of wild rabbit populations, and particularly the gene pool of the two recognized subspecies, *O.c. cuniculus* and *O.c. algiurus*. It is well-known that for almost two million years these two subspecies coexisted in Iberia, and currently the subspecies *O.c. algiurus* is localized in the southwest part of Iberia and the subspecies *O.c. cuniculus* localized in the northeast of Iberia and France. In order to promote the conservation of the genetic integrity of Iberian wild rabbit populations we developed a set of 32 Single Nucleotide Polymorphism (SNP) markers to distinguish wild rabbits from domestic rabbits (n=8), as well as *O.c. cuniculus* from *O.c. algiurus* (n=24). SNPs were chosen based on either their diagnostic pattern between subspecies in individuals sampled away from the hybrid zone in central Iberia, or in marked SNP allelic frequency differences between subspecies (>80%). To be cost-effective and feasible to use in a small number of samples, two snapshot multiplex reactions were optimized. Hybrid index and its cut-off values were inferred using reference samples from the most common domestic breeds and from several wild populations sampled across the geographic distribution range of both subspecies. This novel genetic approach will help to preserve the genetic pool of Iberian wild rabbit populations, in particular the *O.c. algiurus*.

Genetic introgression in red-legged partridge farms in Andalusia

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Key words: red-legged partridge, *Alectoris rufa*, *Alectoris chukar*, genetics, hybridisation.

The red-legged partridge (*Alectoris rufa*) is one of the main game species of the Spanish fauna. Its natural populations have experienced a decline during the last decade due to multiple factors such as habitat loss, pesticides or hunting pressure. To cope with hunting demand, captive breeding in farms and releasing into the wild has increased. Existing legislation prohibits the release of alien species, but partridge farms may have some of their stocks hybridized with the chukar partridge (*Alectoris chukar*) due to historical practices. For this reason, the government of Andalusia region (Southern Spain) implemented a program for checking the genetic status of partridge farms in collaboration with the Wildlife Research Unit (UIRCP-UCO) prior to issuing the permits for restocking in hunting areas. Here we present the results of this survey for most Andalusian farms. We used species-specific SNP (Single Nucleotide Polymorphism) markers. By this procedure, we have analysed 8.857 individuals from 55 farms, which resulted in an overall percentage of hybrid birds of 18.2%. We discuss the results in relation with the procedure used and their relationships with previous data on the introgression found in natural populations in the region

Is the genetic pool of the red-legged partridge (*Alectoris rufa*) threatened in Portugal? The case study of Mértola region

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Key words: *Alectoris rufa*, hybridization, genetic background, Portugal.

The red-legged partridge (*Alectoris rufa*) is one of the most appreciated game birds in the Iberian Peninsula. Over the past decades, natural populations have encompassed steep declines in numbers and distribution as a consequence of habitat loss (i.e. due to intensification of agricultural activities) and overexploitation by hunting. In an attempt to overcome the continuous decline of wild populations, millions of red-legged partridges reared in intensive farms have been released year after year all over Iberia, without any concern regarding the preservation of the genetic pool of wild populations. Although in Spain the anthropogenic hybridization of red-legged and chukar (*Alectoris chukar*) partridges is well-documented and widespread across populations, little is known about the current genetic status of Portuguese populations. To characterize the genetic pool of Portuguese red-legged partridges, we studied the most important hunting region in Portugal for small game species, Mértola (southeast of Portugal). We sampled over 1500 animals hunted between October 2018 and January 2019 in hunting estates located in Mértola and surrounding areas. The number of samples taken per hunting estate varied according its size, and ranged approximately between 10 (for estates of 100-1000 ha) and 40 (for estates larger than 3000 ha) individuals. A set of published nuclear and mitochondrial DNA markers were used to characterize the genetic status of each individual as a pure *A. rufa* or an *A. rufa* x *A. chukar* hybrid. Our results revealed the presence of hybrids in the region of Mértola, with populations from hunting estates showing different levels of hybridization. In addition, an interpolation map with the gradient of hybridization across the study area was obtained and discussed in light of possible management and environmental factors that determine this gradient. Finally, our results highlight the need to implement conservation actions to preserve the genetic pool of Portuguese red-legged populations.

Mite communities in white stork (*Ciconia ciconia*) nests are influenced by host phenology and habitat

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Key words: white stork, nests, chicks, mites, parasite.

The white stork (*Ciconia ciconia*) is a typical trans-Saharan migrant. Recently, storks have adapted to rubbish dumps as reliable food source and have reduced the migration distance or become sedentary. Thus, nests formerly empty during the winter may be occupied continuously nowadays. Stork nests provide microhabitats for mite communities, including parasitic hematophagous mites. We studied if the year-round occupation of stork's nests led to increased burdens of parasitic mites. Mites of 44 samples of nest material were extracted and identified from a total of 21 nests. Additionally, the mite communities were compared between 4 stork nests occupied the entire year and 17 seasonal nests from colonies in central Spain. Samples were obtained before and after the breeding season from nests situated close to a rubbish dump and nests in natural habitats. We also calculated the total and relative abundance for each mite species and the biodiversity indexes for each nest. The nests that had had chicks showed higher mite abundance than nests in which breeding had been unsuccessful. Mite abundance and richness were higher at the end than at the beginning of the breeding season. Both at the beginning and at the end of the breeding season, the nests of sedentary storks located near the rubbish dump had a significantly higher abundance and richness of nest mites than the nests from natural environments. Parasitic mites did not increase in number, potentially due to the high number of predatory mite species. The materials used to build the nests differed between zones, so it is a factor that can determine the mite communities of a nest.

Characterization and molecular identification of spotted fever group *Rickettsia* in ticks from Greece

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Key words: spotted fever group, *Rickettsia*, Greece, ticks, tick-borne diseases.

Spotted fever group (SFG) *Rickettsia* are zoonotic and emerging pathogens with considerable impact in public and animal health, affecting to animal production and causing serious zoonoses. These pathogens are transmitted by arthropod vectors, mainly Ixodidae ticks, and cause emerging and endemic diseases in certain countries. This work aims to evaluate the prevalence of SFG *Rickettsia* in ticks collected from domestic hosts including sheep, goats and dogs in contact with wildlife. The study was carried out in several Greek regions, where it has been confirmed the existence of host species and appropriate environmental conditions to favor the circulation of these pathogens. The presence of *Rickettsias* belonging to SFG circulating in 14 areas of this country was analyzed. Several genetic markers for bacterial genes, such as 16S rRNA, *ompA*, *ompB*, *atpA*, *gltA*, *recA*, *dnaA* and *dnaK*, were amplified and sequenced to accurately classify the rickettsial pathogens in the ticks. Taxonomy and species classification of the *Rickettsia* was achieved by combining phylogenetic and *in silico* digestion analysis of the gene sequences obtained. We obtained a 7.5 % prevalence of ticks (n=187) with SFG *Rickettsial* DNA, including *R. massiliae* (n=3), *R. slovaca* (n=5), *R. raoultii* (n=1) and *R. hoogstraalii* (n=5). These pathogens were detected on *Rhipicephalus sanguineus* (n=3), *Dermacentor marginatus* (n=6), *Haemaphysalis sulcata* (n=3) and *H. parva* (n=2). The results of molecular analysis revealed the presence of novel genetic variants of rickettsia species, evidencing the need to deepen and broaden the study of these zoonotic agents, and to continue implementing the previous information for establishing the prevention and control measures in animal and public health.

Detection and identification of ticks in wild ruminants from the Comunidad Valenciana

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Key words: vector diseases, ticks, wild ruminants.

Hard ticks (Acari: Ixodidae) are considered the most important disease vectors both for humans and animals after mosquitoes. The infections by pathogens transmitted by ticks cause a great number of diseases and have enormous effects both on animal and public health. The apparition of outbreaks of these diseases takes place as a result of changes in the distribution of ticks and their hosts, as well as changes in the interaction of humans and domestic animals in the cycle tick-pathogen-fauna. As for this, wild ruminants act as reservoir hosts of a wide diversity of viruses, bacteria and parasites. The objective of this study was to investigate the diversity of ixodid species present in wild ruminants in the Comunidad Valenciana. To achieve this objective, a total of 802 ticks from 59 wild ungulates captured in the province of Valencia were studied. Ticks were collected from European mouflon (*Ovis orientalis musimon*) (n=30), Iberian ibex (*Capra pyrenaica*) (n=24), red deer (*Cervus elaphus*) (n=4) and fallow deer (*Dama dama*) (n=1). The most frequently identified ticks were those belonging to the genus *Rhipicephalus* (80.9%), identifying *R. bursa*, *R. sanguineus* and *R. pusillus*. Other species found in the studied animals were *Ixodes ricinus* (8.47%), *Haemaphysalis sulcata* (8.42%) and *Dermacentor marginatus* (1.87%). This work reports the diversity of ticks found in the East of the Iberian Peninsula, which can potentially act as host for important pathogens such as *Babesia* sp., *Theileria* sp., *Ehrlichia* sp., *Anaplasma* sp., *Coxiella* sp. and *Borrelia* sp. These results highlight the role of wild ruminants in the natural cycle of ticks in this region, suggesting that wild ruminants can act as relevant reservoirs of potentially zoonotic pathogens transmitted by ixodid ticks. This work has been funded by Iberdrola España S.A. in collaboration with the Universidad Católica San Vicente Mártir (UCV 2017-243-001) (granted to JSM).

Oral vaccination with a formulation combining *Rhipicephalus microplus* subolesin with heat inactivated *Mycobacterium bovis* reduces tick infestations in cattle

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Key words: *Rhipicephalus microplus*, tick, heat-inactivated *Mycobacterium bovis*, oral vaccination, subolesin.

Vaccines are an environmentally friendly alternative to acaricides for the control of tick infestations, in order to reduce tick-borne disease risks affecting human and animal health worldwide and to improve animal welfare and production. Subolesin (SUB, also known as 4D8) is the functional homolog of Akirin2 involved in the regulation of development and innate immune response, and a proven protective antigen for the control of ectoparasite infestations and pathogen infections. Oral vaccination combining protein antigens with immunostimulants has a proven efficacy to increase host welfare and safety but has not yet been used to control of tick infestations. Here we report on the influence of oral vaccination with a formulation combining *Rhipicephalus microplus* SUB and heat inactivated *Mycobacterium bovis* (IV) on cattle tick infestations and fertility. The levels of IgG antibody titers against SUB and *M. bovis* P22, and the expression of selected immune response genes were determined and analyzed as possible correlates of protection. We demonstrate that oral immunization with the SUB+IV formulation resulted in a 51% reduction in the number of female ticks and a 30% reduction in fertility. Considering the cumulative effect on tick survival and fertility in cattle, the overall efficacy in the control of *R. microplus* infestations was estimated at 65%. The *akr2*, IL-1 β , and C3 mRNA levels together with antibody levels against SUB correlated with vaccine efficacy. The effect on tick survival and fertility of the oral immunization with SUB+IV in cattle is essential to reduce tick infestations and extend previous results on the effect of *R. microplus* SUB for the control of cattle tick infestations. These results support the development of oral vaccines formulations to control tick infestations and reduce the incidence of tick-borne diseases.

Functional evolution of Subolesin/Akirin and application in vaccine development

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Key words: immune response, vaccine, interactome, vector, *Anaplasma*.

Ticks are bloodsucking arthropod ectoparasites that transmit pathogens (bacteria, virus and parasites), which cause diseases in humans and animals with growing incidence worldwide. The intracellular bacteria *Anaplasma phagocytophilum* (Ap) is transmitted by *Ixodes* spp. ticks and infects granulocytes causing emerging diseases such as human granulocytic anaplasmosis (HGA) and tick-borne fever (TBF) in ruminants. Besides, Ap has been detected in wildlife, with several animals serving as natural reservoir for the bacteria and facilitating the spread through the tick vector. Subolesin/Akirin (SUB/AKR) are recently discovered orthologous proteins highly conserved in invertebrates and vertebrates, constituting a good model for functional evolution research studies. These proteins are involved in the control of gene expression through the interaction with other regulatory proteins that affect several biological processes, for example, the innate immune response triggered by pathogens like Ap. Our results suggest that SUB/AKR evolved conserving not only its sequence and structure, but also its function and role in cell interactome (network of interactions between proteins and metabolites) and regulome (inter- and intracellular biochemical reactions) in response to pathogen infection and other biological processes. This functional conservation provides a platform for further characterization of the function of these regulatory proteins, and how their evolution can meet species-specific demands. Furthermore, the conserved functional evolution of SUB/AKR correlates with the protective capacity shown by these proteins in vaccine formulations for the control of different arthropod and pathogen species. These results encourage further research to characterize the structure and function of these proteins, and to develop new vaccine formulations by combining SUB/AKR with interacting proteins for the control of multiple ectoparasite infestations and pathogen infection.

Alpha-Gal syndrome: new views in the conflict and cooperation between tick and host

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Key words: tick, allergy, alpha-gal syndrome, vaccine, immune response.

This poster focuses on a recently diagnosed tick-borne allergic disease known as the alpha-Gal syndrome (AGS). Tick bites induce in humans high levels of IgE antibodies against the carbohydrate Gal α 1-3Gal β 1-(3)4GlcNAc-R (α -Gal) present on tick salivary glycoproteins and tissues of non-carnivorous mammals, leading to the AGS syndrome in some individuals. This immune response evolved as a conflict and cooperation between ticks and human hosts. The conflict is characterized by the AGS that mediate delayed anaphylaxis to red meat consumption and certain drugs such as cetuximab, and immediate anaphylaxis to tick bites. The cooperation is supported by the capacity of anti- α -Gal IgM and IgG antibody response to protect against pathogens with α -Gal on their surface. Despite the growing diagnosis of AGS in all world continents, many questions remain to be elucidated on the tick proteins and immune mechanisms triggering this syndrome, and the protective response against pathogen infection elicited by anti- α -Gal antibodies. The answer to these questions will provide information for the evaluation of risks, diagnosis and prevention of the AGS, and the possibility of using the carbohydrate α -Gal to develop vaccines for the control of major infectious diseases.

Recolonization/recovery of feral pigeon populations (*Columbia livia* var. *domestica*) under control

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Key words: captures, pest species, management, cage-traps.

Currently, the feral pigeon (*Columba livia* var. *domestica*) presents naturalized populations all around the world adapted to urban environments. Although it is a hunting species subjected to exploitation, the feral pigeon in urban environments produces problems related to human health, damage to buildings, and unpleasant soiling and foul odor. This is why governments and individuals adopt different measures in order to reduce populations in cities. In this communication, we present the results derived from the research on the evolution of feral pigeon populations in different areas of Malaga province (south of Spain) after finishing the control program by captures with cage-traps. In all cases, the populations of feral pigeons were in isolated buildings of residential areas. The average period of recolonization of the areas where feral pigeons had not remained at the end of the control program is 911.9 ± 983.6 days, while in the zones where there were individuals remaining the recovery of the populations is significantly quicker (390.4 ± 468.6 days) ($X^2 = 208.83$, $df = 1$, $p < 0.01$). On the other hand, we show that there is a positive and statistically significant relationship between the recolonization/recovery period of the populations and the distance from the closest source area ($F_{ANOVA} = 9.033$, $df = 1$, $p = 0.011$). We conclude that the period of recolonization/recovery increases considerably 1) by increasing the distance between the source area closest to the population under control, and 2) by removing all individuals from the population that is being controlled.

Migration of shoveler *Anas clypeata* wintering in Portugal

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Key words: Anatidae, monitoring, movements, tagging, visual efficiency.

The shoveler is essentially a wintering migrant game species in Portugal, although a small breeding population inhabits the country. More than 170 shovelers have been captured and ringed in Portugal since 1993. Almost all these shovelers (more than 160) were nasal-saddled and generated more than 1200 resightings (www.pt-ducks.com). The recoveries, recaptures and resightings, modeled by geographic information systems, allowed the definition of migratory routes from the breeding grounds to the wintering sites in Portugal. All the shovelers used the East Atlantic migratory route, with possible breeding grounds in UK, Holland, Denmark, Poland and Finland. The nasal saddles allowed a 600% increase of data obtained internationally, with several cases of individuals with several observations from different sites. Additionally, since November 2017, two males were marked with GPS/GSM transmitters. The results indicate that shoveler migrates essentially during the night, and the additional information obtained will allow a better future modelling with geographic information systems.

Project RUFA

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Key words: biodiversity conservation, demonstrative hunting estates, game management, red-legged partridge, agricultural sustainability.

During the last 15 years, wild red partridge populations (*Alectoris rufa*) in Spain have decreased by an estimated 33%. The aim of the RUFA project is to contribute to the recovery of red partridge and sympatric farmland bird populations, through the creation of a network of demonstration projects in estates that implement agrarian and hunting management compatible with both agricultural profitability and biodiversity conservation. In addition, the project intends to show the hunting managers that it is possible to recover wild partridge populations without resorting to the release of farm-bred partridges. The actions are based on six pillars. Habitat improvements through a management of agricultural plot margins, where no agricultural practice will be done during the spring and where plant protection products will not be used. Supplementation of water and food through the creation of a network of feeders and drinking stations. Control of generalist predators in accordance with current legislation. Adaptation of the number of individuals hunted to the abundance of birds recorded the previous autumn. Education and training of landowners and managers, promotion of good agricultural and hunting practices. The impacts of these actions are monitored through bird census, photo-trapping and radio-tracking. In all the estates, actions are conducted in an experimental area and a control area is left without actions, in order to evaluate the effect on partridge populations as well as other farmland birds and the community of predators. The project will be conducted between 2019 and 2023 in eight demonstration estates located in the provinces of Álava, Badajoz, Madrid, Ciudad Real, Albacete, Valencia, Granada and Malaga.

The interFIELD project: innovation on agri-environmental measures targeting wildlife

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Key words: conservation, crops, farming, Common Agriculture Policy, United Kingdom.

Broadly speaking, the agri-environmental policies within the Common Agriculture Policy (CAP) have failed to halt the overall biodiversity loss in farmland Europe (especially birds), so the reconciliation of wildlife conservation and farming profit is compromised. The interFIELD project, which has been supported by the Fundación Biodiversidad, from the Ministry of Environment (MITECO), aimed to review scientific and practical knowledge of agri-environmental measures from experts, visit successful management cases in Spain and abroad and suggest a future strategy, especially for those who want to reconcile farming and wildlife conservation, with a focus on game estates. Available research shows that these measures may benefit habitats and species, and it is necessary to reduce farm intensification and conserve extensive farming, the latter less profitable but quite efficient for wildlife conservation. CAP subsidies for extensive farming could contribute to this objective. We conducted two field visits to the United Kingdom, one to Portugal and several to Spain aiming to understand successful cases of agri-environmental measures funded by CAP subsidies (such as the measures implemented in Special Protection Areas for Birds) and also private funded (at small game shooting estates). These cases shared the same view about agri-environmental measures: they are considered as a true crop. In conclusion, we are led to believe that future CAP reforms and schemes should promote agri-environmental measures through crops and set-aside for wildlife, being farmers the main stakeholders. However, other stakeholders should be involved to ensure proper education and research for those interested in these measures, bearing in mind that bureaucracy should be reduced as it is one of the most important problems for farmers.

Wild boar cage traps in urban areas; how effective are they?

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Key words: wild boar, control, captures, cage traps, selectivity.

We analyzed the efficiency, effectiveness, catch rate and selectivity of cage traps used to control wild boar in a urban area of Costa del Sol (Benahavís municipality, Málaga, southern Spain). Between 2015 and 2018 a total of 30 cage traps (three different models) were used (minimum size 170x70x60 cm and different activation systems). The capture effort was 2,337 trap-night. We captured 151 target animals (86 females and 65 males, 111 juveniles [<30 kg] and 40 adults) and 15 non-target animals (mainly cervids). The average weight of the catches was 28.9 ± 2.2 kg (range: 8 to 100 kg). Total catches, sex, age and the average weight of the individuals did not differ significantly between years nor between cage trap models (Kruskal-Wallis test). The ISO efficiency of the cage traps was 0.065; ISO effectiveness (mechanical efficiency) ranged between 8.1% and 32.3% according to the estimate of potential catches (a cage trap can capture from 1 to 4 animals at time depending to the size of the individuals); ISO selectivity was very high (91%). Overall, the cage traps captured animals of both sexes in equal proportion ($p = 0,104$, Chi² test), but the number of younger animals was higher than number of adults ($p < 0.001$, Chi² test). The catches were not homogeneous between seasons, being more frequent in spring and summer ($p < 0.001$, Chi² test). Capture efficiency by sex was not significantly associated with the period of the year ($p = 0.411$, Chi² test). However, we found a significant association in the case of animal age ($p = 0.006$; Chi² test); mostly young animals were captured between spring and summer. Catches of adult breeding animals were very low.

Red deer expansion in an urban environment in the Costa del Sol (Málaga)

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Key words: deer, urban environment, density, colonization.

We analyzed the expansion of a Central European red deer population introduced in a fenced hunting estate (Benahavís municipality, Málaga, southern Spain) in the eighties. The estate was requalified and urbanized in the nineties. Since then, these animals have coexisted with residents and both accidental and intentional escapes have been taking place. The latter escape has been a way to control the damage problems caused by this population, given the difficulty of using other means (the use of firearms in urban areas, administrative obstacles for live capture and animal rights controversies). Using pellet counts, during 2017 and 2018 we estimated the red deer population density in a buffer of 10 km radius around the source estate. The presence of deer has been verified in almost all the sampled area, in places without previous presence of this species. The overall population density around the estate ranged between 17.2 to 18.9 deer / km². We found significant differences ($p < 0.001$, U Mann-Whitney test) in the density values (average \pm SE) between the western (32.6 ± 0.04 deer / km²) and eastern (2.9 ± 0.01 deer / km²) areas. Most of the habitat in the eastern sector is forestry, while in the western zone it is an urban mosaic (isolated dwellings, urbanizations and golf courses) with pine and scrubland patches. The results suggest: 1) a different colonization capacity of the deer, 2) more success in the urban-forest mosaic, and 3) a potential risk of introgression if these Central European deer come into contact with populations of Iberian red deer.

Importance of supplementary feeding for wild boar in fenced hunting areas

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Key words: wild boar, ecology, feeding, fenced areas, reproduction.

The generalized increase of wild boar populations in the Iberian Peninsula, as well as the increasing demand of its hunting in regions like Extremadura, make necessary to improve the knowledge on the ecology of this species. The aim of this work was to estimate the consumption of feed in wild boar from a fenced area with systematic food supplementation and to observe and analyse its effects on the wild boar population. The study was carried out in a wild boar game estate in Extremadura. This estate has a 220 hectare fenced area only for wild boar, in which 4 selective wild boar feeders with a 350 kg feed capacity were installed. The influx of wild boar was recorded using camera trapping. The monthly consumption of feed was recorded from April 2018 to April 2019. The average daily intake per animal throughout the study period was 0.99 kg (\pm 0.37 kg). The highest feed consumption was observed during the month of August (1.51 kg/animal/day) and the lowest in December (0.21 kg/animal/day). A sharp decrease in consumption was recorded between November and January due to the greater availability of natural resources. Between February and March, an increased influx of females with piglets to feeders was recorded. The maximum influx was observed in October, just at the beginning of the reproductive season. The results obtained show that in fenced areas there are two critical periods for supplementary feeding of the wild boar populations: one in spring, coinciding with lactation, and another one during the summer months, until the beginning of the reproductive season. This last period is important in order to guarantee the reproductive success, as well as the sanitary status of the animals.

Myxomatosis in the hare (*Lepus granatensis*): Emerging disease

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Key words: *Lepus granatensis*, myxomatosis, *Oryctolagus cuniculus*, Valencia Region.

Myxomatosis is a disease caused by a poxvirus and transmitted by direct contact between sick and healthy animals, and indirectly by hematophagous vectors. Initially, it is exclusive of the European wild rabbit (*Oryctolagus cuniculus*) in the Iberian Peninsula, although it had been described in hares in England. However, in 2018 in Spain, there were observations of Iberian hares (*Lepus granatensis*) with the typical symptomatology of this disease: blepharitis, anogenital and oronasal myxomas. The first cases were detected in Córdoba (July), extending through the rest of Andalusia, Castilla-La Mancha and Madrid (central-southern Spain), arriving in August to the Valencia Region (eastern Spain). With the collaboration of Hunting Societies and Environmental Officers of the latter region, the Hunting and Fishing Service has performed the necropsy of four killed hares with signs of myxomatosis in the municipalities of Ademuz, Picassent and Camporrobles, sending samples for confirmation: serum to the UASA, eyelid, and mouth and genital myxomas, to the Central Veterinary Reference Laboratory in Algete. The analyses to detect antibodies in serum were conducted, being specific for rabbit myxomatosis, and using Hipra's test *Civtest Cuni Mixomatosis*, and the detection of myxomatosis' nucleic acids by conventional PCR (PESIG/PCR-03+IESIG/PCR-56) on eyelids. Their results were positive in all cases, confirming the disease. We expect to confirm genetically whether it is the virus that affects rabbits or there is a mutation of the same virus for which the disease affects the Iberian hare. The future of myxomatosis in hares is uncertain, as the fall of temperatures has reduced the occurrence of cases in the countryside. Apart from that, the disease has not still been described in the broom hare (*Lepus castroviejoii*), a genuine endemism, that could be labeled as endangered if it affected it.

Project +Coelho: bringing together the scientific community and the hunting sector

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Key words: wild-rabbit, Iberian hare, recovery of leporid populations, National Plan.

The Portuguese Ministry of Agriculture, Forest and Rural Development determined the constitution of a 9-member partnership to implement an integrative approach strategy to counter the effect of rabbit hemorrhagic disease virus 2 (RHDV2), responsible for the abrupt decline of wild rabbit populations in Portugal (dispatch 4757/2017, 31st May). This project, entitled *Action Plan for the Control of Rabbit Haemorrhagic disease virus*, was developed by the National Institute of Agrarian and Veterinarian Research (INIAV IP), the National Authority for Veterinary Health (DGAV), the National Institute for Conservation (ICNF), two private research institutes (CIBIO, OMV) and the main National Hunting Organizations (FENCAÇA, ANPC and CNCP). The project includes also the surveillance of RHD and Myxomatosis in rabbits and hares. The project axes were 1) research program, 2) management practices and 3) sanitary surveillance. The lines of research include, among others, the identification of naturally resistant specimens, the production of an oral vaccine based on viral-like particles to increase the wild rabbit populations' immunity to RHDV2 and the development of an informative and interactive public platform displaying real time cartographic and statistical information related to wild leporids. Twelve months after the plan formulation, practical measures are being prepared for immediate implementation, namely nutritional supplementation with dry feed formulated for wild rabbits in hunting reserves where natural food is scarce, deworming of animals in areas affected by high parasitic loads, identification of resistant populations with high antibody titers for RHDV2, and the creation of genetic sanctuaries. The plan has been financed annually by the *Fundo Florestal Permanente* (<http://www.iniaiv.pt/doenca-hemorragica-viral-dos-coelhos>). The +Coelho project constitutes an example of productive and dynamic interactions between the scientific and hunting sectors and inspired the creation of the *Centro de Competências para o Estudo, Gestão e Sustentabilidade das Espécies Cinegéticas e Biodiversidade*.

Myxomatosis emerges in the Iberian hare after decades to exclusively affect the rabbit: the most striking histopathological differences

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Key words: mixoma virus, Iberian hare, histopathology, wildlife, species barrier.

There was a recent outbreak of Myxomatosis in the Iberian hare (*Lepus granatensis*) in August 2018 in Spain and two months later in Portugal. At the Portuguese National Reference Laboratory, INIAV, the aetiological agent was identified by molecular biology techniques as Myxoma virus (MYXV). This detection was made in the ambit of an ongoing national survey for wild leporids sanitary evaluation that started in 2017 (project +Coelho). The specificity of MYXV for several rabbit species has been known for decades, although a few occasional reports of disease in the European Hare have been published. Currently, the disease affects hares in several districts in the south of Portugal and in more than 15 provinces in Spain, with high mortality and unknown morbidity. On macroscopic examination of affected hares the absence of cutaneous myxomas was the most relevant difference to the nodular form of disease in rabbits. Histopathology showed similar findings to those observed in rabbits, namely moderate epidermal hyperplasia, balloon epithelial degeneration, proliferation of spindle and stellar cells surrounded by an extensive extracellular matrix. However, evidence of higher malignancy in the histopathological lesions is suggested by the presence of spindle cells adjacent to the ulcerated epidermis, with moderate pleomorphism, large nuclei and dense chromatin. Extensive infiltration of heterophilic cells into the dermis was also observed. Nonetheless, the putative contribution of other pathogenic agents to the histopathological presentation is being investigated through hybridisation *in-situ* and immunohistochemistry. Epidemiological and histopathological data suggests that the higher severity of disease in Iberian hares may relate to the recent species barrier jump rather than the long co-evolution of the Myxoma virus in rabbits in which a general tendency towards decreased virulence has been observed. The laboratory and field work was funded by FCT (Grant SFRH / BD / 137067/2018), CIISA, FMV-UL (Project UID / CVT / 00276/2013) and the +Coelho project (Fundo Florestal Permanente, Portugal; Dispatch No. 4757/2017 of 31 May).

Project FIGHT-TWO – Edible bait vaccine for rabbit haemorrhagic disease virus 2 (RHDV2) control in wild rabbits

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Key words: *Oryctolagus cuniculus algirus*, wild rabbit, RHDV2, oral vaccine, VP60-VLPs.

Rabbit haemorrhagic disease (RHD) is a highly contagious, often lethal systemic infection in the European rabbit (*Oryctolagus cuniculus*), and a main factor underlying the species' decline, indirectly impacting on endangered predator species that depend on the rabbit. Presently, the condition is caused by the rabbit haemorrhagic disease virus 2 (RHDV2), which emerged in 2010 and replaced the previously circulating RHDV genogroups (G1-G6). The available RHDV2 commercial vaccines are inactivated, obtained from infected animal liver extracts, to be administered subcutaneously. Further than the risks associated with incomplete virus inactivation, these vaccines are unsuitable for wild rabbits since require handling. The immunity is short and the protection transient. The commercial RHDV vaccines were shown to be ineffective in conferring cross protection against RHDV2. FIGHT-TWO strategic framework (PTDC/CVT-CVT/29062/2017-PT2020) is the development and production of an edible pathogen-free RHDV2 vaccine to be distributed in the field as bait or in dry feed. It has the potential to protect a broad proportion of the wild populations, crucial to reduce virus transmission and control the infection, overcoming the need of capture and manipulation. The virus-like particle based vaccine will be produced in insect cells-baculovirus expression vector system (IC-BEVS) and updated according to RHDV2 evolution (open system). The project partnership includes INIAV, the reference laboratory for animal diseases, two Portuguese Veterinary Universities (Évora and Lisbon) and IBET, a private institute with vast experience in the vaccines production field. FIGHT-TWO will allow to proceed with one of the 12 measures specified in an Action Plan for the Control of Rabbit Haemorrhagic Viral disease in Rabbits (Dispatch 4757/17 of 31 May, Ministry of Agriculture), supporting more generalist management policies towards the recovery of wild rabbit population densities and RHD control, the recovery of ecosystems where the rabbit is keystone and the reactivation of hunting activities in Portugal.

Importance of long-term epidemiological studies in wildlife

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Key words: long term, shared diseases, epidemiology, wildlife.

Understanding temporal dynamics of infection in wild hosts is essential to studies on epidemiology and ecology of shared diseases. These studies require a systematic monitoring that compiles broad temporal and spatial scales information to understand the involved factors. This literature review describes the purposes, main species and pathogens analysed in these broad-scale monitoring studies, as well as the added value provided by their duration. With this purpose, we consulted Scopus, PubMed and Web of Science databases. We selected 547 articles published between 1993 and 2017 that were reporting a study period of at least four consecutive years with a minimum sample size of 10 individuals per year. We observed an increasing trend in the number of studies published from 1993/94 (six articles) to 2016/17 (122 articles). The most studied pathogens were viruses (41.2%), bacteria (38.2%) and protozoa (16%). Regarding the hosts, mammals (85.5%), especially ungulates (40.1%) and carnivores (31.1%), and birds (11.8%) were the most represented. Most of these articles conclude about the effect of the disease on the population dynamics and on the ecology of the species (98.7%), as well as on the shared character (41.2%) or zoonotic character (26.3%) of the disease. There was a significant relationship between the duration of the study and the capacity to conclude on the role of wildlife as a reservoir of the pathogen. In order to design effective control strategies, it is necessary to improve our understanding of the epidemiology of these diseases, which can be achieved through these studies. One of the general aims of the SaBio team at the IREC is to give continuity to the epidemiological studies in different hosts and pathogens as a strategy to generate long-term series of data.

Study of Flavivirus infection in birds along a gradient of interaction between wildlife and livestock

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Palabras clave: flavivirus, aves, mosquitos, equinos, humanos.

West Nile fever virus (WNV) is an emerging arbovirus (arthropod-borne virus) of major importance in Europe; 2,083 human clinical cases were reported in 2018. Its natural cycle involves several species of wild birds as reservoir and *Culex* mosquitoes as vectors. It is pathogenic for horses, in which it can produce severe encephalitis. In addition, WNV can affect humans, although the symptomatology is infrequent and similar to a flu-like illness in most cases. Due to the recent expansion of this Flavivirus to areas of the interior of the Iberian Peninsula, the aim of this study is to determine the prevalence of infection in different bird species in different scenarios of variable interaction between fauna and livestock/human. The study was based on the sampling of birds in five areas of the provinces of Ciudad Real and Toledo, in areas of recent proven WNV expansion. In each sampling area, three points with a variable gradient of interaction between fauna and livestock/human were selected: (1) wildlife zone exclusively, (2) wildlife and domestic livestock interaction zone, and (3) livestock zone (horse farm). In these areas, wild birds were captured by Japanese nets in the summer and autumn of 2018. Bird sampling (n=281) were collected from growing feather canons, blood, and oral and cloacal swabs. The samples were processed for RNA extraction in order to detect the presence of virus genetic material through RRT-PCR. The results will help to better understand the risk of WNV infection in horses and humans interacting with wild birds.

Evolution of sarcoptic mange in red deer in La Rioja

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Key words: mange, red deer, management, zoonosis.

The interest on emerging diseases has increased during the last years, and multiple host scenarios are increasingly relevant for management and conservation of wildlife. Sarcoptic mange is a zoonotic diseases that can affect a great number of domestic and wild species, which makes this disease very important economically, ecologically and from a public health perspective. Sarcoptic mange was detected for the first time in red deer (*Cervus elaphus*) in La Rioja in February 2010 near Ezcaray. Since then, the area affected by the disease has continuously spread as an “oil stain”, with over 800 animals affected by this parasitic disease registered by 2018 in the Regional Game Reserve of Cameros-Demanda and the adjoining private hunting areas. The mean number of cases of mangy red deer was 97 per year, with a maximum of 160. A statistically significant relationship between monthly temperature, number of days with snow cover and the yearly number of mange cases in red deer is observed. The 72.5% of the cases happened in the two first trimesters of the year and were related with sudden temperature changes. The cases of mange where significantly higher in males than in females and in adults than in younglings. According to the epidemiological data collected and to the development of this zoonosis, sarcoptic mange does not seem to be a threat for the populations of red deer in La Rioja. Anyways, continuing with the health monitoring and a correct game management is recommended.

The relative importance of the wild boar and the red deer as tuberculosis hosts in Portugal: preliminary results

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Key words: animal tuberculosis, *Sus scrofa*, *Cervus elaphus*, maintenance hosts, basic reproductive number.

Animal tuberculosis, a zoonosis relevant to large game management, is maintained in the Iberian Peninsula in a system involving multiple hosts. The wild boar and the red deer are the most important maintenance hosts in the wild ungulate community, but their relative importance is unknown. The objective of this study is to quantify the contribution of boars and cervids to the maintenance of animal tuberculosis in five study areas in Portugal (Bragança, Idanha Nova, Castelo de Vide, Moura-Barrancos and Serpa). For each of the species, the tuberculosis basic reproductive number (R0) was estimated in each study area, in a Bayesian framework. Preliminary results suggest that red deer is the main maintenance host in Idanha a Nova (R0 = 3.2 IC₅₀ 1,1-8,6), Bragança (R0 = 19,6 IC₅₀ 4,7-80) and Serpa (R0 = 5,1 IC₅₀ 1.1-21), and the boar is the main maintenance host in Moura-Barrancos (R0 = 1,9 IC₅₀ 0,7-5,8) and Castelo de Vide (R0 = 30,9 IC₅₀ 9.0-124). The probability of both species being maintenance hosts varies between 30% in Castelo de Vide and <1% in Bragança. The relative importance of boar and deer in maintaining tuberculosis in communities of wild ungulates seem to be spatially variable, the causes of which are still unknown. Preliminary results of the quantification of the relative contribution of boar and deer to the maintenance of tuberculosis contribute to the knowledge of the epidemiology of this disease, as well as to the evaluation of potential control strategies in wildlife.

High spread and limited circulation of Pestivirus in wild ruminants in Spain, 2000-2017

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Key words: pestivirus, wild ruminants, seroprevalence, spread, Spain.

Ruminant pestiviruses, Bovine Viral Diarrhoea Virus (BVDV) and Border Disease Virus (BTV), affect several species of artiodactyls causing important economic losses worldwide. The aim of the present study was to determine the seroprevalence and circulating serotypes of pestiviruses in the different wild ruminant species present in Spain, assessing the spatial distribution of these viruses during the period 2000-2017. The 6.6% (123/1874; CI_{95%}: 5.4-7.7) of the analyzed animals were positive to both ELISA and viral neutralization tests. By species, the seroprevalence was 30.0±5.7% (75/250) in chamois (*Rupicapra pyrenaica*), 7.0±2.6% (25/357) in fallow deer (*Dama dama*), 2.5±1.5% (10/401) in red deer (*Cervus elaphus*), 2.4±1.7% (8/330) in Iberian ibex (*Capra pyrenaica*), 1.1±1.1% (4/369) in roe deer (*Capreolus capreolus*) and 0.8±1.5% (1/130) in mouflon (*Ovis aries musimon*), while seropositivity was not found (0/37) in Barbary sheep (*Ammotragus lervia*). Antibodies against pestiviruses were detected in all bioregions (BRs 1-5), with higher seroprevalence ($P < 0.05$) in the north of the country and Mediterranean basin (BRs 1,2,5), identifying serotypes VDVB-1 (BRs 1,2,4) and VEF-4 (BRs 1,2,3,5) in chamois, fallow deer and red deer. The model of generalized estimation equations identified precisely the BR (BRs 1,2,5) as a risk factor potentially associated with pestivirus infection in wild ruminants in Spain. The results obtained in chamois are consistent with the endemic circulation of pestiviruses evidenced in previous studies, while those obtained in the other species suggest their limited implication as natural reservoirs of these viruses in the Iberian Peninsula. However, the high interspecific infection capacity of pestiviruses, as well as the fact that seropositivity was detected in all BRs and most species, show the relevance of identifying and monitoring circulating pestiviruses in order to establish specific control strategies in sympatric livestock species.

Monitoring of diseases between wild ungulates and cattle in the Selva Lacandona, Mexico

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Key words: ungulates, diseases, ticks, cattle, Lacandona.

The present study is carried out in the Selva Lacandona, specifically in the Montes Azules Biosphere Reserve, and in 3 villages surrounding it, in the state of Chiapas, Mexico. The objective is to identify certain diseases, and possible transmissions between wild ungulates and cattle: Baird's tapir (*Tapirus bairdii*), collared peccary (*Pecari tajacu*), white-lipped peccary (*Tayassu pecari*), white-tailed deer (*Odocoileus virginianus*), red brocket deer (*Mazama temama*) and cattle (*Bos taurus*). The main diseases to monitor are: Tuberculosis, Brucellosis, Leptospirosis, Anaplasmosis and Babesiosis. The taking of samples in wild ungulates is through specimens obtained in the subsistence hunt that is carried out in this area by some settlers. In the case of diseases transmitted by ticks; these are collected directly from the host, and from the vegetation, and they are analyzed by PCR techniques. In addition, we use camera trapping to apply the "passage rate" technique, which associates the presence of possible hosts captured in photos, to ticks collected in vegetation on plots. As preliminary results, in relation to species of ticks and endoparasites; the Morisita index showed greater similarities between the 2 cervids, and between both tayassuids, whereas the greater similarity between domestic and wild species was found for cattle and white-tailed deer. Likewise, interviews with hunters and ranchers have been conducted, identifying management practices, species and number of animals mostly hunted, and identifying points and sites of greatest contact between livestock and wild ungulates. The most extracted species during the hunting are: *Cuniculus paca*, *Tayassu pecari*, *Mazama temama* and *Odocoileus virginianus*. The sites of greatest contact between wild and domestic species are the edges of secondary vegetation and grasslands, feeders for salt and corn.

Cephenemiosis in the roe deer. Experience in Galicia and Asturias. Future of the species and its hunting use

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Key words: cephenemiosis, exploitation, roe deer, Galicia and Asturias.

The appearance in the roe deer of the disease known as cephenemiosis in Spain has been an epizooty with catastrophic consequences for this wild species. This epizooty affects both the stability of roe deer populations, so far in expansion, as well as their hunting exploitation and derived consequences (economic impact on the viability of hunting areas, economic repercussion, decrease in sporting expectations and hunting). All of the above supposes a serious risk to the survival of this species in the hunting territories of northwest Spain (Galicia and Asturias). The characteristics and biological peculiarities of the parasite, with a complicated biological cycle and a high infective capacity, its broad distribution and the difficulties for its control and treatment, make this parasitic disease perhaps the greatest real threat to the forest goblin of the last 50 years in Spain, and in particular in the north of the Iberian Peninsula. It is necessary to know the parasite, its cycle, distribution, methodologies for estimation and study. It is also required to update and establish good monitoring methods for deer populations and to know the factors that favour the appearance of the disease and contribute to aggravate the associated mortality, for which a collaboration among all the sectors involved (hunters, environmentalists, owners of game reserves, property owners, scientists and administrations) becomes necessary.

Alfa-herpesvirus and pestivirus seroprevalence in Iberian red deer (*Cervus elaphus*)

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Key words: red deer, ELISA, IBR/IPV, DVB/EF.

Alfa-herpesviruses and pestiviruses are pathogens that cause the infectious bovine rhinotracheitis (IBR)/infectious pustular vulvovaginitis (IPV) and bovine viral diarrhoea (DVB)/border disease (BD), respectively. These diseases may cause great economic losses in infected farms and the epidemiological role of wild animals, such as the Iberian red deer (*Cervus elaphus*), has special interest to eradication programs. In Spain, few works have been carried out about those diseases in this species. The aim of this work was to estimate the seroprevalence of IBR/IPV and DVB/DB in Iberian red deer. A total of 150 blood samples from four game estates and two regional game estates in Extremadura were analysed for the presence of antibodies against IBR/IPV and DVB/DB using two commercial ELISA kits. We observed 25.3% (38/150) of samples positive to IBR/IPV antibodies, with seroprevalence among game estates between 6.7 and 66.7%. Moreover, ELISA resulted in a doubtful outcome for 7.3% of the samples. Regarding to DVB/BD, no positive samples were observed, and only one sample was doubtful. The epidemiological role of the Iberian red deer in Spain is unknown, so further studies are needed to determine its importance. However, these diseases were present in cattle in the same estates and both cattle and red deer frequently interact in the outdoor rearing system. Therefore, biosecurity measures should be implemented in order to control these diseases.

Evaluation of two commercial ELISAs for diagnosing bluetongue in wild ruminants

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Key words: bluetongue, ELISA, diagnostic evaluation, wild ruminants, serology.

Enzyme-linked immunosorbent assays (ELISA) are widely used in bluetongue virus (BTV) surveillance in domestic ruminants to screen for seropositive animals. Using ELISA in wild ruminants has also a great epidemiological interest to detect the persistence of BTV in these species and to monitor areas where livestock is compulsorily vaccinated. However, diagnostic validity of commercial BTV ELISAs in wild ruminants has not been reported yet. The aim of this study is to evaluate two commercial ELISAs for the detection of BTV antibodies in wild ruminants: a double recognition ELISA (DR-ELISA, INGEZIM BTV-DR-12.BTV.K0[®], INGENASA) and a competitive ELISA (C-ELISA, IDScreen Bluetongue Competition ELISA kit[®], IDVET). These tests were evaluated in 57 control sera (animals with known BTV status) and in 264 field sera (with unknown status). In addition, field sera were also analyzed by viral seroneutralization test (SNT) in order to perform a latent-class analysis and determine the diagnostic validity of these techniques. In control sera, the DR-ELISA showed a sensitivity (Se) and specificity (Sp) of 100% and the C-ELISA presented Se = 86.4% and Sp = 97.1%. However, in field sera, the DR-ELISA showed Se = 95.7% and Sp = 85.9%, the C-ELISA showed a Se = 58.2% and Sp = 95.8% and the SNT Se = 84.2% (Sp was fixed at 100%). Our results indicate that commercial ELISAs can be used for bluetongue diagnosis in wild ruminants, although they should be selected regarding to the epidemiological context. The DR-ELISA should be selected when the circulation of the virus in endemic areas is evaluated, while the C-ELISA would be more appropriate for revealing exposure to BTV in free circulation areas.

Seroepidemiology of reproductive diseases in wild ruminants in the Comunidad Valenciana: retrospective study

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Key words: abortive pathogens, seroepidemiology, wild ruminants, Comunidad Valenciana.

Wild ruminants play an important role as reservoir of diseases that can be transmitted both to humans and domestic animals. However, many of these pathogens are not subjected to active epidemiological surveillance, although they are currently considered emerging or of special interest for animal health. Among these diseases, those interfering with the reproductive capability of the animals, either because of their wasting character or by being abortive diseases, are particularly relevant. Motivated by the scarce number of studies carried out in the Comunidad Valenciana, a retrospective seroepidemiological study was performed, assessing the presence of nine pathogens with special relevance in the reproductive success of animals (Lentivirus, Border disease, Schmallenberg virus, *Mycobacterium avium paratuberculosis*, *Mycoplasma agalactiae*, *Brucella ovis*, *Coxiella burnetti*, *Chlamydia abortus* and *Toxoplasma gondii*) in 143 samples from the serum bank of the Game and Fishing Service of the Generalitat Valenciana. The samples were collected from mouflon (*Ovis orientalis musimon*) (n=92), Iberian ibex (*Capra pyrenaica*) (n=45) and red deer (*Cervus elaphus*) (n=6) during the hunting seasons from 2009 to 2016. From all the samples analyzed, 32 (22.3%) presented antibodies against, at least, one disease. The highest seroprevalence found was for *C. abortus* (9.1% CI_{95%}=4.3-13.9), but a high percentage of samples (14.1%) were doubtful for *M. agalactiae*. No antibodies against Border disease, Schmallenberg virus or paratuberculosis were detected. The low seroprevalence observed suggests that the pathogens studied play a secondary role in the population dynamics of wild ruminants in the Comunidad Valenciana and highlights the little role they play as reservoirs of these diseases for domestic livestock. This work has been funded by a grant of the Universidad Católica San Vicente Mártir (UCV 2017-192-001) (granted to JSM).

No effect of inoculation site and injection device on the skin test response of red deer to the intradermal injection of *Mycobacterium avium*-derived purified protein derivative (aPPD)

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Key words: cell-mediated immune response, *Cervus elaphus*, paratuberculosis, tuberculosis.

Mycobacterial diseases are important health issues in farmed deer. The single intradermal tuberculin test (SITT) is the standard test for tuberculosis testing in deer. We studied two factors which might influence the response of deer to skin testing: the inoculation site and the injection device. Deer included in this study were farmed red deer (*Cervus elaphus*) hinds (n=80) born between April and May 2016. Two areas of 3 cm × 3 cm were shaved at the left side of the neck. Site A (SA) was situated about 10 cm caudal to the ascending branch of the jaw, while site B (SB) was 10 cm caudal to site A. All hinds received two 0.1 ml inoculations of *Mycobacterium avium* derived purified protein derivative (aPPD). One inoculation was made by syringe and the other one with the needle-free syringe Dermojet. To test the inoculation site effect, half of the animals were inoculated following two different orders. No differences were recorded for the injection device nor for the inoculation site. Ten hinds had a skinfold increase larger than 3 mm by any injection device. Seven (8.7%) and six (7.6%) hinds were classified as positive by syringe and Dermojet. The same results were obtained for inoculation site: seven (8.7%) positives in SA and six (7.6%) in SB. The distribution of skinfold thickness increases did not differ by injection device. Our findings support the needle-free Dermojet syringe as a suitable tool for skin-testing in red deer and suggest no relevant effect of the position of the inoculation site along the neck in red deer. These results may help to standardize the SITT test in deer, contributing to the control of mycobacterial diseases in farmed cervids.

Effect of systematic vaccination against porcine circovirus type 2 in wild boar populations

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Key words: wild boar, PCV-2, vaccination, tuberculosis.

One of the main symptoms of the pathology associated with porcine circovirus type 2 (PCV-2) infection is lymphoid depletion, which can cause a loss of immunity that facilitates the development of other pathologies such as tuberculosis. The aim of the present study was to compare the antibody response against PCV-2 with the number of viral DNA copies in wild boar populations systematically vaccinated along recent years. For this purpose, four groups were established according to vaccination and deworming schedules. A total of 340 individuals were captured, vaccinated and microchipped, 43 of which were hunted. As controls, animals of the same age not previously captured were used. During the sampling, blood was taken from the retro-bulbar cavernous sinus and lymph nodes were collected for macro and microscopic evaluation. For the determination of IgG / IgM the INgezim circovirus IgM / IgG kit (INGENASA[®], Spain) was used. DNA was extracted using a commercial tissue-specific kit, followed by qPCR-RT with specific primers and subsequent statistical analysis. The animals were classified according to the number of DNA copies of circovirus (500 ng of DNA) as belonging to negative, subclinical and systemic disease categories. The results show an increase in IgG levels in the chipped as well as in the control animals in the different hunting actions, especially in those hunting estates that have implemented specific control measures against the disease for a longer period. The results also highlight a relationship between seronegative animals and a high viral load, which were classified within the category of 'systemic disease', and that coincide with unvaccinated animals. On the contrary, in the rest of the animals, viral replication was either not found or classified as subclinical. In conclusion, the present study highlights the increase in immunity against PCV-2 in systematically vaccinated wild boar populations.

Control of rabbit populations in high-density areas around communication infrastructures

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Key words: rabbit, agricultural damage, infrastructures, control, cage-traps.

The agricultural damages caused by European rabbit are a widespread problem in many Spanish regions, and a common factor in all cases lays in the difficulty of controlling the species in the surroundings of communication infrastructures. In these areas rabbits attain very high densities, as their embankments provide optimal conditions for rabbits to make burrows and also serve as a refuge from predators and hunters (the use of fire weapons is prohibited there because they are "security zones"). We carried out a trial project in one of these areas, the AVE line when passing through Utiel, a Valencian municipality with marked damages since the beginning of the last decade. The project combines the sealing of the infrastructure through different forms of mesh installation, with population control through a selective and innovative live capture system, known as "rabbit drop box trap". We make comparisons between three different trap models, as well as between different control frequencies and intensities. The main objective of the study was to develop a system for the control and minimization of damages that, without using firearms, could be used effectively in infrastructure security zones, and be made available to administrations and affected agents. In a complementary way, the study has served to assess the evolution of populations in areas of very high density (studying, among other parameters, productivity and resistance to diseases), as well as to evaluate how these parameters were affected by periodic extractions, and their capacity for recovery. The results obtained, besides showing varying effectiveness in the systems used, are encouraging with respect to the use of this technique as a control method.

Field evaluation of melatonin implants to improve reproductive performance in yearling Iberian red deer (*Cervus elaphus hispanicus*) hinds

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Key words: *Cervus elaphus hispanicus*, yearling hinds, melatonin, reproduction.

Iberian red deer (*Cervus elaphus hispanicus*) shows a markedly seasonal reproductive pattern shaped by photoperiod, which starts in autumn when the hours of light decrease and the levels of melatonin are higher. Thus, the profitability of deer farming is hampered by not being a continuous production. In addition, if a delay in pregnancy occurs, calves will be born at adverse environmental conditions (summer), having worse survival rates. Thereby, it is desirable that hinds are able to be pregnant before the end of October so the calf birth will take place during spring, the most favorable period of the year. Puberty in female red deer takes place normally during the second autumn of life (around 16 months of age) being constrained by photoperiod and body mass. The low reproductive productivity of young red deer hinds, compared to adults hind, appears to reflect high incidences of puberty failure at 16 months of age. In addition, those yearling hinds that fail to be pregnant in their first year of life, will continue to drag into adulthood their status as “late” hinds and therefore will contribute to reduce fertility of the entire herd. Aforementioned facts, seriously limits the progress of deer farming with this subspecies. In the present study, the effect of melatonin implants administration before breeding season on reproductive performance in yearling Iberian deer hinds was explored. We obtained a significant improvement of fertility rates, an advance of calving date and an increase of calf weight at weaning compared to non-treated yearling hinds. Moreover, differences of fertility found out between adults and yearling hinds disappeared after melatonin treatment on these latter. Our results show that melatonin implants are a useful reproductive management tool in Iberian red deer.

Ultrastructural specializations in hairs from the dark ventral patch in *Cervus elaphus hispanicus*

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Key words: dark ventral patch, specialization, cuticular scales, *osmetrichia*, *Cervus elaphus hispanicus*.

Olfactory signals play a relevant role in communication in many terrestrial mammals. Chemical signals can be deposited in the environment, known as odorous marking, or carried on the animal's body. In polygynous species, where mate competition is intense, communication between rivals is decisive to mediate the intrasexual competition or to avoid the risk of being damaged. In mammals, marking behaviour works synergistically with other odorous secretions to optimize communication. The term *osmetrichia* refers to structural specializations in the hair involved in odorous signalling. There is evidence of such specializations of hairs associated with glands involved in the secretion of compounds related to male-male competition, territoriality and reproduction along mammalian species. In Iberian red deer, a chemical signal revealing an individual's reproductive effort and sexual activity has recently been described. It is a conspicuous dark area present in the ventral area of males, which varies in size depending on the age and dominance status of an individual. The main objective of this work was to describe the morphology and ultra-structure of the hairs that, associated to lipid compounds, compose the dark ventral patch that conforms a sexual signal in this species. Hair from the dark ventral area was examined, as well as samples from other areas of the body and from females as a control. We found a specialized pattern of cuticle scales in the hairs that conform the dark ventral patch, as well as differences in their diameter. All these features point to an adaptation to improve the accumulation and retention of volatile compounds, likely increasing the intensity and duration of the sexual chemical signal during the rutting season.

Effect of sperm concentration on *in vitro* embryo production in red deer (*Cervus elaphus*)

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Key words: *in vitro* embryo, *Cervus elaphus*, sperm concentration.

In vitro embryo production is a technique that offers the possibility of preserving and disseminating the genetics of best individuals. However, for wild species such as Iberian red deer, this reproductive biotechnology is still poorly developed. During co-culture of sperm and oocytes, optimal sperm concentration is crucial to avoid polyspermy, in case the amount is excessive, and also decreased fertilization rate if the sperm count is low. Thus, the objective of this study was to determine the most suitable sperm concentration during *in vitro* fertilization. For that purpose, a total of 584 oocytes were selected from Iberian hind ovaries hunted and eviscerated in an authorized place in their natural habitat (game reserve). The protocol followed to produce *in vitro* embryos was developed by García-Álvarez *et al.*, *Theriogenology* 75:65-7. *In vitro* fertilization was carried out with concentrations of 250×10^3 , 500×10^3 , 1×10^6 , 1.5×10^6 , 2×10^6 spermatozoa/ml. After 8 days of embryo culture, the percentages of embryos obtained at day 2 of culture and from day 6 until day 8 were evaluated. Statistical analysis was performed using a General Linear Model (GLM). When GLM revealed a significant effect ($p < 0.05$), values were compared by Bonferroni test. Sperm concentration did not have an influence on the percentage of embryos obtained at 48 hours post-insemination. Nevertheless, the number of embryos produced from days 6 to 8 was different depending on the sperm concentration used, with higher rates obtained for 1×10^6 (14.6 ± 2.5) compared to the rest of concentrations (from lower to higher: 1.8 ± 2.5 , 3.1 ± 2.5 , 8.6 ± 2.5 and 9.4 ± 2.5). In summary, a sperm concentration of 1×10^6 is required to yield a higher number of *in vitro* produced embryos in Iberian red deer.

Collection and vitrification of chamois (*Rupicapra pyrenaica*) semen from Somiedo Natural Park

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Key words: chamois, sperm, cryopreservation, ultra-fast freezing.

The aim of the present work was to obtain semen from chamois maintained in their natural environment, and its cryopreservation by sperm vitrification technique. Six adult male chamois were net-captured in November and treated with a combination of ketamine-medetomidine for carrying them to the manipulation area. Semen was obtained through the TUMASG technique (ultrasound-guided massage of the accessory sexual glands), and no application electrical stimuli (electro-ejaculation) was required at any time. This technique allowed for obtaining a seminal sample of high quality from all animals. An aliquot of each sample was frozen using a conventional technique with nitrogen vapours and TCG-yh (Tris, citric acid, glucose, egg yolk) supplemented with glycerol (5%, v/v) as diluent. Another aliquot was diluted in TCG-yh medium supplemented with sucrose (100mM), and vitrified using the pellet technique by drop deposition of 50 μ L of sperm suspension directly onto liquid nitrogen. Sperm vitrification allowed obtaining sperm viability ($32.2 \pm 6.2\%$) and acrosome integrity ($69.3 \pm 8.1\%$) rates similar to conventional freezing ($35.7 \pm 7.6\%$ and $52.8 \pm 9.2\%$, respectively). The percentage of motile spermatozoa was higher in those that had been frozen using the conventional technique than in the vitrified ones ($33.3 \pm 5.6\%$ vs $7.5 \pm 2.2\%$). There were no differences between both cryopreservation techniques in the different kinetic variables of rectilinear, curvilinear and medium trajectory velocities. In conclusion, TUMASG technique allows chamois semen collection without the necessity to apply electrical stimuli, preventing capture myopathy development. Sperm vitrification allows for cryopreservation of chamois spermatozoa with an acceptable final quality, which makes it a highly recommendable technique for application in field conditions due to the simplicity of the procedure.

Effect of female presence on sperm quality of pure red-legged partridge (*Alectoris rufa*) and hybridized with chukar partridge (*Alectoris rufa* x *Alectoris chukar*)

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Key words: red-legged partridge, hybridization, presence of females, spermatoc parameters.

The red-legged partridge (*Alectoris rufa*) is a species of great ecological and economic importance. Together with the deterioration of its habitat, agricultural intensification and the use of pesticides, one of the main threats identified in the conservation of the red-legged partridge is genetic introgression with the chukar partridge (*Alectoris chukar*). The hybridisation has determined an alteration in different behavioral and reproductive parameters. In order to deepen the knowledge of the influence of hybridisation on the reproductive activity of this species, we studied the effect of female presence or absence on different sperm variables in one-year old individuals of both red-legged partridge and red-legged x chukar hybrids. The partridges were divided into four groups: paired pure partridges (PP: n = 16), paired hybrid partridges (HP: n = 12), isolated pure partridges (PS: n = 5) and isolated hybrid partridges (HS: n = 17). A total of 142 ejaculates was obtained, in which different quantitative and qualitative sperm variables were analyzed. The PS partridges showed, compared to HS group, a higher sperm concentration (1654.7×10^6 spermatozoa/mL vs 824.1×10^6 spermatozoa /mL), an increment of VCL ($65.19 \mu\text{m/s}$ vs $47.78 \mu\text{m/s}$) and a higher VSL ($46.94 \mu\text{m/s}$ vs $30.91 \mu\text{m/s}$). Female presence led to a significant increase in total sperm motility in PP group in relation to PS group (51.81% vs 32.52%) and in HP group compared to HS group (47.86% vs 27.45%). No significant differences were found between groups in the ejaculate volume or sperm viability. In conclusion, genetically pure partridges show a better spermatoc quality than hybrids, but this difference is reduced in the presence of females.

Carotenoid-based coloration predicts both longevity and lifetime fecundity in male birds, but testosterone disrupts signal reliability

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Key words: carotenoid-based coloration, longevity, red-legged partridges, survival, testosterone.

Sexual selection promotes the evolution of conspicuous animal ornaments. To evolve as signals, these traits must reliably inform about the “quality” of the bearer, a vague concept that often refers to individual fitness. Direct estimates of individual fitness must consider the contribution of longevity and fecundity. However, evidence of a correlation between the level of signal expression and both fitness components are scarce and incomplete, at least among vertebrates. Fitness is difficult to assess in the wild as the death date and extra-pair paternity rates are often unknown. Here, in captive male red-legged partridges, we show that carotenoid-based ornament expression (i.e. redness of the bill and eye rings) at the beginning of the reproductive life predicts both longevity (1-7 years) and lifetime breeding output (offspring number and hatching success). The recently proposed link between the individual capacity to produce red (keto)carotenoid pigments and the efficiency of cell respiration could, ultimately, explain the correlation with lifespan and, indirectly, with fecundity. Nonetheless, in males of avian species, carotenoid-based signal expression in bare parts is also partially controlled by testosterone. Here, we also manipulated androgen levels throughout lifetime by treating males with testosterone or antiandrogen compounds. Treatments made correlations between the signal level and both fitness components disappear, thus making the signals unreliable. This strongly points to the necessity of a tightly-controlled steroid metabolism to allow the evolution of carotenoid-based sexual signals.

Testosterone reduces longevity and reproductive success in male red-legged partridges under captivity

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Key words: breeding success hormones, infection, breeding farms, survival.

Testosterone, the main male sexual steroid, has attracted the attention of many research fields. In ecology, a high population density may trigger testosterone levels due to agonistic interactions. In evolutionary terms, testosterone plays a role in sexual selection favouring the acquisition of mates by improving competition with other males, or favouring the expression of secondary sexual traits used by females to choose their mate. However, maintaining high circulating testosterone levels in males promotes physiological and behavioural costs that could act as selective forces constraining phenotypes. Nevertheless, research work simultaneously testing the impact of high testosterone levels in both the survival and fecundity fitness components are scarce and mostly circumscribed to birds in the wild. Field studies are preferable as individuals are subject to natural selection pressures. However, to determine the exact causes of mortality or the impact of hormonal manipulations on male fertility are difficult tasks when tested in free-ranging animals. We performed a lifespan study in captive red-legged partridges where the androgen function was manipulated by exposing males to high exogenous testosterone levels or anti-androgens during six consecutive breeding seasons. Life history theory predicts that individuals maintaining high androgen levels should obtain higher fitness returns via reproduction, but reduced longevity. Accordingly, testosterone-treated males lived shorter and were more prone to death during an accidental bacterial infection (i.e. *Escherichia coli* outbreak). Same birds, nevertheless, showed a lower capacity to fertilize eggs than controls probably due to endocrine feedback reducing testicular mass. Moreover, males treated with the androgen receptor blocker flutamide did not show increased longevity such as predicted by life history trade-off theory but, throughout their lifetime, sired eggs with higher hatching success than controls. We discuss the implications of these apparently contradictory results.

The flank plumage pattern of the red-legged partridge (*Alectoris rufa*) as a signal of body condition: an experimental study

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Key words: individual quality, melanin, oxidative stress, colour patterns.

The red-legged partridge is possibly the most emblematic small game species of the Iberian Peninsula, playing a central role in the socioeconomic development of rural areas. Apart from this, its role as a study model in the field of evolutionary ecology is increasingly recognized, specifically for the study of the evolution of social signals of individual quality. In the last 15 years, several studies have focused on the function and expression of the red coloration (carotenoid-based) of periocular rings, beak and legs of this species. However, the red-legged partridge also displays conspicuous plumage traits whose function has been barely studied. This is the case of the characteristic barred pattern of the flanks, whose expression depends on the accumulation of two different types of melanin. Previous observational work has suggested that the size of the black bands could function as a signal of individual body condition. However, this hypothesis has not been experimentally tested yet. In this study we conducted an experiment in which a group of birds had limited access to food during moult, whereas another set of birds (controls) had *ad libitum* access to the same diet. Contrary to evidence from previous observational studies, the black pigmentation (eumelanin) increased when the physical condition was experimentally worsened, particularly in the ventral, more visible region of the ornamental flank feathers. This result could be explained by the connection between oxidative stress and melanogenesis, as our treatment probably caused a decrease in a key antioxidant (glutathione) whose concentration inhibits the synthesis of eumelanin.

Factors affecting colour vision in fallow deer (*Dama dama*): colour and tone discrimination

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Key words: fallow deer, *Dama dama*, vision, colour, tone.

The visual performance of eight captive fallow deer was assessed throughout a four-test-battery (colour discrimination, colour inversion, tone discrimination and tone inversion) in which cylindrical plastic buckets of different colours (black, grey, blue and red) were used as a chromatic stimulus. Two different coloured buckets (both fattened with food) were placed in the study platform and it was determined which bucket was assisted first and how long the animals paid attention to each one in the four phases of each test, granting them a maximum time of 300 seconds per phase and a resting time of 60 seconds among phases. Simultaneously, records were taken for two sets of independent variables: six climatological variables and five variables related to the hierarchical structure of the herd. The aim of the present study was to quantify the effect and power that these factors have on the visual discrimination of colour and tone in fallow deer. Within each factor, those conditions related to better and worse visual performance were identified in order to define the influence of the studied factors. The results indicate that these effects vary between 2.1% of the hierarchical status within the herd on the colour discrimination and preference with predominance of the red channel ($P < 0.05$) and 60.2% of the humidity (%) over the time that the animals pay attention to the red colour ($P < 0.001$). Fallow deer vision is dichromatic, with cones of maximum sensitivity to green and blue light. However, when it comes to adequate, natural brightness conditions, fallow deer could be able to discriminate other tones of longer wavelength (red). Males and females showed practically negligible differences in terms of colour discrimination. Although the individuals in lower hierarchical status were significantly superior in their preference for the different colour channels (decreasing preference for green, blue and red, respectively), the animals in the upper scales showed a greater capacity for discrimination and interaction with different tones. This tendency was kept, in an increasing way, taking the growing of the horns into account, being the most dominant animals those that were able to discriminate red tones.

Production and composition of hind's milk as a function of body condition

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Key words: deer, milk production, milk composition, body condition, lactation.

Using data of milked hinds at the UCLM farm since 1998, we have calculated milk production as well as average fat, protein, lactose, energy, total solids, casein B (since 2012) and urea (since 2011) composition throughout the lactation period. Hinds were maintained free within enclosures with artificial meadow that contributed to their feeding. They were milked (after being separated from offspring for 6 hours) at 2, 4, 6, 10, 14 and 18 weeks after parturition. Since 2006, body condition was determined at mating, prepartum, postpartum and end of lactation (week 18 after parturition) by lumbar palpation (scale 1-5). Hind milk production was influenced by body condition ($P < 0.001$), with a determination coefficient at postpartum of $R^2 = 0.84$. The highest production during lactation was 288.73 ± 10.74 l for a body condition of 4.25, decreasing to 187.81 ± 16.42 l for a body condition of 2 or 2.25. Milk composition was also influenced by postpartum body condition; thus, fat ($P < 0.001$), total solids ($P < 0.001$), and energy ($P < 0.01$) showed positive correlations with body condition, while this correlation was negative for proteins ($P < 0.05$), casein B ($P < 0.05$) and urea ($P < 0.01$). Variations in lactose were not significant. In conclusion, body condition is a useful method to evaluate the nutritional status of hinds because, according to these results, it influences milk production and composition, and consequently the growth of fawns, which ultimately affects the subsequent productive capacity and trophies.

Brominated organic pollutants (PBDEs) in wild boars from Extremadura: influence of sex and geographic location

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Key words: PBDEs, fat, wild boar, pollution.

Polybrominated diphenyl ethers (PBDEs) are bioaccumulative, persistent organic pollutants that store mostly in fat tissues. As observed experimentally, they can cause several toxic effects like endocrine disruption and cancer. However, their occurrence in game species like the wild boar has not been studied. The aim of this work was to determine the presence and levels of nine BDE congeners in perirenal fat tissue of 60 hunted boars from two areas in Extremadura. Lipid extraction was achieved with petroleum ether, followed by purification with concentrated sulphuric acid. The extract was analyzed by gas chromatography coupled to mass spectrometry (GC-MS) in SIM-NCI mode. Considering all samples, we detected BDE congeners 28, 47, 99, 100, 153, 154, 155 and 183, but not BDE 209. The most frequent congeners (present in 90% of the samples) were BDE 47 (maximum 24.83 ng/g tissue) and BDE 99 (maximum 18.91 ng/g tissue). The maximum concentration detected corresponded to a measure of BDE 153 (92,6 ng/g tissue), although this congeners occurred in 80% of the samples. The mean concentration of Σ PBDEs was 6.612 ± 16.110 ng/g tissue. Significant differences between zones were detected for BDE congeners 47, 100, 99, 154 and 153, with higher concentrations in Sierra de San Pedro (n=29) compared to Ibores-Villuercas (n= 31) in all cases. However, the frequency of PBDE occurrence in wild boars from Sierra de San Pedro was lower than in Ibores-Villuercas. Sex had no influence in PBDE accumulation. The occurrence of PBDEs in all samples, the scarcity of studies focused on large game species, and the lack of maximum residue levels established for human food safety highlight the relevance of the present study, which is, to the best of our knowledge, the first one of this type in Spain.

The regulation on lead ammunition adopted in Europe and evidence of compliance

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Key words: lead poisoning, contamination, game meat, bullet, shot pellet.

The transition to non-lead ammunition has been enforced by regulations on use and possession of lead shot and rifle bullets. Here we review the scientific and technical literature about this regulatory process in Europe and give some notes of its effectiveness to reduce this source of lead contamination in aquatic and terrestrial environments. Presently, lead shot use has been legally restricted in 23 European countries. Two, Denmark and The Netherlands, have a total ban of lead gunshot use in all types of habitats, 16 countries have a total ban in wetlands and/or for waterbird hunting, and five have a partial ban implemented only in some wetlands. The legal regulation of lead bullets is limited to some German regions. This review also highlights the need to know the level of compliance with the ban on lead ammunition and the subsequent benefits for the susceptible species and for game meat safety.

Effects of pesticides on wildlife: The conservation of the Iberian hare in agrosystems from La Mancha

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Key words: agrosystem, herbicide, *Lepus granatensis*, pesticide.

The gradual change from a traditional towards an intensive agriculture that has taken place in recent centuries has resulted in an important concentration of farmlands and the increasing release of pesticides and chemical fertilizers into the environment. A growing number of studies are linking this agricultural intensification with the loss of plant and animal diversity. In the case of pesticides, although their mode of action is often known in the target species, their secondary or sublethal effects on wildlife are still unknown, particularly at the highest levels of biological organization: populations, communities and ecosystems. We face, therefore, the need to causally link knowledge about the molecular actions of pesticides to their possible interference with biological processes, in order to be able to develop reliable predictions about the consequences of pesticide use. Among the most consumed pesticides, both nationally and regionally, glyphosate, 2,4-D and MCPA stand out. Therefore, this project aims to study the effects of these widely used herbicides on wildlife populations inhabiting agricultural areas, in order to conduct a risk assessment of pesticide exposure and to evaluate the effect that these products may have on the population dynamics of wild species. With this purpose, we chose the Iberian hare (*Lepus granatensis*) as model species because i) it is strongly linked to agrosystems, ii) it is herbivorous, and therefore susceptible to direct exposure of herbicides by ingestion of treated plants, iii) it has a high ecological importance due to their role as prey species of great interest for conservation, and iv) it has a high socio-economic relevance because it is a game species.

Experimental analysis of the impact of seeds treated with triazole fungicides on the red-legged partridge

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Key words: treated seeds, estrogenic disruptors, fungicides, triazoles, *Alectoris rufa*.

The agricultural intensification that has occurred since the mid-20th century and the use of agrochemicals are among the main threats for wildlife associated with farmlands. The treatment of seeds with plant protection products is currently a widespread practice. In particular, triazole fungicides are the most widely used pesticides to treat rainfed cereal seeds, which results in a high exposure risk for granivorous species, such as red-legged partridge (*Alectoris rufa*), which can feed on seeds that remain on field surface after sowing. Triazole fungicides can act as estrogenic disruptors altering the synthesis of steroid hormones and therefore reducing the reproductive output of partridges, which in the long run can cause population declines. This work aims to determine the mechanism of action of these fungicides on partridges by analyzing changes in expression of genes involved in the synthesis of sterols and in steroid hormone levels in exposed animals. With this purpose, we exposed adult partridges to seeds treated with four different plant protection products containing triazoles as active ingredients (flutriafol, prothioconazol, tebuconazole, and a mixture of the latter two). We also tracked reproduction, analyzing clutch sizes, fertilization rates and hatching rates. In addition, we aim at validating non-invasive methods for exposure characterisation and biomarkers of effect from the analysis of feces in order to monitor the impact of these fungicides on wild populations.

Climate change and risks for mountain species. Mosquito vectors and circulation of West Nile virus and avian malaria in territories of Bearded vultures (*Gypaetus barbatus*)

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Key words: climate change, avian malaria, mosquitoes, bearded vulture, West Nile virus.

Among the most feared effects of global warming is the geographic expansion of the hematophagous insect vectors and the pathogens they can transmit. Species living isolated on islands or at high altitudes, like the bearded vulture, are particularly vulnerable to these processes, as their isolation implies also less contact with pathogens and as a consequence less development of specific defenses. Recently, cases of West Nile fever and avian malaria have been observed in bearded vultures kept in captivity, showing their susceptibility to both diseases. However, exposure and incidence of these pathogens in free-living bearded vultures are still unknown. Between May and August 2018 we captured mosquitoes in seven bearded vulture territories in the Pyrenees and Pre-Pyrenees within the Aragon region. Sampling took place during the final part of the nesting period of the bearded vultures, using CDC light traps and Bgsentinel traps, baited with CO₂ situated below the cliff of nesting site. Captures revealed the presence of sand flies and *Culex* gender mosquitoes during this period even in territories located at altitudes over 2000m, but especially and in higher abundance in territories situated at lower altitudes in the Pre-Pyrenees. In one of these territories we also detected carriage of *Plasmodium (P.) relictum* and *P. vaughani* in *Culex* gender mosquitoes and particularly in *Culex pipiens* species. These results suggest the potential exposure of bearded vulture nestlings to *P.relictum*, the main causal agent of avian malaria at least in the territories situated in Guara mountains, in the Aragonese Pre-Pyrenees.