



UNIVERSIDADE DE ÉVORA
MESTRADO EM BIOLOGIA DA CONSERVAÇÃO
DISSERTAÇÃO

Caracterização da diversidade parasitária em carnívoros silvestres e implicações na Conservação

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Orientador: Professora Dr^a. Ludovina Neto Padre

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Évora



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ABSTRACT

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The aim of this study was to collect data in order to update the information related to the parasitic diversity of the predominant mesocarnivores in the southern regions of Portugal and to establish relationships between the parasite dispersal, conservation and human health. Road killed foxes (*Vulpes vulpes*), mongoose (*Herpestes ichneumon*), stone marten (*Martes foina*), common genets (*Genetta genetta*) and euroasian badgers (*Meles meles*) were the considered carnivore-hosts. A sample of hunted foxes was also regarded. All the animals had geo-reference information, ultimately used for spatial analysis. Thorough necropsies were performed and macroparasites collected, identified and preserved. For the first time in Portugal *Ancylostoma martinezi*, a common genets parasite, is recorded.

Statistical species richness and correlation between human and environmental factors were determined. Human population density, water drainage, soil use, minimum distance to head council city's and the infection status of foxes proved no significant statistical relation. Results obtained on zoonotic parasites present in wild animals enhance the necessity of multidisciplinary work between Biology conservation and medical sciences.

Key words: Conservation Biology/Medicine, Iberian MesoCarnivores, Parasitology, Epidemiology, Zoonosis

RESUMO

[**Caracterização da diversidade parasitária em carnívoros silvestres e implicações na Conservação**]

Recolher dados para actualizar os conhecimentos da diversidade parasitária dos mesocarnívoros mais abundantes nas regiões do sul de Portugal e relacionar a dispersão parasitária, a conservação e a saúde humana constituíram os principais objectivos deste estudo. Raposas (*Vulpes vulpes*), sacarrabos (*Herpestes ichneumon*), fuinhas (*Martes foina*), ginetas (*Genetta genetta*) e texugos (*Meles meles*) atropelados foram os carnívoros-hospedeiros em estudo. Um grupo de raposas caçadas foi também considerado parte da amostra. A informação geo-referenciada de todos os animais serviu para executar a análise espacial. Realizaram-se necrópsias meticulosas e procedeu-se à recolha, identificação e preservação dos parasitas encontrados. Pela primeira vez em Portugal é registada a presença do parasita da gineta *Ancylostoma martinezi*.

A correlação entre os factores humanos e ambientais e, a riqueza de espécies foi determinada estatisticamente. Densidade populacional, disponibilidade de água, tipo de uso de solo e distância mínima às sedes de concelho não apresentaram uma relação estatisticamente significativa com a infecção parasitária das raposas. Os resultados obtidos relativamente à presença de parasitas zoonóticos nos animais silvestres em estudo fomentam o trabalho multidisciplinar entre a Biologia da Conservação e as ciências médicas.

Palavras-Chave: Biologia/Medicina da Conservação, Mesocarnívoros Ibéricos, Parasitologia, Epidemiologia, Zoonoses

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INTRODUÇÃO AO PROJECTO

O presente trabalho foi desenvolvido no âmbito do projecto de investigação conducente à tese de Mestrado em Biologia da Conservação e resultou da interdisciplinaridade entre a Biologia e a Medicina Veterinária. Esta ambivalência temática traduz a interligação dos ciclos silváticos, rurais e urbanos e a importância dos indicadores de saúde/sanidade animal, das zoonoses e da Conservação das Espécies (Daszac, 2000).

O estudo relativo às doenças de interface entre animais silvestres/domésticos/Homem tem merecido pouca atenção no nosso país. O conhecimento das condições higio-sanitárias em que se encontram as populações de carnívoros silvestres e as consequências na sua conservação, atendendo ao facto de contactarem com o meio natural cada vez mais humanizado, possibilitaram ao presente estudo um esclarecimento de dados básicos (que parasitas, onde e quando) e a avaliação de suposições instigadas na informação anteriormente disponível.

As espécies em estudo pertencem todas à Classe *Mammalia*, Ordem *Carnívora*, Subordem *Fissipeda*, constando de quatro Famílias distintas – *Carnívora*, *Mustelidae*, *Viverridae* e *Herpestidae* [Correia, 1997, Naturlink site].

Após a identificação dos parasitas externos [Filo *Arthropoda*, Classes *Arachnida* e *Insecta*] e parasitas internos [Filos *Nematoda*, *Platyhelminthes* e *Acantocephala*] encontrados seguiu-se um estudo da bio-ecologia dos hospedeiros como forma de compreensão da menor ou maior contaminação de cada espécie.

O objectivo maior deste estudo é o de contribuir com mais informações que permitam fundamentar programas de monitorização e epidemiovigilância, defendidos como passos primordiais na determinação adequada sobre o estado higiosanitário das populações silvestres [Acha, 2005; Boma, 1996]. E, conseqüentemente estabelecer medidas ajustadas no controle de epizootias e preservação dos equilíbrios naturais [OIE, 2004; Osofsky, 2000; *Europe heath & Who site*]

Segundo Chivian (2002) o período em que actualmente vivemos reflecte uma grande preocupação relativamente às doenças (re)emergentes, doenças zoonóticas, contacto aumentado entre diferentes espécies, agentes, regiões e a danosa acção do Homem. Assim importa conhecer a realidade existente, esclarecer os dados que se assumem em equilíbrio e compreender as implicações globais da alteração desses equilíbrios. Mudanças ambientais, práticas agrícolas, demografia humana, evolução tecnológica, adaptação de agentes patogénicos e a quebra das medidas de saúde pública são alguns dos factores que incorrem na expansão das doenças emergentes e globais [Faust, 1975; OIE, 2004].

O aumento de contacto entre espécies que anteriormente não ocorriam fomenta um acréscimo de infecções cruzadas e potencialmente mais nocivas [Osofsky, 2000]. Os mais afectados são, sem dúvida, aqueles que apresentam menor capacidade de resposta. Os que

coevoluíram e se adaptaram a agentes parasitários próprios, adquiriram um equilíbrio de convivência milenar. O exemplo dos animais silvestres é paradigmático pois por norma apresentam faunas parasitárias muito específicas e portanto têm também maior susceptibilidade e consequências mais gravosas quando confrontados com novos agentes de doença [Gibbs, 2002].

Outra atitude multidisciplinar foi a de usar a geo-referenciação (localização espacial de cada indivíduo da amostra). A utilização dos Sistemas de Informação Geográfica na saúde em Portugal é ainda muito pouco usual' (Pina, 2006) porém o seu inestimável contributo foi reconhecido pela possibilidade de integração de dados anteriormente menos apreciados. Segundo a mesma autora e, tal como já sucedia noutros ramos das ciências Biológicas e Ecológicas 'a utilização de técnicas de análise espacial, seja através dos Sistemas de Informação Geográfica (SIG) seja através da estatística espacial ou de uma combinação das duas, tem despertado muito interesse entre os epidemiologistas e outros profissionais de saúde uma vez que permite ter uma visão abrangente da saúde, dos indivíduos no contexto socio-cultural-ambiental em que estão inseridos'.

À semelhança do que Briones (2007) defende relativamente ao grande exemplo de cooperação realizada entre biólogos, meteorologistas, veterinários, ecólogos, médicos (entre outros) aquando dos preparativos de intervenção da Influenza aviária, outros autores partilham o conceito de integração multidisciplinar de saberes nas diversas áreas da Biologia, Medicina e afins.

Patz *et al* (2004) reforçam este conceito sublinhando que mais de 75% das doenças do Homem são zoonóticas e portanto intrinsecamente ligadas com a saúde de todos os animais, tanto domésticos como silvestres. A chave para a sanidade do futuro é trabalhar sob a égide do conceito 'one health – uma saúde', resultado da contribuição de todos. A Conservação, em última análise, é conquistada pelo equilíbrio entre todos os seres vivos e o meio que os rodeia.

Este documento é a compilação do trabalho que tem vindo a ser realizado desde Setembro de 2007 e apresenta-se em formato artigo, em língua Inglesa. A Introdução reflecte o estado actual dos conhecimentos nesta matéria e os objectivos que nortearam este projecto. Na parte de Material e métodos descrevem-se sucintamente as acções realizadas para a sua consecução e nos Resultados descrevem-se os dados obtidos e estatisticamente calculados. Na Discussão relacionam-se os dados obtidos com os resultados anteriormente descritos por outros autores. Nas Considerações finais (final considerations) manifestam-se as limitações deste estudo e sugestões de desenvolvimento do tema.

INTRODUCTION

Studies relating human factors, effective parasite richness and ecological determinants have revealed interesting results, in many different environments. However in Portugal parasitology studies, in mammals have been related singularly to species richness or presenting parasite infection prevalences. This project tried to assemble different Biological Sciences perspectives in order to provide a baseline database of the parasitic loads of the predominant five mesocarnivores in the southern part of the country – Alentejo and Algarve regions.

An intense bibliography search proved the double perspective of wildlife conservation – on one hand there is the danger of human influence to disturb natural imbalance another vision is that wild carnivores are possible reservoirs and spreaders of zoonotic agents and diseases [Cerbo, 2008; OIE, 2004]. For this matter [Gortázar, 2007] defends that 'Surveillance and descriptive studies are still valuable in regions, species or diseases that have received less attention or are (at least apparently) emerging'.

Like Briones (2007) stated that 'barriers between animal and human diseases are fictitious'. This reality demands a better understanding of the ecological role of host-parasite relations and acknowledge the disturbance on host population dynamics is imperative, in order to eventually prevent disastrous effects as Lanfranchi (2003) argues. The same author instigates awareness and recalls us that wildlife disease managements and veterinary public health must have cooperative measures. And Deem (2001) expresses that 'Veterinarians perform health surveys or assessments and long-term health monitoring that provide critically needed baseline information on species of interest'.

Also Lafferty (2002) justifies an intersection between conservation biology and epidemiology, stating that this bondage can be very fruitful for one can monitor disease 'prevailing behaviour' and help conservation biologists to better understand and minimize disease prevalence and transmission risks. Since Conservation Medicine is still a 'new school' we considered that this project could be a contribution to its materialization. 'Conservation biology and veterinary and human medicine are in the process of joining forces under a common denominator: health, as broadly considered in an ecological context. This coalition has great potential to unite several important but to this point largely separate fields and to ignite a powerful new global awareness: that conservation of biodiversity and of healthy, functional ecosystems is necessary to health of individuals and populations, human and otherwise' [Meffe, 1999].

Deem (2001) declares that infectious and non-infectious diseases are being recognized by conservation biologists as an increasing challenge to the conservation of wildlife. This knowledge and having host mammals available permitted the execution of a parasitology study. Castro (2005) defends that parasites are important determinants of ecological dynamics. Pérez (2006) reinforces

this concept alerting that parasites being organisms with a particular way of life, form an important part of biodiversity; not only regarding the number of known species, but also because of their relevant role within ecological processes.

Still on this matter Valkiūnas (2001) emphasized that even though parasitic diseases have a major role in global health, during the current marked rise of the number of human beings and domestic animals, ecological literature has given it insufficient attention. Attending to the conservation of carnivores Torres (2006) citing Funk *et al* (2001) recalls that the effects of pathogens and diseases are of major concerns and the same author points out that information about parasitic infection is still scarce.

The motion to research the environmental variables and human interference conditions was brought up by many authors. Eira (2006) citing Graczik *in* Conservation Medicine (2002) explains that the association of Humans and Wildlife has been intensified by the increasing urbanization, landscape fragmentation and the relationship between free-ranging/farmed game species and hunting, among other factors. Also Eira (2006) considers that the capability of some wildlife species to act as reservoirs for pathogens with potential risk of zoonotic infections should be kept in mind in order to establish wildlife monitoring guidelines and provide better wildlife management actions. Most of the references in Conservation Biology books, like Primack (2002) consider conservation threats as the results of human activity, e.g. the habitat destruction, habitat fragmentation, habitat degradation (including pollution), the global climate change, the overexploitation of species for human use, the introduction of invasive species, and the increased spread of diseases. The same author explains that these anthropogenic actions threaten species and biological communities by itself, increasing disease transmission, facilitating the spread of disease carrying vectors, with negative impact to human environment and, increasing the interaction with humans, were wild populations of animals acquire diseases from nearby human populations and domestic animals.

It is important to point out that natural cycles are double way vectors, so diseases can spread from domestic animals into wild populations and *vice-versa*. Primack (2002) continues to explain that indirect effects of habitat destruction can increase an organism's susceptibility to disease, by setting a host population into a smaller, crowded area (because of habitat destruction) the habitat quality and food availability will be deteriorated, leading to lowered nutritional status, weaker animals, and less resistance to infection. And, the author states that young, very old, and pregnant individuals are particularly susceptible to disease in such conditions. All this information and also considering Daszak (2001) arguments of a long recognition of wildlife populations to be a link in the chain of pathogen emergence, taking into account the globalization we are living urged the necessity to better understand the role of human actions in wild animals communities.

The main purpose of this project is to establish the influence of human factors (population density, human agglomeration and encroachment) in mesocarnivores parasitic fauna and the magnitude of

wildlife/domestic animals and humans contacts. The resulting information will increase our awareness of natural boundaries and equilibrium to all parts.

To evaluate the sanitary conditions of wild carnivores in the southern part of Portugal, in Alentejo and Algarve regions, in 26 administrative councils, helminths and ectoparasitic fauna were determined in 48 samples of foxes, 6 badgers, 20 common genets, 7 mongoose and 13 stone marten (most commonly found and road-killed carnivores).

These data discarded partial results obtained in other 8 foxes, 3 common genets, 4 mongoose and 3 stone marten (sometimes when unfrozen animals revealed an advanced stage of decomposition, exuberant internal fracture or both). Even though necropsy was performed some organs couldn't be isolated nor the results were considered reliable.

MATERIAL & METHODS

Iberian Mesocarnivores selection and Necropsy procedures

Most wild animals were provided from the MOVE project (collection of killed animals on the roads nearby Évora, mostly EN4 and EN114) and the Euroscut (A22 - Algarve) of specimens recollection. Foxes were also collected from hunting areas (Alentejo – traditional farms that hunt foxes from October to February as part of regional predator control policies).

A thorough necropsy was performed to 94 animals 90 of which had geo-reference details - 47 *Vulpes vulpes* (foxes), 19 *Genetta genetta* (common genets), 11 *Martes foina* (stone marten), 6 *Meles meles* (badgers) and 7 *Herpestes ichneumon* (mongoose). The carnivores regarded in this study are considered not to be in a conservation risk.

Data on body weight, total length, posterior limb and anterior measures were taken. Abnormal or suspect tissues (visual-criteria) were preserved in 10% formol solution. Samples of Muscle tissue (DNA study) were collected and preserved both in alcohol and frozen vials. 'Pool' of organs (spleen, liver, heart) and muscles (tongue and diaphragm) were separately preserved for future micro-parasite analyses.

Sampling of intra-cardiac blood was only possible for a few specimens for future research on haematologic parasites. Likewise stomach content preservation was performed for possible diet studies.

Bones of the anterior and posterior limbs were also tagged (complete identification) and frozen for posterior use in the 'Ostheo' Library (Osteoteca) and further study related to their singular-species composition and structure. Helminthological analysis was methodically performed and confirmed under a magnifier lens. After counted (whenever possible for some parasites were too damaged) helminths were preserved in 70°alcohol vials and for better classification samples were mounted in

Lactofenol d'Amann. Some *Cestoda* were stained with carmin-alcohol-acetic and long-term fixed with the Canadean balsam resin technique.

Foxes of the Iberian Peninsula are considered to be a sub-species, *Vulpes vulpes silacea*, Miller, 1907 (because of different morphological feature). This carnivore prefers heterogeneous habitats for it eats almost everything, they are considered as generalists (diet wise speaking), with an unusual capacity to adapt to every environment [Correia, 1991; Mendonça, 1982].

Genets – *Genetta genetta* prefer forests and dense bush to inhabit, mostly eating rodents and birds, however small reptiles, amphibian and insects can also be included in their diet. Genets and mongoose (*Herpestes ichneumon*) are considered to be naturalized mammals in the Iberian Peninsula, since they are originally from the African continent and have been introduced over 10 century's ago [Correia, 1991; Alvarez, 1990].

Mongoose are also (in similarity to the foxes) very eclectic mammals, dietary wise specking. About habitat preferences these animals are rather found in dense forested , bushy areas even though proximity to human buildings as often been reported. This carnivore is the only one considered to be a day hunter, in opposition to all the other ones resumed in this study [Correia, 1991].

Meles meles, the euroasian badger is the heaviest mammal considered in this study. Being omnivorous eats practically everything, from berry's to worms, including small murids and reptiles. Forest close by pastures make the most desired environment for badger's home range. Although it has been considered the most gregarious *Mustelidea*, in Portugal it is not common to watch social interaction as it is in Great Britain [Correia, 1991; Torres, 2001; Milan, 2004].

Stone marten (*Martes foina*) is also a mustelid and the fact of its small body size is no reason to downsize it fears hunting skills [Correia, 1991; Ribas, 2004].

Species Richness Estimators and Statistical Analysis

Species estimations richness (i.e., the actual number of species present in a given area) are considered as a basic objective of many field studies carried out in community ecology and also of crucial concern when dealing with the conservation and management of biodiversity, according to Bouliner (1998).

Species Richness estimates was performed through EstimateS 8.0 software (EstimateSWin 800, available from R.K. Colwell at www.viceroy.eeb.uconn.edu/estimates), in a similar methodology applied in the 'Endoparasite species richness of Iberian carnivores' [Torres, 2006] were the same program was set to compute parameters with 500 randomized runs (in order to minimize biases of results of such a small sample size). Nichols (1997) recalls that few sampling programs for animal communities provide censuses, and usually some species in surveyed areas are not detected; so, counts of species detected underestimate the number of species present. Accepting this information

and having this sample we used these estimations as a valuable instrument to perceive the real variety observed.

We only considered Chao 2 (a non-parametric incidence-based estimator) and Jackknife 1 (first-order incidence-based estimator) estimations since they have been recommended for this purpose. Estimations were computed with the Classic Formula for Chao 1 & Chao 2 (recommended for small samples, considered as a non-parametric estimator of species richness), for each species (host) the estimation resulted from a 500 run calculation and for only the exceptions of *Herpestes ichneumon* (N=7) and *Meles meles* (N=6) the coverage estimators set for 10 (even though shared species were not considered).

Geo -Spatial analysis and traditional medical statistical measures (Prevalence)

The frequencies of parasitic infection, for each host of different mammal species, has a Medical importance for it can provide information on prevailing parasitism and host responsiveness. This measure was obtained by the simple calculation of the number of cases in the total individual possibilities.

The Spatial analysis was performed by using QGIS and GRASS interface tools. And the spatial variables chosen reflect what Valkiūnas (2001) described as the various ecological problems related to the rise of the world population, such as environmental pollution, disappearance of rare species, shortage of fresh water and habitat destruction.

QGIS was the program used to obtain spatial, environmental and related data for every host individual [QGIS, 2009]. The map layers were obtained from the Agência Nacional do Ambiente (<http://www.iambiente.pt/atlas/est/index.jsp>, 2007) - type of soil use, administrative regions (Concelhos) and their city councils shape-files (Sede_Concelhos) in the Lisbon Grid – gauss system. Also the maps of water drainage also named as useful rain (and regarded as an indicator of water availability) results from the difference of total precipitation and real evapo-transpiration were obtained in this digital environmental atlas.

To the administrative regions we added one more feature to the attribute table - population density (using INE – census 2001 data).

To the type of soil use we used a simplified classification, specifying 4 classes: 1 for every type of agriculture use, 2 for all types of forestry soil use, 3 for a combination forest and agriculture soils and 4 for all the other types (social complexes, salt banks, etc.) by the GRASS comand [v.reclass.attr].

The vectors corresponding to every single host location on a use of soil basis was obtained by the GRASS comand [v.what.vect].

For each host (N=90) the minimum distance to a urban location (head council city) was calculated using the GRASS comand [v.distance].

Then, with the GPS identification for each host (UTM WGS 84, 29N) it was possible to calculate,

through the QGIS/GRASS tools all the characteristics (population density, water drainage, use of soil and minimum distance to a head city council) attributed to each host death location. The Grass [vector update by other maps] command introduced the selected feature in the previously added columns [v.db.addcol].

Statistic significance of data recollected was possible by using SPSS 16.0 [Maroco, 2007].

The initial attribute table considering all the features for each host was not completely analysed since only foxes (*Vulpes vulpes*) had a considerable statistical sample size (N=47). All the other host species had restrained sample size. And for the characteristics of 'truly' accidental sample, non-parametric data, statistic inference was not recommended.

RESULTS

The total number of host-individuals has been considered (94 host individuals) only to present the possibilities of parasitic combination within each host species but the absence of spatial reference made its statistical assessment non-viable, at the end 90 host individuals were statistically validated. The expected parasitic fauna was corroborated by the descriptions found in [Unive site, 2009 & Campillo, 1994]. The Parasitology manuals (Anderson, 1982; Bowman, 1995; Neveu-Lemaire, 1936; Soulsby, 1982; UE & Vila-Viçosa, 2005) permitted the identification of parasites retrieved in this research. The following tables summarizes host characteristics, organized by gender, age group and region of recollection (Table I) and Table II summarizes the observed parasite species richness without naming the different parasite classes.

	Gender		Age group		Region	
	Male	Female	Juvenile	Adult	Alentejo	Algarve
<i>Vulpes vulpes</i> N=48 // Necto=19	36	12	10	38	40	8
<i>Genetta genetta</i> N=20	18	2	3	17	15	5
<i>Martes foina</i> N=13	7	6	2	11	13	-
<i>Herpestes ichneumon</i> N=7	5	2	2	5	7	-
<i>Meles meles</i> N=6	3	3	-	6	4	2

Table I – Host group characteristics - total number of individuals of the intra-species samples divided by Gender, Age group and recollection region.

	Endoparasite species richness							Ectoparasite species richness					
	0	1	2	3	4	5	6	0	1	2	3	4	5
<i>Vulpes vulpes</i> N=48 // Necto=19	3	23	15	4	2	0	1	2	3	8	4	1	1
<i>Genetta genetta</i> N=20	3	7	3	7	-	-	-	11	5	3	1	-	-
<i>Martes foina</i> N=13	6	5	2	-	-	-	-	9	2	2	-	-	-
<i>Herpestes ichneumon</i> N=7	-	6	1	-	-	-	-	2	3	-	2	-	-
<i>Meles meles</i> N=6	3	2	1	-	-	-	-	3	2	-	-	1	-

Table II- Species richness of the total number of individuals (statistically viable)

The next figures present spatial integration of the several variables (layers) and allow visual understanding of host distribution. Note the fact that several foxes are represented in the same point since they were labeled with the same spatial position in the hunting area (equal farm at the same day).

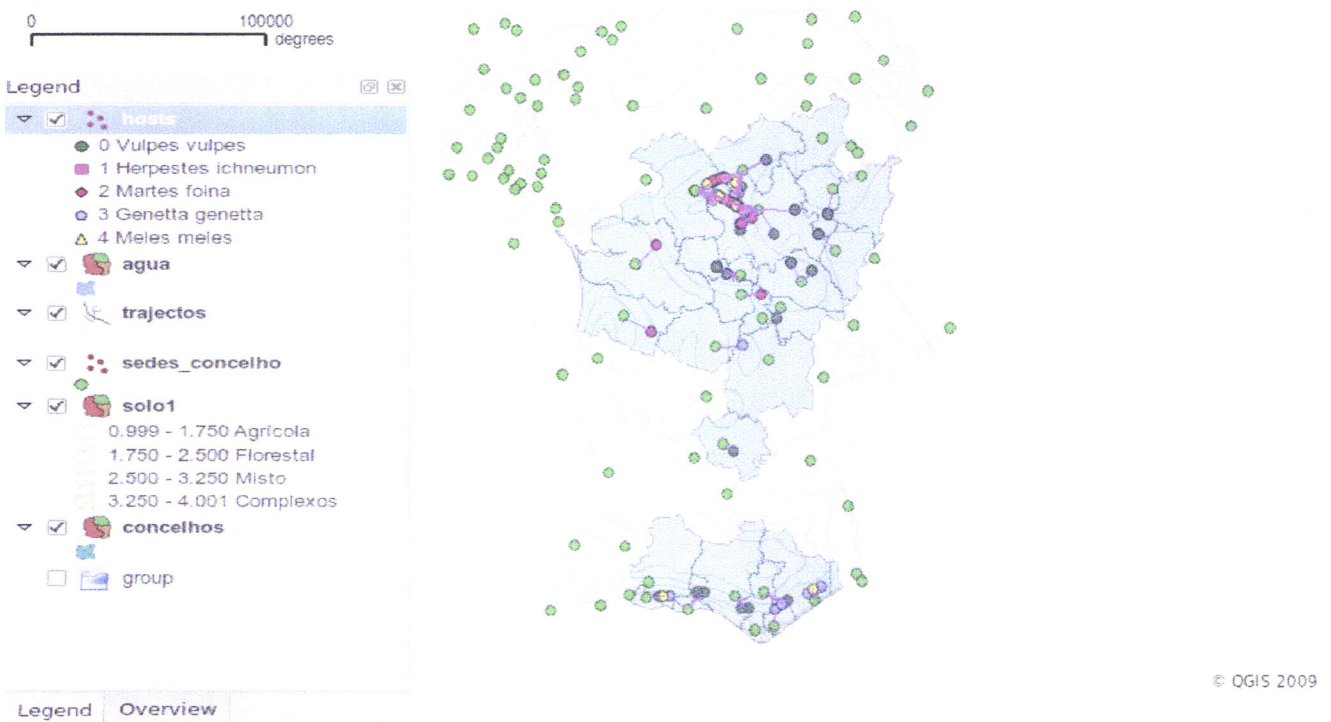


Figure 1 – Host location, water drainage, councils, city's and minimum distance map.

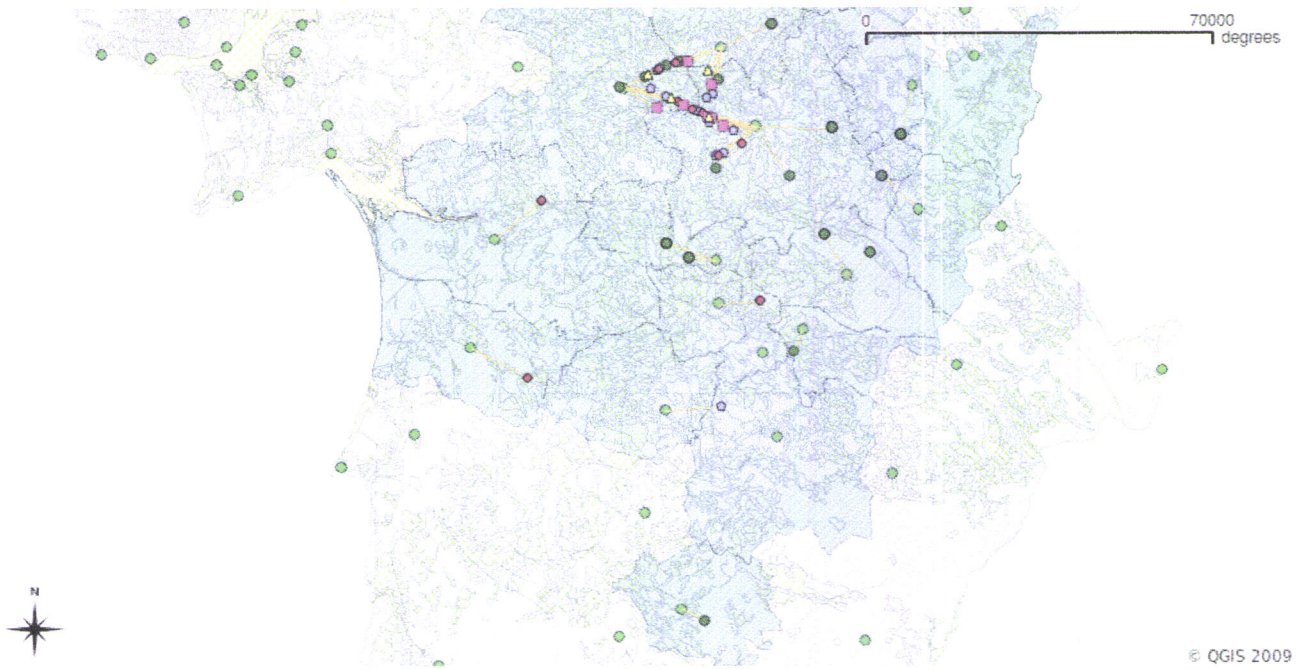


Figure 2 – Alentejo region detail map (common legend with Figure 1).

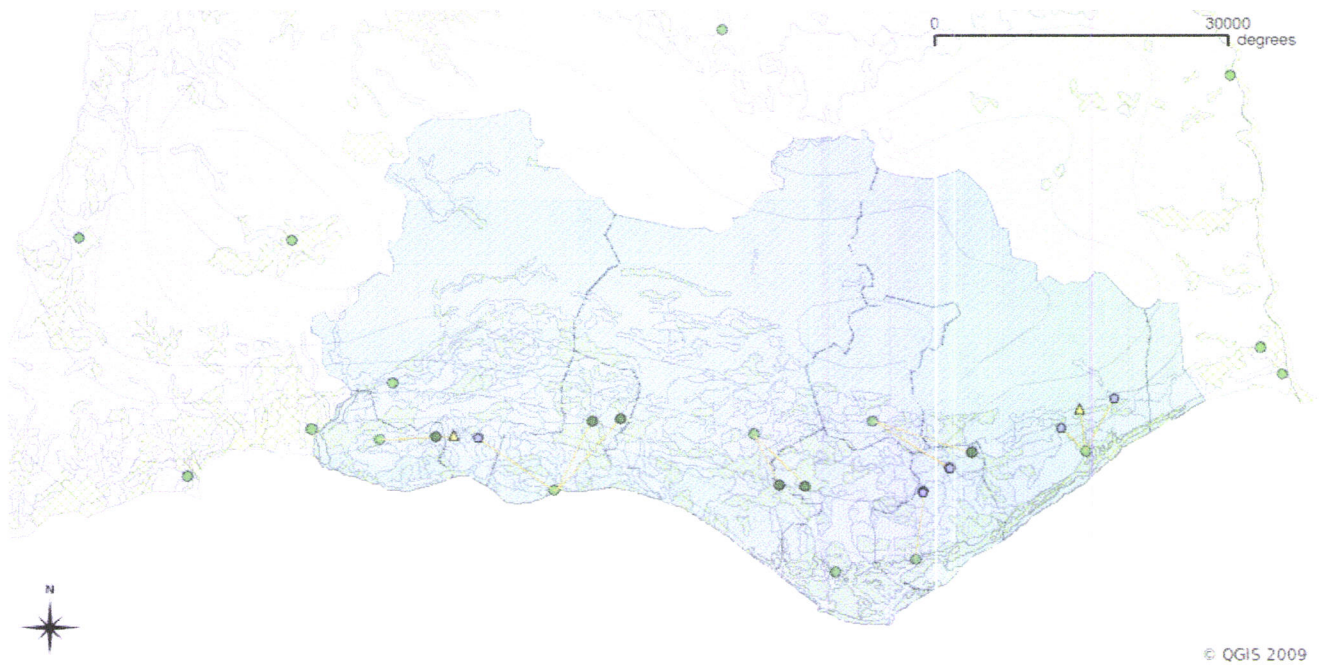


Figure 3 – Algarve region detail map (common legend with Figure 1).

Table III summarizes the number of parasites found and expected. The difference in foxes accounted in the ecto parasite search must be taken in account (only 19 of the 47 statistically treated). Parasite species identification and prevalence, actual presence, for every host groups are presented in Table IV (annexe).

	Individuals in the sample	Total parasite Species / Genus / Family							Estimate of Endoparasite species richness (Chao 2)		Estimate of Endoparasite species richness (Jackknife 1)	
		Geographic reference	Nematoda	Cestoda	Trematoda	Acarico phala	Isotia	Siphonspora	Mitophaga	Mean	SD analytical	Mean
Família Canidae												
<i>Vulpes vulpes</i> Linnaeus, 1758	47	8	4	2	1	1	3	2	20.89	4.83	22.85	3.42
Família Viverridae												
<i>Genetta genetta</i> Linnaeus, 1758	19	5	5	1	-	2	1	2	23.5	17.13	15.73	1.86
Família Mustelidae												
<i>Martes foina</i> Erdleben, 1777	11	2	3	-	-	2	-	2	3.5	1.32	3.90	0.90
<i>Meles meles</i> Linnaeus, 1758	6	3	-	-	-	2	1	2	5.0	3.74	4.66	1.05
Família Herpestidae												
<i>Herpestes ichneumon</i> Linnaeus, 1758	7	2	-	-	-	2	-	2	2.0	0.24	2.0	0

Table III – Observed and estimated species richness. Taxon specification of the parasites found.

Note - There were found *Oxiuridea* (accidental parasites) both in *Vulpes vulpes* and in *Martes foina*. The total number of 30 Helminth species and 12 different ecto-parasites were identified (attending to morphological characteristics). Internal parasites were retrieved from gastro-intestinal gut, air-way organs and heart. From these 30 Helminth species 2 of them were not considered in the forward statistical analysis since they were considered as accidental parasites (resulting from the prey ingestion). It is likely to be from the intake of micromammals (*Muridea*) [Carvalho, 2001], for it has been reported the presence of *Oxiuridea* in these prey species, as equally recognized by Ribas (2004).

Genetta genetta individuals presented us, for the first time reported in Portugal, the *Ancylostoma martinezi* (4 in 19 individuals), a species first described by [Miquel, J., Torres, J., Casanova, J.C. and Feliu, C. (1996)] in Spanish territory. *Toxocara genettae* was present in 2 of the 19 host genets

(confirmed through the morphological data and description available in Alvarez, 1990).

Sample results point out that 85% of common genets had internal parasites and 35% of the them showed to have a simple parasitic infection (one parasite species). From the 19 genets in study three (15%) had 2 internal parasite species and 35% contained an association of three parasite species (*Cestoda* class parasite species were present in all multiple infection cases). Almost half (46%) of the Genets had ectoparasites. A total of eleven (11) different internal parasite species was the observed species richness in common genets sample. The Chao 2 statistical estimations inferred a possible presence of 23,5 endoparasite species with a standard deviation of more than 17 species. In this case Jackknife first-order calculations were very different setting as a mean result an estimation of 15,73 endoparasite species richness, with an 1,86 possible deviation.

Foxes (*Vulpes vulpes*) had the most parasitic variety and possible associations of all the mammals in study, 17 of the 19 foxes (89%) with fur had ectoparasites.

Foxes proved to be highly infected as for 96% (47 foxes) had internal parasites; 48% of these had a simple parasitic infection (one helminth in its organism); 31% revealed a double association of parasite species and 8% (4 foxes) a combination of three parasite species. A total number of 2 foxes had a multiple infection of four different internal parasite species and only a young fox had a maximum parasitism association of 6 species [two of them where actually pulmonary and it was the only host sample with this parasitic combination (*Capillaria aerophila* and *Crenosoma vulpis*). From the entire sample of 48 foxes *Dirofilaria immitis* (pulmonary and vascular parasite) was found only once. *Uncinaria stenocephala* was the most common parasite found (in 42 foxes – 88%) and *Toxocara canis* was present in seven host samples.

Statistical estimations of possible endoparasite richness set a mean infection possibility of 22,89 different species, with a standard deviation of more than 4 species (Chao 2 estimations). First order Jackknife estimations resulted in similar prospectives although the standard deviation resulted in a minor discrepancy ($x = 22,85$; $SD = 3,42$). A total of fifteen (14, not considering *Oxiuridea*, accidental parasite) endoparasite species was the observed species richness in this sample.

Martes foina (stone marten) host samples presented a total of 54% of parasitic infection (7 of 13). And only four individuals had ectoparasitic infestation. The most common parasite found in stone marten was *Spirura ryctipleurites* (4 in 13) and three hosts had parasites from the *Cestoda* class in the intestine. One host revealed the presence of *Oxiuridea*, a parasite not considered to normally live in these mammals, considered to have been ingested with some of its prey (mouse are host recipients of *Oxiuridea*).

Parasite species observed in stone marten produced a total of four different species (internal parasites, disregarding the accidental parasite *Oxiuridea*). Chao's estimations for a total species richness in this *Mustelidae* host-species resulted in a mean 3,5 species combination. Standard deviation calculated to a maximum of 1,32. Jackknife first-order calculations estimated a total of 3,90 species richness and a standard deviation of 0,9 species.

Euroasian badgers (*Meles meles*) sample of six animals revealed a 50% of internal parasitic infection (three host individuals). One badger presented an association of two parasitic species (three species were found in the current research). One badger was infected with only one species (*Spirura ryctipleurites*). Three *Nematoda* parasite species were observed in the badgers sample. Estimations foresaw a five (5) different species possibility of incidence. Standard deviation was set for 3,74 species in Chao 2 calculation. Jackknife's first order estimations also considered mean species richness of 4,66 and a standard deviation of more or less 1,05 species.

Mongoose (*Herpestes ichneumon*) revealed high parasitic infection possibility since six from the seven host individuals revealed internal parasites; five mongoose were infected with *Filaroides martis*, a pulmonary parasite and, three host-individuals had stomach parasites (*Spirura ryctipleurites*). Five mongoose had external parasites and from these two individuals had the maximum of three ectoparasite association.

Two *Nematoda* class internal parasites were observed in the Mongoose sample and, both Chao2 and Jackknife 1 estimations set the mean species richness of 2 possible species for these host mammals (considering the set of conditions). Jackknife considered no standard deviation possible and Chao 2 estimated a more or less 0,24 error in species richness.

The Statistical inference was first performed through an Correlate Bivariate Analysis, considering Species Richness (only for foxes parametric sample), Y (latitude), tipo-uso (soil-use), dens_pop (population density), esco (water drainage) and distance to the nearest human agglomeration as the variables for a Pearson Correlation (PC).

Correlations

		Notes
Output Created		2009-06-04T12:52:26.449
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	90
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
Syntax		<pre> CORRELATIONS /VARIABLES=Species_rich_endo YN110 Toxoc_canN110 tipo_usoN110 dens_popN206 escoN110 dist_sedeN206 /PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE. </pre>

Figure 4 – Correlation data (Source – SPSS, version 16)

Species richness was considered not to be statistically correlated (Correlation significance at 0.05 level) to any environmental variable ($p > 0.05$). Note: Pictures from the SPSS calculations report.

After these primary results we performed a Binary Logistic Regression, by the Forward Conditional method, considering *Toxocara canis* (only presente in foxes sample) as the dependent variable and the categorical independent variable the tipo-uso (Soil-use). The results showed no statistical significance between to the proposed parameter ($p > 0.05$).

Classification Table^a

Observed		Predicted		
		Toxoc. canN110		Percentage Correct
		0	1	
Step 1	Toxoc. canN110	0	1	
		40	0	100.0
		7	0	.0
	Overall Percentage			85.1

a. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1	tipo_usoN110			4.242	2	.120	
	tipo_usoN110(1)	.154	1.222	.016	1	.900	1.167
	tipo_usoN110(2)	1.946	.976	3.976	1	.046	7.000
	Constant	-2.234	.607	13.518	1	.000	.107

Figure 5 – Logistic regression input data (Source – SPSS, version 16)

DISCUSSION

Wild animals killed in human linear structures and hunting processes have been studied for a long time and projects like this are no absolute innovation (e.g.: Eira, 2006; Jordi, 2006). This project used all the available data, from each location, each host species, inside and out. Everything preservable was kept in order to start a baseline database.

In Morand (2000) we can acknowledge that positive relationship between mean parasite abundance and host occupancy (ecological prevalence) is found suggesting that local abundance is linked to spatial distribution across species. The initial concepts that we would find differences between animals killed nearby agriculture fields (the constant revolting of the soil permits little parasite egg/larvae survival) and forestry grounds, as being less handled (serving as reservoir environments for parasites and consequently source of infection to wild animals) were not corroborated by statistical calculation. The Logistic Binary Regression, used to prove make prof of this concept, resulted in no statistical significance regarding *Toxocara canis* presence/absence in different soil-use tips. This parasite preferential transmission mode is by egg ingestion (e.g.: children playing out side and inadvertently taking the hands to their mouths, mothers foxes liking their pups); although resistant to long periods in the ground they do not bare high dessication exposure. Parasitologist experts as Euzéby (1963) and Soulsby (1982) remind us that humidity, temperature and soil conditions interfere greatly with all parasite viability. The fact we narrowed the soil-use types (into 4 categories) makes it impossible to infer that there is absolutely no relation amongst the environmental factor and this parasite. Water availability was another factor little variable in this part of the country (only if we had samples from another bioclimatic region, e.g.: Northwest part of the country could we, possibly, obtain different results as what concerns to parasite diversity and prevalence).

As for host species we could not find all the possible parasites inventoried by many other authors (both in Portugal, Iberian Peninsula or other european countrys) [Eira, 2006; Gortázar, 1998; Manfredi, 2003; Martínez-Carrasco, 2007; Mendonça, 1982; Reperant, 2007; Vervaeck, 2005 & Vila-Viçosa, 1998]. Even the prevalences of parasitic infection were different, as for example: results of parasitic loads and specificity for foxes (*Vulpes vulpes*) although similar with other studies had higher prevalence of *Uncinaria stenocephala*, 88% instead of the 77.42% stated by [Eira, 2006] and lower prevalence of *Toxocara canis* infection, 15% instead of a prevalence of 37.10% demonstrated by the same author (also in Portugal).

Why is the fox such a big "Public transportation for parasites"? the answer lies in the information given us by Lindenfors (2007) that animal with a larger home range size can come into contact with

more parasites, leading to increased parasite species richness and increasing percentage of animals in the diet. The conscience that animals acquire parasites from the environment, including through faecal contamination of water and food sources, exposure to vectors and consumption of paratenic, transport and intermediate hosts recorded by Lindenfors (2007) are aggravated with Meffe's (1999) description of displaced animal populations caused by habitat fragmentation or alteration can provide new niches for pathogens or can carry their pathogens to new areas and new species. The same author remembers that habitat fragmentation can adversely affect populations for all the reasons documented by conservation biologists as well as from increased susceptibility to introduced diseases. This knowledge explains the differences found in similar parasitology studies and novelties found when least expected; also Barbosa (2005) assumes these environmental factors as predictors of foxes parasitic infections.

Foxes are definitely the recognized primary reservoirs of helminthic zoonosis. *Toxocara canis*, *Dirofilaria immitis*, *Uncinaria stenocephala*., *Cappilaria aerophila*, and the ectoparasites as vectors for other pathogens are few examples of parasites shared by foxes and domestic dogs [Gortázar, 1998 & Geocities, 2009]. The same author recalls the possibility of nematodes larvae, including *Toxocara canis* that can infect humans. This matter should be taken in consideration when control policies of zoonotic helminth infections, always keeping in mind the role of the red fox and other wild animals in their ecology.

Daszak (2000) points out that zoonotic diseases find in the *continuum* wildlife-domestic animals-human populations a perfect way to maintain a host-parasite cycle. Since earlier times we have conscience that nothing in this world is isolated so, for every action there is a reaction. But not having a previous contact with a pathogenic agent can be disastrous for the newly infected have no recognition of out to manage with the parasite [Osofsky, 2000 & OIE, 2004]. So, the close contact between wild animals, our pets and the overlap of human and wild animals increments the possibility of cross infection. Schantz (1991) remembers us that potential exposure to zoonotic disease agents of pet animals is greater than ever before. Among these diseases, *larva migrans* due to the common roundworms of dogs and cats, *Toxocara spp.* is probably the most common. Considered a Neglected Parasitic Disease by the World Health Organization (WHO) *Toxocara* infection is known to cause neurological deficits or behaviour disorders in children who may not show obvious signs of infection so this possible transmission must be prevented.

Ancylostomatidea (hookworms) as *Ancylostoma caninum*, *A. genettae*, *martinezi* and *Uncinaria stenocephala* are also serious parasite zoonotic agent since they often parasitize dogs and cats. These maintenance host are capable of producing larvae that invade another mammal through cutaneous invasion and in humans (because the parasite considers the human body an accident

and is not capable to continue its cycle) larvae move erratic under the skin *larva migrans* syndromes (WHO, 2006). Shantz (1991) explains that cutaneous *larva migrans*, characterized by progressive linear eruptive lesions caused by the prolonged migration of the larvae in the skin, is the most common manifestation of zoonotic hookworm infection. Still concerning foxes parasites *Trematodes* as *Alaria alata* are also recognized by their potential to infect humans [Eira, 2006, Merck, 2005 & WHO, 2006]. But not only foxes are zoonosis reservoirs, common genets also have *Ancylostomatidae* (*Ancylostoma martinezi*) and *Ascaridea* (*Toxocara sp.*) parasites that have potential to infect humans; even though there was hasn't been any case reported; the Euroasian badgers (*Meles meles*) are considered to be parasite reservoirs in wildlife and are specially 'blamed' to be Tuberculosis maintenance hosts and spreaders [OIE, 2004]. And mongoose have *Filaroides martis* that in the same way as *Dirofilaria immitis* (earlier cited) are passed to humans through a vector bite (e.g.: Mosquitoes) [Faust, 1975]. *Capillaria spp.* is another example of parasites found on every continent, occurring in dogs, cats and a wide range of wild mammals [Wikipedia, 2009]. The prevalence of *Capillaria aerophila* infection in wild animals is very high although evidence of human infection is very incipient. In the present study *Capillaria aerophila* has a Prevalence of 8% (4 of 48 foxes) and *Capillaria putorii* (badgers) was present in 1 of the six individuals necrosis. *Capillaria putorii* maintains transmission (infection cycle) by intimate contact and oral-nasal discharges remains in social 'dens' [Torres, 2001, Rosalino, 2008 & Milan, 2004].

It is important to keep in mind that many parasites maintain their infectious cycle by using host social preferences and their *modus vivendi* very expertly, as Lindenford (2007) predicts parasites that require close contact among hosts for transmission will be more numerous in species that exhibit a greater level of social contact, while the same is not expected for parasite species that are less dependent on transmission by close contact.

Also ectoparasitic loads and associations are explained by host social contact and use of the same habitat locations [Ribas, 2004]. Refojos (2006) defends that ectoparasite are broad indicators of an animal's sanitary status and may be of important in disease transmission processes. This authors results showed that the frequency of apparition of ticks in genets was minimal in all individuals but two, which were both females; bod had cubs, as deduced by their swollen breasts. Refojos explained the fact that both genets and minks living habits, by changing resting dens frequently, may serve as an explanation for their minimal ectoparasite infestation but bio-sanitary implications of these shared parasite species imposes a need of research since they can act as a vector of illness between wild mammal species.

In our present study 54% of common genets and 69% of stone marten (mink family) had no external (ecto) parasite infestation facts that are very similar to the ones presented by other authors (given similar habitat conditions).

Species richness statistical estimations of stone marten were the most similar to the truly observed results. Chao 2 estimated 3.5 (+/- 1.32), Jackknife 1 estimated 3.9 (+/- 0.9) and 4 species were observed (considering *Oxiuridea* not being this mammals parasite). All the other host carnivores had a higher species richness estimation.

Common genets had a high expected species richness and only for this host species (even though it was not the smallest sample size nor the calculations were less than 500 runs) the Chao 2 standard deviation was too high. Statistically speaking there is a lack of confidence in the Chao 2 estimations.

Jackknife's first-order calculations were very different and with a smaller standard deviation. In fact we can consider that from the expected 15,73 (+/- 1.86) we have found 11 endoparasite species.

These first results made us consider Jackknife's calculations as having less discrepancy. The species recorded are similar to those described by other authors [Miquel, 1995, Alvarez, 1990 & Campillo, 1994]. For *Meles meles* (euroasian badgers) 4.66 (+/- 1.05) were the species richness estimations for badgers infections; we have only recorded three different species. The most recent study upon badgers was published by Rosalino (2008) and describes 17 different helminths. In fact the sample of six badgers is very small, not considered representative so we only present the data without any inference.

Estimations and actual results on endoparasite species richness of mongoose had no difference. Also in this case the host-carnivore sample was very small and that made us to choose for a mean calculation of 500 runs (in order to reduce biased results). Mongoose are eclectic eaters and one could think they would present a great variety of parasites (according to all the statements of habitat occupancy, hunted prey and close contact to other mammals) but surprisingly mongoose proved to give parasites no 'room' for occupancy. Their recent colonization of the Iberian peninsula and the probably high parasite specificity makes them less infected. Ecto parasite infestation, on the other hand, is very different (71%) had external parasites.

Foxes estimations from Chao 2 and Jackknife 1 were also different but also in this case we have considered Jackknife's first-order (smaller standard deviation). So, from the 22.85 (+/- 3.42) species possibly encountered in foxes 14 were actually observed in our study. There are more *helminth* species described by [Eira, 2006] in a recent study performed here in Portugal making us believe that different habitat, prey and the previous preservation of the mammals (frozen immediately after being hunted) might be some of the reasons of such a result discrepancy.

Human population density was assumed to be related to differences in species richness as smaller distances to human agglomerations were also considered responsible to species richness differences. Nor the Pearson Correlation (bivariate analyses) nor Binary Logistic Regression supported this hypothesis, with the sample in study. This conceptual data is pointed out by many authors [Aguirre, 2002; Guégan, 2007, Patz *et al* 2004 & Primack, 2002] by inputting responsibility to

habitat fragmentation that alters host species composition and subsequently microorganisms environmental composition; this forces a lack of preferential prey, overpopulation and overlap of rival predator species provide good opportunities for rapid disease expansion and parasitic transmission. Wildlife is considered as 'zoonotic pools' as they serve as reservoirs for many different pathogens, that will be shared between humans, domestic animals and other wild species whenever the chance poses (e.g. environmental disturbance, anthropogenic pressure, stressful co-habitation) as [Patz, 2004, Daszak, 2001 & Gortazar, 2007] remind us. Our results are not sufficient to make proof of the influence of human intervention in parasite fauna of the studied Iberian mesocarnivores.

The concept that parasite can endanger wild animals already facing tremendous human pressure are not consistent for authors like Castro (2005) acknowledge that this perception of species threatened with extinction is not possible. These authors refer that the simplest deterministic models of parasite dynamics (i.e., of specialist parasites with density-dependent transmission) predict that parasites will always go extinct before their hosts.

All the facts pose new challenges to Conservation Biology for human encroachment is getting more and more pronounced and contact between humans, domestic and wild animals is becoming more constant. Deem (2001) invokes that diseases play a factor limiting role in species survival that can be traced to anthropogenic changes on a global scale that have direct and indirect influences on the health of wildlife species. These changes included human population growth, habitat fragmentation and degradation, the isolation of populations of species, and an increased proximity of humans (and their domestic animals) to wildlife. Once more experts from different scientific areas must be stimulated to work together in order to minimize ecological disruption and maximize natural benefits of respectful co-habitation, as [Gortázar, 2007, Osofsky, 2000 & Aguirre, 2005] required for wildlife disease control measures, proposing an integration of veterinary, ecology and wildlife management expertise.

One can feel some relief because only a small percentage of these zoonotic parasites have a considerable expression on human health. Better hygiene trends gained in the past century, and the human action to make environments hostile by constantly interfere with nature (soil-use) and the deworming actions seasonally applied to our domesticated animals make a difference when it comes to the possibility of parasite transmission. The only animals truly at risk are those who have to live in the gaps of human actions (wild animals).

The more we know about our mammals, parasites, their host-parasite relationship and the influences/impacts they suffer caused by the disturbance of the environment they inhabit are the keys for future preservation and maintenance of life as we recognize.

FINAL CONSIDERATIONS

'As a science, conservation biology has been concerned with biodiversity. Science and medicine have been compartmentalized into a number of equally important categories over the past 100 years. This has allowed for the in-depth examination and subsequent understanding of widespread human and environmental questions' [Spear, 2000].

Deems (2001) and Daszak (2001) advert to the complications resulting from disease routes linking wildlife, humans and domestic animals, and makes proposals of enhanced conservation attitudes that must be better considered in order to minimize potential devastating interactions. Many authors as Chivian (2001), Gortázar (2007) and Guégan (2007) appeal to a better understanding of human health, and ultimately life, for species health and global ecosystems integrity relays on wildlife ecologists, veterinarians and public health professionals awareness.

We hope this embryonic project encourages initiatives of cooperative, multidisciplinary team work. Damaged (road killed) and frozen animals pose great difficulties to the procedures, mostly because frozen process doesn't immediately stop normal autolysis and compromises the preservation of both carnivore and containing parasites. Although there was no explanation found for this fact we had to re-check all the *Cestoda* hook measures; after mounted in Lactofenol d'Amann for at least one week measurements gave different results.

To capture statistical representative samples was purely accidental since we only processed those animals that were killed 'accidentally'. Even though these animals are an endless source of information worthwhile using.

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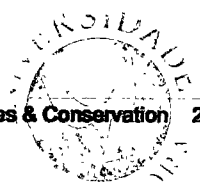
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ANNEXE

Observation	Vulpes vulpes N - 48		Herpestes ichneumon N - 7		Martes foina N - 13		Genetta genetta N - 20		Meles meles N - 6	
	Prevalence	Individuals	Prevalence	Individuals	Prevalence	Individuals	Prevalence	Individuals	Prevalence	Individuals
<i>Nematoda</i>										
<i>Dirofilaria immitis</i> (Lelyst, 1856)	2%	1								
<i>Capillaria aerophilis</i> (Crepin, 1835)	8%	4								
<i>Capillaria putorii</i> (Zeder, 1800)									17%	1
<i>Ceratomyxus vulpis</i> (Rudolphi, 1819)	2%	1								
<i>Filaroides martis</i> (Werner, 1782)			71%	5						
<i>Uncinaria stenocephala</i> (Railliet, 1884)	68%	42								
<i>Ancylostoma marinorum</i> (Miquel, Torres, Cassanova et Fellu, 1894)								20%	4	
<i>Toxocara canis</i> (Werner, 1782)	18%	7								
<i>Torocara genettae</i> (Werner, 1872)							10%	2		
<i>Molineus petersi</i> (Dujardin, 1848)					8%	1				
<i>Spicara rydzikiewitzi</i> (Desingchamps, 1824)	2%	1	43%	3	31%	4	8%	1	17%	1
<i>Physioptera sibirica</i> (Petrov et Gorbunov, 1831)										
<i>Pterygodermatites</i> sp.	4%	2							33%	2
<i>Spiruridea</i> (Carley, 1849)							8%	1		
<i>Acanthidae</i> (Railliet et al, 1912 gen)							8%	1		
<i>Oxyuridea</i> [occidental parasite]	4%	2								
<i>Trichuris vulpis</i> (Frölich, 1788)	2%	1								
<i>Acanthocephale</i> sp.	2%	1								
<i>Cestoda</i>										
<i>Mesocostoides lineatus</i> (Goetze, 1762)	18%	9								
<i>Mesocostoides</i> sp.	2%	1								
<i>Dilepididae</i> (Fuhman, 1807)							16%	3		

<i>Diploplidium nolletii</i> (Sjögabin, 1924)											20%	4			
<i>Diploplidium acanthoteira</i> (Parona, 1888)											6%	1			
<i>Taenia</i> sp.											30%	6			
<i>Taenia taeniformis</i> (Batsch, 1766)											45%	9			
<i>Taenia hidalgana</i> (Péllis, 1766)	4%	2								8%		1			
<i>Taenia pisiformis</i> (Bloch, 1780)	4%	2													
<i>Trematoda</i>															
<i>Alaria aleia</i> (Coasa, 1762)	4%	2													
<i>Ascochyta</i> sp.	2%	1													
<i>Brachylaemus</i> sp.															
Class Arachnida															
<i>Ixodes</i> sp.							14%	1			16%	2	17%	1	
<i>Dermacentor</i> sp.														17%	1
<i>Rhipicephalus</i> sp.	25%	14					43%	3			15%	2	30%	8	
<i>Otodectes cynotis</i> (Sawell, 1861)	18%	9													
<i>Trombicula</i> sp.	2%	1												17%	1
<i>Cheyleiella</i> sp.							43%	3			8%	1			
Class Insecta															
Trichodactidae															
<i>Trichodectes melis</i> (Fabricius [JC] 1805)							29%	2						17%	1
<i>Pulex irritans</i> (Linnaeus, 1766)	18%	9												33%	2
<i>Ctenocephalides canis</i> (Curtis, 1828) & <i>Ctenocephalides felis</i> (Beuchát, 1875)	8%	3												33%	2
<i>Spilopsyllus cuniculi</i> (Dale, 1878)	8%	4													

Table IV – Prevalence of parasite species detected in *Vulpes vulpes* (foxes), *Herpestes ichneumon* (mongoose), *Martes foina* (stone marten), *Genetta genetta* (common genet) and *Meles meles* (euroasian badgers) in the Alentejo and Algarve regions.

