Galemys, 16 (nº especial): 197-206, 2004
ISSN: 1137-8700

HEALTH RISKS IN GAME PRODUCTION: THE WILD BOAR[1]

ÚRSULA HÖFLE, JOAQUÍN VICENTE, ISABEL G. FERNÁNDEZ DE MERA, DIEGO VILLANÚA, PELAYO ACEVEDO, FRANCISCO RUIZ-FONS AND CHRISTIAN GORTÁZAR

Instituto de Investigación en Recursos Cinegéticos (CSIC-UCLM-JCCM). Ronda de Toledo s.n., 13005 Ciudad Real. (gortazar@irec.uclm.es)

[1] Project AGL2001-3947, Plan Nacional de I+D+I and FEDER.

ABSTRACT

The European wild boar (*Sus scrofa*) is a good model for the study of the role of diseases in vertebrate population dynamics, and especially of the impact of various management systems on the health of the species. The sustainable use of this game resource depends on scientific knowledge of the diseases affecting the species. This paper presents the aims, methods, and preliminary results of a multidisciplinary research project that will finish in 2004. To date, the research project has published information on the seroprevalence of antibodies against several viral and bacterial agents, a description of a natural outbreak of Aujeszky´s disease, information on helminth populations, and the pathology and molecular characterization of bovine tuberculosis in the European wild boar. Now, the project focuses on the integration of data obtained through the different disciplines that collaborate in the project with the objective of identifying the risk factors for the prevalence and maintenance of diseases in European wild boar.

Key words: disease, European wild boar, game production, risk factors, Spain, *Sus scrofa*.

INTRODUCTION

Currently, the European wild boar *Sus scrofa* L. is the most widely distributed wild ungulate in the Iberian Peninsula (Rosell and Herrero 2002). Its geographical range is expanding (e.g., Gortázar et al. 2000), and population densities are high, locally, especially in southern Spain (Rosell et al. 2001). Wild boars can have both positive and negative effects on vegetation and wildlife (Focardi et al. 2000; Heinken et al. 2002). Wild boars can cause losses to agriculture and road accidents, but they are a popular and highly sought game species. Additionally, wild boars share some of their parasites and infectious diseases with domestic pigs, and some of them are also shared with other wild and domestic animals, and humans (MacKenzie 1999, Serraino et al. 1999). Given the ubiquity and socio-economic importance of the

species, wild boar is not only a good model to study the role of diseases in vertebrate population dynamics, it also provides an interesting example of conflict among differing factions; in this case, the conflicting interests of pig-breeders, health authorities, hunters, game producers, and conservationists.

We understand that the sustainable use of this game resource depends on the scientific knowledge of the diseases affecting the species; therefore, in 2002, we started a multidisciplinary research project to study the potential health risks associated with the increasingly intensive management of wild boars in hunting areas of Spain. Our main goals are (1) to describe the epidemiology of the most prominent diseases affecting wild boar populations in Spain, emphasizing new or emerging diseases, such as tuberculosis, that are relevant to public health, domestic livestock breeding, game production, and conservation, (2) to study the role of disease in wild boar population dynamics, (3) to identify the main sanitary risks in current game management systems and to promote game production practices that are more compatible with animal health and meat quality, and (4) to reconcile game production with the sustainable use of natural resources and animal welfare (Coon et al. 2002).

METHODS

To address the goals of this project, we are using a multidisciplinary approach that combines contributions from the fields of pathology, genetics, ecology, GIS, public health, and animal production.

Sampling sites

We collected samples from 1,299 hunter-harvested wild boars in the regions of Andalucía, Aragón, Asturias, Castilla – León, Castilla – La Mancha, and Extremadura (Figure 1). In September 2002, relative abundance indexes of wild boar populations were calculated for most sampling sites by counting droppings and rootings on 4-km fixed line transects. The habitat (climate, soil, vegetation) was characterized, and the limits and main reference points of the sampled hunting area (e.g., waterholes or game feeders) were defined using a GPS. To obtain information on game management (annual harvest, artificial feeding, fencing, translocations), we interviewed the owners of the hunting estates and gamekeepers.

In a number of collaborating hunting areas, detailed necropsies were performed on animals found dead or sick and culled, and intensive surveys were used to collect information on any naturally occurring disease-related mortality.

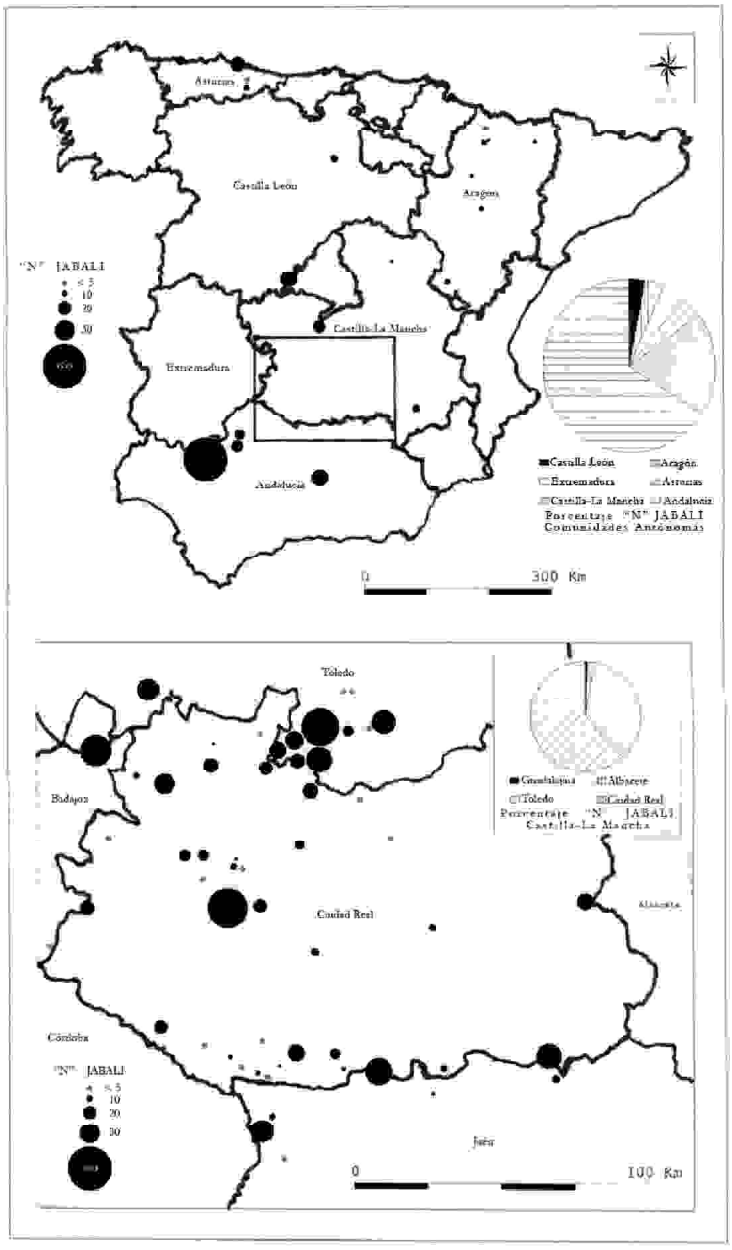

Figure 1. The location of sampling sites used in the study of wild boar in Spain. Circle diameters indicate sample size. Grey circles indicate sampling sites that had ≤5 samples. The two diagrams show the distribution of samples among autonomic communities in Spain and between provinces in the Community of Castilla – La Mancha, respectively.

Samples and measurements

We determined the sex of each animal and used tooth replacement and external appearance to assign each to one of the following age-classes: piglets (up to 6 months old), juveniles (up to 12 months old), subadults (between 12 and 24 months old), and adults (>24 months old) (Sáenz de Buruaga et al. 2001). We recorded the body mass and several morphometric measurements of each animal. Before opening the carcass, we collected a sample of faecal material from the rectum and all of the visible ectoparasites. For the separation of serum, we took blood samples from the heart. Serum was obtained using centrifugation (15 min, 900 G) and stored at -20 °C. For each sample, we calculated a kidney fat index and weighed the spleen and adrenal glands. Prior to genetic analyses (introgression and variability), muscle and liver tissue samples were stored in ethanol. To facilitate the search for necrotic foci and other lesions compatible with tuberculosis (TB), the left and right mandibular, left thoracic, mediastinic, hepatic and several mesenteric lymphnodes were sliced into 1 mm sections. Additionally, we inspected the lungs, spleen, liver, and kidneys of each sample. After macroscopic inspection, one third of the material was stored in formalin, one third was pooled and submitted for bacteriological examination, and one third was frozen for future analysis. For reproductive studies, we retained the ovaries and uterus of female boars.

Analysis of the field data and material

Data on habitat characteristics, including tree, shrub, and grass cover, plant associations and richness indexes, and the *Quercus* spp regeneration rate, were based on the analysis of 20 points located at 200-m intervals along a 4-km line transect. Wild boar faecal droppings tend to be aggregated (Rowland et al. 1984); therefore, to estimate the relative abundance of wild boars in 46 study sites, we used dropping frequency, rather than number. At one site with a known deer and wild boar density, faecal pellet counts were performed monthly from April 2002 to March 2003. Each count consisted of n=40 100-m transects divided into 10 sections, each 10 m in length. We defined dropping frequency (DF) as the average of the number of 10-m sections containing wild boar droppings ($PF=\sum Pi/n$; where ˝P˝ is the number of positive sections (0-10) and ˝n˝ is the number of 100-m transects (usually 40).

Studies of genetic variability using microsatellites are being done in collaboration with Cambridge University, and studies on introgression with domestic pig breeds are being done in collaboration with Instituto Nacional de Investigación Agraria (INIA). A study on population genetics based on mitochondrial DNA is being done by Porto University. Data on adrenal and spleen weight, kidney fat index, and reproductive performance are also being analyzed. Serum and plasma samples from

a number of live-captured wild boars are being analyzed in collaboration with the Universidad Autónoma de Barcelona.

In a preliminary serosurvey, 78 of the serum samples were tested for the presence of antibodies against *Brucella* sp, Classic swine fever virus, influenza virus O antigen, *Erysipelothrix rhusiopatiae*, *Haemophilus parasuis*, *Leptospira interrogans* serovar *pomona, Mycoplasma hyopneumoniae,* pseudorabies virus (PRV), porcine parvovirus (PPV), porcine reproductive and respiratory syndrome virus, *Salmonella* serogroups B, C and D, *Streptococcus suis* and swine influenza virus (SIV) serotypes H1N1 and H2N3 using standard techniques, such as Enzyme Linked Immunosorbent Assay (ELISA), Complement Fixation Test (CFT), Tube Agglutinatioin Test (TAT), Haemagglutination Inhibition Test (HIT), Microscopic Agglutination Test (MAT), Agargel Immunodiffusion Test (AIT) and Bengal Rose (see Vicente et al. 2002). Serum samples of 656 wild boars from 45 sampling sites were analysed for the presence of antibodies against porcine circovirus type 2 (PCV2) using an immunoperoxidase monolayer assay (Vicente et al. 2004). To test 693 of the sera for the presence of antibodies against Aujeszky's disease virus (ADV), we used a blocking ELISA technique (Vicente et al. 2005). Additional serologic analyses, such as the detection of antibodies against porcine reproductive and respiratory syndrome virus (PRRS) and *Brucella* spp., *Toxoplasma gondii* and *Neospora* spp., are in progress.

The pool of lymphnodes and any TB-compatible lesions from other organs were cultured on Coletsos medium for the isolation of mycobacteria, as described by Aduriz et al. (1995). Any growing acid-fast organisms were submitted for spacer oligonucleotide typing.

Faecal samples and a subsample of complete lungs and digestive tracts of hunter-harvested wild boars were used for helminthological analysis (Fernandez-de-Mera et al. 2003, 2004). Macroscopic lesions and selected tissues are being processed for histopathological examination.

Preliminary Results

Wild boar management

Currently, a broad range of approaches is used in the management of wild boar populations in Spain. Most of the intensive management is done in southern Spain, where hunting is of great social and economic relevance, and where the types of land-use and the use of fencing limit crop or traffic accident-related losses. Among the sampling localities used in our study, 28 are limited by high wire fences. Fenced estates are more common in southern Spain, mainly in Montes de Toledo (13 of 17

sites) and Sierra Morena (15 of 19 sites), than in northern Spain (1 of 10 sites). Another open sampling site is located in southeastern Spain (Betic Mountains). Wild boar abundance indexes differed significantly ($F_{1, 29}$= 16.76, P < 0.001, n= 30) among open hunting areas (n=7), which had a DF = 0.05 ±0.05, fenced hunting areas (n=18), which had a DF = 0.44 ±0.2, and intensively managed areas (n=5, which had a DF = 1.48 ±0.8 (Vicente et al. 2004).

Most of the fenced hunting estates offer supplementary feeding to the wild boars or feed other ungulates (e.g., red deer *Cervus elaphus*), which might benefit the wild boar. Supplementary feeding is rare in open areas, except during the hunting season, when many gamekeepers try to attract the animals with food, especially in southern Spain. The practice of translocating wild boars between fenced hunting areas is increasing, and wild boar is being imported from other European countries (e.g. Fernández-de-Mera et al. 2003). The effect of management practices on habitat structure and composition is still being studied.

Parasites and diseases

The prospective preliminary serosurvey (Vicente et al. 2002) conducted on a small number of sera showed that wild boars from southern Spain (Sierra Morena and Montes de Toledo) are in contact with viral agents, such as Aujeszky´s disease virus, porcine parvovirus, and swine influenza virus serotype H1N1. The study of porcine circovirus shows the existence of high seroprevalences and of occasional clinical cases of PMWS. Seropositive wild boars were present in all but one of the geographical regions we considered. Seroprevalence and the titre of PCV2 antibodies were closely related to the management of the wild boar populations (Vicente et al. 2004).

Aujeszky´s disease (pseudorabies), a viral infection, potentially might affect the dynamics of wild boar populations. In 2001, an outbreak of the disease in a fenced hunting estate caused the death of 14% (n=100) of the juveniles and 7.5% (n=40) of the adult boar population (Gortázar et al. 2002). Aujeszky´s disease is a threat to the success of an eradication program currently underway in domestic swine in Spain. Wild boars are a reservoir of the disease (Capua et al. 1997, MacKenzie 1999), which might affect endangered species, such as the Iberian lynx (*Lynx pardinus*) and the southern Iberian wolf (*Canis lupus signatus*) in Sierra Morena. Among the wild boars examined the prevalence of ADV antibodies was 44 ± 4%. All of the seropositive wild boars occurred in south-central Spain, except one individual from central Spain, close to the areas where other positive tests were found. The most intensively managed populations had higher prevalences than did wild boar population living in more natural environments. Seroprevalences were found to be increasing in all of the age-classes, and there was a female bias in seropositivity (Vicente et al. 2005).

The preliminary serosurvey of 78 sera showed that wild boars are in contact with bacterial agents, such as *Leptospira interrogans* serovar *pomona* (11.5%), *Erysipelothrix rhusiopatiae* (5.1%), *Salmonella* serogroup B (3.8%), and *Salmonella* serogroup C (2.6%). Currently, the epidemiology of brucellosis is being investigated. Macroscopic lesions compatible with TB are frequently found in wild boars in southern Spain. In most cases, *Mycobacterium bovis* is the ethiological agent, and it has been shown that strains carried by wild boars are shared with other wild and domestic ungulates (Aranaz et al. 1996, Parra et al. 2003, Gortázar et al 2005). The shedding of mycobacteria likely occurs in saliva because gross lesions were observed in the mandibular salivary gland and were shown to be growing and breaking into the lumen of excretory ducts (Gortázar et al. 2002). Currently, until all of the culture results are available, estimates of prevalence are based on the prevalence of macroscopic lesions. The mean prevalence of macroscopic lesions compatible with bovine tuberculosis (bTb) is closely associated with geographic region and the management of wild boar populations (Figure 2, unpublished data).

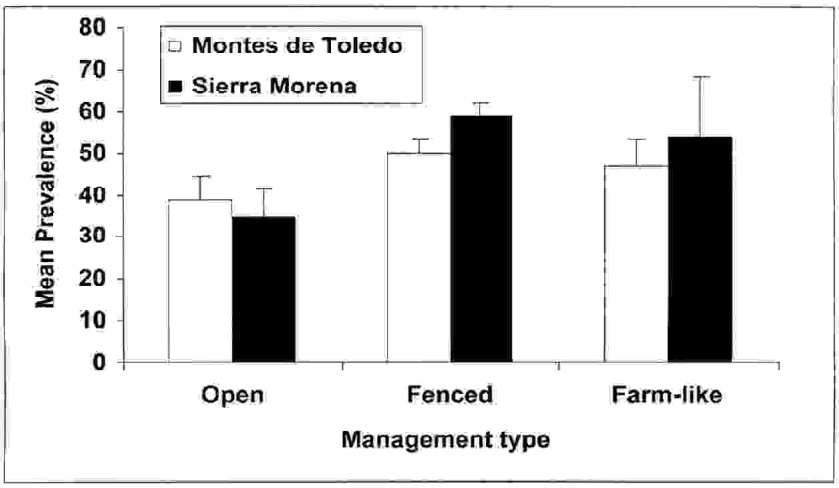

Figure 2. Mean prevalence of macroscopic lesions compatible with bovine TB in wild boars from two geographic areas in south-central Spain among different game-management systems.

The parasites detected in the wild boars of Spain include arthropods (ticks, mites, and lice) and helminths (cestode larvae, one acanthocephalan, and six nematode species). To date, the ticks identified on wild boars include *Hyalomma marginatum*, *Rhipicephalus bursa*, and *Dermacentor marginatus* (de la Fuente et al 2004). The mite *Sarcoptes scabiei* occasionally causes lesions, which are more frequently observed in dorsal areas of the head, the ears, and the back, and are mainly associated

with adult male boars from highly managed populations (unpublished data). The louse *Haematopinus* sp. was found on some of the animals examined. The acanthocephalan *Macracanthorhynchus hirundinaceus* is frequently found in wild boars from Montes de Toledo, and can occasionally cause lesions in severely parasitized individuals. The nematodes identified include *Gongylonema pulchrum*, found in tongue and oesophagus, the pulmonary nematode *Metastrongylus* sp., which showed a high prevalence in our samples, *Ascarops strongylina*, *Physocephalus sexalatus*, and *Simondsia paradoxa*, which were identified in stomach and, *Globocephalus urosubulatus*, which were present in the stomach and small and large intestine. The helminth populations in wild boars from central Spain differed significantly from those found in a group of animals imported from a game farm in France (Fernandez-de Mera et al. 2003).

DISCUSSION

The preliminary observations obtained to date allow to suggest that the high wild boar densities that are common in southern Spain constitute a risk for the transmission of diseases, such as bovine TB, Aujeszky´s disease, PCV2, *Brucella* spp., *Taxoplasma gondii* and several others that might affect human health or can interfere with the control of diseases of domestic livestock (Fernandez de Mera et al. 2003, 2004; Gortázar et al. 2002, 2003, Vicente et al. 2002, 2004, 2005). Furthermore, diseases reduce the profit of game production and even are relevant to the conservation of endangered species, such as the Iberian lynx *(Lynx pardinus)* (Vicente et al. 2005).

This research project will provide methodological improvements in the veterinary inspection of wild boar and game species in general. Additionally, the project will provide suggestions for the improvement of the management of wild boar populations, with attention paid to sanitary risks, especially concerning translocations and supplementary feeding practices. Although it is premature to draw final conclusions, a recommendation to limit the overabundance in wild boar populations is probable.

ACKNOWLEDGEMENTS

This study is supported by project AGL2001-3947, Ministerio de Ciencia y Tecnología and FEDER. This is a contribution to the agreements between Yolanda Fierro and UCLM, and between CSIC and Principado de Asturias. Joaquín Vicente had a grant from Junta de Comunidades de Castilla – La Mancha, and Fran Ruiz from Ministerio de Educación, Cultura y Deporte. We acknowledge the collaboration of Dr. W. Amos from Cambridge University, Dr. L. Silió from the INIA, Dr. P.C. Alves of Porto University, and Dr. J. Lopez from the Universidad Autónoma of Barcelona.

REFERENCES

ADURIZ, J. J., R. A. JUSTE AND N. CORTABARRIA (1995). Lack of mycobactin dependence of mycobacteria isolated on Middlebrook 7H11 from clinical cases of ovine paratuberculosis. *Veterinary Microbiology*, 45: 211-217.

ARANAZ, A., E. LIÉBANA, A. MATEOS, L. DOMÍNGUEZ, D. VIDAL, M. DOMINGO, O. GONZÁLEZ, E. F. RODRÍGUEZ, A.E. BUNSCHOTEN, J. VAN EMBDEN AND D. COUSINS (1996). Spacer oligonucleotide typing of *Mycobacterium bovis* strains from cattle and other animals: a tool for studying epidemiology of tuberculosis. *Journal of Clinical Microbiology*, 34: 2734-2740.

CAPUA, I., R. FICO, M. BANKS, M. TAMBA AND G. CALZETTA (1997). Isolation and characterisation of an Aujeszky's disease virus naturally infecting a wild boar (*Sus scrofa*). *Veterinary Microbiology*, 55: 141-146.

COON, T. G., H. CAMPA, B. F. ALEXANDRA, R. B. PEYTON, S. R. WINTERSTEIN, F. LUPI, M. SCHULZ AND J. SIKARSKIE (2002). Farming captive cervids: a review of social, economic and ecological opportunities and risks in Michigan and North America. *Transactions of the 67 North American Wildlife & Natural Resources Conference:* 251-268.

FERNANDEZ-DE MERA, I., C. GORTÁZAR, J. VICENTE, U. HÖFLE AND Y. FIERRO (2003). Wild boar helminths: risks in animal translocations. *Veterinary Parasitology*, 115, 335-341.

FERNANDEZ-DE MERA, I., C. GORTÁZAR, J. VICENTE, U. HÖFLE AND Y. FIERRO (2004). Efficacy of an in-feed preparation of ivermectin against helminths in the European wild boar. *Parasitology Research*, 92: 133-136.

FOCARDI S., D. CAPIZZI AND D. MONETTI (2000). Competition for acorns among wild boar (*Sus scrofa*) and small mammals in a Mediterranean woodland. *Journal of Zoology*, 250: 329-334.

FUENTE , J. DE LA, M. V. NARANJO, F. RUIZ-FONS, J. VICENTE, A. ESTRADA-PEÑA, C. ALMAZÁN, K. M. KOCAN, M. P. MARTÍN AND C. GORTÁZAR (2004). Prevalence of tick-borne pathogens in oxidid ticks (Acari: Ixodidae) collected from wild boar (*Sus scrofa*) and Iberian red deer (*Cervus elaphus hispanicus*) in central Spain. *European Journal of Wildlife Research*, 50: 187-196.

GORTÁZAR, C., J. HERRERO, R. VILLAFUERTE, AND J. MARCO (2000). Historical examination of the status of large mammals in Aragon, Spain. *Mammalia*, 64: 411-422.

GORTÁZAR, C., J. VICENTE, Y. FIERRO, L. LEÓN, M. J. CUBERO AND M. GONZÁLEZ (2002). Natural Aujeszky's disease in a Spanish wild boar population. *Annals of the New York Academy of Sciences*, 969: 210-212.

GORTÁZAR, C., J. VICENTE, D. GAVIER-WIDEN (2003). Pathology of bovine tuberculosis in the European wild boar. *Veterinary Record*, 152: 779-780.

GORTÁZAR, C., J. VICENTE, S. SAMPER, J. M. GARRIDO, I. G. FERNÁNDEZ DE MERA, P. GAVÍN, R. A. JUSTE, C. MARTÍN, P. ACEVEDO, M. DE LA PUENTE AND U. HOFLE (2005). Molecular characterization of *Mycobacterium tuberculosis* complex isolates from wild ungulates in south-central Spain. *Veterianry Research*, 36 (1): 43-45.

HEINKEN, T., H. HANSPACH, D. RAUDNITSCHKA AND F. SCHAUMANN (2002). Dispersal of vascular plants by four species of wild mammals in a deciduous forest in NE Germany. *Phytocoenologia*, 32: 627-643.

MACKENZIE, D. (1999). Sickly swine - Wild boar are spreading disease to Europe's pig farms. *New Scientist*, 163: 23-23.

PARRA, A., P. FERNÁNDEZ-LLARIO, A. TATO, J. LARRASA, A. GARCÍA, J. M. ALONSO, M. HERMOSO DE MENDOZA, J. HERMOSO DE MENDOZA (2003). Epidemiology of *Mycobacterium bovis* infections of pigs and wild boars using a molecular approach. *Veterinary Microbiology*, 97: 123-133.

ROSELL, C., P. FERNÁNDEZ-LLARIO AND J. HERRERO (2001). El Jabalí (*Sus scrofa* Linnaeus, 1758). *Galemys*, 13 (2): 1-21.

ROSELL, C. AND J. HERRERO (2002). *Sus scrofa*. Pp. 306-309. In: L. J. Palomo and J. Gisbert (eds.). *Atlas de los Mamíferos Terrestres de España*. SECEM – SECEMU, Madrid.

ROWLAND, M. M., C. G. WHITE AND E. M. KARLEN (1984). Use of pellet-group plots to measure trends in deer and elk populations. *Wildlife Society Bulletin*, 12:147-155.

SAÉNZ DE BURUAGA, M., A. LUCIO, AND J. F. PURROY (2001). *Reconocimiento de sexo y edad en especies cinegéticas.* Ed. Edilesa. León. 127 pp.

SERRAINO, A., G. MARCHETTI, V. SANGUINETTI, M. C. ROSSI, R. C. ZANONI, L. CATOZZI, A. BANDERA, W. DINI, W. MIGNONE, F. FRANZETTI AND A. GORI (1999). Monitoring of transmission of tuberculosis between wild boars and cattle: Genotypical analysis of strains by molecular epidemiology techniques. *Journal of Clinical Microbiology*, 37: 2766-2771.

VICENTE, J., L. LEÓN-VIZCAÍNO, C. GORTÁZAR, M. J. CUBERO, M. GONZÁLEZ AND P. MARTÍN-ATANCE (2002). Antibodies to selected viral and bacterial pathogens in wild boars from Southcentral Spain. *Journal of Wildlife Diseases*, 38: 649-652.

VICENTE, J., F. RUIZ-FONS, D. VIDAL, U. HÖFLE, P. ACEVEDO, D. VILLANÚA, I. G. FERNÁNDEZ-DE-MERA, M. P. MARTÍN AND C. GORTÁZAR (2005). Large-scale serosurvey of Aujeszky´s disease virus infection in European wild boar in Spain. *Veterinary Record,* (in press).

VICENTE, J., J. SEGALÉS, U. HÖFLE, M. BALASCH, J. PLANA-DURÁN, M. DOMINGO AND C. GORTÁZAR (2004). Epidemiological study on porcine circovirus type 2 (PCV2) infection in the European wild boar (*Sus scrofa). Veterinary Research*, 35 (in press).