Pathological and serological findings in wild boars (Sus scrofa) from Gran Sasso and Monti della Laga National Park (Central Italy)

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SUMMARY
The Italian Gran Sasso e Monti della Laga National Park represents one of the largest protected areas in Europe, and it is particularly suitable for the preservation of the wild fauna including some endangered species, such as Abruzzi chamois, Apennine wolf and Marsicano brown bear.

Wild boar population residing within the National Park Gran Sasso e Monti della Laga has been sharply increasing and negatively impacts on sensitive ecosystems and human activities. Thus, a management plan, based on wild boar capture by means of catch fences and slaughter, has been implemented. About the 77% of wild boars under study were “young adult” (aged 4-12 months, body weight = 20-60 kg), and the remaining 23% were adult (aged > 12 months).

At slaughterhouse, pathological findings have been recorded from 101 wild boars. Microscopic investigations have been carried out on diseased, as well as on apparently healthy tissues. Blood samples collected from 126 wild boars were tested for Aujeszky’s disease virus, Brucella suis and Leptospira interrogans.

Lesions were mainly caused by parasites, and parasitic bronchopneumonia by Metastrongylus spp. was most frequently observed (92%). Hepatic white spots (28%), lymphoproliferative nodules, hepatic distomatosis (3%), Cysticercus tenuicollis (15%) and hydatid cysts (6%) were also commonly detected. Serology demonstrated a high prevalence of Aujeszky’s disease (35%) and Brucella suis (15%) antibodies in the population under study.

Zoonoses represent about the 60% of emerging public health concerns, and the majority of emerging infectious diseases arise from wild animals. In that context, wild boar is of particular relevance because of its extreme adaptability in different habitats, its wide geographical distribution and its high reproductive rates.

The implementation of this management plan represented a good chance to face the wild fauna living within the Gran Sasso e Monti della Laga National Park. Pathological findings herein described, along with concurrent microbiological, serological and parasitological investigations, contribute to evaluate the health status of wild boar population residing in such a relevant protected area.

KEY WORDS
Wild boar, Central Italy, management, pathology, serology.

INTRODUCTION
Gran Sasso e Monti della Laga National Park (GSMLNP) covers an area of about 150,000 hectares throughout the territories of three regions (Abruzzo, Lazio and Marche) in Central Italy, thus representing one of the largest protected areas in Europe. The extension and the variety of environments make GSMLNP suitable for the preservation of the wild fauna, which includes endangered species such as Abruzzi chamois (Rupicapra pyrenaica ornata), Apennine wolf (Canis lupus italicus) and Marsicano brown bear (Ursos marsicanus arctos)¹.

Wild boar (Sus scrofa) population is widely distributed in Italy and has been progressively increasing and widening its distribution range, a finding which confirms the extraordinary adaptability of that animal species². Wild boar population residing within the GSMLNP has been also significantly increasing (estimated average density = 11 animals/km²) with negative impacts on sensitive ecosystems and human activities. As a consequence, a plan for the management of wild boars within the territory of GSMLNP has been implemented³. We report herein the pathological and serological findings observed in wild boars, which have been kept and slaughtered in the context of such management plan between 2008 and 2010.

MATERIALS AND METHODS
Animals
Wild boars were captured in the provinces of Teramo (Abruzzo region) and Rieti (Lazio region) by means of catch fences, identified by applying ear tags, and then delivered to two abattoirs located near Teramo and Ascoli Piceno (Marche region).
About the 77% of wild boars under study were “young adult” (aged 4-12 months, body weight = 20-60 kg), and the remaining 23% were adult (aged > 12 months). Males and females were equally distributed. Wild boars were usually slaughtered soon after their arrival at the abattoir, with the only exception of 25 animals, which were housed in pens for a few days before slaughtering.

Pathology
A total of 101 wild boars have been investigated. At post-mortem inspection, lesions affecting a wide range of organs were recorded: lungs, liver, spleen, kidneys, heart, palatine tonsils, retro-pharyngeal lymph nodes, stomach.

Pathological samples were promptly fixed in 10% neutral buffered formalin, embedded in paraffin and routinely processed for histopathology (Haematoxylin & Eosin stain, H&E). Likewise, samples from the apparently healthy tissues and organs (lungs, liver, spleen, kidneys, heart, palatine tonsils, retro-pharyngeal lymph nodes; n = 30) were also microscopically investigated.

Muscle samples were taken from the diaphragm pillars and the artificial enzymatic digestion method was carried out for the direct detection of *Trichinella* spp., as required by the European Commission Regulations n. 2075/2005 and n. 1245/2007.

Serology
Sera obtained from blood samples of 126 wild boars - including all those inspected post-mortem - were collected at slaughtering and then tested for the following infectious agents specific antibodies:

a) Aujeszky’s disease virus (ADV), using a commercially available kit (Test Elisa, IDEXX PRV/ADV gB Ab);

b) *Brucella suis* (B. suis), by means of Elisa and agglutination tests11;

c) *Leptospira interrogans*, using the immunological method (Martin & Pettit micro-agglutination technique) specific for different serovars (*L. australis bratislava*, *L. ballum ballum*, *L. canicola*, *L. grippophyphosa*, *L. icterohaemorrhagiae copenhageni*, *L. pomona pomona*, *L. sejroe hardjo*, *L. tarassovi tarassovi*).

RESULTS

Pathology
**Lungs** - Parasitic bronchopneumonia by *Metastrongylus* spp. was observed in 92 wild boars and typically affected the caudodorsal borders of both diaphragmatic lung lobes (Fig. 1). On cut section, the presence of lungworms could be easily appreciated within the terminal airways. Microscopically, parasitic bodies were seen in the bronchial and bronchiolar lumina. Alveolar emphysema, infiltration of eosinophils, granulomas and ectopic lymphoid follicles were additional consistent findings.

A lower percentage of wild boars (12%) showed areas of consolidation affecting the craniocaudal portions of both lungs (Fig. 2A). Microscopically, such lesions corresponded to areas of pulmonary parenchymal atelectasis associated to foci of granulomatous and/or eosinophilic bronchopneumonia with the presence of parasitic larvae (Fig. 2B). Hydatid cysts - the larval stage of the tapeworm *Echinococcus granulosus* - were detected in 4 wild boars, their number ranging from 1 to 5 per subject (Fig. 3).

**Liver** - “White spots”, likely due to the migration of *Ascaris suum* larvae, were reported in 28 animals, often along with ectopic lymphoid follicles (Fig. 4 A-B). *Cysticercus tenuicollis* - the larval stage of *Taenia hydatigena* - and hydatid cysts were seen, respectively, in the 15% and 6% of wild boars. Infections by *Dicrocelium dendriticum* were detected in 3 animals (Fig. 5).

**Spleen** - So-called multiple sub-serosal hernias of the splenic pulp were seen in 5 wild boars, while a single hydatid cyst was reported in one subject.

**Kidneys** - Grossly, scattered pinpoint hemorrhages were observed in a relevant percentage of wild boars (36%); microscopically, such lesions corresponded to recently occurred intra-tubular hemorrhages, most likely due to slaughter technique. Occasionally, renal infarcts were also observed (4%).

Discrete foci of tubule-interstitial nephritis were microscopically seen in all investigated animals, usually in absence of gross lesions; the inflammatory infiltrates mainly consisted of eosinophils and multinucleated giant cells, such findings suggesting a parasitic etiology. “Parasite-induced” ectopic lymphoid follicles were also occasionally detected within the renal parenchyma. Large congenital cysts were recorded in 2 subjects.
Heart - The occasional detection of rounded and hyper eosinophilic cardiomyocytes with protozoal cysts within affected cells are individualized, and diagnosed as sarcocystis, representing the only relevant finding.

Palatine tonsils and retro-pharyngeal lymph nodes - No relevant gross lesion was observed at the level of palatine tonsils. Microscopically, some tonsil crypts were dilated and filled with neutrophils, exfoliated epithelial cells

Figure 2 - Wild Boar. Lung. An entire lobe is consolidated and greyish-greenish in colour (A). Microscopically, a granulomatous lesion with a necrotic core and embedding a number of parasitic larvae is seen (B). H&E stain, final magnification = x200.

Figure 3 - Wild boar. Lung. A single, very large hydatid cyst almost entirely occupies the right diaphragmatic lobe. The simultaneous and overlying inflammatory chronic pleural involvement is likely due to compression.

Figure 4 - Wild boar. Liver. A whitish, firm, round-shaped nodule protrudes from the hepatic surface (A). Microscopically, such nodule (Kisselev’s nodule) mainly consists of lymphoid follicles and contains foci of necrosis and dystrophic calcification (B). H&E stain, final magnification = x50.
and large bacterial aggregates, such findings being considered normal in swine. Sarcocystis were frequently detected within the surrounding skeletal muscle fibers. Reactive lymphoid hyperplasia and the accumulation of exogenous, most likely foodborne pigments, were detected at level of the retro-pharyngeal lymph nodes.

**Muscle** - All wild boars under study proved to be negative for *Trichinella* spp.

**Serology**

Serological results demonstrated the presence of ADV and *B. suis* infections in GSMLNP wild boars and are summarized in Table 1.

**DISCUSSION AND CONCLUSIONS**

Humans and animals always share the same environment, and close interrelationships exist between each with other. Such concept has been exhaustively synthesized within the sentence “one world-one health-one medicine”\(^27\). In this respect, wild animals play a relevant, though often underestimated role. As a matter of fact, zoonoses represent about the 60% of emerging public health concerns, and the majority of emerging infectious diseases (> 70%) arise from wild animals\(^29\).

The study of wildlife diseases is difficult because of the frequent lack of reliable demographic data and of validated laboratory tests\(^30\). However, understanding the epidemiological role played by wild animals is of crucial relevance, mostly in the case of infectious diseases subjected to national eradication plans, affecting endangered animal species, or with zoonotic implications. In that context, wild boar is of particular relevance because of its extreme adaptability in different habitats, its wide geographical distribution and its high reproductive rates\(^30\). Furthermore, the wider extension of both urban areas and agricultural lands, the considerable growth of wild boar populations, as well as the increasing demand for wild boar meat facilitate the interactions between wild boars, on one side, and domestic animals and human beings, on the other\(^22\).

In the present study, lesions were mainly caused by parasites and parasitic bronchopneumonia by *Metastrongylus* spp. was most frequently observed. Hepatic and renal parasitic lesions were also commonly detected. "White spots" liver by *Ascaris suum* are frequent findings in wild boars, their prevalence ranging from 1,5% to 12,5%\(^2,10,16\). Hepatic ascariasis has been also reported in wild boars in Italy, with lower values of prevalence when compared with our data\(^2,16\).

*Hydatidosis/echinococcosis* is endemic in the Mediterranean basin, and still represents a major public health concern in Italy. Wild boar hydatidosis is considered quite frequent in Sardinia (estimated prevalence = 3,7%) and occasionally observed in the Italian peninsula\(^8,12\), always by G1 strain of *Echinococcus granulosus*. The present study confirm the prevalence of hydatidosis in wild boars in the Italian peninsula, but further investigations are needed to evaluate the epidemiological role of wild boars.

Our data about *Dicrocoelium dendriticum* flukes agree with those reported in wild pigs in Italy\(^4\) and suggest that suids could play a role also in the epidemiology of trematode infections.

In the present study, granulomatous lesions due to mycobacterial infections were never observed, although wild boar tuberculosis by *Mycobacterium bovis* has been repeatedly diagnosed in Italy, sometimes with prevalence value higher than 10%, in wild boars\(^5,23\) as well as in feral pigs\(^32\). The role of wild boars in the epidemiology of tuberculosis is still debated\(^33\).

Gastric ulcers are common in domestic pigs\(^14\) but not in wild boars. Reasonably, the confinement severely stressed wild boars, thus raising further questions about the appropriate methods to preserve the welfare of captive wild animals.

Serology indicates that infections by ADV and *B. suis* are widely distributed in GSMLNP wild boar population. ADV infection is endemic in many Italian regions, its seroprevalence ranging between 20-40%\(^34\). Our data fully overlap such findings and confirm the presence of ADV infection in Abruzzo, which was firstly reported about twenty years ago\(^9\). Wild boars and feral pigs should be considered potential reservoirs and source of ADV for domestic pigs, thus representing a serious challenge to the success of eradication programs\(^26\). Furthermore, wild boars could also infect endangered animal species (e.g. Apennine wolf and Marsican brown wolves) without endangering the host species.

![Figure 5](image-url) - Wild boar. Liver. A parasite fluke containing characteristic brown eggs fills a bile duct. H&E stain, final magnification = x100.
bear), which reside within the GSMLNP and are susceptible to ADV with fatal outcome\(^6\).

Evidences suggest that *B. suis* biovar 2 was introduced into Italy through the importation of hares and/or wild boars from endemically infected European countries. *B. suis* biovar 2 infection has been firstly reported in wild boars in Piedmont, with seroprevalence close to 20%\(^2\) and very similar to that observed in the present study. Remarkably, *B. suis* biovar 2 has been recently isolated in a wild boar from Abruzzo region\(^14\). *L. interrogans* infection has been repeatedly reported in wild boars in Italy, with variable and usually low values of seroprevalence\(^12\)\(^\text{,}^13\). *L. interrogans* infections are quite frequent in wild animals (e.g. mustelids) living within the GSMLNP (unpublished data). However, our negative results are not surprising and most likely due to the biology of wild boars in GSMLNP. In fact, puddles used for mud baths, which are potential sources of infection, are usually formed from rain water and exist only for short time.

The estimated prevalence of *Trichinella* spp. infection in wild boars in Italy is also low\(^7\). In particular, a single wild boar proved to be positive for *Trichinella britovi* in GSMLNP during the last six years\(^11\), such infection being quite common in red foxes and wolves (unpublished data).

In conclusion, the implementation of this “management plan” represented a good chance to “face” the wild fauna living within the GSMLNP. Pathological findings herein described, along with concurrent microbiological, serological and parasitological investigations, contribute to evaluate the health status of wild boar population residing in such a relevant protected area.

**CONFLICT OF INTEREST STATEMENT**

Authors disclose any financial and personal relationships with other people or organization that could inappropriately bias their work.

References