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TITOLO TESI

# Epidemiology, Control and Public Health aspects of parasitic diseases

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*In hac Philosophia Propositiones*
*deducuntur ex Phœnomenis et*
*redduntur generales per Inductionem*

**Isaac Newton** (1642-1727)
*Philosophiae Naturalis Principia Mathemat*

# Contents

# Abstract

The present elaborate gathers a three-year route spent on the epidemiology, control and surveillance of parasitic diseases both domestic animals and wildlife. It is divided into eight chapters, where each one, except the prelude and conclusions, concerns different projects regarding to the same topic: Epidemiology and Control of parasitic diseases. It takes into account the ancient issue, but still modern, of cystic echinococcosis (CE), one of the most costly disease to treat and prevent in terms of public health. A puzzle, inside the frame of CE, built during a three-year period. Its prevalence, in cattle, is still the same 8% compared to the one of fifty years back. This tapeworm is still present and also affects exotic animals as ring-tiled lemur (*Lemur catta*) confined in a zoo. Once more, it is stressed the marginal role played by other species, different from sheep and dog, in the *Echinococcus granulosus* (G1 strain) epidemiology. Additionally, it gives updates on the surveillance of the health status of red fox and wild boar populations, offering speculations on the plausible interactions between domestic animal and wildlife. Indeed, wild boar population shows a prevalence of the genus *Metastrongylus* of about 97%, whereas the lungworm *Crenosoma vulpis*, identified based on size and morphology, is recovered from 28.4% of the fox lungs. Helminth community structure in Apennine wolf illustrates also the attitudes to food in terms of predation and parasitic diseases transmission. The first useful data in a surveillance program is to know and count which aetiological agents are present; the experience carried out in some South African preserves, using a tele-diagnosis method in order to find out the relationship between BCS and parasites, offers interesting control strategies. Surveillance is aimed at, demostrating the presence/absence of a disease or infection, knowing its epidemiology and spreading in order to detect as early as possible exotic, emerging or re-emerging diseases as well as to cut costs for eradication and avoid exportation restrictions. In conclusion, the present thesis demonstrates the importance of intersectoral cooperation, where each stakeholder puts in the own knowledges in order to stem the spread of transmissible diseases.

# Chapter 1

## Prelude

The following PhD thesis takes into account some aspects, investigated during the three years PhD path, of epidemiology, control and public health of a number of parasitic diseases.

Before going through the specific scientific experiences, there is the need to give the definitions of epidemiology, control and public health terms, since these three terms are commonly used within the scientific world, but sometimes hard to understand due to their wide meanings.

Epidemiology is "the study of disease in populations and of factors that determine its occurrence; the key word being populations" (Thrusfield, 2007). The translation literally means, based on its Greek roots, *επί* (*epi*-) = upon, *δημο*- (*demo*-) = people, and *λογο*- (*logo*-) = discoursing, is 'the study of that which is upon the people.

The *Office International des Epizooties*' dictionary of Veterinary Epidemiology, edited in 1999, gives the following definition of surveillance: "ongoing systematic and continuous collection, analysis, and interpretation of health data (often designed to detect the appearance of specific diseases), allowing epidemiologists to follow in time and space the health status and some of the risk factors associated with diseases for a given population, for use in the planning, implementation, and evaluation of disease control measures" (Toma *et al*., 1999). The word surveillance arises from the French roots, *sur* = over and *veiller* = to watch (Brachman, 2009).

The history of the definition of public health is fairly different compared to the previous ones. The first one is dated 1920, defined as "the science and art of preventing disease, prolonging life and promoting human health through organized efforts and informed choices of society, organizations, public and private, communities and individuals" by Charles-Edward Amory Winslow (1877-1957). This concept is not unique and has changed across the years due to changes in the population's health status and the determining situations of health. This definition of public health is directly linked to the wider definition of health, in the opinion of the World Health Organisation (1948), referred to as "a state of complete physical, mental and social well-being and not merely the absence of disease". It exists another concept regarding to the veterinary public health idea. This, answering to a practical need for intervention, has been defined by Adriano Mantovani, father of the italian One World, One Health philosophy, in 1997 as "actions that the public and the public administrators expect of Veterinary Medicine in order to protect the health, the economy, the environment and man-animal environment relationships".

The three years PhD path had the main goal to pursue these three aspects of the parasitic diseases, taking in advantage of technical and practical opportunities presented at the Transmissible Diseases and Veterinary Public Health Service of the Department of Veterinary Medical Sciences of the University of Bologna that provides both research and on the land activities. The present manuscript merges the results of these activities, some of those published in peer-reviewed journals or submitted to the Editor.

Three experiences concerning a parasitic zoonosis, the Cystic Echinococcosis (CE), have been joined under the epidemiology, control and public health umbrella. CE is one of the most important zoonotic diseases in the world (Dakkak, 2010) and is currently among the five most frequently diagnosed zoonoses in the Mediterranean Basin (Sadjjadi, 2006). This seems to be the area where the zoonoses are the most numerous, in terms of variety, and widespread, maybe due to the geographical, hystorical and social features that act as the base for the two needful factors: biodiversity and the close relationship between animals and human beings. The significance of the zoonoses in the Mediterranean Basin is also stressed by the WHO that started up, in 1979, the Mediterranean Zoonoses Control Program and its Center (MZCC) established in Athens, Greece. The Programme aimed at the prevention, surveillance and control of zoonoses and related foodborne diseases. An estimted 75% of emerging human pathogens are zoonotic, transmissible from vertebrate animals to human (Graham *et al*., 2008). Indeed, according to Bidaisee and Macpherson, (2014) zoonoses should therefore be considered the single most critical risk factor to human health and well-being, with regard to infectious diseases.

The chapter two, regarding to the Ring-Tailed lemur (*Lemur catta* L., 1758), represents an occasional finding. Described in just other two papers within the scientific web (Shahar *et al*., 1995; Denk *et al*., 2016), it points out the sensitivity of this wild animal to the tapeworm, acting as a dead-end host. Chapters 4 and 5 are related to the aspects of both active and passive surveillance. The experience about the wolf's parasites was based on an active surveillance, in the context of a cooperation with teriologist colleagues and with the Institute of Parasitology of Zurich. The combined approach to this research has ensured to match a sophisticated diagnosis to an ecopathological approach. On the contrary, the survey carried out on the bovine hydatidosis adopted a passive surveillance method, using the data coming from a small abattoir in the Bologna province. The slaughterhouse is specialized in adult bovine butchering bred by the whole italian land. The right running of the National Bovine Register allowed to make a mapping of the bovine CE in our country. Furthermore, bovine, as well as Ring-Tailed lemur, shows basically to be a dead-end host due to the low number

of fertile cysts, although cattle might have a role in the persistence of this zoonosis, especially whether there are specific rearing methods and socio-cultural coexisting conditions.

In the chapters 3 and 7, it has been used the epidemiological approch in order to carry out projects both on the wild boar (*Sus scrofa* L., 1758) and on the red fox (*Vulpes vulpes* L., 1758), respectively. Nowadays, many breeders want to bring back to the outdoor domestic pig farming, so it is important to assess objectively the pulmonary parasitism also in wild boar populations, as a part of the risk assessment concept. Basically, we might summarize the risk within two main aspects, the first one is based on the drastically increased level of exposure to the intermediate host, due to the lack of the ordinary biosecurity barriers, usually used to keep out infectious diseases. Moreover, the absence of treatment protocols act to deal with neglected diseases.

The latest trend, in the field of companion animal diseases, is the one related to the respiratory parasitoses, widely sponsored by pharmaceutical industry. Assuming the risk those parasitoses recognize wild canids as *reservoir*, we tried to investigate a red fox population living on the regional land.

Finally, in the chapter 6, regarding to telediagnosis in african wildlife, it is described the using of a clinical tool, the Body Condition Score (BCS), evaluated remotely through the use of an optical instrument and later on photographed, relating it to the presence of endoparasites, which offers interesting control strategies.

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# Chapter 2

## *Echinococcus granulosus* "*sensu stricto*" in a Captive Ring-Tailed Lemur (*Lemur catta*) in Northern Italy

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Pakistan Veterinary Journal, 2016; 36(1): 121-123.

**Abstract:** Cystic echinococcosis (CE) by Echinococcosis granulosus (Eg) infection was seen in a 13 years old male lemur, found dead in a zoo in Northern Italy. Necropsy revealed several transparent cysts in the lungs and in the abdominal cavity. Freefloating cysts of varying sizes were found in the peritoneal cavity, and no protoscolex was seen microscopically. Histologically, a multifocal severe parasitic granulomatous pneumonia was observed. Confirmation of E. granulosus "sensu stricto" was reached by PCR and sequencing. In view of the absence of definitive host in the zoo, located in nonendemic region for CE, it is speculated that infection introduced through translocation of lemur from endemic region (Southern Italy zoo).

**Keywords:** Captive animals; *Echinococcus granulosus*; Lemur catta; Ring-tailed lemur; Zoo

### Introduction

*Echinococcus* spp., are tiny flatworms belonging to the family Taeniidae and widespread all over the world. Its classification and taxonomy has been very obscuring and controversial and as far as could be ascertained, other than the 'lion strain'10 distinct genotypes (G1-10) of *E. granulosus* have been reported on the basis of mitochondrial DNA sequences (Lavikainen *et al.*, 2003). Currently, based on several morphological, biological and biomolecular criteria, 9 species are ascribed to the genus *Echinococcus*: *E. granulosus* "*sensu stricto*" (s.s.) (G1- G3), *E. multilocularis*, *E. vogeli*, *E. oligarthrus*, *E. shiquicus*, *E. equinus* (G4), *E. ortleppi* (G5), *E. felidis* and *E. canadensis* (G6-G10) (Nakao *et al.*, 2007). The life cycle of *E. granulosus* is indirect and involves as definitive hosts domestic and wild canids, and several species of domestic and wild mammals (humans included) as intermediate hosts for the tissue-invading metacestode stage, the hydatid cyst.

Cystic echinococcosis (CE), represents a public health problem and plays an important socioeconomic role in many areas of the world and in particular in the Mediterranean Region (MR)

where it is currently considered among the five most frequently diagnosed zoonosis, along with brucellosis, rabies, leishmaniasis and food-borne zoonotic infection (Sadjjadi, 2006).

Cystic echinococcosis is endemic in different regions of Italy. Prevalence in livestock is high in Sicily and Sardinia, medium-high in the central and southern regions, and negligible in the remaining areas (Varcasia *et al*., 2011). The reports of CE in wild intermediate hosts are very rare. Our report is referred to the metacestodosis in a captive Ring-tailed lemur (*Lemur catta*). This non-human, arboreal primate is endemic to islands of Madagascar. It is an omnivorous, terrestrial and diurnal animal. The Ringtailed lemur is highly social, living in groups of up to 30 individuals and it is an endangered species according to the IUCN Red List of Threatened SpeciesTM (Andriaholinirina *et al*., 2014).

**Case history and clinical examination**

A 13 years old male lemur, host of a colony in a zoo of the province of Ravenna (Northern Italy: 44°19'35.5''N; 12°16'29.9''E) was found dead. The colony originated from animals previously imported from another zoo in Southern Italy and was composed of 12 animals. The diet was based on vegetables and fruits industrially produced in the area and tap water.

During the necropsy of the dead animal, innumerable transparent cysts were observed in the lungs and in the abdominal cavity. The cysts were of different size from 4 cm to few mm, many were free floating in the peritoneal cavity (Fig.1). The microscopic exam of cystic fluid revealed non-fertile cysts (without any protoscolices).

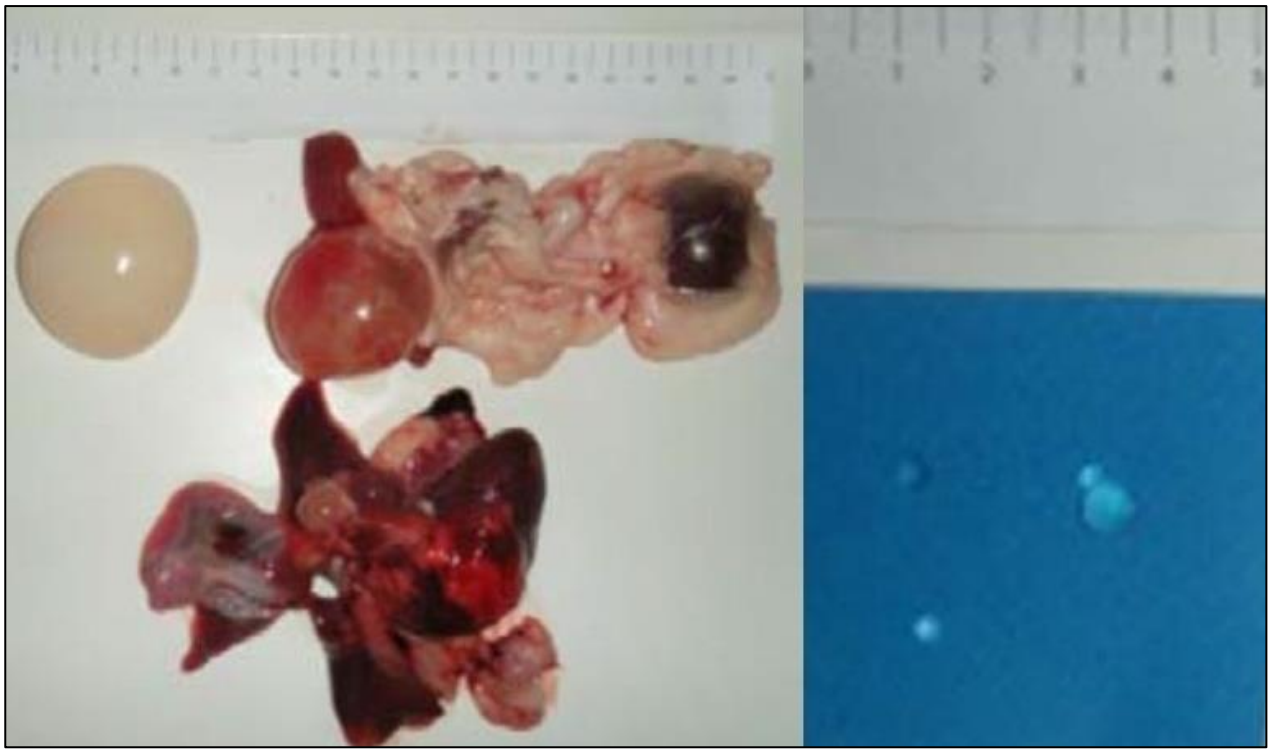

**Fig. 1:** Cysts of different size found during the necropsy.

Histologically, the lung cysts were outlined by a 100-200μm thick lamellar hyaline layer, and were internally lined by a thin germinal layer, but did not contain any protoscolices. Externally, the cysts were surrounded by an infiltrate of mixed inflammatory cells, including multinucleated giant cells, palisading epitheliod cells, macrophages and lymphocytes. Multifocally, there were crumpled fragments of the outer lamellar membrane enclosed by a particularly severe inflammatory infiltrate. The lung tissue showed also scattered peribronchial lymphocytic nodules (Fig. 2).

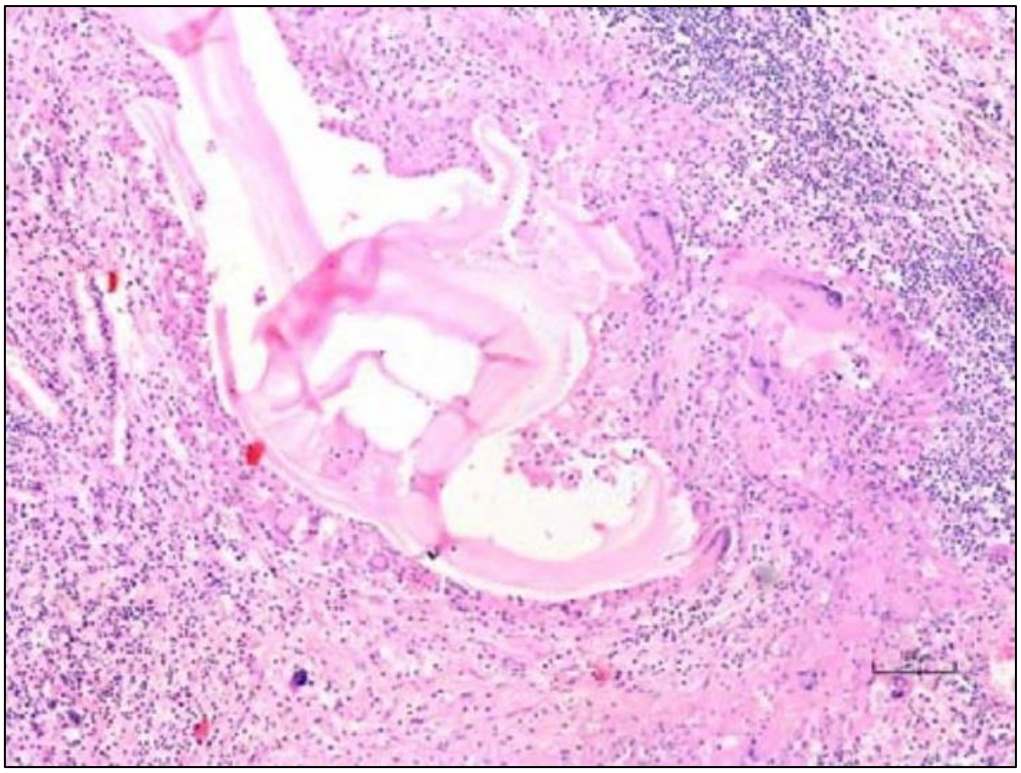

**Fig. 2:** Crumpled fragments of the parasitic membrane surrounded by severe granulomatous reaction, with multinucleated giant cells, palisading epitheliod cells, macrophages and lymphocytes. Lung, HE, bar=100µm.

Molecular identification was carried out by PCR by amplifying fragments within 2 mitochondrial gene cytochrome C oxidase 1 (*cox1*) and NADH dehydrogenase 1 (*ND1*) using DNA extracted from the cysts. PCR products were purified using the High Pure PCR product purification kit (Roche Diagnostics, Mannheim, Germany) and commercially sequenced by MWG-Biotech (Ebersberg, Germany) using the PCR primers. Nucleotide sequences were compared to those available in GenBank® through the use of the basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih. gov/BLAST/).

Molecular analyses of *cox 1* and *ND1* mitochondrial genes respectively shown an homology of 100% with *Echinococcus granulosus s.s.* sequences deposited in GenBank (accession numbers: GQ502212.1; GQ502217.1) revealing an involvement of *E. granulosus s.s.* as cause of CE in lemur examined in the present survey. The Cox1 sequence was deposited in GenBank archive, accession number KT282119.

## Discussion

This, to our knowledge, is the first report of *E. granulosus "sensu stricto"* in a Ring-tailed lemur. Previously, case of hydatidosis in a captive *Lemur catta* has been reported from Zoological Center

of Tel-Aviv (Shahar *et al.*, 1995), however, organism was not genotyped. Contrary to our finding, cysts observed in this case were, fertile, that may be ascribed to involvement of different strain. Other reports in the literature of echinococcosis in *Lemur catta* have been referred caused only by *E. multilocularis* (Palotay and Uno, 1975; Kondo *et al.*, 1996; Deplazes and Eckert, 2001, Umhang *et al.*, 2013). The last three reports were from captive animals in zoo located in endemic zone of Japan, Switzerland and France, respectively, and as infectious route, even if not clear, was suspected eggs-contaminated food or freeroaming foxes. The two cases described by Palotay and Uno (1975) were from a zoo in Oregon (USA) and was supposed this primates were infected in the wild. Moreover, *E. granulosus* (G1) and *E. equinus* (formally G4), have been documented in other genus of lemur, *Cercopithecus ascanius* and *Varecia rubra*, respectively (Boufana *et al.*, 2012). In the latter animal, the presence of free-floating hydatids was also described similarly in our case. These primates were from a zoo of UK, where the *E. granulosus* (G1) and *E. equinus* are known to be endemic however the source of infection was uncertain.

In the present report, no suitable definitive hosts were present in the zoo and the lemurs lived in a status of permanent isolation form other animals. The food-waterborne infection (i.e. with contaminated vegetables/water) was excluded as the lemur were feed with vegetables and fruits produced industrially in the same area and tap water. The zoo is located in an area where CE has very low prevalence, so the most probable way of infection could be ascribed to the fact that infected lemur belonged to a colony that was previously hosted in another zoo in southern Italy, where CE is hyperendemic.

All these considerations allow to reassure staff and visitors of the zoo in respect of this important zoonosis agent but *E. granulosus s.s.* must be taken into account in exotic captive animals, for the possibility to administer to their contaminated food/water from endemic areas.

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# Chapter 3

## Lung parasites of the genus *Metastrongylus* Molin, 1861 (Nematoda: Metastrongilidae) in wild boar (*Sus scrofa* L., 1758) in Central-Italy: An eco-epidemiological study

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Veterinary Parasitology, 2016; 217: 45-52

**Abstract:** The respiratory tracts of 57 wild boars (*Sus scrofa* L. 1758) hunted in central Italy during the 2011/2012 hunting season were examined to detect the presence of lung worms. Fifty-five out of 57 animals (96,5%) were positive. Five species of *Metastrongylus* were detected and their prevalence was as follows: *Metastrongylus asymmetricus* Noda, 1973 (91.2%), *Metastrongylus confusus* Jansen, 1964 and *Metastrongylus salmi* Gedoelst, 1923 (87.7%), *Metastrongylus apri* Gmelin, 1790 (80.7%), *Metastrongylus pudendotectus* Vostokov, 1905 (70.2%). In most cases multi-species infection was observed. The highest parasite load was found in young animals (<1 year old). The *Metastrongylus* genus sex ratio (M/F) had a range from 1:4.8 to 1:1.5 in favor of females. The Simpson and Shannon–Wiener indices showed a moderate uniformity in parasite community composition. The Fager index highlighted a high degree of affinity among all pairs of selected parasites. The whole parasite population showed an aggregate distribution. Our findings confirm that these parasites are widespread in the wild boar population. The establishment of outdoor domestic pig farming in the same area of the game preserve could pose the risk of infection to domestic animals. Further studies will be needed to understand the factors involved in the presence and prevalence of the intermediate host as well as the population dynamics of *Metastrongylus* spp.

**Keywords:** *Metastrongylus*; Wild boar; Lung worms; Epidemiology; Ecological indices; Italy.

### 1. Introduction

During the past century the wild boar (*Sus scrofa* L. 1758) population in Italy fluctuated reaching its minimum after the Second World War. In the 1960s, the species was repopulated through the uncontrolled introduction of Central-Europe wild boars and hybrids characterized by high prolificacy. This fact, together with the depopulation of rural sites and the reduction of natural predators, caused the wild boar population to sprawl in some areas, bringing about losses in agriculture and farming that required official damage control (Riga *et al*., 2011). Furthermore, the repop-ulation led to an alteration of the endemic wild boar population inItaly, and in fact genetic

purity of such species is now very rare(Scandura *et al*., 2005). Nowadays, wild boars are present in 95 out of 107 Italian provinces; their population is estimated at around 300,000-500,000 heads (Carnevali *et al*., 2009). As the practice of outdoor pig farming has been constantly growing over the past years, domestic pigs could interact with wild boars more easily. In order to properly assess the risks for farmed animals, a deeper knowledge of wildlife health is strongly needed. Furthermore, as the wild boar and domestic pig belong to the samespecies and have similar morphology, ethology and diseases, the wild boar can be a valuable model for the study of the host-parasite interaction without the influence of anthropogenic factors.

Verminous pneumonia or metastrongylosis is an important respiratory disease affecting domestic pigs and wild boars. The genus *Metastrongylus* Molin, 1861 includes 6 species reported around the world: *Metastrongylus apri* Gmelin, 1790 (syn. *Metastrongylus elongatus* Dujardin, 1845); *Metastrongylus salmi* Gedoelst,1923; *Metastrongylus pudendotectus* Vostokov, 1905; *Metastrongylus confusus* Jansen, 1964; *Metastrongylus asymmetricus* Noda, 1973 and *Metastrongylus madagascariensis*

Chabaud and Gretillat, 1956. The latter was found only in pigs from Madagascar (Chabaudand Gretillat, 1956); *M. apri*, *M. salmi* and *M. pudendotectus* are commonly reported wordwide with variable frequency (Macchioni *et al*., 1988; Eslami and Farsad-Hamdi, 1992; Manfredi *et al*., 1996; Epe *et al*., 1997; Biddau *et al*., 2003; De Sousa *et al*., 2004; Järvis *et al*.,2007; Nosal *et al*., 2010; Senilk *et al*., 2011; Da Silva and Müller, 2012). *M. confusus* and *M. asymmetricus* are not that widespread and usually associated with low prevalence and abundance (Macchioni *et al*., 1988; Eslami and Farsad-Hamdi, 1992; Manfredi *et al*., 1996; Epe *et al*., 1997; Biddau *et al*., 2003; De Sousa *et al*., 2004; Järvis *et al*., 2007; Nosal *et al*., 2010; Senilk *et al*., 2011; Da Silva and Müller, 2012).

Lung parasites of the *Metastrongylus* genus produce fully larvated eggs in the host respiratory system.

Eggs come up to the boar's mouth when the animal coughs or sneezes and are then swallowed and passed with the feces; in the soil the eggs will hatch and release L1 larvae that are ingested by earthworms, which are the intermediate hosts. The final host acquires the parasite by ingesting infected earthworms which are very abundant in their environment (Anderson, 2000).

Currently, *Metastrongylus* spp. is uncommon in intensively farmed pigs whereas its prevalence is very high in wild boars (Gadomska, 1981; Macchioni *et al*.,1988; Hubert and Henry, 1989; Manfredi *et al*., 1996; Epe *et al*., 1997; De-La-Muela *et al*., 2001; Biddau *et al*., 2003; De Sousa *et al*., 2004; Järvis *et al*., 2007; Nosal *et al*., 2010; Senilk *et al*., 2011; Da Silva and Müller, 2012; García-

González *et al.*, 2013; Navarro-Gonzalez *et al.*, 2013). The infection is considered one of the most important selective factors in wildboar, increasing the mortality of weaker young and adult animals, causing dyspnoea, bronchopneumonia and permanent weight loss. In addition, infection inflicts tissue damage that allows the co-occurance of opportunistic infections from viruses and bacteria (Da Silva and Müller, 2012). In this study, we investigated lungworms of a wild boar population from Central-Italy using an eco-epidemiological approach.

## 2. Material and methods

### *2.1 Study area and sampled population*

During two consecutive hunting seasons (2011 and 2012, from November the 1st up to January the 31st), a total of 57 wild boars (36 male and 21 female) were collected from hunters in a game preserve in the Central-Italy, in the province of Viterbo. The boundaries of the hunting preserve (2415 ha), located near Musignano (42°25’53.54’’N; 11°43’01.42’’E), are irregular in shape: they cover about 10 km from East to West, and reach a maximum width of 2 km from North to South. The area has irregular orographic landscape features as well, varying from 71 to 437 m above sea level (a.s.l.) (Fig.
1).

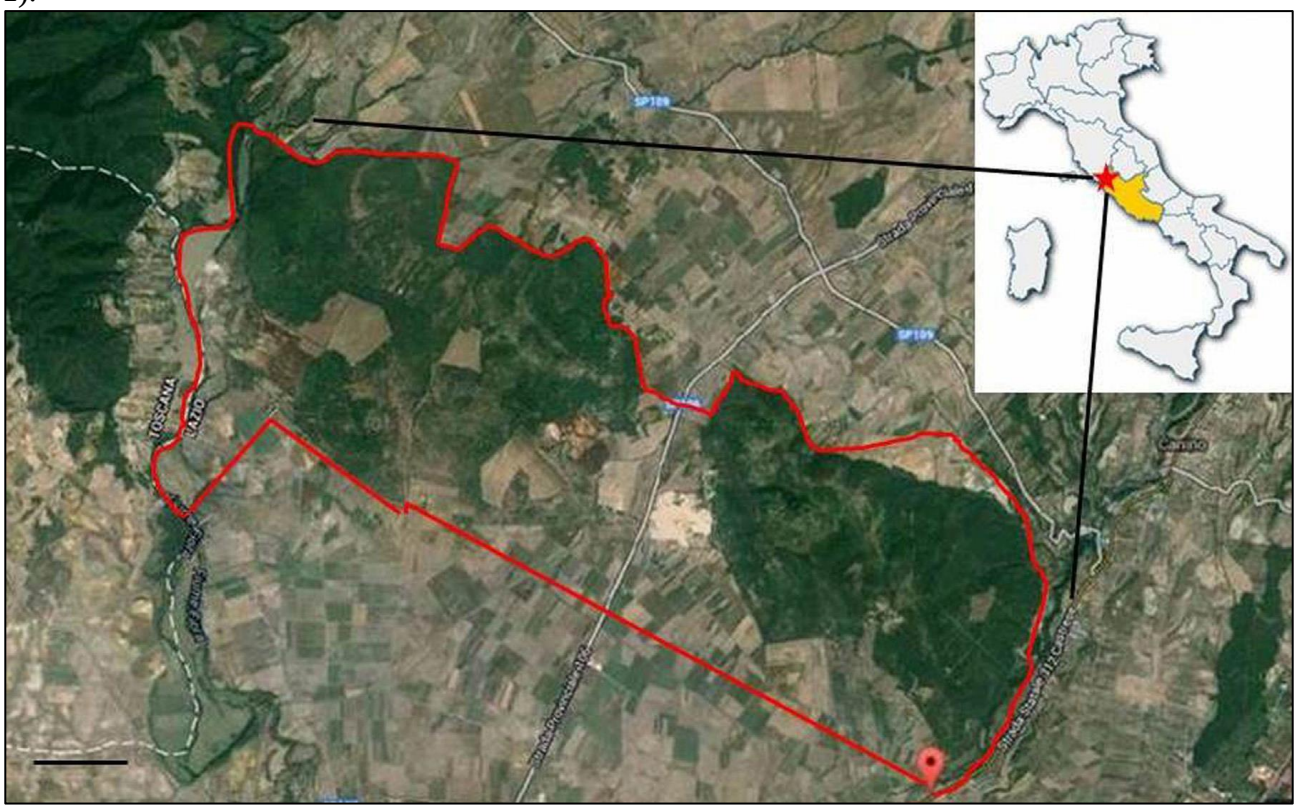


**Fig. 1:** The study area is located in Musignano, Lazio region, Central Italy (red marker: 42°25’53.54’’N; 11°43’01.42’’E); bar = 1 Km.

Nineteen percent of the territory is zoned for agricultural use (cereals and fodder), while the remaining areas is zoned both for extensive breeding (cattle and horses) and hunting purposes (wildboar, roe deer, red deer), or for growing oak forests (*Quercus* spp.).

Surrounded by fences, the preserve hosts approximately 970 wild boars (0.4 heads/ha) that are supplementarily fed with cereals and fodder produced in the same preserve. Feeding is provided on the ground; no feeders are used. Data from each wild boar (culling site, sampling time, sex and age) were recorded. Animals were divided into 3 age groups juveniles (<1 year), subadults (1–3 years) and adults (>3 years) (Tab. 1), both on the basis of coat color and of tooth development (Matschke, 1967; Boitiani and Mattei, 1991; Genov and Massei, 1991). Animals weight ranged from 8 to 120 kg.

**Table 1**
Prevalence, mean intensity and mean abundance of *Metastrongylus* spp. in the wild boar population examined in Central Italy: gender and age relation.

| Gender | N° animals (%) | N° Positive animals n. | Prevalence (%) | Mean intensity* | Range | Mean abundance* |
|---|---|---|---|---|---|---|
| Male | 36 (63.2) | 34 | 94 | 173.3 | 6–812 | 163.7 |
| Female | 21 (36.8) | 21 | 100 | 151.7 | 5–1328 | 151.7 |
| Age classes | | | | | | |
| <1 year | 8 (14) | 8 | 100 | 332[a] | 25–812 | 332[a] |
| 1–3 years | 20 (35.1) | 20 | 100 | 171.9[ab] | 5–1328 | 171.2[ab] |
| >3 years | 29 (50.9) | 27 | 93.1 | 111[b] | 6–446 | 103.3[b] |
| Total | 57 (100) | 55 | 96.5 | 165 | 5–1328 | 159.2 |

* Significant differences between age classes at Kruskall–Wallis test (Mean Intensity: $H = 6.31$; $p = 0.046$; Mean abundance: $H = 7.25$; $p = 0.027$. Different letters mean significant difference.

*2.2 Parasites collection*

The lungs from each animal were collected, then labeled and stored in plastic bags at −20°C until processing. After thawing at room temperature for 24h, the samples were inspected to detect gross lesions. The lungs were then divided into left and right lung and the weight of each lung was recorded. The bronchial tree was completely dissected from the main bronchi to the smallest bronchioles; all nematodes found were fixed in 70% ethanol and cleared with lactophenol to allow for gender and species identification as well as counting, according to specific morphometric keys (Dujardin, 1845; Gedolest, 1923; Jansen, 1964; Noda, 1973).

*2.3 Data analysis*

The infection parameters described (prevalence, mean abundance, mean intensity) were based on the terminology of Bush *et al*., (1997). The sex ratio, related to the whole parasite community and to each single species, were calculated. Pearson's chi-squared ($\chi^2$) test was applied to compare prevalence among sex, age classes and left/right lung. The non-parametric Kruskall-Wallis and Wilcoxon Signed-Rank tests (Ròzsa *et al*., 2000) were used to calculate the differences between quantitative variables (mean abundance and mean intensity).

A linear regression analysis was performed to evaluate the influence of the parasite load on the number of *Metastrongylus* species found: the number of parasite species found in each sample was used as dependent variable, while the whole number of parasites was considered an independent variable.

Furthermore, in order to describing and analyzing the parasite community ecology, as in the Magurran (1988) study, the following ecological indices were calculated: species richness (S), Simpson index

(D) (Simpson, 1949) and Shannon-Wiener index (H) (Shannon, 1948) to evaluate the degree of community diversity; Fager index to evaluate the affinity between different species of parasites (Fager, 1957); *k* value as an indicator of parasites distribution (Poulin, 2007).

The data were analyzed using SPSS® ver.16. Values of $p \leq 0.05$were considered statistically significant.

## 3. Results

### *3.1 Gross examination of lungs*

The left lung had a mean weight of 180.53g (±13.97 SE) and a range of 57-425g, while the right lung had a mean weight of 215.7g (±14.96 SE) and a range of 71-413 g.

Hemorrhagic lesions due to fire arm wounds were the most frequently observed lesions. Areas of lobular emphysema and fibrotic lesions were found, in some cases, on the edge of the caudal lobes. Solid and greyish scattered nodules between 1 and 3 mm in diameter were observed in the heavily infected lungs. The carefully dissected nodules harbored one or more adult worms. Bronchial wall thickening and catarrhal inflammation were observed. Fibrinous pneumonia or atelectasis were identified, in some cases, in the apical part of the lobe (Fig. 2).

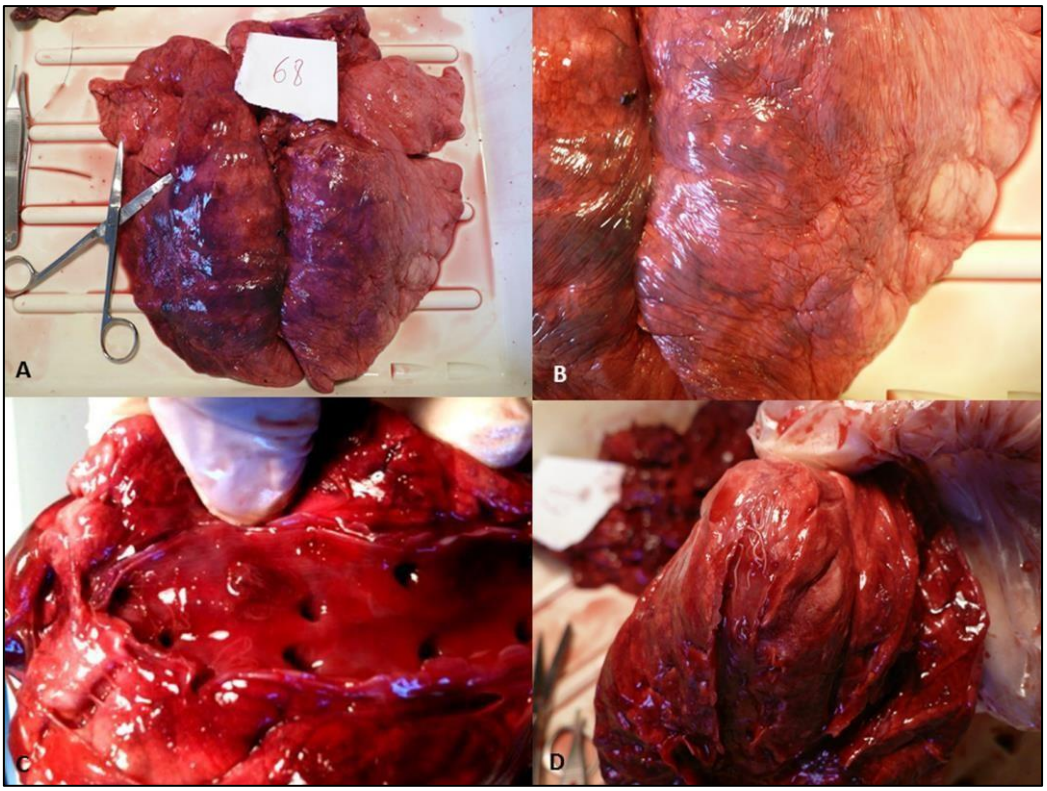


**Fig. 2:** Lungs of wild boar with hemorrhagic lesions (A), areas of lobular emphysema and fibrotic lesions (B) solid nodule (C) and adult worms in bronchi (D).

*3.2. Helminth species, prevalence, mean abundance and mean intensity*

Fifty-five out of 57 (96.5%) wild boars were positive for lungworms; 9077 helminths belonging to the genus *Metastrongylus*, were collected. Five species were morphologically identified (Fig. 3) and their prevalence was as follows: *M. asymmetricus* 91.2%, *M.salmi* 87.7%, *M. confusus* 87.7%, *M. apri* 80.7%, *M. pudendotectus* 70.2%. The prevalence, mean abundance, mean intensity of lung worms for different sex and age classes and data on the parasitepopulation are summarized in Tables 1 and 2.

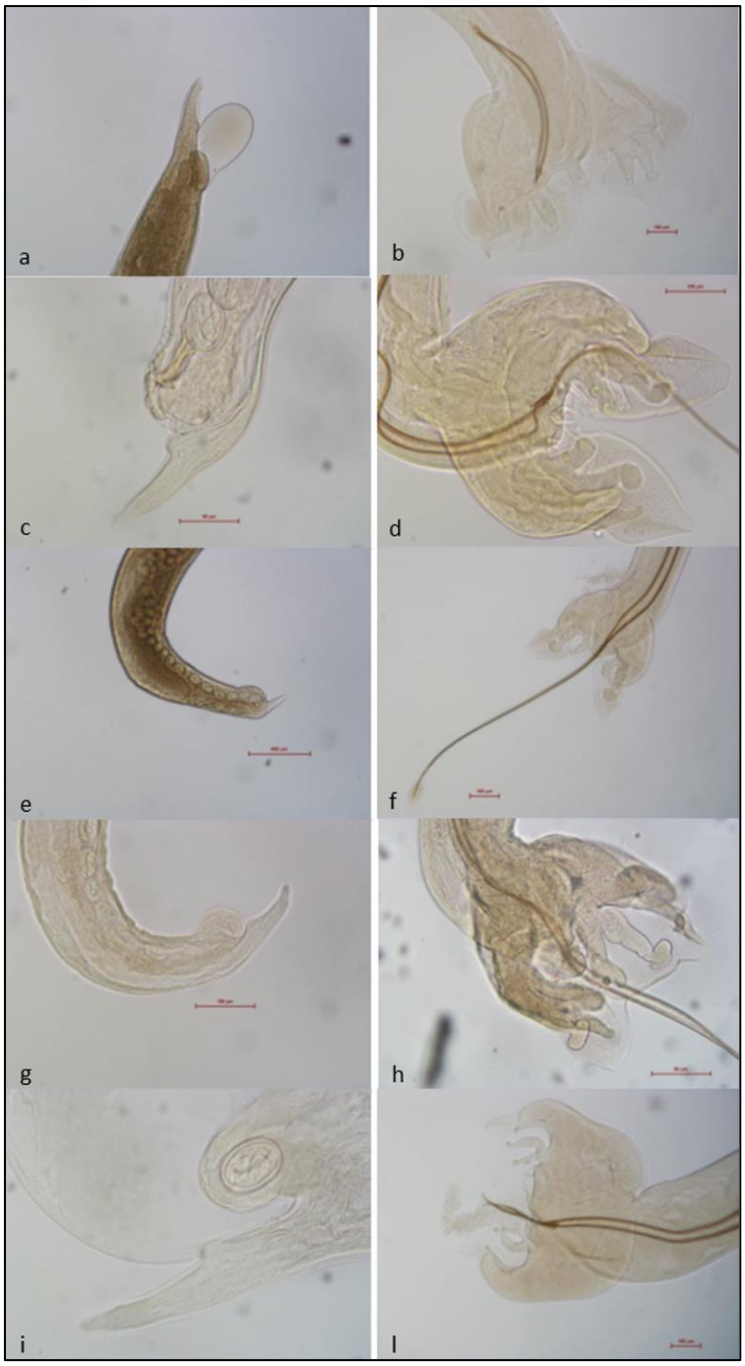


**Fig. 3:** Morphological features of the caudal end of the five *Metastrongylus* species collected. *M. asymmetricus* (a: female, b: male); *M. salmi* (c: female, d: male); *M. confusus* (e: female, f: male); *M. apri*, (g: female, h: male); *M. pudendotectus* (i: female, l: male). Scale bars = 100 µm.

**Table 2**
Prevalence, relative frequency, mean intensity, mean abundance, sex-ratio and k value of different species of *Metastrongylus*.

| | Prevalence (%) | Relative frequency (%) | Mean intensity | Range | Mean abundance | Sex-ratio (M/F) | *k* value |
|---|---|---|---|---|---|---|---|
| *M. asymmetricus* | 91.2 | 22.4 | 39.1 | 1–249 | 35.7 | 1:2.1 | 0.54 |
| *M. salmi* | 87.7 | 27.3 | 49.6 | 1–253 | 43.5 | 1:1.8 | 0.81 |
| *M. confusus* | 87.7 | 8.4 | 15.3 | 1–68 | 13.4 | 1:1.5 | 0.75 |
| *M. apri* | 80.7 | 7.5 | 14.9 | 1–72 | 12.0 | 1:4.8 | 0.51 |
| *M. pudendotectus* | 70.2 | 34.4 | 77.8 | 1–940 | 54.6 | 1:1.6 | 0.12 |
| Total *Metastrongylus* spp. | 96.5 | 100 | 165.0 | 1–9077 | 159.2 | 1:1.9 | |

Since most of the population was infected, no statistically significant differences in prevalence of *Metastrongylus* spp. in different gender or age classes were found.

However, the Kruskall-Wallis test showed significant differences in the overall mean infection intensity/abundance (H=6.31; p=0.046 and H=7.25; p=0.027 respectively) between the age classes, being higher in juvenile animals (<1 year) then in adults (>3 years). The differences between genders were not statisticallysignificant (Tab. 1).

Nematodes were found in the left lung in 87.7% of the animals (50/57) while they were found in the right lung in 96.5% of the animals (55/57), the infection was bilateral in 87.7% of the cases (50/57). The difference between left and right lung was not statistically significant at $\chi^2$ test ($\chi^2$=3.02; p=0.082). The mean infection intensity was markedly lower in the left lung (122.96; range 1-696) than in the right one (327.20; range 1-1336); this difference was statistically significant at the Wilcoxon Signed-Rank test (Z=-2.397; p=0.017).

No statistically significant differences in the number of parasite species involved among the age classes were observed. However, *M. apri*, *M. confusus* and *M. pudendotectus* showed statistically significant differences at Kruskall-Wallis test (p≤0.05) in the parasite load among the age classes, being higher in juvenile animals thenin adults (Tab. 3).

**Table 3**
Mean abundance of different species of *Metastrongylus* according to age classes of wild boars.

| | *M. apri** | *M. salmi* | *M. asymmetricus* | *M. confusus** | *M. pudendotectus** |
|---|---|---|---|---|---|
| < 1 year | 25.9[a] | 53.2 | 55.2 | 31.6[a] | 166.0[a] |
| 1-3 years | 13.7[ab] | 43.9 | 31.5 | 12.9[b] | 69.1[ab] |
| > 3 years | 7.0[b] | 40.5 | 33.1 | 8.8[b] | 13.9[b] |

Different letters mean significant difference.
* Statistically significant at Kruskall–Wallis test. (*M. apri*: $H = 7.38, p = 0.025$; *M. confusus*: $H = 8.8$, $p = 0.012$ *M. pudendotectus*: $H = 8.9, p = 0.012$

*3.3 Frequency distribution*

Eighty percent of positive animals (44/55) harbored less than 250 worms while in 16.4% (9/55) the number of nematodes ranged from 250 to 750. Only 3.6% of the animals (2/55) harbored more than 750 parasites.

*3.4 Parasite community analysis*

Data relative to parasite population are reported in Table 2. Sixty-five percent (5933/9077) of the lung worms were female and34.6% (3144/9077) males. The overall sex ratio (M/F) was 1:1.9 infavor of females. The most frequent species was M. pudendotectus (3112/9077; relative frequency = 34.4%) that showed the highest 0.012 mean intensity (77.8) while *M. apri* showed the lowest relative frequency (685/9077; 7.5%) and mean intensity (14.9) (Tab. 2).

Among positive wild boars, 5 parasite species were found in 61.8% (34/55), 4 species in 23.7% (13/55), 3 species in 1.8% (1/55) and 2 species in 10.9% (6/55). The only case of monospecific infection was detected in 1.8% wild boars (1/55) and was caused by *M. asymmetricus*.

The linear regression analysis showed that as the parasite load increased there was a significantly greater number of parasites pecies found ($R^2$=0.134; p=0.006). The species richness index was in fact 5.

*K* value showed an aggregate distribution for all parasite species. A distribution is defined as aggregate when a high proportion of parasite is concentrated in a few host individuals. *M. pudendotectus* was shown to be the most aggregated, while *M. salmi* was shown to be the least aggregated (Tab. 2).

The Simpson index was equal to 0.255; this signals a 25.5% probability of a random finding of two parasites belonging to the same species in the total parasite community. The value of uniformity or evenness (E), obtained by normalizing the Simpson index through the ratio between 1/D (=3.92) and S (=5), was of 0.784; the value obtained suggested a medium-high degree of abundance homogeneity among the species in the parasite community. The Shannon–Wiener index (H) was 0.907. Such value, which is close to the maximum value for its range (0-1), suggested a high degree of homogeneity among the parasite populations in the habitat. The Fager index was >0.5, this indicating a high degree of affinity among all species (Tab. 4).

**Table 4**
Fager index between different species of *Metastrongylus*.

| A species | B species | Fager index |
|---|---|---|
| *M. pudendotectus* | *M. salmi* | 0.77 |
| *M. pudendotectus* | *M. asymmetricus* | 0.80 |
| *M. pudendotectus* | *M. confusus* | 0.77 |
| *M. pudendotectus* | *M. apri* | 0.74 |
| *M. apri* | *M. asymmetricus* | 0.85 |
| *M. apri* | *M. confusus* | 0.84 |
| *M. apri* | *M. salmi* | 0.84 |
| *M. salmi* | *M. confusus* | 0.88 |
| *M. salmi* | *M. asymmetricus* | 0.85 |
| *M. asymmetricus* | *M. confusus* | 0.87 |

## 4. Discussion

In Europe the prevalence of *Metastrongylus* spp. infection in wild boars is generally high, ranging from 41.1% (García-González *et al.*, 2013) to 100% (Epe et al., 1997) (Tab. 5). Our study of *Metastrongylus* infection showed it to be highly widespread (prevalence: 96.5%), this confirming other authors' data and studies, i.e. Manfredi *et al*., (1996) referring to Liguria (North-Western Italian

Region) and by Biddau *et al*., (2003) referring to Sardinia (Center-Italian Island Region). However, the prevalence we noticed was shown to be higher than the values indicated by Macchioni *et al*., (1988) referring to Tuscany (Center-Italian Region).

**Table 5**
Literature summary of reported prevalence of different species of *Metastrongylus* genus in wild boars.

| Authors | Country | Overall prevalence (%) | *M. asymmetricus*(%) | *M. salmi*(%) | *M. confusus*(%) | *M. apri*(%) | *M. pudendotectus*(%) |
|---|---|---|---|---|---|---|---|
| Gadomska, 1981 | Poland | 92.0 | – | N.S. | – | N.S. | N.S. |
| Macchioni et al., 1988 | Italy | 71.0 | 9.0 | 61.0 | 53.0 | 23.0 | 98.0 |
| Humbert and Henry, 1989 | France | 92.0 | N.S. | N.S. | N.S. | N.S. | N.S. |
| Eslami and Farsad-Hamdi, 1992 | Iran | N.S. | – | 14.0 | – | 16.0 | 14.0 |
| Manfredi et al., 1996 | Italy | 96.5 | 5.1 | 63.7 | 44.2 | 47.6 | 65.9 |
| Epe et al., 1997 | Germany | 100 | – | 80.0 | 24.5 | 91.1 | 93.3 |
| De La Muela et al., 2001 | Spain | 85.0 | N.I. | N.I. | N.I. | N.I. | N.I. |
| Biddau et al., 2003 | Italy | 92.7 | – | 81.8 | 46.4 | 50.9 | 80.0 |
| De Sousa et al., 2004 | Portugal | 42.1 | – | 42.1 | – | 42.1 | – |
| Järvis et al., 2007 | Estonia | 82.0 | – | 77.0 | – | 41.0 | 78.0 |
| Nosal et al., 2010 | Poland | 80.0 | 40.0 | 72.0 | 76.0 | 64.0 | 76.0 |
| Senilk et al., 2011 | Turkey | 74.0 | – | 52.0 | – | 59.0 | 52.0 |
| Da Silva and Műller, 2012 | Brasil | 60.0 | – | 7.5 | – | 52.5 | 20.0 |
| Navarro-Gonzalez et al., 2013 | Spain | 53.4 | – | N.S. | – | N.S. | N.S. |
| García-González et al., 2013 | Spain | 41.1 | – | N.S. | – | N.S. | N.S. |
| Present study | Italy | 96.5 | 91.2 | 87.7 | 87.7 | 80.7 | 70.2 |

N.S.: Not Specified; this indicates cases in which the authors of a paper report the presence of a given species, but do not list its individual prevalence; N.I.: Not identified; this indicates cases in which the authors of a paper report the presence of *Metastrongylus* spp., but have not identified specimens to species level.

When comparing the prevalence obtained in different surveys, it is important to consider some aspects related to population management, such as fences and food supply. Fences marking boundaries could lead to a concentration of the final hosts and thus an increase of parasite infective stage in the environment, although the most limiting factor appears to be the presence and concentration of the intermediate hosts (Anderson, 1993). The idea that food supplied by man could reduce rooting and therefore the ingestion of intermediate hosts by boars attracted by the large amounts of man-supplied food does not prove to be effective, because the rooting behavior is a high priority exploratory behavior developed during species evolution and is not influenced by the presence of food. In fact, Humbert and Henry, (1989) found the feeding areas provided by man to be high risk for acquiring pulmonary nematode infections due to a higher density of infected earthworms. In the NavarroGonzalez *et al*., (2013) study, the feeding areas were built on a concrete foundation and this seemed to dramatically reduce the presence of the intermediate hosts. In our study, the distribution of food took place directly on the ground, so the presence of the intermediate host would be higher.

With respect to the prevalence of the infection in the different age classes, we did not find significant differences, unlike what reported by Humbert and Henry, (1989). This was probably due to the high prevalence of the infection in the examined population.

The parasite mean abundance and mean intensity of the infection were higher in the young animals as observed by some authors (Humbert and Henry, 1989; Manfredi *et al*., 1996; Biddau *et al*., 2003; Navarro-Gonzalez *et al*., 2013; García-González *et al*., 2013). In experimental infections, maximum susceptibility was detected in 2-8 weeks-old pigs (Dunn, 1956; Mackenzie, 1958), after their 12th

week of life pigs seem to acquire an increased resistance to parasite infections; it could be assumed that the same pattern holds true in wild boars.

The infection intensity, mean intensity and mean abundance of infection were moderate. We noticed that 80% of the analyzed lungs harbored less than 250 worms and only two had more than 750 worms. Indeed, the *k* values calculated for the different species confirms the trend of parasites to assume an aggregate distribution (Crofton, 1971). It is possible, as Rousset *et al*., (1996) suggested, that the animals with the highest parasite loads were not sampled because of their high mortality, but in the case of a private game preserve, the wild boars with higher number of parasites would bemore easily hunted.

The higher parasite load in the right lung was most likely related to the bigger size of the right lung as compared to the left one; indeed, the mean weight of the right lung resulted 35.18g heavier than the left one. In addition, the difference between lungs could be explained with difference in blood flow between the right and left pulmonary arteries (Barone, 2003) and consequently, different concentrations of larvae that reach the two lungs.

The species richness index was 5, thus in accord with other studies (Macchioni *et al*., 1988; Humbert and Henry, 1989; Manfredi *et al*., 1996; Nosal *et al*., 2010).

Such species richness can be influenced by many factors, both internal and external to the community, such as the environmental conditions that could facilitate the presence of the intermediate hosts and influence the development of different parasite species. The number of parasite species simultaneously present increases significantly in proportion to the intensity of infections. Indeed, in an ecosystem where different species are present, the greater the number of parasites per host, the greater the likelihood of finding the less represented species as well.

According to Poulin (2007), the species with lower prevalence are present only in infracommunities having higher species richness (stacked model). In our study, *M. asymmetricus* was found to have high prevalence (91,2%), with relatively high mean abundance and mean intensity. The only case of monospecies infection found in our study was indeed caused by *M. asymmetricus*. Other studies did not report *M. asymmetricus* (Eslami and Farsad-Hamdi, 1992; De Sousa *et al*., 2004; Järvis *et al*., 2007; Senilk *et al*., 2011; Da Silva and Müller, 2012; Navarro-Gonzales *et al*., 2013; Garcia-Gonzales *et al*., 2013); for instance, in Italy, Biddau *et al*., (2003) reported the absence of *M. asymmetricus* in the island of Sardinia, while other authors found it elsewhere in Italy with a prevalence lower than10% (Macchioni *et al*.,1988; Manfredi *et al*., 1996) (Tab. 5). Probably the source of this difference may be found in the environmental conditions that can stimulate the development of a species, rather than another.

*M. pudendotectus* was the species with the lowest prevalence, but the most abundant one, especially in the young and sub-adult animals and seems to have a greater tendency to the aggregate distribution than the other species ($k$ =0.12). Our results are partially discordant with other studies conducted in Italy which reported higher values of prevalence and mean intensity of this infection (Macchioni *et al*., 1998; Biddau *et al*., 2003). Unlike *M. pudendotectus*, *M. salmi* seem to be more equally distributed within the sample ($k$ =0.81).

The sex ratio related both to the whole community and to each single species was always in favor of females, as previously reported by other authors. This evidence could be explained by the polygamy of nematodes (Roche and Patrzek, 1966) and by a different life expectancy between male and female worms (Poulin, 1997). The sex ratio among nematodes seems to be related to the size of the parasite population. Poulin *et al*., (1997) reported the sex ratio to be less in favor of females in infections of high intensity. This is only partially confirmed by our findings. Indeed in *M. confusus* the sex ratio was also only slightly in favor of females, but its mean intensity was low. This suggests that the sex ratio is only partially influenced by intensity and distribution of the infection.

We calculated the diversity of species through the Simpson index and the Shannon-Wiener index; this evenness was shown by the Simpson index. The results showed a high degree of homogeneity among populations within the community. The Fager index showed the presence of a considerable degree of affinity among all species. The Fager index was also calculated by Manfredi *et al*., (1996); they reported high affinity between all species except for *M. asymmetricus*, which was involved in the only case of mono-specific infection. Ewing *et al*., (1982) speculated about a mutualistic association between *M. apri* and *M. pudendotectus*. In our study, different *Metastrongylus* species seem to be present within the same host at random, without any kind of dependence or competition. The same conclusions were reached by Manfredi *et al*., (1996) in Liguria and Biddau *et al*., (2003) in Sardinia (Italy). It cannot be ruled out, however, that instances of mutualism can occur between different species in cases of high frequency of mixed infections.

## 5. Conclusions

This eco-epidemiological study examined the lung worm population of wild boars in an area where there was no previous information concerning this matter. It is worth noting that the population we analyzed was reared for hunting purposes; this can surely influence the population dynamics and probably the dynamics of the parasites in that environment as well. The ecological study approach, although limited to a few indices, proved to be very useful in performing a more complete analysis of the parasite community.

We observed an aggregate distribution for *Metastrongylus* spp., showing a well-adapted host-parasite relationship. Our findings confirmed that these parasites are widespread in the wild boar population. The introduction of outdoor domestic pig farming in the same area could pose the risk of infection to domestic animals. Further research about earthworms within the investigation area is still needed to assess their environmental concentration and further clarify the ecology of the different species of parasites.

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# Chapter 4

## Italian wolves (*Canis lupus italicus* Altobello, 1921) and molecular detection of taeniids in the Foreste Casentinesi National Park, Northern Italian Apennines.

G Poglayen, F Gori, B Morandi, R Galuppi, E Fabbri, R Caniglia, P Milanesi, M Galaverni, E Randi, B Marchesi, P Deplazes International Journal for Parasitology: Parasites and Wildlife, 2017; 6: 1-7

**Abstract:** After centuries of massive decline, the recovery of the wolf (*Canis lupus italicus*) in Italy is a typical conservation success story. To learn more about the possible role of parasites in the wolves' individual and population health and conservation we used non-invasive molecular approaches on fecal samples to identify individual wolves, pack membership, and the taeniids present, some of which are zoonotic. A total of 130 specimens belonging to 54 wolves from eight packs were collected and examined. Taeniid eggs were isolated using a sieving/flotation technique, and the species level was identified by PCR (gene target: 12S rRNA and *nad1*). Taeniid prevalence was 40.7% for *Taenia hydatigena*, 22.2% for *T. krabbei*, 1.8% for *T. polyachanta* and 5.5% for *Echinococcus granulosus*. The prevalence of *E. granulosus* is discussed. Our results show that the taeniid fauna found in wolves from the Foreste Casentinesi National Park is comparable to that described for other domestic and wild italian canids and provides insights into the wolves' diet and their relationship with the environment.

**Keywords:** *Canis lupus italicus*; National park; Non-invasive genetics; Molecular identification; Parasites; Taeniids

### 1. Introduction

After centuries of massive decline, several populations of large carnivores (brown bear, wolf, lynx, and wolverine) are now recolonizing parts of their historical ranges in many European countries thanks to the implementation of active adaptive conservation efforts (Chapron *et al*., 2014). The wolf in Italy is a typical conservation success story (Randi, 2011).

At the end of the Second World War, Italian wolves were close to extinction, surviving at their historical minimum population size in two isolated areas in the Southern Apennines (Zimen and Boitani, 1975; Boitani, 1984, 1992). However, since the late eighties socioecological changes and the increase in wild ungulates in natural areas have favored a spontaneous re-expansion of Italian

wolves along the Apennines to the Western Italian and French Alps (Breitenmoser, 1998; Boitani, 2000;

Valière *et al*., 2003; Fabbri *et al*., 2007; Marucco and McIntire, 2010). On one hand, the impact of this rapid recovery can increase conflicts with hunters seeking the same prey, livestock breeders suffering economic losses caused by wolf predation on domestic herds (Milanesi *et al*., 2015), and the general public many of whom have a historical fear of wolves, which are still perceived as a potential threat to human safety (Linnell and Boitani, 2011; Glikman *et al*., 2012). On the other, the wolf arouses positive harmonies as a flagship species whose biology, ecology and population dynamics remain poorly known in the Italian ecological context.

During the last 40 years, many studies have investigated the distribution and expansion of the Italian wolf population (Zimen and Boitani, 1975; Fabbri *et al*., 2007), its abundance (Marucco *et al*., 2009; Caniglia *et al*., 2012; Galaverni *et al*., 2016), composition and home ranges of packs (Ciucci *et al*., 1997; Apollonio *et al*., 2004; Scandura *et al*., 2011; Caniglia *et al*., 2014), its genetic variability (Randi *et al*., 2000; Randi and Lucchini, 2002; Lucchini *et al*., 2004), the threat posed by hybridization with domestic dogs (Caniglia *et al*., 2013; Randi *et al*., 2014) and the impact on wild and domestic ungulates (Gazzola *et al*., 2005). A number of studies have investigated Italian wolf parasites (Arru *et al*., 1988; Guberti *et al*., 1991, 1993, 1998, 2004, 2005; Gori *et al*., 2015) because of the recognized role of wildlife parasites in shaping individual host fitness (Hudson, 2002) and their public health significance as zoonoses (Thompson, 2013). All these studies paid particular attention to *Echinococcus granulosus* "*sensu stricto*" (sheep strain genotype 1), an important emerging and reemerging zoonotic agent, above all in the Mediterranean basin (Sadjjadi, 2006). *E. granulosus* is a small tapeworm approximately 3 mm in length, endemic in this region since the appearance of sheep farming and hence a close relationship has developed over the centuries between the domestic dog (definitive host) and small ruminants as the main intermediate hosts. The official data of sheep cystic echinococcosis (CE) in Italy is summarized in Deplazes *et al*. (2017), while the prevalence in adult sheep is at least around 40% (Poglayen *et al*., 2008a,b). The low prevalence of cystic echinococcosis (CE) in wild ruminants, the main wolf prey, has prevented the establishment of a purely wild animal cycle so far (Guberti *et al*., 2004). The low number of wolves (n ~1300-1800) (Galaverni *et al*., 2016), a high prevalence of infected sheep (40%), and many positive dogs, allow the wolf to be considered in a parallel epidemiological context, closely linked to the domestic cycle (Guberti *et al*., 2004). The other species of tapeworm give rise to speculation in attempts to understand and know more about the wolf diet, as the larval stage of each cestode has a specific host

range (i.e. *Taenia hydatigena*: wild and domestic ungulates; *T. krabbei*: only wild ungulates; *T. polyacantha*: micromammals).

The aim of this molecular study was to evaluate the presence of taeniid tapeworms in the wolves of the Foreste Casentinesi, Monte Falterona e Campigna National Park (FCNP), Northern Italy. This area provides opportunities to better understand the ongoing expansion of the Italian wolf population as some (Cagnolaro *et al*., 1974; Apollonio *et al*., 2004) claim that the wolf never disappeared from the FCNP, which acted as a natural ecological corridor along the Apennines guaranteeing the link between wolves from Central Italy and those of the Western Alps (Fabbri *et al*., 2007; Caniglia *et al*., 2014).

Most of the studies on wildlife intestinal parasites depend on standard methodologies based on postmortem examination (Wobeser, 2007). As the wolf is a protected and elusive species these techniques are not a feasible option, so we used fecal analyses (Carbonell and Rodriguez, 1998) combining parasitological analysis with individual host genotyping based on fecal DNA (e.g. Zhang *et al*., 2011). This approach allowed us to identify each fecal sample's taxonomic affiliation (e.g. wolf, dog or hybrid), genetic profile, sex and, thanks to the pedigree reconstruction, the family group to which it belonged (Lucchini *et al*., 2002; Fabbri *et al*., 2007; Marucco *et al*., 2012; Caniglia *et al*., 2014).

## 2. Materials and methods

### *2.1 Study area*

The study area includes the Foreste Casentinesi, Monte Falterona e Campigna National Park (FCNP) located in the Northern Italian Apennines (43°51’34.26’’N; 11°44’38.39’’E) and covers a surface of about 36,000ha, ranging from 400 to 1658m a.s.l. (Fig. 1).

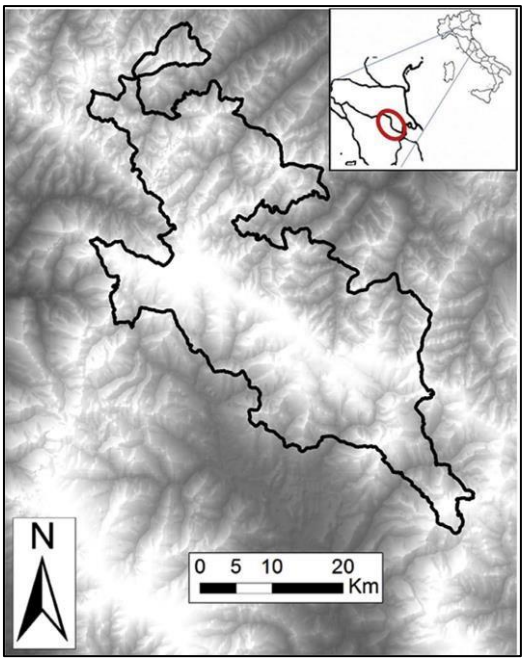


**Fig. 1:** The study area is located on the two sides of the Apennine watershed between Romagna and Tuscany, including the whole territory of the Foreste Casentinesi, Monte Falterona and Campigna National Park (FCNP).

Much of the area is woodland, characterized by some of the oldest European secular forests of silver fir (*Abies alba* Miller, 1759) and deciduous mixed woods of oak (*Quercus* spp.), beech (*Fagus sylvatica* L.), sycamore (*Acer pseudoplatanus* L.) and chestnut (*Castanea sativa* Miller). The area is densely populated by wild ungulates, including wild boar (*Sus scrofa* L., 1758), red deer (*Cervus elaphus* L., 1758), roe deer (*Capreolus capreolus* L., 1758), fallow deer (*Dama dama* L., 1758) and mouflon (*Ovis musimon* Pallas, 1762). The park lies between two regions, Emilia-Romagna and Tuscany. The protected area includes roads and 13 villages, with an average human density of 41.05 people/km$^2$. Few domestic ungulates (cattle, sheep and goats) are reared inside the park and hunting is strictly forbidden.

*2.2 Sample collection and individual genotyping analysis*

From 2001 to 2008 the Environmental Section (CTA) of the Italian Forestry Corp (CFS) and Institute for Environmental Protection and Research (ISPRA) started an intensive genetic monitoring program based on the non-invasive collection of scat samples to investigate the presence, status and distribution of the wolf population in the FCNP. The project was carried out in the framework of a wider regional study, whose results are reported in Caniglia *et al*., (2014).

During the genetic monitoring project, 1433 non-invasive presumed wolf biological samples were collected in the FCNP and analyzed at the ISPRA Genetic Laboratory to identify the genetic profile of individual wolves. Feces were collected along trails or country roads chosen opportunistically to

maximize the probability of finding fresh samples and covering the entire study area. Roads and trails were surveyed at least once per month and the geographic coordinates of every sample were recorded by GPS.

Small samples from the external portions of scats were individually stored in 10 vials of 95% ethanol. Before any manipulation, as a safety precaution they were stored for 10 days at -80 °C (Eckert *et al*., 2001), then at -20 °C until DNA extraction.

DNA was extracted using the MultiPROBE IIex robotic liquid handling system (Conquer Scientific, San Diego, CA, USA) and the QIAGEN QIAmp DNA stool extraction kit (QIAGEN Inc., Hilden Germany). The individual genotype of each sample was identified using a multiple-tube approach (Taberlet *et al*., 1997) at 12 unlinked autosomal canine microsatellites (short tandem repeats, STRs) selected for their polymorphism and reliable scorability, and a restriction fragment length polymorphism to the ZFX gene to gender identification. Maternal haplotypes were identified by sequencing 350 base pairs of the mitochondrial DNA (mtDNA) control region and paternal haplotypes by typing 4 Y-linked microsatellites (YSTR). DNA sequences and microsatellites were analyzed in a 3130XL ABI automated sequencer (Applied Biosystems, Foster City CA, USA), using the ABI software SEQSCAPE 2.5 for sequences and GENEMAPPER 4.0 for microsatellites (Applied Biosystems). GIMLET was used to reconstruct the consensus genotypes for each sample, compare them and control the good attribution of several samples to the same individual. The reliability of the reconstructed multilocus genetic profile was assessed using RELIOTYPE (Miller *et al*., 2002) and a threshold of 0.95. Only genotypes with a probability of reliability to ≥0.95 were retained. For details on PCR conditions and primer references, multi-tube protocol, reliability and match tests, see Caniglia *et al*., (2014).

### *2.3 Species identification from genetic profiles*

We used STRUCTURE v.2.3 (Falush *et al*., 2003) to assign the individual genotypes as wolves, dogs, or wolf *x* dog hybrids. Reference wolf (n=168) and dog (n=160) genotypes were randomly selected from the ISPRA Canis database. We ran STRUCTURE with five replicates of 104 burn-in followed by 105 iterations of Markov chain Monte Carlo sampling, with the ADMIXTURE model and assuming independent allele frequencies. According to previous studies (Caniglia *et al*., 2014), the optimal number of populations was set at $K$=2 (the value that maximized the posterior probability of the data). At $K$=2, we assessed the average proportion of membership (Qi) of the sampled populations to the inferred clusters. Then we assigned genotypes to the Italian wolf or dog clusters

at threshold $q_i$ =0.95 (individual proportion of membership; Randi, 2008), or identified them as admixed if their $q_i$ values were intermediate.

*2.4 Pack identification and pedigree*

We determined the spatial distributions by 95% kernel analysis using the ADEHABITATHR package for R (Calenge, 2006) for all the genotypes sampled in restricted ranges (<100 $km^2$) at least four times and for periods longer than 24 months, and mapped them in ARCGIS 10.0. We performed parentage analyses considering candidate parents all the individuals sampled in the first year of sampling and more than four times in the same area, and candidate offspring all the individuals collected within the 95% kernel spatial distribution of each pack and in a surrounding buffer area of approximately 17km radius from the kernel center (for details, see Caniglia *et al*., 2014).

*2.5 Taeniidae identification*

From the ISPRA genetic bank 130 specimens belonging to 54 wolves, chosen according to the genetic profile (not dog or wolf *x* dog hybrid) and to abundance of material, were examined for taeniidae eggs.

Up to 2 g of feces were sieved in a filter (mesh 150 mm) and washed several times in a cup. The filtrate was centrifuged (1600 *x* g) for ten minutes and the pellet collected. Taeniid eggs were isolated from the pellet using the flotation and sieving method described by Mathis *et al*., (1996) and subjected to morphological identification under an inverted microscope. In egg positive samples and in 14 of the negative samples, DNA extraction was carried out with the complete sieving fraction as described by Štefanić *et al*., (2004). The 14 negative samples were included as negative controls. A total of 69 samples were analyzed using a multiplex-PCR to discriminate between *E. granulosus* and *E. multilocularis* and other cestodes including *Taenia* spp. (Trachsel *et al*., 2007). To obtain clear sequences of the *E. granulosus* positive samples, the PCR was repeated but only using *E. granulosus* primers (Cest5 and Cest4) keeping the same conditions as described above, and therefore amplicons were sequenced. Another PCR targeting *nad1* gene (Armua-Fernandez *et al*., 2011) was used in samples without clear sequencing results to confirm the species of those samples. The amplicons were directly sequenced after purification of the PCR products using the MinElute® PCR purification kit (Qiagen). Sequencing was performed by Synergene Biotech GmbH, Biotech Center Zurich, Switzerland (http://www.synergenebiotech.com). Primer Cest5 and Cest5seq was used for non*Echinococcus* cestodes including *Taenia* spp. and Cest5 for *E. granulosus*, while primer *nad1*T-

Rv for *Taenia* spp. for amplicons obtained with multiplex-PCR and *nad1* PCR, respectively. Sequences were compared with the one present in GenBank using Blast tool (http://www.blast.ncbi.nlm.nih.gov).

## 3. Results

### *3.1 Genetic individual identification and pack reconstruction*

From the 1433 analyzed samples, 544 were successfully genotyped (38%), belonging to 137 individuals: 117 wolves and 20 dogs (no wolf *x* dog hybrids were identified). The kinship and spatial analyses identified eight packs within the FCNP park (Fig. 2a), for which complete genealogies were reconstructed and are available in Caniglia *et al*., (2014).

### *3.2 Parasite identification*

We examined 130 fecal samples from 54 different wolf individuals, of which 35 individuals (and 90 corresponding samples) belonged to the eight packs (Fig. 2) and 19 were not assigned to any known pack (40 samples). Fecal samples were examined only for Taeniidae eggs, and showed a 42.1% frequency (55/130) and a prevalence of 61.1% (33/54) (Tables 1 and 2, respectively). Among the five parasite species isolated, *T. hydatigena* was the most common in terms of frequency in all samples (23.8%) and prevalence in the population examined (40.7%), followed by *T. krabbei* with a frequency of 10.7% and a prevalence of 22.2%. One sample (0.7%) corresponding to one animal (1.8%) was positive for *T. polyacantha*. *E. granulosus* "*sensu stricto*" (G1-G3) was found in three samples (2.3%) belonging to three different wolves (5.5%).

The nucleotide sequence was not obtained from six positive samples, so the taeniids could only be identified as other cestodes including *Taenia* spp., while no sample was positive for *E. multilocularis*. Of the 33 positive wolves, 22 were sampled only once (66.6%), five twice (15.1%) and of these only one was positive for the same parasite (*T. krabbei*) in the second sampling. Four wolves were sampled three times (12.1%) and three of these maintained positivities for *T. hydatigena*. One wolf (3%) was sampled four times and another five times. Two samples were simultaneously positive for *T. hydatigena* and *E. granulosus* (Fig. 2b).

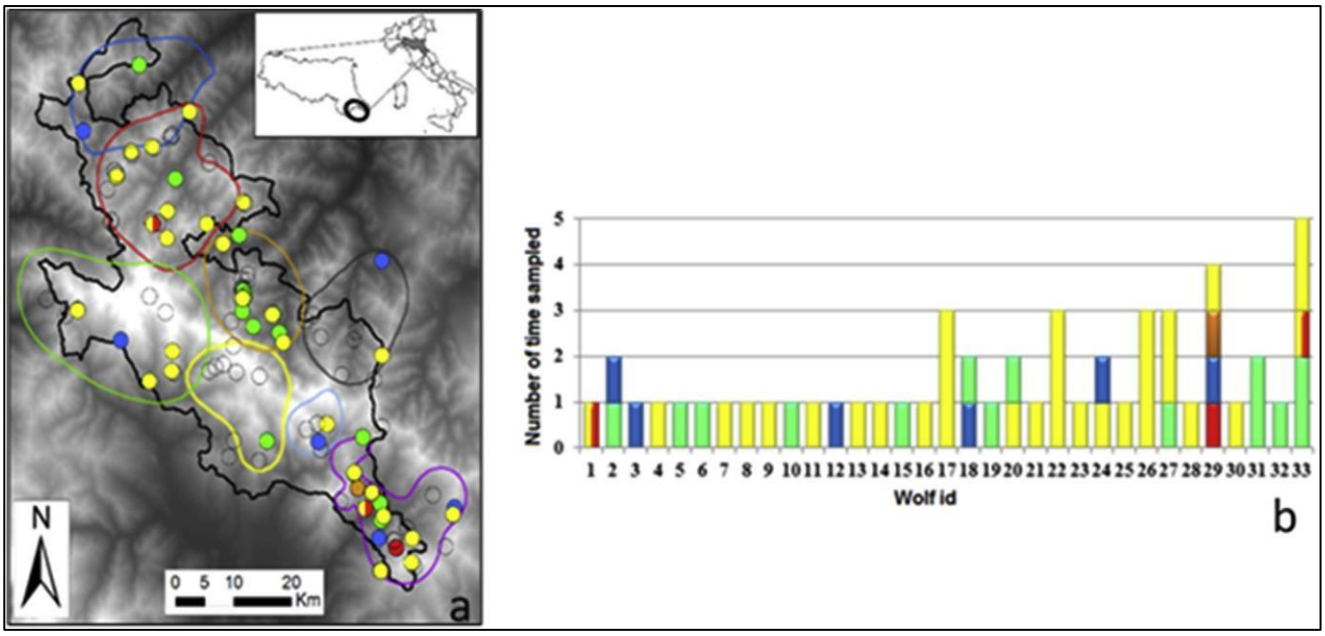


Fig. 2. a) Boundaries of eight wolf packs (see Caniglia *et al*., 2014) in blue, red, green, orange, gray, yellow, light blue and purple lines. Occurrence of species of parasites in wolf scats in red (*Echinococcus granulosus*), yellow (*Taenia hydatigena*), green (*T. krabbei*), orange (*T. polyacantha*) and blue (non-*Echinococcus* cestodes including *Taenia* spp. no sequence). Yellow-red dots indicate the occurrence of both *E. granulosus* and *T. hydatigena*. Empty dots indicate the absence of parasites. b) Frequency of sampling in positive animals.

**Table 1**
Frequency of taeniid species findings in fecal samples from Foreste Casentinesi National Park (Italy).

| Total samples n = 130 | Taeniid species | Number of positive samples (Frequency %) | Confidence interval (95%) |
|---|---|---|---|
| | *Taenia hydatigena* (Pallas, 1766) | 31 (23.8) | 16.5–31.1 |
| | *Taenia krabbei* (Moniez, 1879) | 14 (10.7) | 5.4–16 |
| | *Taenia polyacantha* (Leuckart, 1856) | 1 (0.7) | 0.0–2.1 |
| | *Echinococcus granulosus* (G1-G3) | 3 (2.3) | 0.0–4.8 |
| | non-*Echinococcus* cestodes including Taenia spp. | 6 (4.6) | 1–8.2 |
| Total | 4 Taeniid species | 55 (42.1) | 33.7–50.5 |

**Table 2**
Prevalence of different taeniid species found in the sampled population.

| Total wolves n = 54 | Taeniid species | Number of positive animals (Prevalence %) | Confidence interval (95%) |
|---|---|---|---|
| | *Taenia hydatigena* | 22 (40.7) | 27.6–53.8 |
| | *Taenia krabbei* | 12 (22.2) | 11.2–33.2 |
| | *Taenia polyacantha* | 1 (1.8) | 0.0–5.3 |
| | *Echinococcus granulosus* (G1-G3) | 3 (5.5) | 0.0–11.5 |
| | non-*Echinococcus* cestodes including *Taenia* spp. | 6 (11.1) | 2.8–19.4 |
| Total | | 33 (61.1) | 48.1–74.1 |

## 4. Discussion

Our 130 fecal wolf samples showed a taeniid prevalence close to 60%, the most common being *T. hydatigena* with a prevalence of 40.7%. None of the eight family packs presented the same composition of taeniid fauna, and only one had all four isolated species. As expected, since no sample was positive for *E. multilocularis*, the prevalence of the other zoonotic cestode, *E. granulosus* (G1G3), is not surprising because of its wide diffusion in Italy. In fact, when slaughterhouse data

were matched with the national ovine registry to identify the geographical origin of animals all over the country, CE prevalence was at least 40% in adult sheep (Poglayen *et al*., 2008a,b). The lower detection rate compared with Gori *et al*.'s findings (2015) should be ascribed to the particular environment of our wolves, a national park with a high wild prey density and virtually echinococcosis-free. No wild cycle of *E. granulosus* has been described in Italy and the *E. granulosus* prevalence in carnivores in this park is probably linked to predation on domestic animals. The information on wolf attacks stems from a Regional program to refund damaged breeders and thereby contribute to wolf conservation. These attacks appear very close to the park boundaries with three cases even inside a wolf pack (Fig. 3). The presence of *E. granulosus* in wolves far away from the reported attacks could be attributed to the wolves' mobility also to reduce energetic hunting efforts.

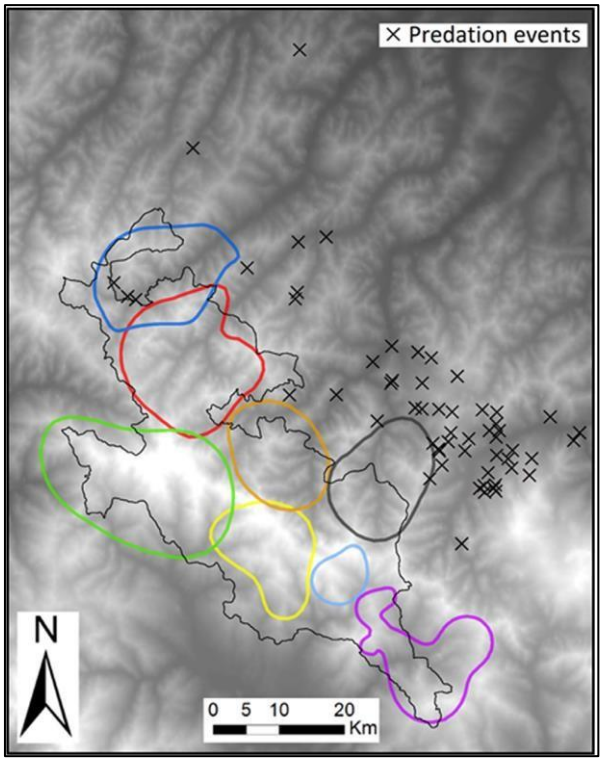


**Fig. 3:** Distribution of attacks on livestock in the Emilia-Romagna region, near and inside the FCNP wolf packs. (Data from Emilia-Romagna attacks control program).

According to Guberti *et al*., (2005), the low prevalence of *E. granulosus* is further confirmation of the absence of a wild cycle of this parasite. A deterministic model was adopted to simulate a purely theoretical sylvatic cycleand demonstrate that even if both the wolf and the wild ungulate population are increasing, the wolf is still part of the parasite's main dog/sheep cycle (Guberti *et al*., 2004). To confirm this stochastic model, an active surveillance program on wild fauna hunted or found dead in our region has been implemented by public research laboratories (Istituto Zooprofilattico

Sperimentale) with the main aim to protect livestock. No CE has ever been detected in the wild ruminants of the area (Tosi pers comm).

The presence of taeniids is always related to the host diet. Although the wolf is a carnivore, its diet is varied and well suited to the different trophic niches offered in southern Europe where this canid has apparently adapted to feed on fruit, rubbish and livestock as well as small and medium-sized mammals (Meriggi and Lovari, 1996). The lack of specific surveys on parasite fauna in different potential prey means we can only speculate on the presence of metacestodes.

*T. hydatigena* was the most common species detected in stools and wolves in our survey. This parasite is also common in wolves in Europe (Craig and Craig, 2005; Moks *et al*., 2006; Bagrade *et al*., 2009; Ćirović *et al*., 2015) whose intermediate hosts belong to wild and domestic ungulates. The second commonest parasite species was *T. krabbei* whose lower presence should be linked to a strictly wild cycle as the main intermediate host and wolf prey in the FCNP is roe deer. Before the advent of molecular biology tools, *T. ovis* and *T. krabbei* were difficult to distinguish morphologically, Lavikainen *et al*., (2008) suggested possible identification mistakes in old studies which may have included the isolation of *T. ovis* in Italy (Guberti *et al*., 1993). *T. krabbei* is also common in Europe (Craig and Craig, 2005; Bagrade *et al*., 2009). *T. polyacantha* reflects the presence of intermediate hosts (micromammals), and its low prevalence (1.8%) suggests wolves make scarce use of these kinds of prey. According to Scaravelli, (2001), 19 micromammal species are present in the FCNP. Our study differs from the other six national studies on taeniids. One adopted an epidemiological approach (Gori *et al*., 2015), while the other five were both parasitological and epidemiological (Guberti *et al*., 1991, 1993, 1998, 2004, 2005) and referred to 119 dead wolves collected throughout the Apennines range of species distribution (Tab. 3). Therefore the results were obtained by total worm count followed by morphological identification of parasites. The only possible comparison between these and our data is in terms of taeniid presence/frequency. More recently, Gori *et al*., (2015) reported the molecular results for cestodes from 179 fecal samples attributed to wolf, evaluating size, shape, smell and composition according to Bassi *et al*., (2012) in the Liguria region. In this case, the same parasitological approach was adopted, but lacking the species identification from the genetic profile the stool recognition is less accurate: in fact, stray dogs in the Emilia- Romagna region are confined in kennels, whereas in Liguria many stray dogs are still free-ranging.

This would create some bias on the actual species sampled. Furthermore, the Liguria study area is larger and included the whole region (540,000ha) where scattered protected and hunting areas are mixed, whereas our area includes only a small National Park (36,000ha). The comparison with last

survey can be done only in term of taeniids frequency (Tab. 3). In our surveys, each scat is molecularly referred to a single wolf of a single pack (Fig. 2a).

**Table 3**
Difference in taeniid frequency between our results and those of Gori et al. (2015).

| Taeniids | Gori et al. (2015) | Present study |
|---|---|---|
| *Taenia hydatigena* | 19.6 | 23.8 |
| *Taenia krabbei* | 4.5 | 10.7 |
| *Taenia ovis* | 2.2 | Not found |
| *Taenia crassiceps* | 0.6 | Not found |
| *Hydatigena taeniaeformis* | 0.6 | Not found |
| *Echinococcus granulosus s.l.* | 5.6 | – |
| *Echinococcus granulosus s.s.* | – | 2.3 |
| *Taenia polyacanta* | Not found | 0.7 |

In summary, the main differences between previous national experiences are by parasitological and epidemiological approach with Guberti et al, (1991, 1993, 1998, 2004 and 2005), while only epidemiological with Gori *et al*., (2015). All the differences are difficult to explain because of the different sampling (a whole region vs a small National Park) and the different stools identification. The taeniids found are common parasites of Italian wolves with different prevalence rates (Guberti *et al*., 1991, 1993, 1998, 2004, 2005; Gori *et al*., 2015). The only data from the same area on carnivore taeniids, isolated by necropsy, referred to foxes in which taeniid species were the same (Poglayen *et al*., 1985, 1988; Fiocchi *et al*., 2016).

From a public health perspective, it is important to emphasize the absence of *E. multilocularis* in the Apennines. In recent years, this taeniid has become an important parasite in Northern Europe, also in an urban context. The only stable small focus is present in foxes of North-Eastern Italy with no human cases (Casulli *et al*., 2005; Dellamaria *et al*., 2014).

Among the taeniids detected, our study focused on *E. granulosus*. In Italy, CE is widely prevalent in livestock (Garippa, 2006; Deplazes *et al*., in press), making wolf infection a negligible aspect in the public health context. Efforts to combat CE target the domestic cycle using well known tools of proven efficacy. National abattoirs ensure the destruction of positive offal so that no infectious material may enter the meat production cycle. The problem arises with frequent illegal home slaughter which favours the spread of the parasite among shepherds and farm dogs and contributes to maintain high infection rates in these dogs, ruminants and humans. The lack of a national CE control program is solely responsible for the Italian situation. Some local efforts are of no use in a general context. As *Canis lupus italicus* is a species subject to conservation, the involvement of wolves in Italy in *E. granulosus* transmission in the absence of a wild animal parasite cycle can be considered a downstream phenomenon of the domestic cycle.

Since Guberti *et al*.'s first paper (1991), the wolf population has increased to approximately 1800 heads and expanded to reach the North-Eastern Alps, but the taeniid fauna has remained the same. Therefore, these parasite species do not pose a risk for wolf conservation.

The combined non-invasive method adopted in this study confirms its importance in the study of ecology, behavior and parasitology without interfering with the sensitive population dynamics of Italy's most important carnivore. In addition, our global approach involved collaboration with theriologists and genetists expert in the Italian wolf population sharing our parasitological expertise. Nature is a complex mosaic and in-depth study of one tile alone will not shed light on the whole picture.

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# Chapter 5

## Retrospective study on Cystic Echinococcosis in cattle of Italy

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**Abstract:**

Introduction: Cystic Echinococcosis (CE) is one of the most widespread zoonosis of veterinary and medical importance still constituting a sanitary, economic and socio-cultural problem in Italy. Methodology: The aim of this study was to update epidemiological data on cattle CE in Italy. Data on CE positivity of 5,336 cattle were acquired from abattoir registers between January 2009 and July 2010. Morphobiological characterization of hydatids was performed by direct examination of liver and lungs of 1,664 animals butchered in the same slaughterhouses in 2010. Strain typing of parasites was carried out through the amplification and sequencing of nd1 and *cox1* mitochondrial genes. Results: Overall CE prevalence was of 8.1% (430/5,336). Parasitological examination of hydatids showed an overall prevalence of 8.6% with a fertility rate of 0.7% (12/1,664). Regarding localization, hydatids were found in 8% of the livers and in 7.6% of the lungs, respectively. Among positive animals, higher prevalence was observed in the liver (93%) compared to lungs (88.1%) ($p > 0.05$). Conclusion: The economic loss due to organs condemnation related to CE in cattle amounted to almost € 24,000 per year in the examined abattoir during 2010. Sequence analysis showed the presence of G1 (sheep strain) or *Echinococcus granulosus sensu strictu* in all examined samples. The G1 confirmed, once more, its possible development into several intermediate hosts such as cattle, especially in areas like southern Italy and Sardinia where the lifecycle of the parasite is still to date carried on by sheep and dogs.

**Keywords:** *Echinococcus granulosus*; hydatidosis; cattle; Italy; G1 strain**.**

### 1. Introduction

Cystic Echinococcosis (CE) is a parasitic infection that occurs worldwide causing considerable public health problems and substantial economic loss in animal productions (Scala *et al*., 2004a). It has been defined one of the most important parasitic zoonoses in several countries of the Mediterranean basin (Battelli *et al*., 2004; Dakkak, 2010) .The economic damage caused by CE has

a special significance in developing countries where sheep production is particularly important (Torgerson *et al*., 2001; Scala and Mazzette,2009) and it is calculated as the sum of costs incurred by the National Health Service for human hospitalization and losses in animal production (Torgerson and Heath, 2003). CE is caused by the larval stage of the tapeworm *Echinococcus granulosus* "*sensu lato*" (s.l.) (Cestoda, Taeniidae) that requires two mammal hosts (definitive and intermediate) to complete its lifecycle. The definitive hosts are carnivores (canids) that harbour adult tapeworm and excrete the parasite eggs with their faeces. Intermediate hosts, as sheep, goats, cattle, camels, buffaloes, pigs, horses and donkeys can be infected by eggs ingestion. The dog-sheep cycle has been reported to be predominant in Southern Europe and Mediterranean Basin (Daryani *et al*., 2009). Humans, considered as "deadend" host, can be accidentally infected acting as intermediate hosts. The ability of *E. granulosus* to fit into a wide range of hosts species and its great genetic variability contribute to the universal distribution of this parasite (Thompson and McManus, 2002). Different methods based on morphology, physiology, biochemistry and immunology have been used to characterize the genetic variants or strains of *E. granulosus* (Eckert *et al*., 2001; Romig *et al*., 2006). Through molecular studies, 10 different genotypes (G1-G10) of *E. granulosus* have been identified in the past decades (Thompson and McManus, 2002; Lavikainen *et al*., 2003). G1 genotype (sheep strain) is the most widespread around the world, infecting sheep, cattle, pig, goat, buffalo and humans. Generally, cattle have been considered a poor suitable host for the G1 genotype, although some studies have demonstrated that cattle could play a role as a reservoir of the G1 genotype in some areas of northern Africa (Andresiuk *et al*., 2009).

Some years ago, several authors proposed a revision of the genus based on phylogenetic studies (Romig *et al*., 2006; Nakao *et al*., 2006; Schneider et al., 2010), including strains G1–G2–G3 into a single specie, *E. granulosus sensu stricto,* and to elevate the strains G4 and G5 to the level of species: *Echinococcus equinus* and *Echinococcus ortleppi,* respectively. In addition, the closely related and apparently monophyletic group of genotypes (G6 - G10) has been grouped into a single species, named *Echinococcus canadensis* (Romig *et al*., 2006; Nakao *et al*., 2006; Schneider *et al*., 2010). Until now, in Italy the G1 (Scala *et al*., 2004b; Varcasia *et al*., 2004), G2 and G3 strains (Rinaldi *et al*., 2008) were isolated in cattle; these usually determine variable values of prevalences with low fertility levels. In 2008, the cattle strain G5 has been identified for the first time in Italy from a bovine coming from Northern regions (Casulli *et al*., 2008). No other finding of this genotype has been reported in Italy until present date.

Moreover, epidemiological survey on CE diffusion in cattle in Italy are quite scarce and sometimes dated.

CE tends to be underestimated in Italy, due to under-reporting and to the lack of compulsory notification at abattoirs; furthermore, official information about diffusion of the infection in human and animals is often incomplete and dated to assess properly the epidemiology of the disease. The role of abattoirs as epidemiological observatory could be very important to monitor this parasitosis in endemic countries like Italy.

The aim of this study was to investigate the CE in cattle in Italy in order to update the epidemiological and biomolecular data in such important farm animals.

## 2. Methodology

The survey was performed in an abattoir (Emilia-Romagna Region, Northern Italy) that collects cattle from all over the country. Data on CE prevalence in slaughtered animals were acquired from the slaughterhouse official veterinary register in 2009 and 2010. Information on identity, age and origin of the cattle were obtained from the National Bovine Register (National Information System, https://www.vetinfo.sanita.it/).

Between January 2009 and July 2010, 5,336 cattle coming from 1,250 farms located in 13 different regions of four geographical Italian areas were examined, as detailed in Table 1.

**Table 1.** Number of slaughtered animals per year and their origin.

| Year | Number of slaughtered cattle | Average age | Northern Italy | Central Italy | Southern Italy | Sardinia |
|---|---|---|---|---|---|---|
| 2009 | 3,672 | 6.36 (SD ± 0.7) | 1,823 | 1,139 | 404 | 306 |
| 2010 | 1,664 | 6 (SD ± 0.95) | 876 | 329 | 299 | 160 |
| Total | 5,336 | 6 (SD ± 0.56) | 2,699 (50.6%) | 1,468 (27.5%) | 703 (13.2%) | 466 (8.7%) |

SD means Standard Deviation.

### *2.1 Parasitological examination*

In 2010, hydatids cysts were counted and their anatomical distribution was registered. Cysts were classified as fertile, sterile and degenerate (calcified or caseous). Fertility was evaluated by using light microscopic observation (400X) of protoscoleces; vitality was assessed by muscular movements and motility of flame cells, and through methylene blue exclusion test (Casado *et al*., 1986).

### *2.2 Statistical analysis*

Data was analyzed accepting a confidence level of 95%: a P-value $\leq 0.05$ was considered statistically significant. Prevalence *per* year, origin, age class was calculated and differences between the proportions were assessed using Chi-square test ($\chi^2$). Mean intensity, topographic location, typology

and fertility of CE cysts were estimated and described. All the statistical tests were performed with software EpiInfo v 6.0.

*2.3 Molecular study*

Thirty hydatid cysts (10 fertile and 20 sterile), each sampled from different animals, were stored at 20°C for biomolecular analysis. DNA was extracted using a commercial kit (Roche DNA template extraction kit). The protocol established by Dinkel *et al*., (2004) was performed on all DNA samples in order to discriminate with a first screening the G1 strain of *E. granulosus* from the G5 and G6/7 strains with four different PCR reactions. After amplification, 10µl of the amplification products were detected and photographed on a 1.5% stained agarose gel. At the same time sequencing of NADH and *cox1* mitochondrial genes was performed on the same samples as described by Bowles and McManus, (Bowels and McManus, 1993a; Bowels and McManus, 1993b). Nucleotide sequence analysis was undertaken using the National Center for Biotechnology Information BLAST programs and databases. Multiple sequence alignments were made with Mega 7.0 software and compared also with GenBank sequences.

## 3. Results

*3.1 Abattoir data*

In the study period, hydatids were found in 8.1% of examined animals (430/5,336); specifically, a prevalence of 7.8% was recorded in 2009 (287/3,672) and a prevalence of 8.6% in 2010 (143/1,664), even though for the latter only the first six months were monitored. The overall farm prevalence observed was 14.5% (181/1,250), with a prevalence of 15.6% (126/810) in 2009 and of 12.5% (55/440) in 2010. All positive animals were adults (age ≥ 1 year). Age based prevalence showed a statistically significant variation: the prevalence rate increases when the age of cattle advances ($\chi^2$ with 2 degrees of freedom = 84.93; $p < 0.001$) (Tab. 2).

**Table 2.** Positivity for Cystic Echinococcosis (CE) in age classes.

| Age classes (years) | Percentage of slaughtered cattle in the biennium | Prevalence per class of age (%) | CE positive (%) |
|---|---|---|---|
| < 1 | 1% (53/5336) | 0% (0/53) | 0% (0/430) |
| ≥ 1 - ≤ 3 | 21.4% (1,142/5,336) | 1.8% (20/1,142) | 4.7% (20/430) |
| > 3 | 77.6% (4,141/5,336) | 9.9% (410/4,141) | 95.3% (410/430) |

Infections rates, both in 2009 and 2010 were higher in cattle from Sardinia (45.9%) and Southern Italy (20.8%) than in animals from Northern and Central Italy (Tab. 3). Differences among the prevalence in each geographical area (Northern Italy, Central Italy, Southern Italy and Sardinia) were found to be statistically significant ($\chi^2$ with 3 degrees of freedom = 1290.92; $p < 0.001$).

**Table 3.** Geographical distribution of the prevalence.

| | 2009 | 2010 | 2009 + 2010 | |
|---|---|---|---|---|
| | Prevalence % | Prevalence % | Prevalence % | Odds ratio |
| Northern Italy | 0.9% (16/1,823) | 0.8% (7/876) | 0.9% (23/2,699) | 1.00 |
| Centre Italy | 3.5% (40/1,139) | 2.1% (7/329) | 3.2% (47/1,468) | 3.85 |
| Southern Italy | 20% (81/404) | 21.7% (65/299) | 20.8% (146/703) | 30.50 |
| Sardinia | 49% (150/306) | 40% (64/160) | 45.9% (214/466) | 98.80 |
| Italy | 7.8% (287/3,672) | 8.6% (143/1,664) | 8.1% (430/5,336) | / |

*3.2 Parasitological examination*

Parasitological examination of hydatids in 2010 pointed out an overall prevalence of 8.6% (143/1,664) with an overall fertility rate of 0.7% (12/1,664). A prevalence of 8% was recorded in the liver and a prevalence of 7.6% in the lungs. Difference between prevalences in the two anatomical districts was not statistically significant ($\chi^2 = 0.21$; $p > 0.05$). Among positive animals, hydatids cysts were found in 93% of livers (133/143) and 88.1% lungs (126/143) ($\chi^2 = 2$; $p > 0.001$); the 81.8% of positive cattle (117/143) harboured cysts in both organs.

The total number of cysts and the mean intensity *ratio* found in livers and lungs are reported in Tables 4 and 5, respectively. More cysts were recovered in lungs than in livers (53.2% *vs* 46.8%) and significative difference was found between these values ($\chi^2 = 39.28$; $p < 0.0001$). The mean intensity (MI) of infection in lungs was 20.3, with a number of cysts ranging between 1 and 140; in livers MI of infection are of 16.9 with a maximum number of 98 cysts (range 1-98).

**Table 4.** Distribution and mean intensity of CE in livers.

| Cystic distribution in livers | Number of cysts | Prevalence (%) | Mean Intensity | Odds Ratio | Statistical Analysis of data |
|---|---|---|---|---|---|
| Left hepatic lobe (diaphragmatic surface) | 520 | 23.1% | 3.9 (520/133) | 8.84 | |
| Right hepatic lobe (diaphragmatic surface) | 767 | 34.1% | 5.8 (767/133) | 15.22 | |
| Left hepatic lobe (visceral surface) | 382 | 17% | 2.9 (382/133) | 6.02 | |
| Right hepatic lobe (visceral surface) | 230 | 10.2% | 1.7 (230/133) | 3.35 | |
| Quadrate lobe (visceral surface) | 273 | 12.1% | 2.1 (273/133) | 4.06 | |
| Caudate lobe (visceral surface) | 74 | 3.3% | 0.56 (74/133 ) | 1.0 | |
| Total Diaphragmatic Surface | 1,287 | 57.3% | 9.7 (1,283/133) | | |
| Total Visceral Surface | 959 | 42.7% | 7.2 (959/133) | | |
| | 2,246 | | 16.9 (2,246/133) | | |

**Table 5.** Distribution and mean intensity of CE in lungs.

| Cystic distribution in lungs | Number of cysts | Prevalence (%) | Mean Intensity | Odds Ratio | Statistical Analysis of data |
|---|---|---|---|---|---|
| Left lung – Cranial lobe | 437 | 17.1% | 3.5 (437/126) | 1.00 | |
| Left lung – Caudal lobe | 642 | 25.1% | 5.1 (642/126) | 1.63 | |
| Right lung – Cranial lobe | 254 | 9.9% | 2.0 (254/126) | 0.53 | |
| Right lung – Middle lobe | 640 | 25.1% | 5.1 (640/126) | 1.62 | |
| Right lung – Caudal lobe | 504 | 19.7% | 4.0 (504/126) | 1.19 | |
| Right lung – Accessory lobe | 76 | 3% | 0.6 (76/126) | 0.15 | |
| TOTAL LEFT | 1,079 | 42.3% | 8.6 (1,079/126) | | |
| TOTAL RIGHT | 1,474 | 57.7% | 11.7 (1,474/126) | | |
| TOTAL UPPER LOBES | 991 | 38.8% | 7.9 (991/126) | | |
| TOTAL LOWER LOBES | 1,562 | 61.2% | 12.4 (1,562/126) | | |

The liver diaphragmatic surface was the most involved by cystic lesions; a statistically significant difference was found between prevalence rates of infection referred to the diaphragmatic (57.3%)

and visceral (42.7%) surface in positive livers ($\chi^2 = 85.15$; $p < 0.0001$) (Tab. 4). Right lungs (57.7%) were more parasitized than left side (42.3%) ($\chi^2 = 122.23$; $p < 0.0001$); the lower segments (61.2%) resulted more affected than the upper (38.8%) ($\chi^2 = 255.42$; $p < 0.0001$) (Tab. 5).

The visceral districts were more affected by unilocular cysts than septate ones, both in liver ($\chi^2 = 1058.55$; $p < 0.0001$) and lungs ($\chi^2 = 2541.01$; $p < 0.0001$) (Tab. 6).

Fertile and viable hydatids were found in 8.4% of positive cattle (12/143), in 1.5% of the positive livers (2/133) and in the 7.9% of the positive lungs (10/126), respectively. Protoscolices were found in 2.1% of the total hepatic cysts (47/2,246) and in 7.1% of the total pulmonary cysts (183/2,553): the differences between these percentages resulted statistically significant ($\chi^2 = 67.45$; $p < 0.001$). Degenerated cysts (calcified and caseous) were found more frequently in livers (60.1%) than in lungs (30.4%); the *chi*-square test for this difference was significant ($\chi^2 = 429.87$; $p < 0.001$). Number and MI of infection in fertile, sterile and calcified hydatid cysts in organs are reported in Table 7.

**Table 6.** Number of hydatids and mean intensity of infection in liver and lungs on the basis of morphological characteristics of cysts.

| | Number of cysts (Prevalence %) | Mean intensity of infection |
|---|---|---|
| Total hepatic cysts | 2,246 | 16.9 (2,246/133) |
| Unilocular | 883 (39.3%) | 6.6 (883/133) |
| Septate | 12 (0.5%) | 0.1 (12/133) |
| Total pulmonary cysts | 2,553 | 20.3 (2,553/126) |
| Unilocular | 1,700 (66.6%) | 13.5 (1,700/133) |
| Septate | 2 (0.08%) | 0.02 (2/133) |

**Table 7.** Number of hydatids and mean intensity of infection in liver and lungs on the basis of degenerative process of cysts.

| | Number of cysts | Mean intensity of infection |
|---|---|---|
| Total hepatic cysts | 2246 | 16.9 (2,246/133) |
| Degenerate | 1,351 (60.1%) | 10.2 (1,351/133) |
| Sterile | 848 (37.7%) | 6.4 (848/133) |
| Fertile | 47 (2.1%) | 0.4 (47/133) |
| Total pulmonary cysts | 2553 | 20.3 (2,553/126) |
| Degenerate | 775 (30.4%) | 6.2 (775/126) |
| Sterile | 1,595 (62.5%) | 12.7 (1,595/126) |
| Fertile | 183 (7.1%) | 1.5 (183/126) |

The molecular surveys carried out both with strain specific PCR through the protocol by Dinkel *et al*., (2004) and Mt-DNA sequencing have shown the presence of G1 strain (sheep strain) or *Echinococcus granulosus* "*sensu stricto*" in all examined samples.

### *3.3 Economic losses*

The estimation of the economic losses caused by *E. granulosus* in parasitized organs was evaluated by collating the number of condemned cattle livers, the official number of slaughtered animals provided by the abattoir during the first semester of 2010 and the average price of the liver in the market (€ 15,00/kg). In the estimation were not included the losses due to lungs condemnation because this organ has a relatively low value on the market, even if it should be taken into account

that lungs, if not parasitized, could be used to be transformed into other products such as pet food. Due to Italian legislation, every single organ with CE should be completely destroyed and cutting and toileting operations are forbidden (Reg. Ce 854/2004).

The formula used to estimate total gross economic losses due to condemnation in the examined abattoir was calculated as follows:

$$CLC = \frac{NSC * PLC}{100 * LC}$$

Where: CLC = Cost of liver condemned; NSC = number of slaughtered cattle for the considered period (1,664); PLC = Percentage of liver condemnation (found in the present survey = 8%); LC = Mean price of cattle liver in Italian markets [(€ 15.00/kg) × 6kg = € 90]. Where 6kg is the average weight of a cattle liver.

In addition, the cost of the disposal of condemned offal as recommended by the government, which in Italy amounts to € 0.40 per kg was included into the estimation of economic losses caused by *E. granulosus*.

Economic losses due to liver condemnation as a result of CE detection amounted to € 11.980,8 for the six months of the survey carried out in 2010. The cost for the proper disposal of condemned livers was € 319.2, which should be added to the cost of disposal of parasitized lungs, that amounted to € 504 for the considered period (six months). The overall cost, summing the loss of income from sales of condemned livers plus the cost of disposal of livers and lungs related to *E. granulosus* infection for the first six months in 2010 was € 12,804, that considering the lack of seasonality of the disease might be at least 24,000 Euros for the whole year 2010.

## 4. Discussion

The CE overall prevalence of 8.1% reported in cattle in this study is noteworthy if compared to official data published by EFSA in (2011) (0.2%) and to the prevalence reported in cattle by several authors in the past, like Schiavo *et al*., (1979) (1.5%), Romboli *et al*., (1980) (2.4%) and Fattori *et al*., (2000) (0.6%). Observing this data, it can be assumed a maintenance of the epidemiological conditions that allow the perpetuation of *E. granulosus* life cycle even after decades; in addition, the absence of a statistical significance between the prevalence in 2009 and 2010 contributes to highlight a stability of the infection in the country in recent years.

As highlighted by other authors (Garippa *et al*., 2004; Garippa and Manfredi, 2009), the variation of infection rates within different Italian areas is a common finding. In the biennium, the highest prevalence rates were found in animals coming from Sardinia (45.9%) and Southern Italy (20.8%),

classified in the past as hyper-endemic areas (Scala *et al*., 2004b; Varcasia *et al*., 2004). Our data agree with those reported in the same areas by other Italian authors: specifically, 41.5% (Garippa and Manfredi, 2009) and 20.1% (Fattori *et al*., 2000) for Sardinia and Southern Italy, respectively. Cattle coming from Northern Italy showed the lowest value of positivity as reported by Fattori *et al*., (2000), confirming the sporadic trend of CE in this part of the country (Romboli et al., 1980; Garippa and Manfredi, 2009). In central Italy, the overall prevalence (3.2%) was lower than values reported by Garippa and Manfredi, (2009) (7.3%-15.3%).

According to the chronic nature of hydatidosis, a lower rate of infection was observed in young cattle (1- 3 years) compared with older animals (Scala *et al*., 2004b). This might be explained by the longer exposure time of the aged animals to eggs of *E. granulosus* (Himonas, 1987).

In this study most of examined animals showed cystic lesions in liver and lungs as also reported elsewhere (Rinaldi *et al*., 2008; Giannetto *et al*., 2004; Azlaf and Dakkak, 2006; Fikire *et al*., 2012). In positive animals, parasitological lesions occurred more frequently in livers (93%) than in lungs (88.1%) as also found by Haridy *et al*., (2006) in Egypt and by Ibrahim, (2010) in Saudi Arabia; this is conceivably explained considering that the oncospheres primarily meet the portal vein route during their migration in the host (Kebede *et al*., 2009). In livers, a higher number of cysts were found in the right hepatic lobe (diaphragmatic surface) (34.1%, OR = 15.22).

The MI of infection was higher in lungs than in livers probably due to the soft texture of this organ (compared to liver). The infection observed in the right lung was higher than in left one: this could be caused by its greater size and to the anatomical structure of the tracheal bronchus in relation to its respective vessels that have a second smaller branch in the right lung (Dyce *et al*., 1999).

In this study, unilocular cysts were more common than septate ones in both organs as also reported by Dalimi *et al*., (2002), Rinaldi *et al*., (2008) and Ibrahim, (2010).

The higher number of degenerate cysts in liver (60.1%) may be attributed to relatively higher reticuloendothelial cells and abundant connective tissue reaction of the organ (Regassa *et al*., 2010). Fertile cysts were found both in liver and in lungs with a greater prevalence in positive lungs (7.9%); in this case also, this is probably due to the relatively softer consistency of lungs tissues that allows an easier development of cysts (Himonas, 1987). These findings are in accordance with results reported for the same parasite (*E. granulosus s.s.*; G1) in sheep and in cattle by other authors (Scala *et al*., 2004b; Varcasia *et al*., 2004; Scala *et al*., 2006), where a higher prevalence in liver was reported, while the highest fertility value was observed in lungs.

Molecular results showed the presence *Echinococcus granulosus* "*sensu stricto*" or former G1 strain

(sheep strain) in all examined samples. This data is consistent with results reported by other surveys in Italy, where cattle seems to be mostly infected by sheep strain (G1). The G5 specific cattle strain or *E. ortleppi* was found only once in one cattle imported from Switzerland to Northern Italy (Scala *et al*., 2004b; Rinaldi *et al*., 2008; Casulli *et al*., 2008; Busi *et al*., 2007).

Although 8.6% of the infected cattle examined in the laboratory were positive to CE, only 0.7% of animals harboured fertile cysts; this confirms that G1 infection in cattle is characterized by low fertility values as this parasite seems not to find cattle as a good host (Fikire *et al*., 2012). This value is also consistent with what described by other authors in Italy ranging from a complete absence of fertile cysts (Rinaldi *et al*., 2008) to 0.76%, and 2.6% in Sardinia (Scala *et al*., 2004b; Varcasia *et al*., 2004] to 1.3-4% (Garippa and Manfredi, 2009), depending on the geographical area considered. Fertility rates reported in cattle by international literature are higher, ranging from 12% in Uruguay (Hernandez *et al*., 2011) to 27.7% in Ethiopia (Kebede *et al*., 2009). The variation in fertility rate in different geographical zone could be explained by the presence in these locations of other species of *Echinococcus* spp., for example by the presence of *E. ortleppi* (McManus and Smith, 2006).

The loss of income from sales of condemned livers plus the cost of disposal of livers and lungs related to *E. granulosus* infection was at least 24,000 Euros for the year 2010, highlighting another important economical factor of CE for farmers and generally for the economy of this farming sector.

The prevalence rate together with the fertility values found in this survey reveal how CE in cattle, especially in some Italian areas such as Sardinia and Southern regions is still of a public health concern. In some cases in which the presence of a huge number of hydatids was detected, also a decreased of production due to disfunction of organs involved can be hypothesized (Kern, 2006).

## 5. Conclusion

The G1 sheep strain confirmed, once more, its great adaptability to several intermediate hosts, particularly to cattle. Some authors (Scala *et al*., 2004b; Rinaldi *et al*., 2008) consider cattle not important in the maintenance of the *E. granulosus* cycle, related to the frequent findings of sterile hydatids in infected organs. Despite that, the results obtained in this study, related to the finding of fertile and viable cysts, leads to consider the bovine as an active host for the G1 strain, even if considerably less than in sheep. This suggests that cattle might have a role in the persistence of this zoonosis, particularly where specific rearing methods and socio-cultural conditions coexist: the use of the same pasture for cattle and sheep in extensive production system and the practice of homeslaughtering. Another factor predisposing the maintenance of the biological cycle of *E.*

*granulosus* is the presence of a high number of stray or free-ranging dogs in an area, that could become infected by ingestion of viscera from infected intermediate dead hosts (especially sheep) or offal discharged after home slaughtering (Manfredi *et al*., 2011). When these conditions occur, cattle might be useful as an indicator of CE infection in a specific area providing information on the level of taeniid eggs contamination in the environment and allowing to identify territories potentially at risk (Pellegrini and Cilli, 1955).

Despite the wide spread of *E. granulosus* in intermediate hosts in Italy as observed in this study especially in Southern regions and major islands, and despite the severity of this disease in human, CE is considered a neglected zoonosis and still causes scarce interest in media and in the National Health System (Poglayen *et al*., 2008). Although since 1964 the record of CE cases at slaughterhouse has been imposed to veterinary officers (O.M. 21 April 1964), nowadays the official information are not representative of the national epidemiological situation. The under-reporting of hospital and abattoir data and the lack of compulsory notification cause the underestimation of the real diffusion of infection. Comparing our data with Pellegrini and Cilli, (1955) we may confirm that after more than fifty years despite the decrease of prevalence in Northern and Central regions it has to be reported a constant and important presence of the disease in Italy, mainly in Southern regions and Sardinia. This study contributed to update and integrate the epidemiological information on CE in Italy and confirms that the slaughterhouse, if well managed, is an important epidemiological observatory, especially for neglected parasitosis.

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# Chapter 6

## Telediagnosis: Parasitological experiences in wild ruminants of South African preserves

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**Abstract:** A survey on wild ruminants' health status of any South African preserves was attempted, assessing body condition score (BCS) through tele-diagnosis. The wildlife BCS was linked to the presence of gastrointestinal parasites that should be recognized, counted and statistically evaluated. For this purpose, we examined 103 faecal samples of wild ruminants from 6 South African preserves. For practical reasons, the animals were divided into two macro-categories: small and large ruminants. The results obtained showed a prevalence of 78.1 and 15.6% in large ruminants for gastrointestinal strongyles (GIS) and coccidian, respectively, while small ruminants showed 92.3% due to GIS and 30.8% for coccidia. No statistically significant difference in the prevalence among the preserves was detected; on the other hand, a low value of BCS corresponds to a greater presence of parasites with statistics difference in the macro-categories (small ruminant $\chi^2$=5.238; p=0.020; large ruminant $\chi^2$= 15.215; p<0.001) and sex classes (male $\chi^2$=5.409; p=0.020; female $\chi^2$=17.350; p <0.001). For these reasons, our results provide a practical feedback for the management preserves. The present paper is fully part of the limited experiences of telediagnosis in a conservation perspective. Based on the results obtained, we decided to organize a project that could limit and assess the risk factors in the management of these activities in the South African context.

**Keywords:** Wild ruminants; telediagnosis; parasites; body condition scores; South African preserves.

### 1. Introduction

In recent past, Veterinary Medicine has focused its interest on involving wild animals not only as single head fenced in captivity and therefore clinically similar to domestic one, but also as free-living populations. All these are meant to protect biodiversity and curtail the possible spread of pathogens, and zoonotic diseases. These preliminary considerations suggest transferring the clinical approach proposed by Bologna Academy (Messieri and Moretti, 1982) and more recently by Cambridge Academy (Jackson and Cockcroft, 2002), simplifying and adapting them to wild ruminants in game

preserves of South Africa. These are wild farms suitable for the conservation, including breeding of species of local wildlife particularly valuable, from economic, touristic or endangered point of view. Their management is quite particular: wild ruminants are fenced on many hectares of land and continuously exchanged with other preserves. Considering that from this wild farm parasitological information are lacking and also domestic ruminants are raised close to wild ones, we suggested transferring the clinical approach cited adapting them to wild ruminants by a visual system for scoring body condition (telediagnosis). In the international literature, we have found four specific papers of this non-invasive method to define health status: two in Asian Elephants (*Elephas maximus* L., 1758) (Ramesh *et al*., 2011; Wijeyamohan *et al*., 2015) and two on wild ruminants, in particular Bassano *et al*., (2003) on *Ovis canadensis* (Shaw, 1804) and *Capra ibex* (L., 1758) and Pfeifer, (2015) on *Cervus elaphus* (L., 1758).

The aim of this study was to survey the health status of wild ruminants by telediagnosis. This was evaluated by scoring body condition. Body condition score (BCS) is a subjective tool to assess the amount of metabolizable energy stored in body fat (primarily subcutaneous) and muscle tissues of a live animal (Edmonson *et al*., 1989; Burkholder, 2000; Alapati *et al*., 2010). Body condition is an index of an animal's health (Terranova and Coffman, 1997). An increase or decrease in body condition could mean a change in quality of management or environment in which an animal lives (Fig. 1).

**Fig. 1:** Images of animals and environments in the South African preserves investigated.

The wildlife BCS should be linked to the presence of gastrointestinal parasites that should may be recognized, counted and statistically evaluated.

These described assumptions have had to adapt to the preserves logistical and laboratory requirements provided. Another purpose to study the parasitism of wild ruminants should be to help their management by rangers.

## 2. Materials and methods

### *2.1 Study area*

Our survey was conducted in 6 preserves in the Eastern region of Garden Route, Republic of Sud Africa (Fig. 2) during February 2016. The area has soil and weather characteristics that allow arid lands mixed with wetlands, characterized by particular kind of bush (named "*fynbos*"), especially suitable for game preserve activity aimed to the conservation of autochthonous flora and fauna.


**Fig. 2:** Study area with the six investigated preserves: red star (Garden Route, 34°12'31''S; 21°38'00''E), white (Wolwedans, 34°01'48''S; 21°59'40''E), yellow (Gondwana, 34°04'51''S; 21°54'40''E), orange (Hartenbos, 34°02'41''S; 21°59'41''E), light blue (Bergsig, 34°05'32''S; 22°02'06''E) and green (Plettenberg, 33°56'43''S; 23°21'00''E).

### *2.2 Animals*

Overall, we have had the opportunity to work with 103 animals belonging to 15 different ruminant species (Tab. 1). The adjustment of the clinical procedures applied to domestic animals provides general appearance and physical examination, excluding the medical history, since in wildlife it is impossible to know the history of individuals. The animals were identified through an optical

instrument (field glass Olympus 10x50) at dropping time, later they were photographed and then classified according to sex (male, female) and category (small or large ruminants). The sex was determined in 102 animals, 34 males and 68 females, in one instance it was not possible because it was a very young individual and hidden from the herd. BCS was evaluated analysing the ribs, spine, hip bone/rump, tail head and belly, according to the method described by Pfeifer, (2015). Randomly, the classification was simplified by grouping the animals into two main categories: emaciated/medium and good/excellent. Faecal samples were collected off the ground, marked with a serial number, scientific and common names of the species. Collected samples were stored in a cooler, transported in a few hours in a refrigerator (+ 4°C), and then in the laboratory examined.

**Table 1.** Animal species and categories considered.

| Category | Species | Number |
|---|---|---|
| **Large ruminant** | Giraffe (*Giraffa camelopardalis* L., 1758) | 9 |
| | Blu Wildebeest (*Connochaetes taurinus* Lichtenstein, 1812) | 10 |
| | Waterbuck (*Kobus ellipsiprymnus* Ogilby, 1833) | 3 |
| | Orix (*Oryx gazzella* L., 1758) | 3 |
| | Eland (*Taurotragus oryx* Pallas, 1766) | 20 |
| | Buffalo (*Syncerus caffer* Sparrman, 1779) | 7 |
| | Kudu (*Tragelaphus strepsiceros* Pallas, 1766) | 2 |
| | Sable Antelope (*Hippotragus niger* Harris, 1838) | 7 |
| | Black Wildebeest (*Cannochaetes gnou* Zimmermann, 1780) | 3 |
| | Total large ruminant | 64 |
| **Small ruminant** | Bontebok (*Damaliscus pygargus* Pallas, 1767) | 11 |
| | Gray rhebok (*Pelea capreolus* Forster, 1790) | 1 |
| | Red Hartebeest (*Alcelaphus buselaphus* Pallas, 1766) | 4 |
| | Impala (*Aepyceros melampus* Lichtenstein, 1812) | 16 |
| | Springbok (*Antidorcas marsupialis* Zimmermann, 1780) | 6 |
| | Blesbuck (*Damaliscus pygargus phillips* Harper, 1939) | 1 |
| | Total small ruminant | 39 |
| | Total | 103 |

*2.3 Examined samples*

Stool samples were referred for qualitative and quantitative coprological evaluation. It was realized with an alternative tool that stocks parasitic forms without centrifugal step (Mini- FLOTAC, Silva *et al*., 2013; Godber *et al*., 2015), using a floatation solution (specific gravity 1.3).

*2.4 Statistical analysis*

The study of prevalence for coccidia and gastrointestinal strongyles (GIS) was evaluated by comparing the sampling area, sex, and category (small or large ruminants) using chi-square test ($\chi^2$). All statistical analyses were performed using the software SPSS 23.0 (IBM SPSS Statistics, New York, United States).

## 3. Results and Discussion

### *3.1 Qualitative results*

Overall, 86 of 103 (83.5%) analysed faecal samples were positive for parasites. Specifically, 86 samples were positive for gastrointestinal strongyles (GIS); and 22 (21.85%) of these were also positive for oocysts of coccidia. Two samples tested positive for whipworm and tapeworm eggs respectively (0.97%). Parasites prevalence was not statistically different ($p > 0.05$) between small ruminants and large ruminants (Tab. 2).

Statistically significant difference in the prevalence among the preserves was not detected (Tab. 3). However, there was a lower prevalence, albeit without statistical significance, of GIS in Wolwedans and lack of coccidia in Hartenbos. Even between sexes the parasitism seems to be equal.

**Table 2.** Relationship between the four macro-categories considered.

| Ruminant | GIS (Prevalence%) | Coccidia (Prevalence%) |
|---|---|---|
| Large ruminant | 50/64 (78.1%) | 10/64 (15.6%) |
| Small ruminant | 36/39 (92.3%) | 12/39 (30.8%) |

**Table 3.** Prevalence of the two different parasites categories in the investigated preserves.

| Game preserve visited (animals sampled) | GIS (Prevalence %) | Coccidia (Prevalence %) |
|---|---|---|
| Bergsig (14) | 12 (85.7%) | 3 (21.4%) |
| Garden Route (29) | 26 (89.7%) | 8 (27.6%) |
| Gondwana (35) | 29 (82.9%) | 6 (17.1%) |
| Hartenbos (9) | 7 (77.8%) | Not found |
| Plettenberg (8) | 7 (87.5%) | 3 (37.5%) |
| Wolwedans (8) | 5 (62.5%) | 2 (25.0%) |

### *3.2 Quantitative results*

If we take into account the quantitative results, positivity at least one parasite (egg/oocyst), a statistically significant difference emerges for BCS levels and sex (Tab. 4 and Tab. 5). In one head only positive for GIS we observed diarrhoea.

The lack of previous surveys, the preserves management characteristics and the logistic difficulties led as to modify our initial project. This resulted during data elaboration to consider only the macro categories of ruminants (large and small) and other parasites (GIS and coccidia). For this purpose, it was particularly useful having available a diagnostic tool that allowed a field activity. Both macro categories created reflect the reality of the hosts/parasite/environment situation in the surveyed areas. The absence of the lower category of BCS supports the hypothesis of a natural predation by carnivore. Despite this simplification, our experience allows validating some results by the statistic help, which excludes the results randomness.

Also without the statistic help, the two parasites categories' prevalence in large and small ruminants was higher anyway. This outcome should be justified in that large African ruminants like diet of trees and bushes that do not favor oro-faecal transmission cycle, characteristic of gastrointestinal parasites. According to the preserves' situation, the different parasites' prevalence could depend on Wolwedans in that it is organized like a true breeding unit (few hectares and small yards) with all characteristics management procedures, while the particularly dried environment of Hartenbos could limit the coccidian transmission that needs humidity to reach the infectivity stages. We did not find prevalence differences between sexes, but this was evident in both categories when related to BCS linked parasite prevalence both for GIS and coccidia. The presence of these parasites is significantly associated in both sexes. This data appears particularly interesting for the characteristics of the preserves studied; one could benefit from the information relative to the crucial influence of parasites and BCS being able to hypothesize specific control activities.

**Table 4.** Statistically significant differences between animal categories related to BCS.

| Animal categories | Emaciated/Medium (%) | Good/ Excellent (%) | |
|---|---|---|---|
| Small Ruminant | 31 (96.9%) | 5 (71.4%) | $X^2$=5.238; P=0.020 |
| Large Ruminant | 33 (97.1%) | 17 (56.7%) | $X^2$= 15.215; P<0.001 |
| Total | 64 (97%) | 22 (59.5%) | $X^2$=24.207; P<0.001 |

**Table 5.** Statistically significant differences between sex related to BCS.

| Sex | Emaciated/Medium (%) | Good/Excellent (%) | |
|---|---|---|---|
| Male | 14 (93.3%) | 11 (57.9%) | $X^2$=5.409; P=0.020 |
| Female | 49 (98.0%) | 11 (61.1%) | $X^2$=17.350; P<0.001 |
| Total | 63 (96.9%) | 22 (59.5%) | $X^2$=23.827; P<0.001 |

## 4. Conclusion

For this reason, our results although limited in numbers and of simplified approach could have a practical feedback for the preserves management. In fact, if a bad BCS is related to the higher parasites presence same animals should be treated avoiding its loss and at the same time not interfere with the natural distribution of the parasites (Wilson *et al*., 2002). For a practical purpose, the animals that could benefit from treatment could be those fenced in small pens or captured for transport.

Future updating should reduce the two macro categories correctly recognising the host species and identify parasites found in dead animals. In this regard, it is extremely interesting the experience carried out in the Limpopo National Park (South Africa) by Van Wyk and Boomker, (2011) where it was possible to isolate and identify the parasites species and the conclusions refer to the importance of parasites in the transfer animal, well known at our latitudes (Lanfranchi *et al*., 2003).

The present paper is full part of the limited experiences of telediagnosis in a conservation perspective. Based on the results obtained, we decided to organize a project that could limit and assess the risk factors in the management of these activities in the South African context.

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# Chapter 7

## Surveillance on pulmonary helminth parasites of red fox (*Vulpes vulpes*, L. 1758), in Northern Italy

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**Abstract:** *Crenosoma vulpis,* the fox lungworm, is a nematode parasite of wild and domestic canids belonging to the super-family *Metastrongyloidea*. A survey of infection was carried out examining 88 red foxes (*Vulpes vulpes*) obtained during the regular hunting season (2014-2015) from the EmiliaRomagna region of Italy. Carcasses were stored frozen (-21°C) prior to necropsy. Lungs were examined for the presence of adult worms by dissection of the trachea, bronchi and bronchioles and then the tissue was examined for first-stage larvae (L1) by the Baermann method. No adult stages were detected, but larvae (L1), identified based on size and morphology as *Crenosoma vulpis* were recovered from 28.4% (25/88) of the fox lungs. No significant differences in infection were not found based on sex or geographical distribution. Results are compared with those of similar surveys on pulmonary helminths of red foxes carried out in Italy and into other European countries. The spread of foxes in urban areas and the high prevalence of lungworms may enhance the presence of these nematodes in domestic dogs as well. This survey might be useful even for clinician collegues, who often underestimate the importance of parasites during routine physical examination on owned pets.

**Keywords**: Red fox, *Vulpes vulpes*, Survey, *Crenosoma vulpis*, Emilia-Romagna, Italy.

### 1. Introduction

Red fox (*Vulpes vulpes*, L. 1758) is a wild canid worldwide in distribution, belonging to the Order *Carnivora*, Family *Canidae*. It is a common species in Italy and Emilia-Romagna region as well. It is highly adaptable, permitting adjustment to a wide range of habitats from urbanized lowlands to hilly and mountainous territories (Spagnesi and De Marinis, 2002). Despite the hunting pressure and several rabies outbreaks in Europe, fox population density has been rapidly growing (Zimen, 1990). Foxes are opportunistic predators, whose varied diet includes: lagomorphs, small and medium-sized rodents, domestic and wild birds, reptiles, invertebrates but also utilizes fruit and vegetables especially in summer and autumn (Kidawa and Kowalczyk, 2011). If available they feed on rubbish, carrion of domestic and wild ungulates, and aquatic animals such as fish and amphibians. Prey availability and geographical setting could affect the parasitic fauna of these animals, including

helminthofauna of the respiratory tract (Segovia *et al*., 2004; Martìnez-Carrasco *et al*., 2007), such as *Crenosoma vulpis* (Dujardin, 1845) (Nematoda, Metastrongyloidea) and others. The life cycle of *C. vulpis* is well known being indirect (Anderson, 2000), involving gastropod intermediate hosts, for instance the terrestrial red slug *Arion rufus* L., 1758 (Beugnet *et al*., 2018). Paratenic hosts (reptiles) have been reported just for *Crenosoma mephitidis* (Anderson, 2000). This fox lungworm infects wild and domestic canids and several other carnivores (Levine, 1980). It dwells in the bronchioles, bronchi and trachea of the definitive hosts, mainly foxes, but sporadically has been reported in dogs (Cobb and Fisher, 1992; Shaw *et al*., 1996; Bihr and Conboy, 1999; Reilly *et al*., 2000; Unterer *et al*., 2002; Conboy, 2004; Barutzki and Schaper, 2011; Latrofa *et al*., 2015). Nevertheless, its presence, in companion animal, could be underdiagnosed (Traversa *et al*., 2010), with infection misdiagnosed as allergic respiratory disease. Since treatment of allergic respiratory disease with longterm corticosteroids is symptomatic, misdiagnosed lungworm infection dogs show a positive response to therapy making it extremely difficult to discern the true etiology (Bihr and Conboy, 1999). Additionally, temporary alterations are caused by migrating larvae in the parenchyma of liver and lungs. L5 and adult worms cause eosinophilic bronchiolitis and bronchitis with a tendency to chronicity and spreading to involve the lung parenchyma resulting in broncopneumonia (Deplazes *et al*., 2016). It is known to be endemic and evenly spread in areas of North America (Bihr and Conboy, 1999) and Europe with a temperate climate (Deplazes *et al*., 2016).

It is important to consider the possible role played by fox as *reservoir* for domestic animals, as suspected by Tolnai *et al*., (2015) and Hodžić *et al*., (2016). Bihr and Conboy, (1999) suggested a significant difference in the infection proportions in dogs residing in rural and urban areas, assuming an overlapping in the wild/domestic interface. If on one hand the red fox could be an important *reservoir* for domestic dogs, on the other hand we cannot forget that in Italy there is also present a frail protected wolf population, slowly numerically increasing (Poglayen *et al*. 2017).

According to the faunistc and zoogeographical maps (Toso *et al*., 1999), the red fox is widely distributed on the regional territory with peaks of 1.38 pairs/km$^2$ in some area of Forlì-Cesena province.

The Emilia-Romagna region is the most important area for the pig breeding and the trade by its products. To protect this area, defined as “pork valley”, we have to demostrate the total absence of *Trichinella* spp. even in wildlife, in order to avoid exportation restrictions. Consequently, to this activity, we collected the respiratory tracts, the importance of the surveillance, to monitoring and studying the evolution of the diseases, is well known since the times B.C. (Choi, 2012). Due to this, more scientific informations on the epidemiology of lungworms in Italy are needed. This study aimed

to provide further data to better understand the ecological factors determining the distribution and diffusion of these helminths. Results are compared with other surveys carried out in other italian regions and european countries.

## 2. Materials and methods

### *2.1 Study area*

The survey was realized in the provinces of Forlì-Cesena (2,378.4 km$^2$ 44°13'N; 12°02'E), Rimini (864.88 km$^2$ 44°03'N; 12°34'E) and Ravenna (1,859.44 km$^2$ 44°25'N; 12°11'E) for a total area of 5,102.72 km$^2$ about a quarter of whole Emilia-Romagna region (22,452.78 km$^2$, see fig.1). The northern side of these provinces, which is the more populated and industrialized, is characterized by cultivated and flat lands (Pianura Padana) whereas the southern one is rich in hills and mountains, the top altitude is 1654 m above sea level (a.s.l) reached by Falterona mount, belonging to the ForlìCesena province. These territories have a subcontinental temperate climate which turns into fresh temperate in some valleys of the Apennines showing cold winters and sultry summers.

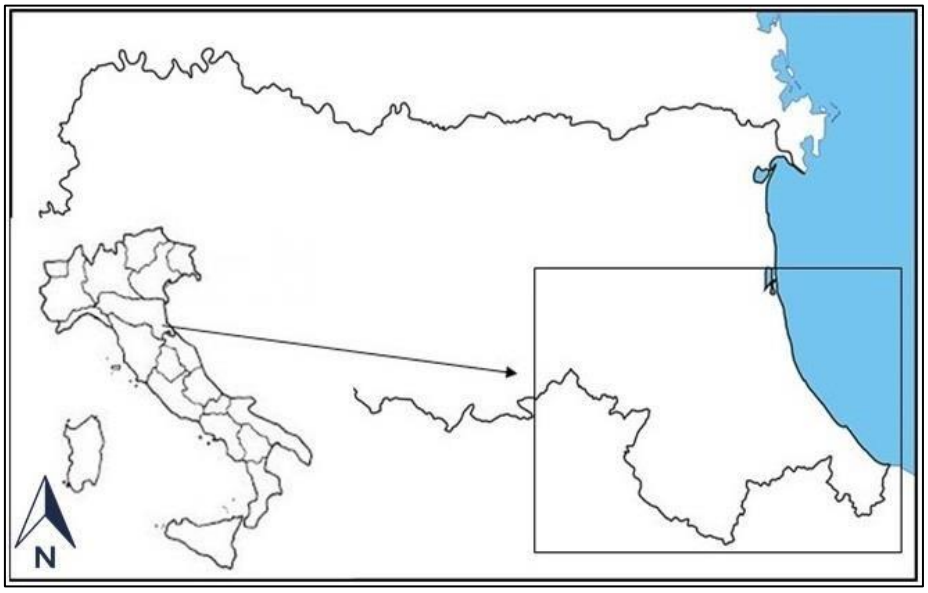


**Fig. 1:** Study area, Emilia-Romagna region, Forli-Cesena, Rimini and Ravenna provinces, located on the easthern side of the region. 44°N 12°E.

### *2.2 Parasites collection*

From September 2014 to Jenuary 2015, 88 carcasses of red foxes, either shot during the regular hunting season or found dead, were delivered to Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini" sect. Forlì, and then the respective respiratory tracts were handed to the service of Transmisible Diseases and Veterinary Public Health at the

Department of Veterinary Medical Science, *Alma Mater Studiorum* University of Bologna. Afterwards, these ones were stored at -21°C before being processed. Each specimen was labeled with information regarding the geographic origin and sex when available. Fifty-one red foxes came from Forlì-Cesena province, thirty-five from Rimini province and just two from Ravenna province. Before analysis, trachea and lungs (fig.2) were thawed at room temperature and the trachea was inspected for macroscopic lesions and then bronchi and bronchioles were cut, open and carefully observed for the presence of adult parasites. Lungs were then chopped into small pieces (5mm) and nematode larvae (L1) were collected using the Baermann method according to Eysker *et al*., (1990).

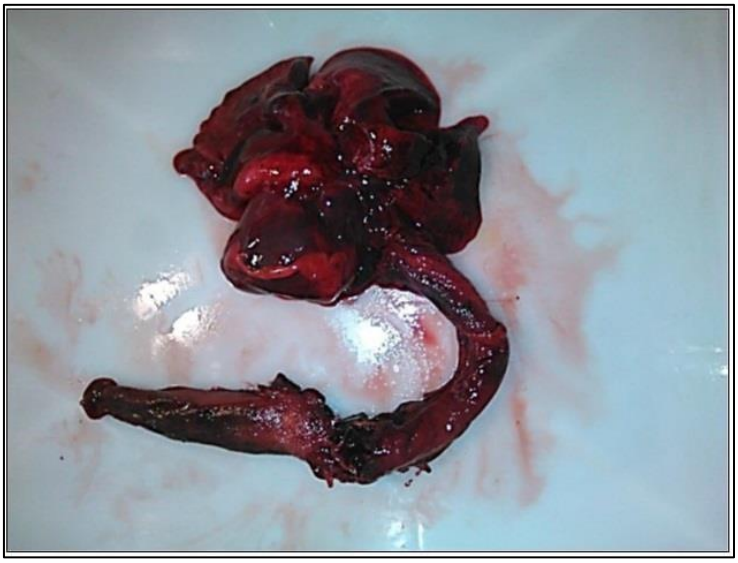

**Fig. 2:** Entire respiratory tract delivered to the Lab. of the service of Transmisible Diseases and Veterinary Public Health at the Department of Veterinary Medical Science, *Alma Mater Studiorum*, University of Bologna, before being processed.

*2.3 Morphological identification*

Larvae recovered by Baermann examination were identified based on size and morphology. The L1 were measured and the tails were examined under an oil immersion lens and photographed. All microscopic image and measures were taken using a digital software image processing system NISElements D 4.10.01 64-bH. Each element was identified according to the morphological keys as suggested by McGarry and Morgan, (2009) and Conboy, (2009).

*2.4 Data analysis*

Prevalence with a 95% Confidence Interval (CI) was estimated. Range, mean length, standard deviation and meadian of the detected larvae were also computed. Chi-squared test was used in order to assess any statistical difference between sex, provinces and geographical features based on the

frequency of parasites, using the software EpiInfo3.5.1, accepting a statistical significance level for p-value less than 0.05.

## 3. Results

Gender was known for 63 of the 88 foxes collected, with 32 males and 31 females. No adult parasites were found but 271 first-stage metastrongyloid larvae (L1) were recovered. A majority of the larvae appeared dead, but many were recovered alive showing vigorous motility. The species was identified as *C. vulpis* (fig.3). The mean length of the detected larvae (including the non-viable L1) was 274.48 μm (SD 44.29 μm), with a range from 116.75 to 333,77 μm. There was a cephalic button at the anterior-end, a sub-dorsal mouth opening, the esophagus was about a third to almost half the length of the larvae and the tail ended in a simple point but had a slight deflection (Fig. 3).

**Fig. 3:** *Crenosoma vulpis* larva (scale bar = 50 μm). It is important to note tail having a sharpened end. Look at the detail as indicated by the arrow.

Larvae were recovered from 25 of the 88 foxes giving a prevalence of 28.4% (95%CI: 19-37.8). With respect to gender, L1 were detected in 9/32 males (28%) and 7/31 females (22.6%), with no significant differences indicated between the sexes based on chi-square test ($\chi^2$= 0.26, p=0.613). No statistical difference ($\chi^2$=0.41; p=0.523) was also found comparing the prevalence based on the provinces. For this purpose, we considered just two provinces, Forli-Cesena and Rimini, out of three because only two foxes were from Ravenna province. In addition, we look for any difference about the geographical features, dividing the sample in coming from either flatland or hill/mountain. Once again, no statistical differences were showed $\chi^2$= 1.85, p=0.174 (see tab.1).

**Table 1.** Foxes found positive for *C. vulpis* larvae, stratified on the basis of gender, province and geographical features. $\chi^2$ test does not show any statistical difference

| | strata | n° for strata | n° of positive | frequency. | | |
|---|---|---|---|---|---|---|
| **gender** | male | 32 | 9 | 0.28 | $\chi^2$= 0.26 | p=0.613 |
| | female | 31 | 7 | 0.23 | | |
| **province** | Forli-Cesena | 51 | 11 | 0.22 | $\chi^2$= 0.41 | p=0.523 |
| | Rimini | 35 | 13 | 0.37 | | |
| **geographical features** | flatland | 37 | 12 | 0.32 | $\chi^2$= 1.85 | p=0.174 |
| | hill/mountain | 51 | 13 | 0.25 | | |

## 4. Discussion

No adult helminths were macroscopically recovered in the trachea, bronchi and lower airways, as already reported in dogs by Bihr and Conboy, (1999), moreover no macroscopic lesions were observed. Not having found adult worms stages, we couldn't taking into account any ecoepidemiological aspect such as mean intensity, abundance or sex ratio.

Survival of first-stage larvae recovered from frozen tissue was first reported for *Crenosoma goblei* in raccoons (Snyder, 1985). As already described by Jeffery *et al*., (2004), Saeed *et al*., (2006), Hodžić *et al*., (2016) and Conboy *et al*., (2017) despite the freezing at -21°C for more then two weeks first stage larvae were found being still alive, in our case this is particularly true because Baermann technique is able to concentrate just living larvae. This could explain survival even in colder climates, for instance Prince Edward Island (Shaw *et al*., 1996; Bihr and Conboy, 1999; Nevárez *et al*., 2005; Conboy, 2009; Conboy *et al*., 2013) and Newfoundland (Jeffery *et al*., 2004).

Overall, high prevalence values for pulmonary helminths in foxes have been detected in several European countries, as Spain (Segovia *et al*., 2004), Great Britain (Morgan *et al*., 2008), Netherlands (Borgsteede, 1984; Franssen *et al*., 2014), Germany (Schöffel *et al*., 1991; Steinbach *et al*., 1994; Manke and Stoye, 1998), Austria (Lassing *et al*., 1998), Denmark (Saeed *et al*., 2006), Norway (Davidson *et al*., 2006), Lithuania (Bruẑinskaitė-Schmidhalter *et al*., 2012) and Hungary (Sréter *et al*., 2003), whereas the differences among the *C. vulpis* prevalence in our survey and in Europe are summarized in table 2, which range from 4.5% in the Netherlands (Borgsteede, 1984) to 58% in Norway (Davidson *et al*., 2006).

**Table 2.** Reported different prevalences of *C. vulpis* in different European country.

| European country | *C. vulpis* | | Sample size | Study area |
|---|---|---|---|---|
| | % | 95% (CI) | | |
| Borgsteede, 1984 | 4.5 | - | 139 | Netherlands |
| Carvalho-Varela and Marcos, 1993 | 1.3 | - | 306 | Portugal |
| Rajković-Janje et al., 2002 | >20 | - | 85 | Croatia |
| Sréter et al., 2003 | 24 | - | 100 | Hungary |
| Saeed et al., 2006 | 17.4 | - | 748 | Denmark |
| Davidson et al., 2006 | 58 | - | 181 | Norway |
| Bružinskaitė-Schmidhalter et al., 2012 | 53.8 | 43.8-63.7 | 310 | Lithuania |
| Tolnai et al., 2015 | 24.6 | 23.2-26 | 937 | Hungary |
| Hodžić et al., 2016 | 45.7 | 39.3-52.3 | 221 | Bosnia and Herzegovina |
| Present study | 28.4 | 19-37.8 | 88 | Italy |

Before comparing our results, we must consider that other Italian surveys cover a long time period, precisely 32 years. High prevalences were found in Trentino-Alto Adige (Manfredi *et al*., 2003): 17.3% followed by Magi *et al*., (2014) in Liguria and Piemonte (15.8%), furthermore Magi *et al*., (2009) found a prevalence of 14.7% in Tuscany (tab.3). Our prevalence 28.4% is the highest compared to the others Italian experiences even if our sample size was not really huge.

**Table 3.** Are here summarized the different prevalences for *C. vulpis* reported in the studies conducted all over the italian country from 1983 up to date.

| Italy | *C. vulpis* | | Sample size | Study area |
|---|---|---|---|---|
| | % | 95%:(CI) | | |
| Rossi et al., 1983 | 9.1 | - | 33 | Piomonte |
| Poli et al., 1985 | 3 | - | 355 | Tuscany |
| Manfredi et al., 2003 | 17.3 | - | 42 | Trentino-Alto Adige |
| Magi et al., 2009 | 14.7 | - | 129 | Tuscany |
| Magi et al., 2014 | 15.8 | 10.2-21.3 | 165 | Liguria and Piemonte |
| Latrofa et al., 2015 | 8.7 | - | 138 | Southern Italy |
| Present study | 28.4 | 19-37.8 | 88 | Emilia-Romagna |

Our measures of morphological features differ from those observed by McGarry and Morgan (2009), our length range of *C. vulpis* is larger, maybe due to the number of larvae computed, just 26 compared to ours 271, making it more accurate. The small end of the size range (116.75 μm) is likely due to the ones that may have been released from dead worms in a premature stage.

The results of the present survey differ from many European studies on pulmonary helminths of red foxes, but is the highest reported prevalence in Italy despite a modest number (88) of examined lungs. Furthermore this study confirms *C. vulpis* infection to be widespread in the European red foxes population. The absence of noticeable macroscopic lesions in pulmonry parenchyma suggests the

perfect balance host-parasite. The primary goal for parasites is that not to harm their hosts, for having the best chance of long-term survival.

The spread of foxes in urban areas of European and North American countries and the high prevalence of lungworms may enhance the presence of these nematodes in companion animals in which, though neglected in the past, has recently been detected and/or recognised as a cause of chronic respiratory disease in different countries (Barutzki and Schaper, 2011; Barutzki, 2013; Conboy *et al*., 2013; Maksimov *et al*., 2017). In our country the importance of this phenomena is increasing, the first report was described by Rinaldi *et al*., (2007). In two recent surveys, one dog out of one-thousand scored positive (Morelli *et al*. 2018) as well as five dogs out 1748 (0.3%) found positive by Brianti *et al*., (2018). In pet animals, respiratory signs may be misdiagnosed as allergic repsiratory disease resulting in administration of anti-inflammatory drugs and/or antibiotic therapy. Under-diagnosis also occurs because fecal flotation lacks detection sensitivity and Baermann examination is infrequently performed. Furthermore, to increase the sensitivity, a single fecal sample often results in a false negative diagnosis, so repeated sampling and Baermann examination are useful, usually three faecal samples collected for three days consecutively are needed.

## 5. Conclusion

This study examined the presence of lungworm infection in red foxes from a previously unexamined area. Many foxes are legally culled from the population each year as part of the annual animal control plan for safegarding wildlife, the environment and public health, offering the opportunity to conduct studies on the parasitic fauna.

Although the red fox population is expanding, even spreading into urban areas, there are relatively few surveys in Italy on the parasites of this animal. Few are also the awarenesses about the intermediate hosts, although one experimental study on *Cornu aspersum* (Müller, 1774), a common terrestrial gastropod, was carried out (Latrofa *et al*., 2015). The new era of molecular technique opens unpredictable views so far with the identification of 4 haplotypes based on the 12s rDNA (Colella *et al*., 2015).

It is worth noting that this survey might be useful even for clinician collegues, who often underestimate the importance of parasites during physical examination on owned pets. In the close future may be interesting take into account the health status of those dogs sharing the same environment with the foxes such as hunting or truffle dogs, in order to deeply understand the role of foxes in the epidemiology of lungworms, the relative risk factors for companion animal and the

relationship degree existing among domestic and wildlife canids. All of these stressed aspects force us to deepen the studies on *C. vulpis*.

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# Chapter 8

## Conclusions

Epidemiology, control and Public Health acted as the main roots that inspired the activities during the three-year PhD path. Wildlife transmissible diseases, referring either to zoonotic agents or in order to prevent domestic animal diseases, in addition to updates on the spreading of CE and its adult form *Echinococcus granulosus*, have added some tile to the complex epidemiological mosaic on the parasitic diseases in the italian country.

From a public health perspective the absence of a national CE control program is the solely responsible for the Italian situation; indeed it is still reported a constant and important presence of the disease in Italy. In endemic areas, other species can enter in the common domestic dog/sheep cycle, such as Italian wolves, captive ring-tailed lemur and bovine, acting etiher as definitive or intermediate hosts, however their role is once more demonstred fully negligible in the epidemiology of CE. The increasing trend in demand of organic and environmentally sustainable products might make rearising neglected diseases. For instance, the introduction of outdoor domestic pig farming where the wild boar population is extremely large could pose a risk for domestic animals. At the same time, veterinary clinicians may need to consider the possibility of lungworm infection not only in those dogs sharing a high degree of environmental over-lap with foxes (such as hunting or truffle dogs) but also in urban and suburban dogs.

The assesment of BCS allowed to demonastrate how coccidia and gastrointestinal strongyle affect the performances also in African wildlife, leading benefits to the management activity.

In conclusion, the present thesis demonstrates the importance of intersectoral cooperation, where each stakeholder puts in the own knowledges in order to stem the spread of transmissible diseases.