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Review

Insights on *Spirocerca lupi*, the Carcinogenic Dog Nematode

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***Spirocerca lupi* is a nematode transmitted by dung beetles that infects domestic and wild canids in tropical and subtropical regions and is associated with neoplasia. It produces a distinctive pathology with the formation of esophageal nodules classified as inflammatory, preneoplastic, or neoplastic with metastasis to distant organs. Aberrant central nervous system migration of this nematode is also responsible for severe neurological manifestations. Reports of spirocercosis have increased over the last two decades showing spread of this canine helminth in five continents. *S. lupi* from different geographical locations is genetically distinct with two genotypes, genotype I from Africa, Asia, and Australia, and genotype II from Europe, and recently separated from *Spirocerca vulpis*, a new species described in red foxes from Europe.**

Highlights

S. lupi induces the formation of esophageal nodules in dogs that range from nonneoplastic to malignant.

The nodule progresses from an inflammatory fibrocytic lesion to a preneoplastic nodule that is characterized by the presence of active fibroblasts and may eventually undergo neoplastic transformation to sarcoma.

The reported number of *S. lupi* infections in domestic and wild canids has increased in the last decade.

Microsatellites and rDNA analyses have demonstrated a complex population dynamic in *S. lupi* and classified two genotypes segregated by geographic location. Studies in wild canids questioned whether infection is caused by different *S. lupi* genotypes or separate species.

Spirocerca lupi: A Unique Cause of a Range of Severe Pathologies in Canines

S. lupi is a parasitic nematode of canids, mainly domestic dogs, which causes a disease known as spirocercosis. It is transmitted to dogs by the ingestion of infected dung beetles that have fed on dog feces containing *S. lupi* eggs. This parasite is distributed mainly in tropical and subtropical areas around the world, with sporadic cases in temperate regions [1,2]. The number of dogs suffering from this infection has significantly increased in the last two decades, as reported from Israel [3], Hungary [4], and South Africa [5]. A special interest in spirocercosis stems from the unique pathological phenomena that *S. lupi* induces in its canine host, which range in severity from regurgitation of undigested food due to partial blockage of the esophagus by nodules containing worms, to fatal neoplastic transformation of the nodules and development of malignant sarcomas with diffuse metastases.

Substantial research in the last decade has improved understanding of the genetics and pathology associated with *S. lupi* and has assisted in improving the diagnosis and treatment of this infection. Moreover, reports of *S. lupi* infection in domestic and wild canid species have highlighted the role of potential **reservoirs** (see Glossary) of this parasite in wildlife. In addition, novel information on the **phylogenetic** relationships of *Spirocerca* spp. from different geographical regions and hosts [6] has revealed the diversity of species within the genus *Spirocerca* [7].

Life Cycle and Developmental Stages of *S. lupi*

The life cycle of *S. lupi* involves canids and coprophagous beetles as definitive and intermediate hosts, respectively (Figure 1). Domestic dogs and other canids are infected by ingesting dung beetles or **paratenic hosts** carrying encapsulated third larval stages (L3) of *S. lupi*. L3 are released within 2 days in the dog's stomach [8], penetrate the gut wall, and start migrating through the gastric artery walls, potentially causing irritation and vomiting [9,10]. By day 10 postinfection, worms can be found in the caudal thoracic aorta wall and migrate cranially through the vessel wall for up to 100 days [11]. In this period, the L3 stage molts to the L4 preadult stage [11]. *S. lupi* developmental stages migrate towards their final niche through the intima of the aortic wall and do not reach the aortic lumen where they would face a strong counter blood flow [11]. The final niche of *S. lupi* adults is the esophagus, although aberrant migration in the thoracic cavity [12,13], nervous system [14], subcutaneous tissues [15], or urinary tract [15,16] can also occur. By day 93 postinfection, *S. lupi* reaches the esophagus at a position where it is adjacent to the aorta in the mediastinum and matures in the esophageal submucosa and subadventitia, inducing the formation of a nodule that protrudes into the esophageal lumen. Adult male and female *S. lupi* complete their development and their anterior and posterior organs are fully mature approximately 161 days after infection of their canine host (Figure 2). Fully developed females release **embryonated** and infective eggs into the esophageal lumen after

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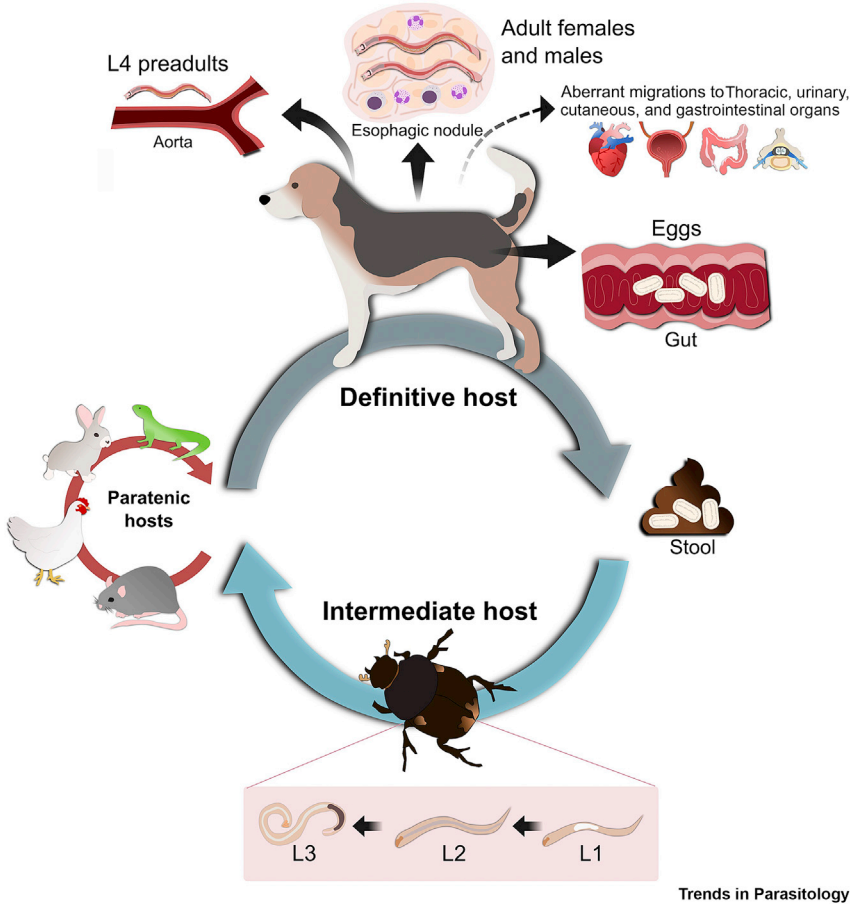

Figure 1. Life Cycle of *Spirocerca lupi* in its Definitive Canine (Gray Arrow), Intermediate Dung Beetle (Blue Arrow), and Paratenic (Red Arrow) Hosts.

Dogs become infected when they ingest L3 larvae contained in an intermediate or paratenic host. The L3 larvae are released and start migrating through the stomach tissue to the aorta wall (as L4 stages) until reaching the esophageal mucosa. In the esophagus, adult females and males develop, mate, and start shedding embryonated eggs that pass through the stomach and intestine into the dog's feces. The eggs are ingested by the intermediate hosts, usually dung beetles, and L1 hatch in them and complete their development to the L3 stage. In addition, L3-infected arthropods can be preyed upon and ingested by paratenic hosts, which maintain these infective stages until potentially ingested by a canine final host.

perforating an opening in the esophageal mucosa. The eggs continue to the stomach and the small and large intestines to be shed in the dog's feces [17,18]. The **prepatent period** from the dog's infection to the initial shedding of infective eggs is 121–124 days [13], and adult *S. lupi* can then survive in their canine host for 2 years or more [9], thus, disseminating infective eggs for prolonged periods.

Dung beetles of the family Scarabaeidae are the most common natural intermediate hosts of *S. lupi* [19,20]. The Scarabaeidae mouthparts allow the passage of food particles in the range of 2–150 μm, and are thus compatible to uptake *S. lupi* eggs which are 35 μm long and 15 μm wide [21]. Various Scarabaeidae species have been confirmed as natural intermediate hosts of *S. lupi*, including *Onthophagus sellatus* in Israel [19], and *Onthophagus pugionatus*, *Onthophagus ebenus*, and *Gymnopleurus virens* in South Africa [20]. Nevertheless, it has been experimentally documented that other arthropod species, including millipedes can support the development of L3 stages after feeding on *S. lupi* eggs [22]. Eggs **hatch** in the beetle's buccal cavity and midgut approximately 8 h after

Glossary

Admixture: presence of DNA in an individual from a distantly related population.
Aneurysm: weakening and bulging of a blood vessel that may lead to abnormal blood flow and eventual tearing of the vessel wall.
cox1: mitochondrial gene extensively used for inferring phylogenetic relationships between specimens.
Cryptic species: specimens with high genetic differences but morphologically identical.
Embryonation: process of development of the morula to a larva inside an egg.
Genetic structure: a population that contains subgroups in which gene flow is restricted as a consequence of social, geographic, ecological, or biological barriers.
Hatching: release of a larvae from the eggshell.
Infrapopulation: all individuals of the same parasite species that occur simultaneously in the same host.
Paratenic host: a host that carries viable larval stages without further development and is able to infect a new suitable host.
Phylogenetics: evolutionary study of the relationships among biological entities, such as species or specimens of the same species.
Prepatent period: time elapsed from the infection of an animal and the recovery of the infective stage of the parasite in a blood, fecal, tissue, or urine sample
Reservoir: an animal host infected by a parasite that serves as a source of infection to the main host or host suffering the disease caused by the pathogen.
Spondylitis: inflammation of the vertebra resulting in deformation and in some cases ossified bridging between vertebrae.
Thromboembolism: blood vessel obstruction due to the dislodgement of an embolic blood clot from a distant site.

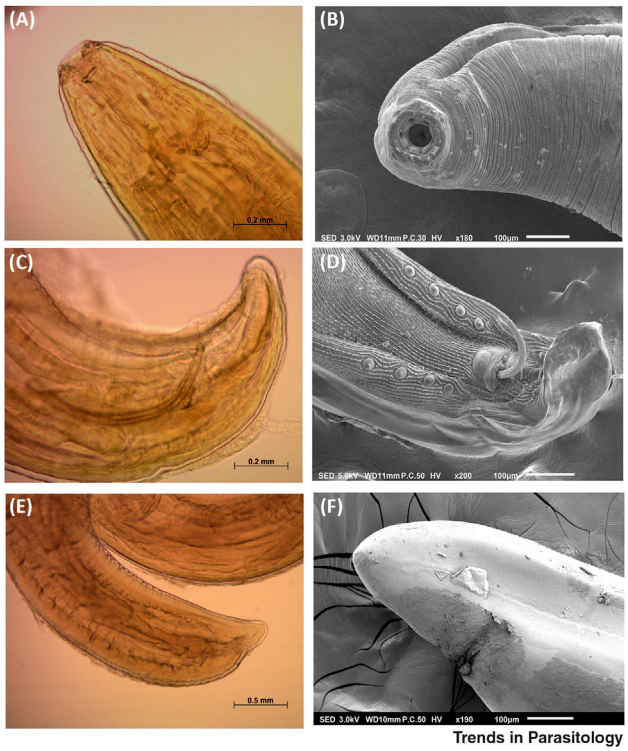

Figure 2. Morphology of *Spirocerca lupi* Adults by Light and Scanning Electron Microscopy.
The hexagonal buccal capsule with a pair of amphids and two pairs of cephalic papillae are evident in (A) and (B). Analysis of the posterior ends of adults allows sexing of the worms. (C) shows the minor spicula of a male, and (D) describes further details of the posterior end of a male with the minor spicula, parallel striation of the cuticle, four pairs of equidistant preanal papillae and gubernaculum. The posterior end of females (E) and (F) is characterized by a blunted end and terminal anus where the digestive tube deposits.

infection [17]. This process is facilitated by the mechanical force induced by the beetle's mouthparts [17,18] and a change in the conditions found within the intermediate host such as pH, temperature, CO_2 concentration, and proteolytic enzymes [17]. Then, hatched L1 larvae develop to L2 and L3 in the beetle's hemocoel [18]. The surface of L2 and L3 is rich in carbohydrate molecules and their composition varies between the two larval stages, as demonstrated by binding of several lectin types to the larvae [23]. L3, which are the stage infective to the definitive host, are encapsulated in the beetle's hemocoel in glycoproteic structures of 0.5–1 mm, which have been shown experimentally to be degraded by gastric contents [11]. Chicken, rabbits, lizards, and mice can serve as paratenic hosts by ingesting infected dung beetles and maintaining the L3 larvae vital and infective mainly encysted in their gastric wall [24].

S. lupi eggs, L3 larvae, and adult worms have been found to harbor bacterial symbionts of the genus *Comamonas* [25]. The role of *Comamonas* sp. in the worm is currently not well understood and it has been suggested that they aid in the digestion and degradation of blood by *S. lupi*, due to their localization in the gut epithelia and their phylogenetic proximity to other *Comamonas* spp. found in blood-feeding arthropods [25].

Epidemiology and Distribution

Although domestic dogs are known as the main hosts of *S. lupi*, several wild carnivores (Box 1) have been also reported to be infected with *S. lupi*. According to surveys and reports from dogs and wild canids, *S. lupi* is present in all continents, except for Antarctica (Figure 3). Stray, urban, adult, large

Box 1. Wild Carnivores and *Spirocerca lupi*

Wild carnivores have been reported to be infected with *S. lupi* (Figure 3 in main text, and Table I). Most of the reports have been based on microscopic identification of fecal eggs or the macroscopic observation of adults recovered from lesions, and not on detailed morphometric, histopathological, and molecular analyses [60,77–81]. Thus, the true taxonomical status of some specimens is uncertain. A study performed by our group demonstrated the importance of such analyses, since worms that were previously identified as *S. lupi* in red foxes, were found to belong to a novel species named *S. vulpis* based on morphometric differences, presence of adult worms mainly in stomach nodules and not the esophagus, and an average genetic variation of 9.2% in the *cox1* DNA sequence compared with *S. lupi* [7]. The diversity of the genus *Spirocerca* is likely to be greater than reported currently.

A study on genetically confirmed *S. lupi* infecting black-backed jackals (*C. mesomelas*) in South Africa suggested that these hosts are less susceptible than dogs to *S. lupi* due to the low pathogenicity of the infection and lesser degree of inflammation described in their lesions [65]. This suggests that black-backed jackals may not be major definitive hosts for *S. lupi*. The role of this and other carnivore species in the epidemiology of *S. lupi* should be studied further.

Table I. Summary of Carnivore Species Other Than the Domestic Dog (*Canis lupus familiaris*) in Which *S. lupi* Has Been Reported

Carnivore species	Country	Anatomical location of lesions	Prevalence	Refs
Vulpes vulpes	Azerbaijan	N.A.[a]	40.1%	[82]
	Greece	Stomach	N.A.	[78]
	Italy	Stomach	N.A.	[77]
	Portugal	Stomach	80%	[83]
	Spain	Nodules primarily in stomach, also in great omentum, mesenterium, pericardium	22.03%	(G. Sanchis-Monsonis, DVM thesis, University of Murcia, 2015)
	Ukraine	Nodules on stomach and esophagus walls	0.6%	[79]
Lycalopex culpaeus	Peru	Esophagus and aorta[b]	N.A.	[66]
Urocyon cinereoargenteus	USA	Aorta	20%	[81]
Canis latrans	USA	Esophagus and aorta[b]	83%	[80]
Canis mesomelas	South Africa	Aorta	17%	[65,84]
Canis aureus	Azerbaijan	N.A.	22.4%	[82]
	Iran	N.A.	2.5%	[60]
Canis lupus	Azerbaijan	N.A.	54%	[82]
	Poland	N.A.[c]	11.5%	[85]
	Slovakia	N.A.[c]	0.8%	[61]
Speothos venaticus	Argentina	Aorta	N.A.	[59]
Chrysocyon brachyurus	Brazil	Lung	N.A.	[62]
Procyon lotor	Poland	N.A.[c]	8.8%	[63]
Felix rufus	USA	Aorta	35%	[81]
Felis catus	United Kingdom	N.A.[c]	N.A.	[2]
Lemur, several species	Madagascar	N.A.[c]	N.A.	[86]

[a]Abbreviation: N.A., not available.
[b]Molecular analysis was done
[c]Diagnosis based on fecal examination.
N.A., not available.

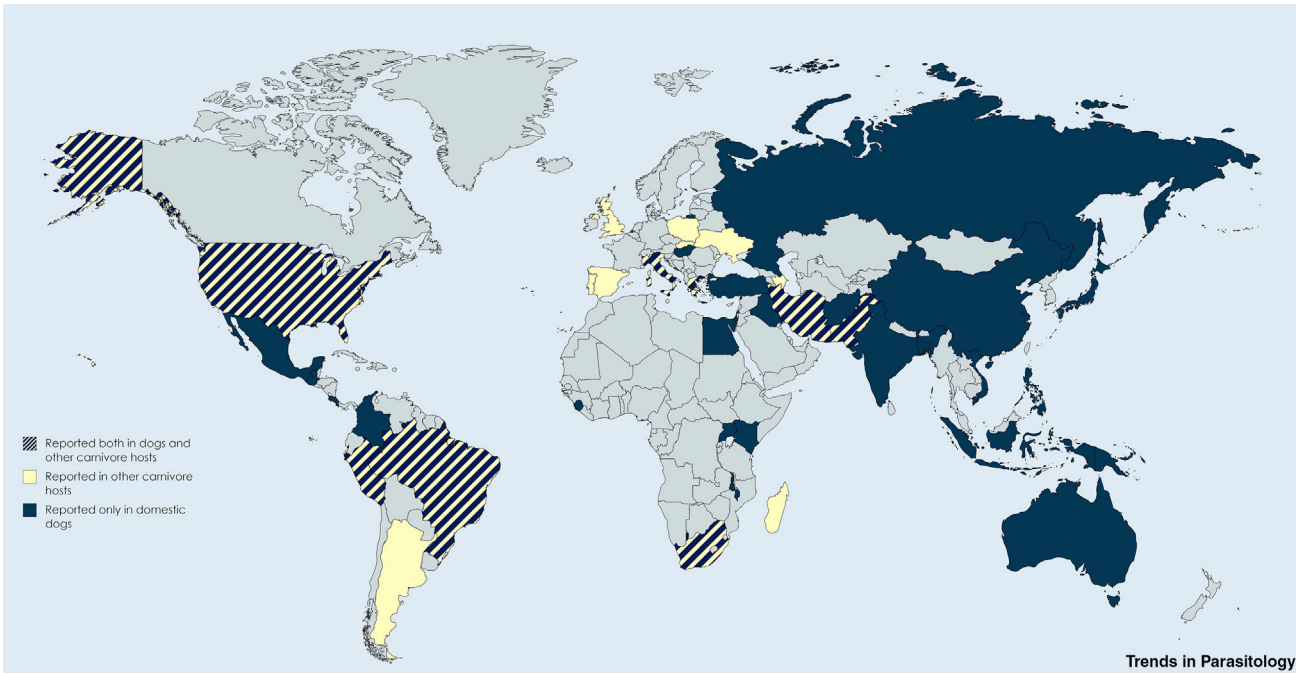

Figure 3. Global Distribution of *Spirocerca lupi*. Reports of *S. lupi* in domestic dogs, other carnivores, and both domestic dogs and carnivores are color coded.

References according to continents, countries, and regions are numbered in the reference list. Americas: USA [13], Mexico [7,80,98], Guatemala (LP. Palencia-Vides, DVM thesis, University of San Carlos, Guatemala, 2013), Costa Rica [87], Grenada [26], Colombia [88], Peru [66], Brazil [27,62], and Argentina [59]; Europe: Portugal [83], Spain (G. Sanchis-Monsonis, DVM thesis, University of Murcia, 2015), Italy [1,77], England [2], Greece [27,78], Poland [63,85], Hungary [4], Slovakia [61], Ukraine [79], and Turkey [27]; Asia: Azerbaijan [89], Afghanistan [90], Russia [91], Israel [6], Iran [60], Iraq [9], Uzbekistan [89], Pakistan [27], India [27,89], Bangladesh [92], Mainland China [52], Indonesia [13], Philippines [93], Japan [13], and Vietnam [94]; Africa: Sierra Leone [95], Egypt [13], Kenya [27], Uganda [96], Malawi [13], South Africa [64,65], and Madagascar [86]; Oceania: Australia [6] and Papua New Guinea [97]. This map was created using the web application www.mapchart.net.

breed, and hunting dogs have higher incidences of infection compared with household pets, small breed dogs, and puppies [3,26,27]. This probably reflects the fact that *S. lupi* infection is associated with the canine host's behavior, exposure to and ingestion of infected intermediate hosts, and the length of its prepatent period. The reported prevalence of *S. lupi* infection found in different studies is influenced also by the method of diagnosis, with higher incidences in necropsy surveys than in fecal coproscopy studies [28,29].

An increase in the distribution of *S. lupi* in dogs has been reported in recent decades in some countries, such as Israel [3] and Hungary [4], in which four- and sevenfold increases in the annual number of clinical cases has been reported, respectively. Contrasting results regarding the seasonality of *S. lupi* infection in dogs have been reported from different geographical regions. While studies from South Africa [30] and Hungary [4] demonstrated no seasonality patterns with a similar prevalence of infection during all seasons, in Israel [3] and Iran [31] ,most spirocercosis cases are detected during winter, whereas infections of the intermediate hosts are greater during summer [19,32].

Pathology, Inflammation, and Carcinogenesis

S. lupi induces several types of lesions in different tissues of its canine host, including aortic scarring with **aneurysm** formation, thoracic vertebral **spondylitis**, and caudal esophageal inflammatory nodule formation that may progress to sarcoma [27]. During its migration through the aortic wall, *S. lupi* induces tissue damage, inflammation, and subsequent scarring of the aortic wall. Mineralization of the scarred aortic wall causes increased rigidity with pockets of decreased resistance which form

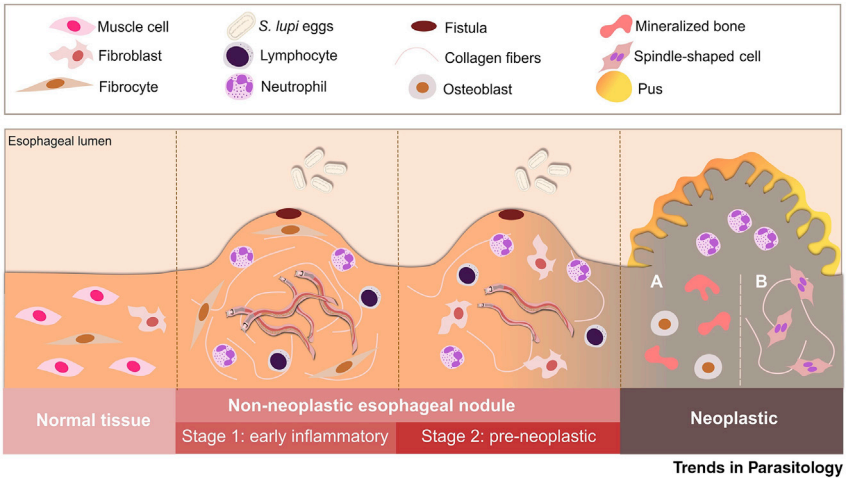

Figure 4. Progression of the Esophageal Lesions Caused by *Spirocerca lupi*.
In the early inflammatory stage, abundant male and female adults can be observed within the nodules with a large number of fibrocytes, neutrophils, and collagen deposition, and a smaller number of lymphocytes. In the pre-neoplastic stage, worms can still be observed accompanied by fibroblast infiltration, neutrophils, and lymphocytes. Finally, in the neoplastic stage, adult worms are rarely found in the lesions and progression to osteosarcoma (A) or fibrosarcoma (B) is usually observed. In osteosarcomas, osteoblasts and mineralized bone deposition are common in histological sections, whereas spindle-shaped cells with collagen fibers are detected in fibrosarcomas.

aneurysms [28]. Subsequently, blood clots that form in the aortic aneurysms can be released and lead to aortic iliac **thromboembolism** [29]. The rupture of aneurysms may cause rapid and fatal bleeding into the chest cavity (hemothorax) [32].

Spirocercosis is associated with osteoproliferative abnormalities in distant sites. Thoracic vertebral spondylitis is a unique lesion associated with *S. lupi* infection characterized by a proliferative periosteal reaction of the ventral thoracic vertebrae body with new bone formation that may bridge between thoracic vertebrae. In addition, hypertrophic osteopathy of the thoracic limbs is observed in dogs, in which the periosteal membrane around the long limb bones proliferates and creates new bony prominences. This presentation is frequently reported in dogs with neoplastic spirocercosis [33]. Since there is no evidence for *S. lupi* migration into these anatomical sites, it has been suggested that the lesions are induced by osteoproliferative growth factors released by the worm or inflammatory mediators [34].

S. lupi infection is also associated with salivary gland enlargement and manifests clinically as excessive drooling of saliva or sialorrhea [33]. Histopathological analyses of enlarged salivary glands revealed acinar hyperplasia hypothesized to be induced by stimulation of the vagal nerve during *S. lupi* insult to the esophagus. This insult leads to the formation of tissue response in the mediastinal space where this nerve also passes [35].

The esophageal nodule formed during *S. lupi* infection progresses from early inflammatory to pre-neoplastic and eventually neoplastic (Figure 4). The non-neoplastic (early inflammatory and pre-neoplastic) esophageal nodule initially develops in the location where the adult worm is primarily located in the mediastinum, and part of it protrudes into the esophageal lumen. The luminal portion of the nodule is smooth and possesses a protuberance with a crater-like structure from which the adult female lays its eggs. Histopathological studies have shown that in early inflammatory lesions, the connective tissue is composed mostly of fibrocytes and large amounts of collagen, whereas in pre-neoplastic nodules, it contains immature proliferating fibroblasts and a reduced amount of collagen. Moreover, pre-neoplastic nodules show an increased mitotic index and higher numbers

of multinucleated cells. In addition, 69% of non-neoplastic *S. lupi* esophageal nodules contain adult worms [36]. The worms are found in connective tissue surrounded by pus pockets that contain neutrophils and a smaller number of lymphocytes that are frequently organized in small foci [37]. Non-neoplastic *S. lupi*-induced esophageal nodules have sometimes been incorrectly referred to as granulomas [9]. However, the main feature of a granuloma is the presence of organized macrophages and granulation tissue [38], while *S. lupi* nodules contain predominantly neutrophils, and B and T lymphocytes with no evidence of granulation [36]. Osseous metaplasia is observed in the transition between the pre-neoplastic and neoplastic stages and it is an indication of the progression from benign to malignant nodules in spirocercosis [36].

Over time, approximately 25% of the esophageal nodules undergo neoplastic transformation, with subsequent metastasis to other organs [39]. The malignant nodule loses its smooth appearance and becomes lobulated and necrotic and may reach up to 11 cm in length [3,36]. In geographical regions considered as spirocercosis free, malignant neoplasms of the esophagus are rare in dogs and are usually classified as carcinomas. Thus, spirocercosis is considered a major cause of malignant esophageal neoplasia in domestic canines and therefore a natural model for helminth-induced cancer [40].

Four types of malignant sarcomas have been described in spirocercosis, namely osteosarcoma, fibrosarcoma, undifferentiated sarcoma [36,41], and chondrosarcoma [42]. Histological analyses of malignant esophageal sarcomas in spirocercosis show abundant malignant spindle cells and a high mitotic index and can be clearly distinguished from non-neoplastic nodules. Furthermore, adult worms are observed only in 15% of the sarcoma cases [36]. Osteosarcomas are the most common sarcomas associated with *S. lupi* infection [36] and the histological characteristics of these tumors include foci of polygonal osteoblasts, variable numbers of multinucleated osteoclasts and presence of osteoid matrix, with or without foci of chondroid differentiation. In addition, fibrosarcomas associated with *S. lupi* infection are histologically characterized by intercellular collagenous matrix, bundles of pleomorphic cells that vary in their matrix appearance, and a high mitotic index [43]. Undifferentiated sarcomas are characterized by the presence of malignant spindle cells without the presence of abundant intercellular matrix [36], and *S. lupi*-associated chondrosarcomas have been described as containing proliferative atypical chondroblasts within a cartilaginous matrix [42].

Studies have shown that different disease markers are associated with the presence of *S. lupi*-induced neoplastic nodules as compared with dogs with non-neoplastic lesions. Dogs with neoplastic nodules have higher blood neutrophils counts [44], higher levels of serum C-reactive protein [45,46], vascular endothelial growth factor (VEGF) [44,47], and interleukin (IL)-8, a proinflammatory chemokine produced by macrophages and other cell types [48]. Additionally, several growth factors associated with other proliferation processes have been shown to be expressed in *S. lupi* esophageal nodules, including VEGF, fibroblast growth factor, and platelet-derived growth factor [44,47]. Altogether, these markers indicate a strong proinflammatory response when spirocercosis is associated with neoplasia.

Aberrant migration of *S. lupi* and its manifestations in different organs have been widely reported in canine spirocercosis. It has been described to affect multiple organs from the rectum [49] to the central nervous system [14,50]; most commonly involving thoracic organs as well the urinary system [16] and subcutaneous tissues [15]. Aberrant migration to the spinal cord has been reported to produce a unique clinical syndrome of painful, asymmetric, progressive paralysis with eosinophilic infiltrate in the cerebrospinal fluid [14,50]. In addition, treatment with milbemycin oxime has been found to interrupt the worm's migration route and thus, treatment and incomplete elimination has been suggested as one of the causes for aberrant migration [51].

Genetics of *S. lupi*: Population Studies and Phylogenetic Analysis

Sequencing of the mitochondrial genome of *S. lupi* and some of its major genes has improved the understanding of its phylogenetic relationships with other relevant human and veterinary nematodes.

The mitochondrial genome of *S. lupi* [52] revealed details of genes encoding 12 proteins, 22 tRNAs, and two rRNAs. Since its description, several of these genes have been widely used for diagnostic purposes [48,53], population studies [54], and phylogenetic analyses [6]. Furthermore, the gene composition and its synteny have proved to be conserved and closely related to similar genes in other members of the Thelaziidae and Onchocercidae families, which include the canine and human pathogens *Thelazia callipaeda* and *Wuchereria bancrofti* [52].

Phylogenetic and population studies have increased the understanding of the epidemiology, phylogeny, and evolution of *S. lupi*. This knowledge has ultimately led to the description of a novel *Spirocerca* species and the detection of *S. lupi* genotypes. It has been shown that *S. lupi* possess a large genetic variability in its mitochondrial genes, rDNA, and microsatellite markers [6,54,55]. This diversity has been used to study *S. lupi* specimens from the same geographical locations [54,55] as well as from different countries [6]. *S. lupi* specimens of dogs from South Africa separated up to 25 km apart showed an overall low **genetic structure** and high differentiation within **infrapopulations** compared with the infrapopulations of different hosts [54]. This indicates that in a restricted geographical area, the genetic composition of the worm homogenizes in the newly infected hosts, leading to high homozygosity of *S. lupi*. In addition, specimens obtained from dog populations separated over 100 km in South Africa seem to undergo frequent **admixture** when hosts become infected and the subsequent adults mate randomly in them [55]. This genetic structure analysis accompanied by mathematical modeling has proposed that if a new mutation conferring drug resistance arises in *S. lupi*, a rapid spread will occur over short and long ranges [55].

Analyses of *S. lupi* from dogs in different continents have provided a wide perspective of the phylogenetic interactions within this worm species [6]. Nucleotide differences in the ***cox1*** and internal transcribed spacer (ITS)-1 loci separated *S. lupi* into two genotypes: genotype I, grouping specimens from Israel, India, South Africa, and Australia; and genotype II, including European specimens from Hungary and Italy [6]. The South African and Israeli specimens studied seemed to share a common ancestor that might have derived from the migration of intermediate, paratenic, or definitive hosts between these countries, potentially via African animals imported to a safari park in Central Israel, near which an outbreak of this infection started in dogs [56]. Furthermore, the large intergenotypic variability found among *S. lupi* specimens suggest that *S. lupi* genotype II might represent a **cryptic species**, since there are no evident differences in morphological structures, but a high nucleotide variation compared with genotype I. The circulation of two *S. lupi* genotypes among dog populations might have originated from the migration of infected hosts to new regions. Subsequently, species diversification might have occurred due to the low prevalence or reduced gene pool within each geographical region, which could lead to genetic drift of specific markers in parasitic populations [57].

It has been suggested that *S. lupi* is a parasite of domestic dogs that has spilled over to wild animals (Box 1) [58]. However, it is unknown whether *S. lupi* originated from dogs and later infected other wild canids, or vice versa. Additionally, it is uncertain if some *S. lupi* reports in wild canids [59–63] represent different *Spirocerca* spp., since most studies lack genotypic screening, with the exception of reports from the red fox from Spain and Bosnia and Herzegovina [7], the black-backed jackal from South Africa [64,65], and the Andean fox from Peru [66]. A comprehensive anatomical and genetic characterization of *Spirocerca* specimens collected from the red fox (*Vulpes vulpes*) in Spain and Bosnia and Herzegovina revealed that these nematodes represent a novel *Spirocerca* species, *Spirocerca vulpis* [7]. In addition to the genetic differences found in the *cox1* and ITS-1 loci DNA sequences between *S. lupi* and *S. vulpis*, which were 6.8% and 6.1%, respectively, a morphological trait that aids in the differentiation between *S. lupi* and *S. vulpis* is the presence of teeth-like structures emerging from the buccal capsule in *S. vulpis* and their absence in *S. lupi*. This is evident by light as well as electron microscopy (Figure 2A,B) [7]. Moreover, *S. vulpis* is predominantly located in the gastric mucosa, whereas *S. lupi* adults are mostly found in the esophageal nodules. Exceptionally, *Spirocerca* sp. worms reported from the Andean fox, *Lycalopex calpaeus*, in Peru are genetically closely related to *S. lupi* from Israel and also located in esophageal nodules [6]. These findings pose question marks on the evolutionary and migratory path of *S. lupi* from the Old World to the New World, or vice versa. *Spirocerca* sp. eggs were found in coprolites of a puma, *Puma concolor*, or a jaguar, *Panthera onca*,

from about 1000 BC in a cave in Argentina [67]. This constitutes the oldest record of *Spirocerca* sp. in animals and further suggests that *Spirocerca* spp. might have originated in the American continent and then spread to the Old World.

Based on the observation of shared haplotypes in specimens of *S. lupi* collected from domestic dogs and black-backed jackals, *Canis mesomelas*, in South Africa, it is possible that both species can be infected by *S. lupi* [64]. However, the role of black-backed jackals as definitive hosts of *S. lupi* is still unresolved since only immature stages of the helminth have been recovered from the aorta and gastric mucosa of jackals and these lesions are associated with mild pathology [64,65]. Hence, a meticulous morphological study of worms and genetic analysis, especially in those collected from wild animals and those that show high nucleotide variations in comparison with other well-characterized specimens, is required for a better understanding of the distribution, evolution, and ecology of *Spirocerca* spp.

Advances in Diagnosis of Spirocercosis

Spirocercosis is usually diagnosed by interpretation of the dog's clinical history and signs, the observation of nodules by image-based methods (i.e., radiology, endoscopy, and computed tomography) as reviewed elsewhere [68], and the microscopic identification of *S. lupi* eggs in fecal samples of infected dogs. These diagnostic tools used to date have their advantages and disadvantages [68]. For instance, endoscopy is currently the gold standard to detect cases of canine esophageal spirocercosis since it detects non-neoplastic nodules as well as tumors, and is usually able to distinguish between them, but the requirement for complete anesthesia of the dog and the high cost of performing it may hinder its large-scale implementation in non-affluent settings. By contrast, microscopy methods are inexpensive and fast to perform. However, the identification of *S. lupi* eggs either by microscopic or molecular assays is useful only in cases of esophageal nodules containing viable female worms, are subject to the intermittent shedding of eggs into feces, and are unable to detect cases of single-sex infections [48,53]. Shedding of eggs may be absent in the presence of neoplastic esophageal masses induced in spirocercosis. Furthermore, it has been observed that sera obtained from *S. lupi*-infected dogs may crossreact on commercial serological assays which detect circulating antigens of *Dirofilaria immitis* [69]. Thus, sensitive, specific, and inexpensive assays are needed to overcome these challenges, and to assist the detection of esophageal *S. lupi* and its aberrant migration.

In the last decade, three DNA-based assays targeting microsatellites and rDNA have been used to accurately identify different life stages of *S. lupi* [48,53,70]. A multiplex PCR targeting nine polymorphic microsatellites [70] found only in *S. lupi* has been used to identify and genotype *S. lupi* adults and immature stages collected from canid hosts [55,65]. Additionally, two high-resolution melt analysis real-time quantitative PCRs (HRM-coupled qPCRs) targeting fragments of the *18S* and ITS-1 loci have demonstrated high sensitivity for the detection, confirmation, and quantification of *S. lupi* eggs from the feces of naturally infected dogs at concentrations as low as 0.2 eggs/g. Moreover, these HRM qPCRs have also been useful in identifying *S. lupi* in spinal cord tissues [14] and cerebrospinal fluid [71] of dogs with intraspinal spirocercosis.

Treatment

Benign spirocercosis is successfully treated with avermectins, such as doramectin (Dectomax, Zoetis, France) 400 μg/kg subcutaneously at 2-week intervals [72]. As treatment with doramectin is off-label for dogs and toxic for those who have multidrug resistance gene mutations [73], other drugs have been studied including milbemycin oxime [74] and a spot-on combination of 10% imidacloprid/2.5% moxidectin (Advocate, Bayer, Germany; Advantage Multi, Bayer Animal Health, USA) [75]. These drugs have shown a good degree of success as therapeutic and prophylactic agents [74,75] compared with an apparently higher success rate with doramectin [75].

Malignant esophageal neoplasms can be treated by surgical excision and additional chemotherapy [41]. Surgery is recommended only when there is no evidence of metastasis and the tumor is located in an excisable position in the esophagus. Partial esophagostomy has shown good results, whereas

total esophagostomy and end-to-end anastomosis has resulted in poor outcome [41]. Endoscopic laser-assisted esophageal tumor excision has been described, with the same prognosis as surgery, fewer complications, and reduced expense [76].

Concluding Remarks

Studies on canine spirocercosis in the last decade have resulted in improved understanding of the life cycle of its causative agent and the pathological events that take place during the formation of parasitic nodules in the host's esophagus and ensuing malignant tumors. Highly sensitive and specific molecular assays have been developed to detect *S. lupi* DNA in feces and tissues and improved treatment of both benign and neoplastic spirocercosis has been described. Genetic studies have characterized parasite genotypes and indicated the separation of *S. lupi* from the new species *S. vulpis* infecting red foxes in Europe. Genetic evidence suggests that *S. lupi* might be a species complex, composed of several genotypes, and cryptic species, and that there are potentially additional different *Spirocerca* spp. infecting wild canids (see Outstanding Questions). Additional studies are required to investigate the immunomodulation exerted by *S. lupi* during the induction of neoplastic transformation in dogs, the possible role of its excreted–secreted molecules in this process, and to further improve the diagnosis and prevention of infection.

Outstanding Questions

What are the molecular mechanisms underlying the progression of *S. lupi* nodules towards osteosarcoma, fibrosarcoma, and other neoplastic conditions?

Does the genome of *S. lupi* encode for pro-oncogenic proteins that participate in the neoplastic transformation of nodules?

What key molecules secreted by *S. lupi* effect the immune response towards the parasite in dogs?

Is there a difference in pathogenicity among *S. lupi* genotypes?

Do additional *Spirocerca* species infect domestic dogs and wild canids?

Does the endosymbiont *Comamonas* sp. have a role in the course of the immune response against *S. lupi*?

Is *Comamonas* sp. present in all *S. lupi* genotypes?

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