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SPIROCERCOSIS IN DOGS IN ISRAEL

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ABSTRACT

***Spirocerca lupi* is a nematode causing *spirocercosis* disease that affects mostly carnivores and especially *canidae*. The life cycle of *S. lupi* includes a coprophagous beetle as an obligatory intermediate host and a variety of facultative paratenic hosts. In Israel, spirocercosis is considered to be a serious condition with a variety of clinical signs comprising a great risk to canine populations. The diagnosis at an early infective stage is unreliable and the vast majority of infected dogs are diagnosed only when the disease has advanced. In advanced stages of the disease, treatment is difficult and there is a high risk for complications. A study was carried out to compare the prevalence of *S. lupi* in the central region of Israel with a previous investigation and by that consequently try to estimate the efficacy of preventative treatment used nowadays in Israel. The study was done by the use of two different methods: looking for the infective larvae (L3) in the main intermediate host in Israel, *Ontophagus sellatus*, and searching for the eggs of *S. lupi* by performing flotation methods on faecal samples. Beetles and faecal samples were collected from four different locations in the winter and summer of 2017, 2018, and 2019. According to the literature review and collection of data from case studies, the prevalence of spirocercosis is increasing in Israel, despite the negative results from the dissections of beetles and faecal samples.**

Key words: coprophagous beetle; diagnosis; nematode; *Spirocerca lupi*

INTRODUCTION

Spirocerca lupi is a nematode causing spirocercosis which is a disease considered to be a serious condition with a variety of clinical signs, mostly as a result of the migration and persistence of the larvae or adult worms in the final host. The most commonly reported clinical signs are regurgitation and vomiting [10]. Sudden deaths may occur and are associated with the rupture of the aortic aneurysms caused by the parasite migrations. *Spirocercosis* is diagnosed based on the history, clinical signs, thoracic imaging,

conventional and molecular coproscopy, endoscopy and necropsy [18].

The life cycle of *S. lupi* includes a final host (mostly dogs, foxes, wild canids, wild felids and occasionally domestic cats), an intermediate host (coprophagous beetles), and many other vertebrates such as rodents, birds, chickens and reptiles which can act as paratenic hosts. Larval stages L1—L3 are in the intermediate host and larval stages L3—L4, L5 (young adult) and sexually mature adults are in the definitive host [17].

After ingestion of the intermediate or paratenic host by the final host, the L3 are liberated in the stomach and penetrate the gastric mucosa 2 hours after ingestion. The *larvae* migrate in the walls of the gastric arteries and reach the caudal thoracic aorta approximately 10 days after hatching and remain there from days 10 to 10^9 while maturing to L4. About 3 months post infection the *larvae* leave the aorta and migrate to the oesophagus where they cause irritation and as a result, the beginning of the development of granulomas as they develop to the adult stage in a further 3 months (days 93—227 post infection) [18]. The adult worms are found coiled in nodules in the submucosa and adventitia of the oesophagus. The female worm perforates the mucosa, and by that making an opening to the lumen of the oesophagus through which future eggs can pass, then moves back to the submucosa or muscular layers to complete development. A nodule eventually develops around the worm. Mature oesophageal nodules containing adult worms are present by 3—9 months post infection. The adult worm can remain in the oesophagus for up to 2 years. The eggs do not hatch until ingested by coprophagous beetle. In the intermediate host, the larvae encyst within the tissues and develop to the infective L3. Depending on the beetle and the environmental conditions (temperature, pH, RH), this process can take between 7 days to 2 months. Paratenic hosts may also be involved if the beetle is ingested by any of

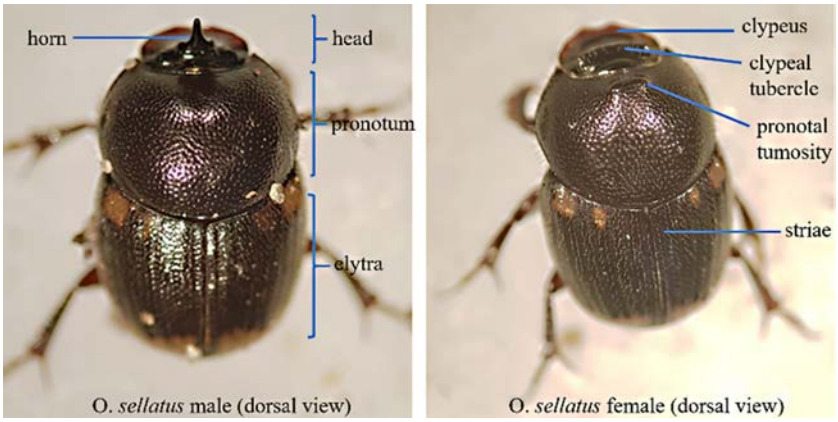

Fig. 1. *Onthophagus sellatus* dorsal view, male and female
Source: Neta Geva

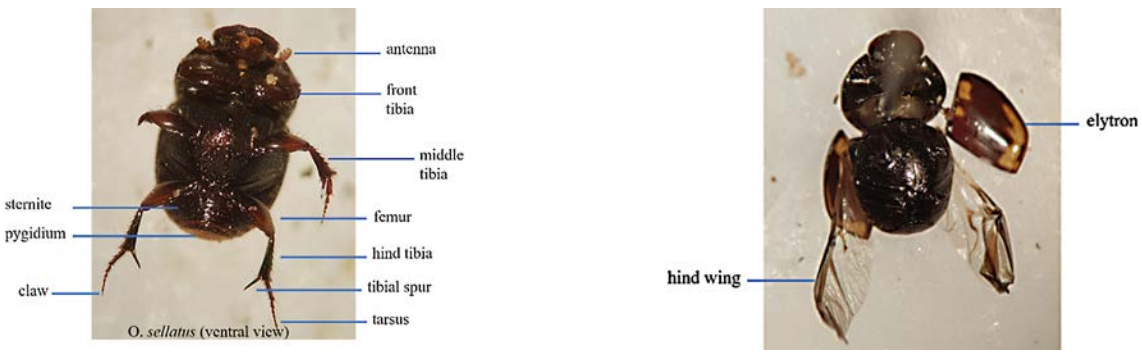

Fig. 2. *Onthophagus sellatus* ventral view and wings
Source: Neta Geva

a variety of other animals. In the paratenic hosts L3 excyst and then re-encyst within host tissue where they appear as small white nodules. In the initial 4 days of infection, larval migration is non-directional and they may be found within the veins and lymphatics of the gastric wall. This can result in aberrant migration to other organs like the stomach, intestine, mediastinum, diaphragm, heart, lung, kidney and anal mucosa. Migration to the nervous system is confined to the spine involving extradural and intradural reported cases [3, 10, 15, 17, 18].

Onthophagus sellatus is the most prevalent dung beetle species, with the highest numbers recorded in parks in Israel, between July and October and was the only coprophagous beetle species to harbour *S. lupi larvae*, indicating its role as the intermediate host for *S. lupi* in Israel [5]. *O. sellatus* is a 6 mm long beetle. It has a black coloured elytra with striate and typical four (square looking) orange spots on the upper elytra and orange pigment on the lower extremities of the elytra. The hind wings are fully developed and it bears three pairs of fossorial legs. The outer edge of the front tibia possess 4 tibial teeth intended for digging [8]. The male is differentiated from the female by cephalic horn which is distinctly angled at its base (Figs. 1, 2).

S. lupi is found worldwide especially in tropical and subtropical regions. The majority of reports are from Israel, Greece, Turkey, India, Pakistan, the southern United States, Brazil, Kenya and South Africa. Furthermore, in the last few years it has become of growing concern in Europe after records of *Spirocerca lupi* have been described in Belarus, Bulgaria, Hungary, Italy, Portugal, The Netherlands, Spain, Ukraine and the European parts of Russia. In 2005 for the first time, *S. lupi* was identified in Poland in grey wolves and in 2016 in Slovakia [2, 14, 16].

The main problems regarding *spirocercosis* is the lack of medication for prevention against the parasite, and the fact that diagnosis in early stages can be challenging and most animals are only diagnosed once the advanced disease is present. The raising occurrence and distribution of its obligatory intermediate host (the coprophagous beetle) is another reason for concern.

The aim of this study was to compare the prevalence of *S. lupi* in central Israel with the previous situation and, on the basis of the results, try to estimate the efficacy of preventative treatment used nowadays in Israel. Comparison was done by dissections of *Ontophagus sellatus* coprophagous beetles and faecal examination of dogs.

MATERIALS AND METHODS

All samples were collected in Israel on four different occasions and in different locations in the centre of Israel. Faecal samples were collected in December 2017, August 2018 and January 2019. Coprophagous beetles were collected in August 2018.

Collection of coprophagous beetles

Around 200 beetles were collected in a period of one week from four different parks in the centre of Israel: Park Yom Hkipurim in Ramat Gan, Park Josef, Ein Yhav and Hharnav mamushi in Holon. These parks are known to be endemic for *spirocercosis* based on previous surveys [5]. Coprophagous beetles were collected in an area where dog faeces were left in the park. The beetles were found in a few hours in old dog faeces left on the grass. Dog faeces with beetles were collected with a garden shovel and placed into plastic buckets with 20 cm of soil that had been dug from beneath the faeces. After collection from the parks, the beetles were counted and transferred to other plastic buckets. Altogether nine plastic buckets were prepared in advance in order to provide suitable conditions for the survival of the beetles until dissection. Small holes were made in the lid and the soil was filtered and dampened. Twenty five beetles were placed to each bucket. Approximately 20 cm layer of soil and a small portion of dog faeces were placed in the buckets. They were kept at 25 °C with cycles of light: dark—14 h: 10 h. Every three days the beetles were fed with dog faeces and the humidity was kept by spraying water on the surface of the soil.

Collection of faecal samples

A total of 35 faecal samples were collected: 10 during December 2017, 5 during August 2018, and 20 during January 2019. Twenty samples were collected randomly from four public parks in three cities in the centre of Israel: Holon, Tel-Aviv and Ramat Gan. An additional 15 samples were brought to a veterinary clinic in Holon, Belinson for Animals, by clients. The samples brought to the clinic in Holon for examination were collected in plastic bags. Every sample was checked on the day of arrival to the clinic.

Coprological examination

In order to identify *S. lupi* eggs in the faeces, a sugar flotation method was used for all 35 samples [9].

Dissection method

To identify encysted L3 of *S. lupi* in the coprophagous beetles, 150 beetles were dissected. The aim of the dissection was to determine whether the beetles harbour *S. lupi* larvae. Individual beetles were recorded as being either positive or negative for infection (Fig. 3).

Table 1. Results from dissection of beetles

Number of *O. sellatus*	L3 *Spirocerca lupi*	Mite	*Sudhausia crassa*
Male — 82	–	2	13
Female — 68	–	5	8

Results from coprological examination

RESULTS

The dissection was performed in Prof. Gad Baneth laboratory in the Hebrew University of Jerusalem in Rehovot, over a period of eight days. Approximately 20 beetles were dissected per day with a total of 150 beetles dissected. In the tissue of 21 beetles, very small live nematodes were observed during dissection and were diagnosed as *Sudhausia crassa*. Microscopical examination identified *S. crassa* in 14 % of the beetles. Mites from the genus Poecilochirus in the family Parasitidae, were found on 7 beetles (4.67 %). All 150 beetles were negative for *S. lupi* larvae.

Altogether 35 faecal samples of random dogs were tested and were negative for *S. lupi* eggs.

DISCUSSION

Spirocercosis in Israel is a problem occurring over the years with the first cases reported in 1934. *Spirocercosis* was common in dogs mainly in the area of Jerusalem. No cases were recorded between 1948 and 1980, and only 6 cases were reported between 1980 and 1989. Suddenly in 1989, *S. lupi* was found in 4 dogs from Ramat Gan (a town bordering with Tel Aviv in the centre of Israel), and since then the infection incidences had sharply increased. Additional cases were reported later on [1]. *Spirocercosis* was considered an endemic problem during 1990—1999, with 50 cases diagnosed at the Hebrew University of Veterinary Teaching Hospital (HUVTH) [10].

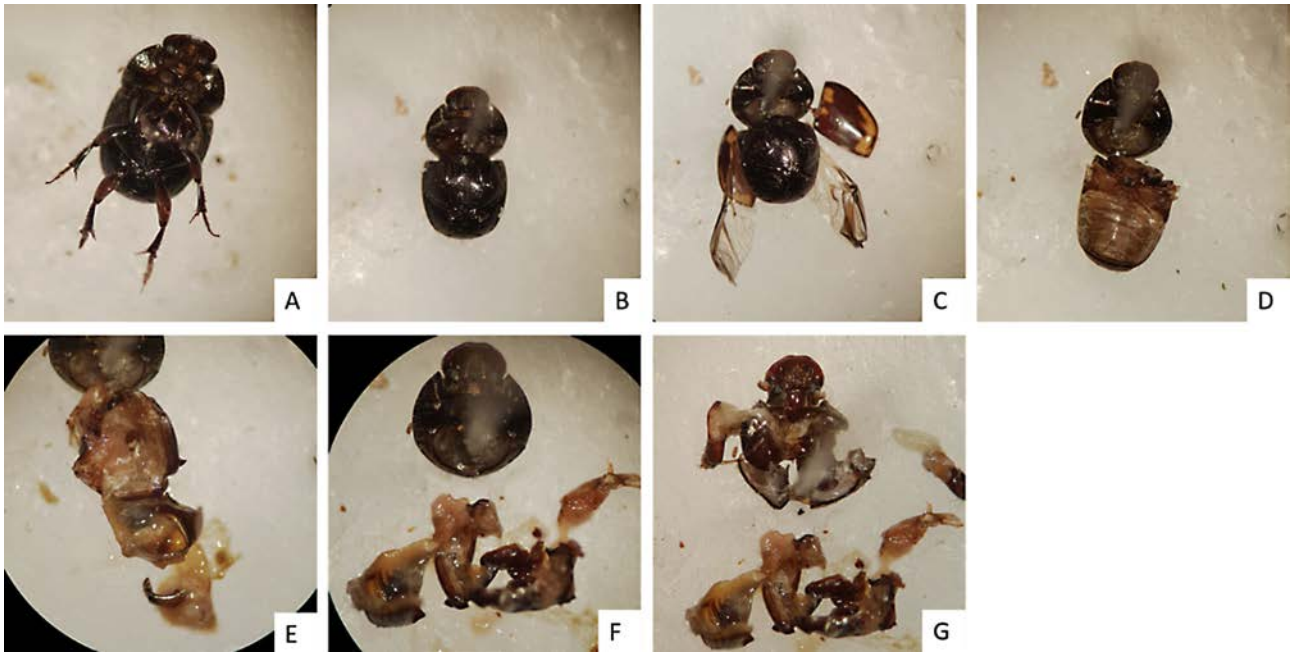

Fig. 3. Dissection of beetles procedure
Source: Neta Geva

A) Stabilization of beetle on petri plate by a holding pin in the pronotum; B) Removal of the legs; C+D) Removal of the elytron and wings; E) Cut opening of the abdomen and spreading of content; F) Opening of pronotum; G) Opening of head

From 1993 the infection started to spread to other areas in the country, first to the neighbouring cities of Tel Aviv and Givataim and later to other cities nearby. By the year 2000, infected dogs were diagnosed with *spirocercosis* in the whole central part of Israel, spreading to a range of 60 km, with the southeast occurrence in the town of Ashdod and the northern in the town of Natanya. Between 2005 and 2008 the infection had spread as far as Haifa and Naharya in the north of the state, and as far as Beer Sheva and Arad in the southern part of Israel. These towns are more than 100 km from Ramat Gan, where the infection was first diagnosed in the early 1989 indicating that most of the populated area of Israel is infected and the entire dog population is at risk of infection [1]. *Spirocerca lupi* affects mainly canines in many countries worldwide. Clinical cases were reported in a large number of countries including South Africa, Israel, Argentina, Brazil, Kenya, Iran, India and the southern USA.

Of the factors affecting the global prevalence of the parasite, the most important are the intermediate and paratenic hosts proximity to the definitive host and their population density [13].

Regarding the other factors affecting the prevalence of reported cases, there is no relation to the sex of the dog. However, with regard to age, dogs more than 5 years of age are at higher risk of infection compared to dogs below 1 year of age, reflecting the increased probability of exposure. Moreover, infected dogs under 6 months of age do not develop oesophageal disease and the classical clinical signs of *spirocercosis* are absent [1].

Clinical *spirocercosis* occurred more in large breeds and there seems to be a breed predilection with higher incidences in German Shepherd and Labrador Retrievers as shown in studies conducted in South Africa and Israel [12]. Stray dogs were found more infected than households pets presumably because of higher exposure to the intermediate or paratenic hosts [10]. Between the years 2000—2006 a study was made in Slovakia to determine the prevalence rate of certain parasites in red foxes from the entire territory of the Slovak Republic. A total of 1198 faecal samples were collected from the rectum or colon of foxes (*Vulpes vulpes*) and the samples were investigated using sugar centrifugation flotation technique. *Spirocerca lupi* eggs were found in 11 (out of 1198) samples with the highest positivity (3.6 %) in Žilina region bordering with Poland [11]. In another study performed in 2016 in Slovakia, 256 faecal samples were collected from grey wolves (*Canis lupus*) in three areas in Slovakia and examined using flotation method with zinc sulphate solution. Out of the 256 samples, 2 were positive for *S. lupi*. This study provides important findings for further epidemiology research in the grey wolf population [2].

The first time *S. lupi* was diagnosed in Poland was during the studies of the helminth fauna of wolves in the autumn of 2005. Eighty six faecal samples were examined. *Spirocerca lupi* was detected with the flotation techniques demonstrating a prevalence of 2.32 %. This was the third case of *S. lupi* occurrence in the wolf (*Canis lupus*) within its distribution range. The infected wolf populations came from two distant regions in Poland: Rzepin Primeval Forest and Roztocze [16].

A retrospective study that was done in the years 2007—2016, investigated the prevalence and treatment outcome of Hungarian dogs with oesophageal *spirocercosis* in 30 cases. More than two thirds of the cases were diagnosed in Budapest and the surroundings. The yearly distribution of case numbers varied between 0 and 8, with 2007 being the year with the least (0) cases, whereas 2015 being the year with the most diagnosed cases (8). The study showed a significant increase in the number of dogs with *spirocercosis* in Hungary between 2007 and 2016 [14].

The definitive diagnosis of *spirocercosis* usually consists of the detection of the characteristic eggs by faecal flotation. Due to the very small size of the eggs, it is difficult to detect them in the direct faecal preparations [18].

The sensitivity of the faecal flotation could be low due to unpredictable and intermittent egg shedding. Eggs can be found for a relatively short period, between 140 to 205 days post infection, and only if the adult female have perforated the oesophageal nodule. In the case of a negative result, it is recommended to repeat the faecal flotation examination after several days [10]. The eggs of *Spirocerca lupi* are elongated and with a thick smooth shell. The egg size is small with typical measurements of 20—37 µm by 11—18 µm and have a unique shape of a "paper clip" [4]. When laid, the eggs contain a larva (L1). The eggs can be found mostly in the faeces and occasionally in vomitus [17].

To this date, no drug is effective in killing both adult and larval stages of *S. lupi* without causing side effects to the host. Diethylcarbamazine was the first anthelmintic used for the treatment of *spirocercosis*. It was shown to be effective in reducing the clinical signs of vomiting and regur-

gitation in dogs with oesophageal nodules and suppressed egg shedding. However, it did not affect the adult worm [1].

Disophenol killed adult worms in nodules, but the drug was not effective against larval stages and had a narrow margin of safety and is no longer available for use. A combination of nitroxynil and ivermectin administered subcutaneously was reported to be successful in treating infected dogs in Reunion in 81.6 % of the cases. Doramectin was shown to have good efficacy under clinical conditions and to this day it is administered as an injection of 400 $\mu g.kg^{-1}$ subcutaneously every 14 days until resolution [1].

Prevention includes periodic prophylactic treatment of dogs with avermectins and other macrolidic lactons, prohibiting dogs from preying on paratenic hosts or eating faeces and collecting dog faeces [6].

Surgery is only required in cases where the nodules have undergone neoplastic transformation. There are high complication rates in oesophageal surgery including: excessive tension at the suture line, lack of serosa, constant motion of the suture site, passage of undigested food or saliva over the suture site, segmental blood supply and lack of the omentum. The survival rates are low, and usually this procedure is not recommended [18].

Doramectin is the main medication used today for prevention. However, it does not have a 100 % efficacy. In a study that has been done to evaluate the prophylactic effect of doramectin, dogs were injected subcutaneously with doramectin (400 $\mu g.kg^{-1}$) once every month for three months, and then inoculated with infectious *S. lupi larvae* (L3) one month after the last doramectin treatment. Doramectin did not completely prevent *spirocercosis*, however it resulted in fewer oesophageal nodules, reduced clinical signs and reduced significantly the egg shedding [7].

In another study performed in 2010, a monthly application of spot-on, with a combination of imidacloprid 10 % and moxidectin 2.5 %, was applied on puppies aged 2—4 months, for a period of 9 months and was shown to be effective and well tolerated for the prevention of *spirocercosis* [7]. In Israel, doramectin or ivermectin at 200 $\mu g.kg^{-1}$ is given as preventative therapy, administered subcutaneously every two or three months. The efficacy of this preventative treatment is uncertain and more investigation need to be done regarding its effects on the incidence and prevalence of *spirocercosis* in Israel [1].

CONCLUSIONS

Efficient prevention and control depends on understanding the interactions between the organisms involved in the epidemiology of *spirocercosis*. This includes periodic prophylactic treatment of dogs with avermectins and other macrolidic lactons, prohibiting dogs from preying on paratenic hosts or eating faeces. Control is difficult because of the wide distribution of the intermediate and paratenic hosts. Control of coprophagous beetles is not easy to perform due to its high distribution and variety of suitable hosts that can transmit the infection.

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